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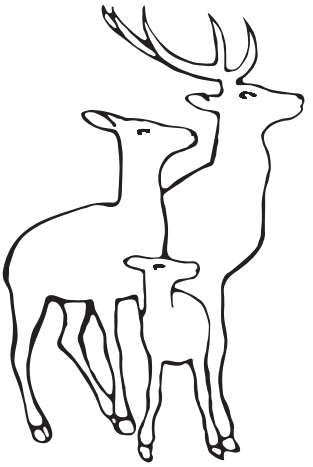

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Review

Ophthalmologic approach to babies with cerebral visual impairment

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ABSTRACT

Background. Cerebral visual impairment (CVI) is an increasingly common type of visual disturbance in infants and children. The increased incidence is associated with improved neonatal intensive care services and survival of premature infants, especially in developed countries and our country. For accurate ophthalmological evaluation, it is important to know the behavioral responses to visual stimuli that are unique to CVI such as color preference, need for movement, visual latency, visual field preference, and difficulty in visual complexity.

Methods. This review is prepared to draw attention to the subject within the scope of Ankara University Vision, Artificial Vision and Low Vision Rehabilitation training programs.

Results. The most common causes are hypoxia and perinatal ischemia. Ocular structures are generally normal or are not sufficient to explain the visual impairment. Diagnosis and habilitation methods differ from visual impairment of ocular origin. As a result, early diagnosis of CVI in infants and toddlers and an effective visual habilitation with a multidisciplinary approach where ophthalmologists and pediatricians lead the team is very valuable.

Conclusions. Early diagnosis and early visual habilitation will increase the quality of life of babies and will provide important gains for families and therefore the whole society. Pediatricians should be familiar with this group of disturbances and the available resources, as they are best placed to refer the child for evaluation and rehabilitation and encourage the family for follow-up.

Key words: visual impairment, cerebral, low vision, visual habilitation.

Visual impairments in infants and toddlers can be seen due to ocular and cerebral causes or both. Cerebral visual impairment (CVI) is a disorder that we have encountered more commonly in infants and toddlers, recently. This situation is closely related to the increase in neonatal care services and keeping the infants alive that were previously lost in the early period, especially in developed countries and our country. In addition, many factors such as scientific developments in infertility treatment, increased preterm births, multiple gestations and ongoing high consanguineous marriage rates affect the frequency of CVI. The frequency of CVI differs in developed and developing countries. In the United States, CVI was found in 2.4% of 7200 children who applied to the pediatric ophthalmology clinic[1], while it was present in 19% of children with severe visual impairment.[2] In the U.K., 40-48% of children under 15 years of age with visual impairment had CVI.[3,4] In New Zealand, 30% of childhood blindness is reported to be associated with CVI,

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and the calculated prevalence was 0.02%.[5] In India, isolated CVI was found in 33% of children with low vision, and both cerebral and ocular pathologies were detected in 11%.[6] Among children with severe visual impairment, CVI prevalence was reported as 2.5% in Malaysia and 0.3% in China.[7,8]

To date, this condition has been expressed with definitions such as cortical blindness, cortical visual impairment, central blindness, neurological visual impairment, and cerebral visual impairment. Since the involvement is not limited to the cortex, it is preferable to use the word 'cerebral' instead of cortical. Since the vision can be improved in this age group and even only the presence of light perception is considered as a developmental emergency, it is preferable to use 'visual impairment' instead of blindness.

Vision is a complex system, only part of which is created by the eye. The primary visual center in the brain is the occipital cortex (striate cortex). However, the extrastriate cortex-related pathways of the brain are required for the cognitive visual function. With the dorsal stream that connects the visual cortex to the parietal lobes, movement function (the 'where' stream); with the ventral stream that connects the visual cortex to the temporal lobes, recognition function (the 'what' stream) is performed. Frontal brain areas perform the task of managing by using this complex tool.[9,10]

All babies learn to see after they are born. In order to learn to see, adequate visual stimulation is required in addition to healthy eyes, visual pathways, and related centers in the brain. Brain plasticity is extremely important for gaining vision in infants and toddlers. Although plasticity continues until the age of ten, the best time for proper stimulation is the early infancy period. Therefore, it is vital to start visual habilitation early.[11,12] Diagnosis and rehabilitation of CVI differ from visual impairments caused by other reasons.[13,14]

The causes of CVI are congenital or acquired (Table I).[15-18] The most common cause is perinatal hypoxia and ischemia. Neurological and ophthalmological deficits are seen together in most of the children with CVI. Frequent neurological deficits are seizures (requiring anticonvulsant therapy), cerebral palsy, hemiparesis, hydrocephalus, hypotonia, hearing loss, microcephaly, and learning difficulties.[1] In addition, disorders that may present with symptoms and findings similar to those of CVI, such as autism spectrum disorders, developmental coordination disorders, delayed visual maturation, learning difficulties, bilateral central scotoma, dyskinetic eye movement disorders, and mental retardation, should be kept in mind in the differential diagnosis.[19,20]

In CVI, ocular structures are generally completely normal, or the pathologies are not sufficient to explain visual loss. In these patients, some parts of the brain, such as the visual cortex or visual pathways, are damaged, and abnormal findings can be detected by imaging methods. However, CVI may be accompanied by refractive errors, strabismus, ocular motor apraxia, and optic atrophy. Nystagmus is rare and should alert the physician in terms of anterior visual pathway damage. Optical atrophy can be seen in the case of severe hypoxia and ischemia and its presence should alert the physician in terms of the severity of the condition and poorness of the prognosis.

I. OPHTHALMOLOGICAL EVALUATION IN CVI

Appropriate tests and methods should be used regarding the age and underlying pathology. Behavioral responses against visual stimuli specific to CVI are of great importance. Detailed anamnesis should be taken with the company of the family or child's caregiver and observations of the family about the child's behavior should be evaluated. To avoid stimulating other senses, the examination room should be quiet and dull, the physician performing the examination should be dressed in a plain solid color if possible, and avoid stimuli such as odors. The fixation object/toy should be selected according to the child's

Table I. Causes of cerebral visual impairment.

Congenital and Perinatal Causes	
Asphyxia and perinatal hypoxic encephalopathy	Placenta previa, abruptio placentae, uterine rupture, umbilical cord prolapse, severe shoulder dystocia, maternal diabetes, maternal infection, rapid labor
Intraventricular hemorrhage	Post-hemorrhagic hydrocephalus
Hydrocephalus-shunt malfunction	
Periventricular leukomalacia	
Post-natal hypoxia-ischemia	Neonatal stroke
Cerebrovascular problems, cerebral artery occlusion	
Prematurity	
Structural anomalies	Occipital or parietal encephalocele, meningomyelocele (spina bifida), Dandy-Walker syndrome, primary microcephaly, schizencephaly, primary congenital hydrocephalus, polymicrogria, corpus callosum agenesis
Neurological diseases	Cerebral palsy
Infections	TORCH infections
Multiple gestation	Premature birth, periventricular leukomalacia
Hereditary diseases	HANAC syndrome, Aicardi-Goutieres syndrome, Walker-Warburg syndrome, MELAS syndrome, ornithine transcarbamylase deficiency, Fabry disease, X-linked adrenoleukodystrophy
Acquired Causes	
Trauma, accident	
Metabolic and neurodegenerative conditions	Hypoglycemia, kernicterus, carbon monoxide intoxication, uremia, hypovolemia
Hypoxia	
Infections	Sepsis, encephalitis, meningitis
Tumors	
Toxic causes	Vincristine, cyclosporine etc.

age and interest (Fig. 1a, 1b). Cerebral pathology leading to visual impairment, other sensory and neurological deficits that may affect the child's expected visual behavior and response, should be considered.

Evaluations made during the detailed ophthalmological examination;

a. Binocular vision: Although it is difficult to assess binocular vision in these children, it is considered to be defective. Strabismus in infants with CVI, disrupts the formation of the fusion mechanisms and prevents binocularity, and development of depth perception, and three-dimensional vision.[21,22]

b. Oculomotor evaluation: Before starting the examination, it should be observed whether the child has a head position. Binocular and monocular eye movements should be evaluated. In assessing the ocular misalignment, it is appropriate to use tests evaluating the corneal light reflex, such as Hirschberg and Krimsky tests, due to the lack of cooperation in these patients. The presence of optokinetic nystagmus response is evaluated by rotating the optokinetic drum, a tool with alternating black and white vertical stripes, in front of the patient. The response consists of slow eye movements in the direction of the stimulus, followed by fast corrective eye movements, indicates an intact visual pathway.

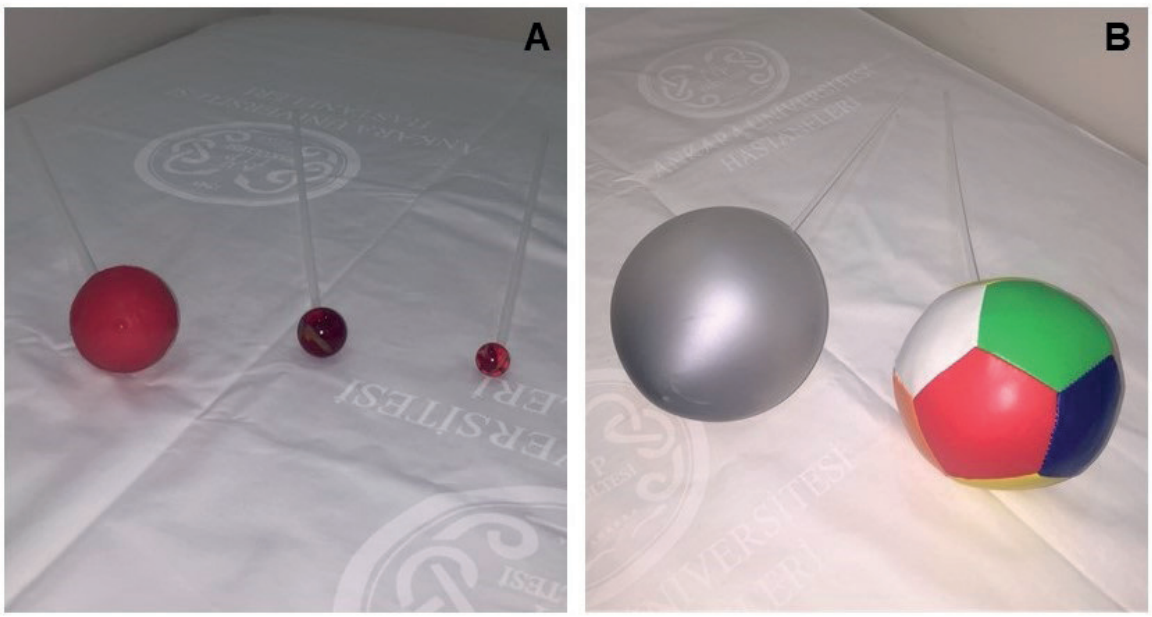

Fig. 1. Red objects with a diameter of 1 cm, 2.5 cm and 5 cm (1a), gray and colored objects with a diameter of 25 cm (1b).

c. Visual acuity: Preferential-looking tests, based on observing the child's behavior when encountering different visual stimuli, and visual evoked potentials (VEP) are reliable and valid methods used frequently. In preferential-looking test, the child who is presented with two stimulus areas, one with stripes and the other with a homogeneous gray area, is expected to prefer to look at the striped one. Although VEP provides vision measurement without the influence of motor ability, behavioral vision measurements are more useful in defining the function.[23]

d. Contrast sensitivity: Contrast sensitivity is an important function that affects daily activities such as communication, access to information, orientation and mobility skills. Besides many factors related to the object, environment, and eyes, contrast sensitivity is expected to be mainly affected in CVI.[24]

e. Color vision: In most children with CVI color vision is normal and color selection is preserved. All color vision tests should be carried out in daylight.

f. Visual Field: Visual fields are examined with a method suitable for the child's age and cooperation. The confrontation method should be preferred between 4 months- 6 years of age and automated visual field tests, for older children. The confrontation method, which is applied by the physician moving the object slowly from the periphery to the center in the nasal, upper, temporal, and lower quadrant, respectively, is the most widely used.[25,26]

g. Electrophysiological methods:
Electrophysiological tests detect neurological and concomitant ocular pathologies and may determine the prognosis of CVI. For this purpose, VEP tests (pattern, flash, sweep) have gained priority in CVI.[19] However, the interpretation of pattern and flash VEP results in young children is difficult and debatable.[23,27] Sweep VEP test is more suitable for quantitative measurement. Although thought to define visual acuity correctly, limitations such as concomitant neurological disorders, general status of health, medications make it difficult to acquire an exact match.[28,29]

II. BEHAVIORAL RESPONSES TO VISUAL STIMULI IN CVI

The presence of behavioral responses can vary depending on the region and the severity of the cerebral pathology. Besides, factors pertaining to the child (fatigue, post-seizure period, overstimulation), the target (color, complexity) and the environment (simple, crowded) may affect these behavioral responses and may differ from the child's home where he/she feels more comfortable. All conditions in the examination must be recorded.

If these behavioral answers are summarized;

a. Color preference: Color vision is close to normal for most children with CVI because color is represented bilaterally in the visual cortex and these areas are generally preserved. Children with CVI are more interested in visual targets with distinct colors. Most prefer red and yellow. However, having a red/yellow color preference does not lead to the diagnosis of CVI, or any other color selection does not exclude CVI diagnosis.[30]

If the child has a specific color preference, it is important to use this color during the child's habilitation. Attaching an object with the preferred color to the spoon or the feeding bottle while eating can increase the motivation of the child, increase the vision, and improve visual learning.[31] Children with CVI first see one-color objects. As the severity of the disease decreases, they begin to see more colors and patterns, and color and pattern preferences do not remain as the CVI improves.[30]

b. Requirement for Movement: The majority of children with CVI tend to turn towards moving objects, or react differently to these objects, such as smiling or remaining silent.[32] These children usually like to watch the traffic from the window or watch television for a long time.

Perception of movement in children with CVI differs according to the damaged area of the brain. The pathways mentioned above are necessary for cognitive visual functions.[33] The occipitoparietal area detects motion and damage to the posterior periventricular white matter may alter perception of motion.[34] This type of lesions are often due to prematurity; these babies have problems detecting fast-moving objects and prefer slower moving objects.[10] In children with damage is mainly in the occipital lobe and not in the occipitoparietal pathway, perception of movement is normal but visual impairment is present.[35] These children try to create the feeling of seeing with head shaking, or turning around themselves.

Habilitation may therefore make use of moving objects to initiate and maintain seeing. As the severity of CVI decreases, moving is only needed when the infant is distracted, and may even be left out with further improvement.

c. Visual latency: Visual latency is the time between looking at a target and seeing the target. Visual responses are slow and delayed in children with CVI. They can ignore the target at first. After enough time, they can focus on the target and turn towards the target. The presence and duration of visual latency varies from one child to the other.[36]

With the improvement of CVI, latency is shortened, and a delay may occur only when the child is tired, stressed, or overstimulated. With full recovery, the latency may be eliminated completely.

d. Visual field preference: Most of these children have a visual field preference that varies depending on the brain's damaged area.[30,37] There is a dorsal stream dysfunction in hypoxic-ischemic encephalopathy and periventricular leukomalacia. Especially in periventricular leukomalacia, the lateral ventricles are enlarged and the upper fibers of the optical radiation are affected: this creates defect in binocular lower visual field as the most frequent finding[38,39] The right or left visual field and rarely, a central visual field may be preferred.[30] The visual field preference may not be available in approximately 30% of cases.

These children often prefer either the right or left visual field at the initial stage of the disease (Fig. 2). At this stage, training should be started by showing the toy from the preferred visual field. As the severity of CVI decreases, these children start using both the right and left visual fields, but still, one is dominant. Significant improvement results in the disappearance of any preferences for visual fields.

e. Difficulties discriminating or interpreting complex visual patterns: This is one of the most prominent, long lasting, and resistant characteristics. It may persist for a lifetime to some degree. These children need support in academic life.[30,40] The problem is thought to be related to the damage of the dorsal stream and the intersected areas of the occipital and temporal lobes which serve as the 'image library'.[41] Difficulties discriminating or interpreting complex visual patterns can be related to objects (multicolor objects), sequences (multiple toys), the environment (crowded environments), as well as sensations (simultaneous stimulation of multiple senses). Patients seem to be restless in crowded and varied environmental conditions, or prefer to sleep to avoid complexity. These children initially prefer to look at a single object. The next step would include showing a second toy or a multi-color sample of the one-color toy. As the disease recovers, the child can see objects ranging from simple to complex. The visual activity can be improved from fully controlled environments to complex environments including other stimuli.[30,42]

Fig. 2. A child with a diagnosis of cerebral visual impairment and left visual field preference.

f. Light-gazing and non-purposeful gaze: Staring at the light is an impulsive behavior. Parents define this behavior by saying that their children are looking at the light that leaks from the window or the lamp on the ceiling for a long time, but not the target.[30] The non-purposeful gaze is defined as the child not being able to look at the target shown or focusing when there is no target.[30] While looking at the light is normal in newborn babies, the continuation of this behavior in children with CVI signs, indicates that visual development is delayed.[30] In the classroom, instead of looking at the blackboard, they may aimlessly stare at the light coming from the window, or turn their heads to the other side and look as if they are not interested. The purpose of this action is to try to accomplish perception by using senses other than vision.[43]

The behavior of looking at the light was observed in 60% of children with CVI. In computed brain tomography findings in children with or without his behavior, no definite neuro-anatomical difference could be demonstrated.[44] The behavior of looking at the light and non-purposeful gaze is not related to the severity and etiology of the disease and visual level. When there is even a slight impact on the striate cortex, not causing a significant

decrease in vision, may result in this behavior. The intensity of light-gazing decreases as CVI improves.[37]

g. Difficulty with distance viewing: As difficulties discriminating or interpreting complex visual patterns, this is also thought to be related to the dorsal stream. Therefore, problems with complex visual patterns may be associated with those in distance viewing.[10,41,42] The latter is one of the resistant features of CVI. The possibility of viewing distant objects increases with the simplicity of the environment. Initially, he/she can only look at objects at a distance of 40-45 cm, but as the disease improves, the distance s/he can see increases, but nevertheless, it will still be difficult to see in a complex environment.

h. Atypical visual reflexes: Most children with CVI respond atypically to two reflexes, called blink reflexes and threat responses, which are expected to exist from birth. Normally, when a child's nasal bridge is tapped gently, s/he blinks simultaneously. The threat response is the blink when a hand is moved quickly in the midline of the baby's face. In most of these children, these reflexes are either absent or delayed. In the recovery process, a delayed blink reflex may appear first, and then starts to gain continuity; the response to threat is observed intermittently. In the advanced phase of recovery, both reflexes are constantly present.

i. Difficulty in visual innovations: Since they have difficulties to process the information they see these children prefer familiar objects that can be easily remembered.[30] The same object is offered repeatedly during training. The child is then introduced to a new object with similar properties. Initially, s/he shows interest only in the familiar objects, and with recovery, new objects that have common features with the familiar ones. In the case of improvement, there is no limitation in object preference.[45]

j. Visually guided reach: Reaching and catching a target are motor behaviors, but they are also based on the dorsal and ventral stream of the visual system. Ventral stream functions enable to us to see and recognize the target while dorsal stream functions allow us to reach and take it. Therefore, achieving a target is a multi-step process taking place within the brain.[46,47] Any impairment in this complex system may affect ordinary movements. In children with CVI, seeing and reaching a target cannot be performed simultaneously. Characteristics of the target and the environment also affect the reaching function.[30] During these evaluations the child's motor functions should also take into account.

With the improvement of CVI, firstly, reaching to familiar and preferred color objects on a plain background is seen. Then, looking and reaching may be done in quick succession. With full recovery, looking and reaching appear simultaneously.[30]

Habilitation

Except for hydrocephalus and metabolic diseases, there is no etiological treatment for CVI. The presence of multiple disabilities can adversely affect the habilitation. The crucial point is to ensure the communication and interaction of the child with the external world and to increase participation in daily life. Various clinical studies have been conducted to improve the quality of life of children with CVI, but the improvement of visual functions has not been sufficiently emphasized.[48,49]

Vision re/habilitation therapy can be used for both ocular and cerebral visual impairment. However, rehabilitation strategies vary depending on the underlying cause. The strategies used in ocular low vision will not be effective in CVI. In ocular problems, the desired results may not be achieved with rehabilitation. However, in CVI where an ophthalmological examination is usually normal, vision is more likely to improve.[50] Groenveld et al.,[14] stated that children with CVI always tend to recover and there is always some residual vision. Infants with brain damage may benefit from appropriate stimulation and experience an

increase in vision.[11] This was explained by brain plasticity, a concept first mentioned by Hubel and Wiesel in 1981 and it made them win the Nobel Prize.[51] In CVI, stimulation for visual development should be applied within the crucial time window for visual development, or the 'critical period'.

In summary, CVI in infants and toddlers can benefit from early diagnosis and effective visual habilitation by a multidisciplinary team where ophthalmologists and pediatricians are collaborating.

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Original Article

Mucormycosis in a pediatric population: a review of 20 cases from southern Turkey

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ABSTRACT

Background. Mucormycosis is a fatal invasive fungal infection seen most often in patients with compromised defense mechanisms. The aim of this article was to review the data of pediatric mucor in the South of Turkey.

Methods. Twenty pediatric cases with biopsy proven mucormycosis were reported, between January 2007 through January 2017. Data were extracted from the medical charts of patients retrospectively.

Results. Underlying conditions were hematological malignancy (75%), in whom 93% had acute leukemia, aplastic anemia (15%), diabetes mellitus (5%) and other malignancies (5%). The main sites of infection were sinus (85%); alone (29.4%) or with cerebral (17.6%), and orbital involvement (17.6%). Pulmonary involvement was reported in 11 patients (55%), two of them had the alone form and nine cases were associated with nasal sinus involvement. Disseminated mucormycosis was documented in 45%. Fever and pain/swelling of organs were the most commonly encountered signs and symptoms. Treatment compromised of am-photericin B monotherapy in five patients. All patients except one received liposomal formu-lations (LAmB). A combination of surgery and antifungal therapy was performed in 75%. Crude survival was 55%; among 15 cases treated with a combination of surgery and antifun-gal therapy, survival rate was 8/15 (53%). The overall mortality rate was high in patients diagnosed with disseminated infection (100%).

Conclusions. Mucormycosis in pediatric cases requires a high index of suspicion and urgent evaluation of clinical samples. Surgical debridement should be considered when feasible. Initial medical therapy should include an amphotericin preparation with or step-down to posaconazole.

Key words: mucormycosis, zygomycosis, children, immunosuppressive, amphotericin B.

Mucormycosis (zygomycosis) is the third emerging important invasive fungal infection dur-ing the past decade, it is associated with a worse outcome when compared to other invasive fungal infections such as candidiasis or aspergillosis.[1-4] The increase in the incidence may be attributed to a better outcome in the survival of immunocompromised patients.[5] Depending on the underlying condition, such as the withdrawal or reduction of corticosteroids, impair-ment of neutropenia, hematological malignancies, hematopoietic stem cell transplantation (HSCT), adequate control of glycemia in cases of diabetes, and the portal of entry, they can cause rhinocerebral, pulmonary, cutaneous, gastrointestinal or even disseminated infection.[6,7] The rapid initiation of antifungal therapy is the cornerstone due to the highly difficult treat-ment of this destructive infection.

We report retrospective results in the present study, where we reviewed clinical characteristics, risk factors, treatment and outcome of pediatric mucormycosis, diagnosed by histopathology at our center from 2007 until 2017, a University Hospital in Southern Turkey.

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Material and Methods

The study was conducted in a tertiary medical college hospital (Çukurova University Faculty of Medicine). Our patients were managed by a multidisciplinary healthcare team comprised of oncologists, infectious disease specialists, surgeons, pathologists, radiologists and inten-sivists. The study was approved by the Institutional Ethics Committee (Approval number: 04-09-2019/91).

All pediatric patients (aged 0-18) that were diagnosed histologically as mucormycosis be-tween January 2007 through January 2017, were included in the study. All cases were proven as mucormycosis based on a histopathologic examination of a needle aspiration and/or a bi-opsy specimen which revealed hyphae and evidence of associated tissue damage from the nasal cavity and/or paranasal sinuses and/or palate, lung or dermis.

Histopathologic diagnosis: Mucor infections are one of the correctly detectable fungi by biopsy and they are determined as angioinvasive, broad, without septas and 90 degrees branching hyphae in tissue sections. Although routine hematoxylene-eosin tissue sections are generally sufficient some histochemical stains like PAS and GMS (Gomori's methanamine silver) can be used, its particularly predominant appearance is necrosis. Sometimes fungal hyphaes can be few, or degraded or folded in tissue sections.

All patients underwent computed tomography (CT) or magnetic resonance imaging (MRI) scans of paranasal sinuses, orbita, craniocerebrum and lungs.

The sites of infection were classified according to those utilized in the study of Roden et al.[4] Accordingly, sinusitis was defined as an infection involving the paranasal sinuses; those with disease in paranasal sinuses and orbital infiltration were defined as sino-orbital infec-tion; those with disease in the paranasal sinuses and the brain as rhinocerebral infection; those with disease in the paranasal sinuses and lungs were defined as having sinopulmonary infection; pulmonary infection was defined as infection confined to lung tissue and as deep extension when invading adjacent tissues, and dissemination was defined as two or more non-contiguous locations of Mucorales infection.

The demographic characteristics, type of underlying conditions, risk factors, the site of in-fection, clinical signs and symptoms of infection, radiological findings, treatments, and out-come were extracted from the medical charts of patients.

Results

The hospital pathology records identified 24 individual cases of mucormycosis in pediatric patients during 10 years. Of these, four cases were excluded from the database because they did not meet the stringent predefined inclusion criteria or the patient file could not be reached. During the study, 20 patients with proven mucormycosis (14 male, 6 female) were recorded. Demographic characteristics and clinical features are summarized in Tables I and II. The age at diagnosis ranged from 2 to 16 years (average age was 9 years) and 7 patients were aged ≤ 5 years. Two patients out of 20 were foreign (Syrian) nationals.

The patients' underlying conditions are listed in Table II. Fifteen patients (75%) with he-matological malignancy composed the largest group, in whom 93% had acute leukemia (one underwent HSCT). Three patients had aplastic anemia (2 were Fanconia anemia and one underwent HSCT). Eighteen patients were neutropenic and 8 patients were known as being on antifungal prophylaxis (5 patients with fluconazole and 3 patients with itroconazole) while 7 patients were receiving co-trimaxazole. Two patients had chronic renal failure and one patient had metabolic acidosis.

Nasal involvement was identified in a majority of the cases (17/20; 85%) patients, alone in 5/17, with cerebral involvement in 3/17, and with orbital involvement in 3/17 (Table III). Pulmonary involvement was reported in 11/20

Table I. Overview of our 20 pediatric mucormycosis cases (2007-2017).

Number	Age/sex/year City	Underlying medical problem predisposing factor	Prophylaxis	Symptoms and signs	Co-infection	Radiological findings	Clinical form	Surgical treatment (count)	First line antifungal therapy/duration (days, mg/kg)	Survival outcome (follow-up, cause of death)
1	13/F/2007 Osmaniye	AML(relapse)/ CHB Neutropenia	FLU (20 days)	Facial pain, blackish necrotic debris on nose	*Aspergillus*	CT: sinusitis, pneumonia, pericarditis	Sino-pulmonary, tracheitis, pericarditis/ deep extension/ dissemination	Only biopsy	LAmB (24 days, 10mg/kg)	Died (fungal infection)
2	3/F/2008 Maraş	ALL Neutropenia	FLU, KO	Fever, ulcerative palate lesion	*Aspergillus, Candida*	CT: sinusitis, sinus wall destruction,	Sinusitis	Surgery (1)	cAmB (90 days) then POS (7 months)	Cured
3	5/M/2008 Gaziantep	ALL (relapse) Neutropenia, hyperglycemia	?	Fever, nasal pain, facial swelling, epistaxis, cough	-	CT: sinusitis, air crescent sign (right)	Sino-pulmonary	Surgery (1)	LAmB	Cured (transferred to another center)
4	11/M/2008 Adana	AML(relapse) Neutropenia	?	Fever, voice loss, chest pain	-	CT: sinusitis, pulmonary nodule, consolidation	Sino-pulmonary tracheitis/ deep extension/ dissemination	Only biopsy	LAmB (31 days)	Died
5	3/M/2008 Urfa	ALL (refractory) Neutropenia, cerebral mucor history	?	Fever, facial pain and swelling, epistaxis, ulcerative palate lesion, cough	*Pseudomonas*	CT: sinusitis, sinus wall destruction, pneumonia MRI: cerebritis, leptomeningitis	Rhino-cerebral, pulmonary/ dissemination	Surgery (3)	LAmB (40 days)	Died (fungal infection)
6	8/M/2009 Adana	ALL (relapse) Neutropenia	I, KO	Fever, facial swelling	-	CT: sinusitis, pneumonia	Sino-pulmonary	Surgery (2)	LAmB (165 days, 10 mg/kg) +caspofungin, VOR, then POS	Cured (14 months)
7	8/M/2010 Urfa	ALL Neutropenia Mucor history (10 months)	FLU, KO	Fever, facial swelling, sepsis	-	CT: sinusitis, MRI: frontal lobe infiltration	Rhino-cerebral/ dissemination	Surgery (2)	LAmB (60 days, 7 mg/kg) POS (5 months)	Died (fungal infection)

ALL: acute lymphoblastic leukemia, AML: acute myeloid leukemia, cAmB: conventional amphotericin B, CHB: chronic hepatitis B, CRF: chronic renal failure, CT: computerized tomography, FAA: Fanconi aplastic anemia, FLU: fluconazole, HSCT: hematopoietic stem cell transplantation, I: itraconazole, KO: co-trimoxazole, LAmB: liposomal amphotericin B formulation, MRI: magnetic resonance imaging, PNET: primitive neuroectodermal tumor, POS: posaconazole, V: voriconazole.

Table I. Continued.

Number	Age/sex/year City	Underlying medical problem predisposing factor	Prophylaxis	Symptoms and signs	Co-infection	Radiological findings	Clinical form	Surgical treatment (count)	First line antifungal therapy/duration (days, mg/kg)	Survival outcome (follow-up, cause of death)
8	5/M/2010 Hatay	ALL (induction) Neutropenia	FLU	Fever, facial swelling, oral blackish necrotic debris, ulcerative palate lesion	-	CT: sinusitis, sinus wall destruction	Sinusitis	Surgery (2)	LAmB (163 days) POS (52 months)	Cured
9	12/M/2012 Osmaniye	ALL (induction) Neutropenia	-	Fever, blackish necrotic debris in nose, ulcerative palate lesion	-	CT: sinusitis, sinus wall destruction	Sinusitis	Only biopsy	LAmB (34 days) then itraconazole	Cured
10	2/M/2013 Urfa	ALL-HSCT Neutropenia	FLU, KO	Fever, cutaneous lesion	-	Ultrasound: cutaneous abscess	Cutaneous/deep extension	Surgery (1)	LAmB (22 days)	Cured
11	7/F/2013 Antakya	FAA and CRF Neutropenia, metabolic acidosis	KO	Fever, epistaxis, sepsis, periorbital swelling, sepsis	-	CT: sinusitis, periorbital cellulitis, hepatic calcification	Sino-orbital/ dissemination	Surgery (1)	LAmB (29 days) +VOR	Died (fungal infection)
12	16/M/2014 Urfa	PNET (metastatic) Neutropenia	I	Fever, mucositis, throat ache, nasal congestion headache, diarrhea	-	CT: sinusitis, sinus wall destruction, multiple pulmonary nodule (metastasis)	Sinusitis	Surgery (1)	LAmB (21 days) POS (5 months)	Cured
13	15/M/2014 Osmaniye	AML(relapse) Neutropenia	I	Fever, epistaxis	*Aspergillus*	CT: pansinusitis, air crescent sign (multiple, bilateral)	Sino-pulmonary/ dissemination	Surgery (1)	LAmB (28 days)	Died (fungal infection)
14	11/F/2014 Hatay	ALL(induction) Neutropenia	-	Facial swelling, palate lesion	*Candida*	CT: pansinusitis	Sinusitis	Surgery (1)	LAmB (60 days) POS	Cured

ALL: acute lymphoblastic leukemia, AML: acute myeloid leukemia, cAmB: conventional amphotericin B, CHB: chronic hepatitis B, CRF: chronic renal failure, CT: computerized tomography, FAA: Fanconi aplastic anemia, FLU: fluconazole, HSCT: hematopoietic stem cell transplantation, I: itraconazole, KO: co-trimoxazole, LAmB: liposomal amphotericin B formulation, MRI: magnetic resonance imaging, PNET: primitive neuroectodermal tumor, POS: posaconazole, V: voriconazole.

Table I. Continued.

Number	Age/sex/year City	Underlying medical problem predisposing factor	Prophylaxis	Symptoms and signs	Co-infection	Radiological findings	Clinical form	Surgical treatment (count)	First line antifungal therapy/duration (days, mg/kg)	Survival outcome (follow-up, cause of death)
15	5/F/2014 Adana	ALL(induction) Neutropenia	KO	Fever, cough	-	CT: air crescent sign (left)	Pulmonary	Surgery (lobectomy)	LAmB (75 days) +VOR POS (2 years)	Cured
16	11/M/2015 Adana	FAA-HSCT (1.5 years ago) and CRF	-	Cough	-	CT: air crescent sign (right)	Pulmonary	Surgery (lobectomy)	LAmB	Cured (transferred to another center)
17	16/F/2016 Adana	Diabetes mellitus Hyperglycemia	-	Pain of tooth and nose, nasal congestion, facial swelling, headache, vomiting	-	CT: sinusitis, MR: frontal lobe abscess (1 cm)	Rhino-cerebral	Only biopsy	LAmB (44 days) POS (43 day)	Cured
18	16/M/2016 Elazığ	Non-Hodgkin lymphoma (relapse)/ Neutropenia	KO	Fever, facial swelling, sepsis	*Aspergillus, Candida*	CT: pansinusitis, parotitis orbital cellulitis, pulmonary nodule and effusion	Sino-orbital, pulmonary, / dissemination	Surgery (2)	LAmB (15 day) +VOR	Died (fungal infection)
19	5/M/2016 Syrian	ALL(relapse) Neutropenia	-	Fever, facial swelling	-	CT: pansinusitis, periorbital cellulitis, pulmonary nodules (bilateral)	Sino-orbital, pulmonary/ dissemination	Surgery (4)	LAmB (60 days) / caspofungin (when LAmB not available)	Died (fungal infection)
20	107M/2016 Syrian	Aplastic anemia Neutropenia, hepatitis	-	Fever, facial and periorbital swelling, ulcerative palate lesion, tooth pain, sepsis	-	CT: pansinusitis, sinus wall destruction, tonsillar abscess, air crescent sign (right)	Sino-pulmonary, tonsillar abscess/ dissemination	Only biopsy	LAmB/ caspofungin (when LAmB not available) (105 days)	Died (fungal infection)

ALL: acute lymphoblastic leukemia, AML: acute myeloid leukemia, cAmB: conventional amphotericin B, CHB: chronic hepatitis B, CRF: chronic renal failure, CT: computerized tomography, FAA: Fanconi aplastic anemia, FLU: fluconazole, HSCT: hematopoietic stem cell transplantation, I: itraconazole, KO: co-trimoxazole, LAmB: liposomal amphotericin B formulation, MRI: magnetic resonance imaging, PNET: primitive neuroectodermal tumor, POS: posaconazole, V: voriconazole.

Table II. Patient demographics, predisposing factors, clinical findings and outcomes.

Clinical features	N (%)	Mortality, n/N (%)
Age		
<5 years	3 (15)	1/9 (11)
≥5-18 years	17 (85)	8/9 (89)
Male/Female	14/6	7/14; 2/6 (50; 33)
Co-morbidity		
Diabetes mellitus	1 (5)	-
Haematological malignancy	15 (75)	9/15 (60)
ALL	11 (55)	3/11 (27)
AML	3 (15)	3/3 (100)
Non-Hodgkin lymphoma	1 (5)	1/1 (100)
Other malignancies		
PNET	1 (5)	-
Haematological disease		
Aplastic anemia	3 (15)	2/3 (67)
Predisposing factor		
Neutropenia	18 (90)	9/18 (50)
Cancer chemotherapy	16 (80)	7/16 (44)
Co-infection with *Aspergillus*	5 (25)	2/5 (40)
Co-infection with *Candida*	3 (15)	2/3 (67)
Antifungal prophylaxis medication		
Fluconazole	5 (25)	2/5 (40)
Itraconazole	3 (15)	1/3 (33)
Co-Trimoxazole	7 (35)	
Signs and symptoms		
Fever	16 (80)	
Swelling of face/periorbital	12 (72)	
Blackish necrotic debris of nose/oral	8 (40)	
Ulcerative palate lession	6 (30)	
Facial/nasal/tooth/throat pain and tooth infection	6 (30)	
Bloody nasal discharge	4 (20)	
Cough	4 (20)	
Sepsis	4 (20)	
Headache	2 (10)	
Nasal congestion	2 (10)	
Voice loss	1 (5)	
Chest pain	1 (5)	
Surgery	15 (75)	
Outcomes		
Mortality	9 (45)	

(55%) patients, 2 of them had alone form and 9 associated with nasal sinus involvement. Disseminated mucormycosis was documented in 9/20 (45%) patients. There were unusual presentations, including 2 patients with parotitis and hepatitis.

Table III. Distribution of the sites of involvement.

Involvement	Sinusitis (%) [case no]		Dissemination (%) (case no)	Sex (M/F)	Malignancy	Aplastic anemia	Diabetes mellitus	Surgery	Mortality
	Yes	No							
Alone	5 (25%) [2, 8, 9, 12, 14]	-		3/2	5	-	-	4	0
Orbital	3 (15%) [11, 18, 19]	-	2 (10%) [11, 18]	2/1	2	1	-	3	3
Cerebral	3 (15%) [5, 7, 17]	-	2 (10%) [5, 7]	2/1	2	-	1		
Pulmonary	9 (45 %) [1*†, 3, 4*, 5, 6, 13, 18, 19, 20]	2 (10%) [15, 16]	7 (35%) [1, 4, 5, 13, 18, 19, 20]	7/2	9	2	-	8	8
Cutaneous	-	1 (5%) [10]	-	1/-	1	-	-	-	-
Total	17 (85%)	3 (15%)	9 (45%) #	14/6	16 (80%)	3 (15%)	1 (5%)	15 (75%)	9 (45%) #

*: tracheitis, †: tonsillar abscess, #: case 5 had cerebral and pulmonary involvement; case 18 had orbital and pulmonary involvement.

Generally, fever and pain/swelling of organs were the most commonly encountered signs and symptoms (Table II). None of the patients were culture positive. In cases accompanied by *Aspergillus*, 3 of the 4 cases showed death and worsened prognosis.

Treatment regimens prescribed for patients diagnosed with mucormycosis are shown in Table II. All patients were treated with a form of antifungal therapy, resulting in a survival of 55%. All patients except one (whom had conventional amphotericin B, cAmB) received lip-osomal formulations (LAmB). Fifteen of these cases were treated with a combination of sur-gery and antifungal therapy, resulting in a survival rate of 8/15 (53%) (Table II). Posaconazole has been used successfuly for step-down therapy in seven patients. The overall mortality rate of patients identified in this study was 45%, with a high mortality rate occur-ring in patients diagnosed with disseminated infection (100%).

Discussion

Mucormycosis is a life-threatening fungal infection characterized by a highly aggressive (angiotropic) progression that occurs mostly in immunocompromised patients.[8] Data concerning patient sex, age at onset of disease, symptoms and signs, radiological findings, treatment modalities, and outcome were analyzed retrospectively, for a 10-year period. To our knowledge, there have been a limited number of reports from Turkey in the pediatric population diagnosed with mucormycosis, therefore, our report is a large case series reported from our country.

In several studies, mucormycosis was reported primarily in males (65%). In case reports from Turkey, most of the patients were male (52%).[9] Although Pana et al.[1], in 63 children diagnosed with mucormycosis, reported the rate of girls as higher, in our study, a significant proportion of the patients were male. In our series, the majority of children (85%) were older than 5 years, with a mortality of 78%.

For older infants and children, Francis et al.[10] has recently reported that hematologic malignancy is a significant risk factor, as is being a hematopoietic stem cell transplant or solid organ transplant recipient, these were followed by diabetes. Kömür et al.[11] conducted a retrospective study evaluating 51 cases of mucormycosis in our city in adult patients and reported that malignancy was the most common co-morbidity affecting 59% of the subjects, followed by diabetes. In our series of 20 pediatric mucormycosis cases, the most prominent identified underlying conditions were hematological malignancies, especially acute leukemia (70%), and this was followed by aplastic anemia (15%). In patients with prolonged neutropenia, aggressive evaluation should be performed in case of fever.[12] The majority of patients in our series had neutropenia (90 %). Fever was observed in almost every neutropenic case. Although, we have a well working transplantation unit, only two (10%) of our pediatric mucor patients were transplant patients; the distribution of infection is different from the literature. In our experience, the mucor infection occurred in four patients in the early stage of chemotherapy, during induction, and that contrasts with the data in the literature of pediatric mucor cases in which this was reported to occur later on during chemotherapy.[13] Seven of the ALL cases were either relapsed or refractory and clinically unwell. In our study, in only one of 20 cases, the patient had uncontrolled diabetes mellitus.

Seasonal variation in atmospheric concentration of fungal spores has been documented for some molds in several geogaphical locations.[14,15] As reported in India and in Middle East countries such as Iran, events are reproduced in August and in tropical and subtropical sea-sons when spores are most intense in the air.[16] It is necessary to evaluate the contribution of this climate zone and the seasonal weather conditions we have in our city. The fact that our region is moist might be an effective risk factor for disease development.

The authors' prior and most recent experience with hematological malignancy patients suggests that mucormycosis is most likely in cases when the patient has been receiving As-pergillus-active antifungal (especially voriconazole) prophylaxis.[17-19] Although in our series, 8/20 patients who had received antifungal prophylaxis with fluconazole or itraconazole, did not have Mucorales activity.

For Mucorales the portals of entry in the human body are the respiratory tract through inhalation of fungal spores. In most series[20-25], such as the studies of Chakrabarti et al.[3] and Roden et al.[4], rhino-orbito-cerebral mucormycosis was reported as the most frequent clinical mani-festation and this can quickly progress to disseminated form with disastrous consequences if not diagnosed and treated early. This is consistent with the global trend, the most commonly identified condition was sinus involvement (75%) alone in 5/20 and with concomitant in-volvement in 12/20 in our study, followed by the pulmonary form, have been found to be the most prevalent in our study. Pulmonary mucormycosis has been reported at a frequency be-tween 44-64% in children.[26-29] Although rhinoorbitocerebral sinus disease is mostly common in diabetic patients, pulmonary disease predominates in pediatric patients with malignancy and hematopoietic cell transplantations (75%).[1] We detected that eleven cases (55%) had pulmonary involvement (9/11 had added involvement of other forms). Seven (7/11, 78%) with pulmonary involvement occurred as the disseminated form. From a recent review of Francis et al.[10] the dissemination form of the disease occurs in 32% to 38% of pediatric cas-es, which was 45% in our cases, higher than the literature. The patients with a single in-volvement form had a better outcome than those with dissemination.[30]

Histopathologic examination of clinical specimens and culture are recommended for the diagnosis of mucormycosis. Although tissues frequently are not available for biopsy, because of thrombocytopenia or hemodynamic instability, definitive diagnosis is made most frequently

on the basis of direct microscopic examination. However, tissue identification is a very important diagnostic tool, since it distinguishes the presence of the fungus as a pathogen in the specimen from a culture contaminant. Direct microscopy of clinical specimens allows a rapid presumptive diagnosis and differentiation of mucormycosis from aspergillosis and other hyalohyphomycoses and phaeohyphamycoses-and is strongly recommended for treatment decisions. In recent registries of mucormycosis, histopathology led to the diagnosis in 63% and 66% of cases.[31,32] The diagnosis of 75 cases from an Indian tertiary-care hospital was based on histopathology.[33]

Culture of a clinically relevant isolate enables identification and susceptibility testing of the pathogen. Culture is poorly sensitive because Mucorales hyphae are friable in nature, hence may be damaged easily during sample collection (avoidance of excessive tissue homogenization is recommended before culturing). Additionally, some species fall to sporulate in standard media, precluding a timely and easy morphological identification. Better recovery is seen if slices of minimally manipulated tissue are placed onto the culture medium or baited with bread to promote mycelial growth.[34,35] As a result, approximately only one-third of all histopatologically proven specimens result in a positive fungal culture.[36,37] Countless reports of negative culture results are scattered throughout the literature.

Imaging techniques are helpful; although they are non-specific and do not correlate well with surgical and pathological findings. According to the revised version of EORTC/MSG pub-lished in 2008[38], all of our patient's diagnosis were documented with proven mucormycosis by histopathology and none of them were culture positive. As our opinion, in our center neg-ative culture may be explained by various factors, such as aggressive processing of the spec-imen and inappropriate storage of samples before plating. Although the limitation in our study was biased by the selection of cases that were only proven by biopsy, the diagnosis of our patients was confirmed by histopathological and radiological examination in addition to clinical findings. Despite the limitations to the study, we retrospectively gave a good esti-mate for the burden of mucor infections in pediatric cases in Turkey, highlighting the index of clinical suspicion and the important role played by histology especially from suspected cases in the diagnosis that will be able to guide towards early surgery.

The mucormycosis treatment with antifungal medicine is an important factor affecting the outcome. According to the literature, the mainstay of therapy for treating mucor remains as amphotericin B, primarily in its liposomal formulation.[39] Furthermore, delayed antifungal therapy would increase mortality of mucormycosis among patients with neutropenia. In our series, the first line treatment was cAmB or LAmB in all patients, at different daily doses up to 10 mg/kg. In our experience, higher doses of LAmB were well tolerated. For combination therapy, Pagano et al.[41] reported the beneficial effect of posaconazole in addition to LAmB in hematological patients failing to response to LAmB monotherapy.[40-43] Though posaconazole was not available to give in the first years in our study. In recent years, 7 patients were given parenteral AmB followed by descalation to oral posaconazole. The optimal total duration of antifungal drug administration required for mucormycosis is controversial and varies depending on the extent of the disease.[44] In children successfully treated in our series, we discontinued antifungal therapy only when clinical resolution was evident and adequate im-mune recovery had occurred.

Because of the risk of rapid progression to dissemination of pediatric mucormycosis cases, when feasible, surgery should be considered as a treatment choice. Surgery and antifungal combination therapy are mostly the mainstays of management of invasive mucormyco-sis.[4,7,44] Children who received combined therapy had a mortality rate of 18.5% compared

with 60% for those who received antifungal therapy alone.[30] The majority of the patients suffered from serious underlying conditions (thrombocytopenia, pulmonary infection) limiting the possibility for surgery. Surgery was just performed on 15/20 patients in this study. Of these cases, there was a 60% response rate.

Despite aggressive surgical intervention and intensive antifungal treatment, mucormycosis is associated with a greater mortality rate (47-56%). It rises to range from 50-100% depending on the disease form, which is in agreement with our findings in the current study.[5,32,45,46] Our survival rate was 55%. Despite the fact that most of the disseminated cases received a combined treatment, the high mortality rate shows that this treatment is not sufficient.

Empirical treatment for mucormycosis is emergent if there are suspicions.The cases in our study were definitively diagnosed cases. In fact it is certain that the rate is higher than it is in this series, because we think most cases have not been appropriately diagnosed. Although surgery was performed in most of the cases, the mortality rate was high due to the severity of the disease. Perhaps an earlier intervention should be made. Unfortunately, we did not have a chance for postmortem examination to prove this claim.

In conclusion, a steady increase in the reports of mucormycosis during the last decades may be due to increased awareness of a fungal infection in risk groups, and early diagnosis and treatment of these invasive fungal infections can improve the outcomes of children. The recommended management for overall survival of invasive mucormycosis has been surgical debridement combined antifungal therapy and restoration of the underlying immune status should be considered a key factor for a better outcome of the disease. As can be seen from these, good management of risk factors, especially neutropenia and hyperglycemia, prevents this disease from occurring.

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Original Article

Comparison of four different non-invasive respiratory support techniques as primary respiratory support in preterm infants

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ABSTRACT

Background. The use of non-invasive ventilation methods in neonatal intensive care units has been increasing in recent years. Non-invasive ventilation techniques are lung preserving methods and they reduce the risk of volutrauma, barotrauma, and atelectotrauma.

Methods. The effect of heated humidified high-flow nasal cannula (HHHFNC), continuous positive airway pressure (CPAP), nasal intermittent positive-pressure ventilation (NIPPV), and nasal high-frequency oscillation ventilation (NHFOV) were compared in preterm infants with respiratory distress.

Results. Between December 2015 and February 2017, a total of 76 preterm infants (gestational age <32 weeks) with respiratory distress were enrolled in this study. Of the patients, 20 received HHHFNC, while 20 received nasal CPAP (NCPAP), 19 received NIPPV, and 17 received NHFOV for respiratory support. The primary outcome was intubation requirement during non-invasive respiratory support. The secondary outcome included duration of non-invasive ventilation, air leak syndrome, abdominal distension, intraventricular hemorrhage, necrotizing enterocolitis (NEC), nasal injury, increased secretions, agitation, and mortality rate. The intubation ratio was higher in the NCPAP (40%) and NHFOV (29.4%) groups when compared with the NIPPV (10.5%) and HHHFNC (11.8%) groups. More nasal injury had developed in the NIPPV (78.9%) and NHFOV (82.4%) groups when compared with the NCPAP (40%) and HHHFNC (35%) groups. Moreover, the viscous secretion that blocked the cannulas was higher in NIPPV (78.9%) and NHFOV (76.5%) groups than NCPAP (25%) and HHHFNC (40%) groups. There were no significant differences in the duration of non-invasive ventilation methods, abdominal distension, NEC, air leak syndrome or mortality in the 4 groups.

Conclusions. The NIPPV and HHHFNC methods can be useful as a primary mode of respiratory support for respiratory distress. However, doctors need to be careful with regard to the complications that may develop.

Key words: non-invasive ventilation, preterm, respiratory support, different techniques, efficiency.

New developments in neonatal intensive care units (NICUs) regarding ventilation techniques, antenatal steroid treatment, and surfactant therapy have decreased lung disease-related morbidity and mortality in newborns.[1-3] Despite the use of surfactant treatments with high-frequency oscillation or volume guarantee ventilation methods, bronchopulmonary dysplasia (BPD) is still the most important cause of pulmonary morbidity in preterm infants.[2,4] Invasive mechanical ventilation is an important environmental risk factor for BPD due to volutrauma, barotrauma, and atelectotrauma.[4] In recent years, various non-invasive respiratory support (NRS) techniques have been used in neonates.[5] The aim of this study was to compare the effects of heated humidified high-flow nasal cannula (HHHFNC), continuous positive airway pressure (CPAP), nasal intermittent

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positive-pressure ventilation (NIPPV), and nasal high-frequency oscillation ventilation (NHFOV) in preterm infants with respiratory distress.

Material and Methods

This study was performed at Hacettepe University NICU between December 2015 and February 2017. Ethical approval was obtained from the institutional Hacettepe University, Clinical Research Ethics Committee (IRB number: 16969557-1327; GO 17/38-11) and participation involved informed consent. A total of 76 preterm infants (gestational age <32 weeks) with respiratory distress were enrolled in this study. All of the infants were supported with NCPAP (positive-end expiratory pressure (PEEP): 5 cm H_2O) in the delivery room and admitted to the NICU with CPAP. The newborns were randomly allocated into a nasal treatment mode of either HHHFNC, NCPAP, NIPPV, or NHFOV. During the study, 20 patients received HHHFNC, while 20 patients received NCPAP, 19 patients received NIPPV, and 17 patients received NHFOV for respiratory support. Newborns who required mechanical ventilation were excluded from the study. Moreover, newborns with congenital malformations or inherited metabolic diseases were also excluded.

All of the infants were loaded with caffeine citrate on their first day of life (10 mg/kg) and this continued each day. Data of the maternal characteristics (age, gestational age, mode of delivery, prenatal corticosteroid administrations, premature membrane rupture (PPROM), chorioamnionitis), and neonatal characteristics (birth weight, gender, 5-min Apgar score, etc.) of the patients were collected.

NCPAP

The NCPAP support was delivered via the bubble CPAP system (Fisher & Paykel Healthcare, Auckland, New Zealand), which generates continuous positive airway pressure, applied through short binasal prongs used as an interface (Optiflow Junior 2 nasal cannula). The respiratory pressure of the NCPAP was 5–6 cm H_2O.[6]

NIPPV

The NIPPV support was delivered via a conventional ventilator device (Drager Babylog 8000; Lübeck, Germany), which generates intermittent positive pressure ventilation, applied through short binasal prongs used as an interface (Optiflow Junior 2 nasal cannula). The initial ventilator parameters were PEEP: 5–6 cm H_2O; peak inspiratory pressure: 15–20 cm H_2O; inspiratory time 0.4–0.5 s; respiratory rate 25–30 breaths/min.

NHFOV

The NHFOV support was delivered via a high-frequency oscillation ventilator (Drager Babylog 8000), applied through short binasal prongs used as an interface (Optiflow Junior 2 nasal cannula). The initial ventilator parameters were mean arterial pressure: 6 cm H_2O; Delta P: 100% and rate: 10 Hz.

HHHFNC

The HHHFNC support was delivered via a precision flow device (Precision Flow, Vapotherm, Inc, Exeter, NH, USA), applied through the small bore cannula in the Vapotherm as an interface. The initial nasal flow parameters were: flow: 5 L/min, heat: 37 °C.

The fraction of inspired oxygen (FiO_2) was set in all of the NRS techniques with the target pulse oximeter rate of 90%–95%.

Non-invasive ventilation failure criteria included acidosis and hypercarbia (pH <7.20 and pCO2 >65 mmHg), apnea (≥2 episodes/h), and necrotizing enterocolitis (NEC) in the gastrointestinal tract. The criteria of surfactant administration included a FiO2 requirement higher than 0.4. The first dose of surfactant was 200 mg/kg (Curosurf, Chiesi, Parma, Italy) and an additional dose of 100 mg/kg was given at least 6 h after the previous administration. A

total of 54 patients had received minimally invasive surfactant therapy.

The primary outcome was the requirement of intubation during NRS. The secondary outcomes comprised the duration of non-invasive ventilation (days), air leak syndrome, abdominal distension, intraventricular hemorrhage (IVH), NEC, nasal injury, increased secretions, agitation, and mortality rate. N-PASS (Neonatal Pain Agitation and Sedation Scale) was used for diagnosis of pain and agitation and to determine the necessity of sedation.

Statistical analysis

For the calculation of the sample size, the rate of intubation requirement was used as the main primary outcome. A confidence level of α = 0.05 We used; the power level desired was 0.80, and consequently, 15 patients were needed for each group. Parameters of the 4 groups were compared using 1-way ANOVA for the continuous variables and the chi square test was performed for the categorical variables. Statistical significance was accepted as $P < 0.05$. Statistical analysis was performed using IBM SPSS Statistics for Windows (SPSS Inc., Chicago, IL, USA).

Results

The birth weight, gestational week (GW), maternal age, Apgar score, type of delivery, PPROM, and infection were evaluated as maternal and gestational properties. The median birth weight was 1190 g (600–2010) and the median GW was 28 weeks (26–32) in the HHHFNC group, while the median birth weight was 1240 g (580–2010) and the median GW was 28 (26–32) weeks in the NCPAP group. Moreover, the median birth weight was 1130 g (530–2550) and the median GW was 28 weeks (26–32) in the NIPPV group, while median birth weight was 1250 g (800–2240) and the median GW was 29 (27–32) weeks in the NHFOV group. There were no significant differences in the maternal and gestational features ($P > 0.05$) (Table I).

Of the patients, 54 needed surfactant therapy, but there were no significant differences in the surfactant necessity between the 4 groups ($P > 0.05$) (Table II).

The mean duration of NRS was 6.2 days in the HHHFNC group, 5 days in the NCPAP group, 3.4 days in the NIPPV group, and 4.2 days in the NHFOV group ($P > 0.05$). Additionally, there were no significant differences in the abdominal distension, NEC, air leak syndrome,

Table I. Maternal and gestational features of NRS groups.

	HHHFNC (n=20)	NCPAP (n=20)	NIPPV (n=19)	NHFOV (n=17)	p value
Birth weight (g) Median(min-max)	1190 (600-2010)	1240 (580-2010)	1130 (530-2550)	1250 (800-2240)	0.69
GW (week) Median(min-max)	28 weeks (26^2-32)	28 weeks (26^3-32)	28 weeks (26^2-32)	29 weeks (27-32)	0.58
Maternal age (year) mean ± SD	30.4 ± 5.8	29.9 ± 4.7	32.2 ± 4.4	30.2 ± 5.3	0.51
Apgar (5^{th} min)	9 (3-10)	9 (3-10)	8 (3-10)	9 (3-10)	0.48
C/S, n (%)	19 (95)	20 (100)	19 (100)	16 (94)	0.59
PPROM, n(%)	1 (5)	3 (15)	1 (5)	2 (12)	0.65
Chorioamnionitis, n(%)	1 (5)	1 (5)	0 (0)	0 (0)	1.0
Antenatal steroid, n(%)	6 (30)	5 (25)	7 (37)	11(65)	0.07

HHHFNC: Heated humidified high-flow nasal canula, NCPAP: Nasal continuous positive airway pressure, NIPPV: Nasal intermittent positive pressure ventilation, NHFOV: Nasal high-frequency oscillation ventilation, SD: Standart deviation, GW: Gestation week.
Apgar: Apgar score. C/S: Cesarian section. PPROM: Preterm premature rupture of membranes.

Table II. Surfactant necessity of infants in NRS groups

	HHHFNC	NCPAP	NIPPV	NHFOV	p value
Single dose surfactant	7	8	5	5	0.84
Two doses surfactant	2	3	2	4	0.67
Three doses surfactant	5	4	7	2	0.35

HHHFNC: Heated humidified high-flow nasal canula, NCPAP: Nasal continuous positive airway pressure, NIPPV: Nasal intermittent positive pressure ventilation, NHFOV: Nasal high-frequency oscillation ventilation.

Table III. Duration of ventilation and NRS related complications in NRS groups.

	HHHFNC (n=20)	NCPAP (n=20)	NIPPV (n=19)	NHFOV (n=17)	p value
NRS duration (day)	6.2 (2-25)	5 (2-15)	3.4 (2-12)	4.2 (2-8)	0.11
Air leak n(%)	0 (0)	0 (0)	1 (5.3)	1 (5.9)	0.22
Abdominal distension n(%)	8 (40.0)	7 (35.0)	5 (26.3)	8 (47.0)	0.62
NEC, n(%)	2 (10)	0 (0)	1 (5.3)	0 (0)	0.50
N-PASS score n(score>3)	5/20	5/20	5/19	4/17	0.54
Mortality, n(%)	0 (0)	1 (5.0)	0 (0)	0 (0)	1.0

HHHFNC: Heated humidified high-flow nasal canula, NCPAP: Nasal continuous positive airway pressure, NIPPV: Nasal intermittent positive pressure ventilation, NHFOV: Nasal high-frequency oscillation ventilation, NRS: Noninvasive respiratory support, NEC: Necrotizing enterocolitis, N-PASS: Neonatal pain agitation and sedation scale.

and mortality in the 4 groups (P > 0.05). Patients who had a N-PASS score of more than 3 required sedation. There were no significant differences between the groups (P > 0.05) (Table III).

The intubation ratio was higher in the NCPAP (40%) and NHFOV (29.4%) groups when compared with the NIPPV (10.5%) and HHHFNC (11.8%) groups (P < 0.05). Viscous secretion that blocked cannulas and required recurrent aspiration was present in the NIPPV (78.9%) and NHFOV (76.5%) groups when compared with the NCPAP (25%) and HHHFNC (40%) groups (P < 0.05). More nasal injury (nasal bleeding and ulceration) developed in the NIPPV (78.9%) and NHFOV (82.4%) groups when compared with the NCPAP (40%) and HHHFNC (35%) groups (P < 0.05). Moreover, more IVH was observed in the NIPPV (21.1%) and HHHFNC (10%) groups than in any of the other groups (P < 0.05) (Table IV).

We also separately evaluated small preterm infants whose gestational ages were below 28 weeks in the study group (Table V). The mean birth weight was not different between groups (P > 0.05). The intubation ratio was higher in the NCPAP (60%) and NHFOV (37%) groups when compared with the NIPPV (9%) and HHHFNC (15%) groups (P < 0.05). Viscous secretion was present in the NIPPV (90%) and NHFOV (80%) groups when compared with the NCPAP (30%) and HHHFNC (61%) groups (P < 0.05). There is no significant difference between the groups in terms of other morbidities (P > 0.05).

Discussion

In recent years, NRS methods have become the first respiratory support strategies in NICUs to prevent the development of BPD.[7,8] Hence, there are many studies comparing NRS methods and their results in the literature.[9-12] In this context, the use of different NRS methods has been proposed in different studies.

In this study, it was found that the HHHFNC and NIPPV groups had lower failure rates than the NCPAP and NHFOV groups. The intubation requirement was higher in the NCPAP and NHFOV groups. In a meta-analysis including 10 trials, NIPPV was stated as more efficient than NCPAP concerning the ratio of respiratory failure and intubation requirement.[13] Furthermore, in previous

Table IV. Non-invasive ventilation failure and procedure comorbid complications.

	HHHFNC (n=20)	NCPAP (n=20)	NIPPV (n=19)	NHFOV (n=17)	p value
Number of intubated infants after NIV n(%)	2(10)	8(40)	2(10.5)	5(29.4)	0.04
Viscous secretion n(%)	8(40)	5(25)	15(78.9)	13(76.5)	0.001
IVH n(%) (Grade 1)	2(10)	0(0)	4(21.1)	0(0)	0.04
Sepsis n(%)	7(35)	8(40)	9(47.3)	6(35.2)	0.85
PDA n(%)	3(15)	5(25)	5(26.3)	3(17.6)	0.81
Nasal injury n(%)	7(35)	8(40)	15(78.9)	14(82.4)	0.002
BPD n(%)	6(30)	3(15)	10(52.6)	6(35.2)	1.0

HHHFNC: Heated humidified high-flow nasal canula, NCPAP: Nasal continuous positive airway pressure, NIPPV: Nasal intermittent positive pressure ventilation, NHFOV: Nasal high-frequency oscillation ventilation, IVH: Intraventricular hemorrhage, PDA: Patent ductus arteriosus, BPD: Bronchopulmonary dysplasia.

Table V. Comparison of patient group findings under 28 weeks of gestation.

NRS Groups	GH: 28 weeks under cases(n=42)				
	HHHFNC (n=13)	NCPAP (n=10)	NIPPV (n=11)	NHFOV (n=8)	p value
NRS duration (day)(mean)	7.9	4.1	6.1	4.5	0.15
(min-max)	(5-25)	(3-15)	(2-12)	(3-8)	
GW (week)	27 weeks	27 weeks	27 weeks	27 weeks	0.79
Median(min-max)	(26^{2}-27^{4})	(26^{3}-27^{5})	(26^{2}-27^{4})	(27-27^{6})	
Birth weight (g)	1066.5± 328	1180 ± 396	1032.7 ± 370	1145 ± 265	0.59
mean ± SD	(600-1660)	(580-1770)	(530-1760)	(800-1510)	
Apgar (5th min)	8 (3-10)	7 (3-10)	7 (3-10)	8 (3-10)	0.95
C/S, n (%)	12(92)	10(100)	11(100)	8(100)	0.51
PPROM, n(%)	1(7)	3(30)	1(9)	1(12)	0.43
Antenatal steroid, n(%)	5(38)	4(40)	5(45)	5(63)	0.72
Abdominal distension, n(%)	6(46)	5(50)	3(27)	4(50)	0.67
N-PASS score n(score>3)	4 (30)	4(40)	3(27)	3(37)	0.23
Number of intubated infants after NIV n(%)	2(15)	6(60)	1(9)	3(37)	0.02
Viscous secretion, n(%)	8(61)	3(30)	10(90)	7(87)	0.01
Sepsis, n(%)	7(53)	6(60)	7(63)	4(50)	0.93
PDA, n(%)	3(23)	5(50)	5(45)	3(37)	0.55
Nasal injury, n(%)	7(53)	5(50)	10(90)	7(87)	0.08
BPD, n(%)	5(38)	3(30)	5(45)	3(37)	0.69

HHHFNC: Heated humidified high-flow nasal canula, NCPAP: Nasal continuous positive airway pressure, NIPPV: Nasal intermittent positive pressure ventilation, NHFOV: Nasal high-frequency oscillation ventilation, IVH: Intraventricular hemorrhage, PDA: Patent ductus arteriosus, BPD: Bronchopulmonary dysplasia.

studies with preterm infants, intubation rates were lower with NIPPV than with NCPAP.[14-16] Li et al.[17] compared NCPAP and NIPPV as the primary mode of respiratory support. They stated that there was a significant decrease in the intubation need and invasive ventilation in the NIPPV group, as in the current study. But we already know that synchronisation in NIPPV has much better results concerning the requirement for intubation and lung damage that will lead to the development of BPD.[18] One of the limitations in our study was that we could

not do synchronized NIPPV. However, there are studies in the literature showing that BPD, mortality, NEC and IVH rates do not change when synchronization is achieved.[19] Also no significant difference was found in the BPD and mortality in patients below 28 weeks GA.

According to previous studies, NHFOV does not require synchronization and caused less barotrauma.[20-22] However, similar to the current findings herein, Czernik et al.[23] determined a high risk of respiratory failure in extremely preterm babies. Moreover, a survey of 5 European countries described the side effects of NHFOV as agitation, viscous secretions, and upper airway obstruction associated efficiency problems. As a result of the higher mean pressures with NHFOV, abdominal distention becomes another side effect of this technique.[24] Viscous secretions and the need for very frequent aspiration was observed in the NHFOV group in the current study. It was also speculated that the use of a relatively low frequency at high amplitude with NHFOV causes excessive viscous secretion formation in the upper airway.[25] On the other hand, in babies below 28 weeks GA, nasal secretion rates were found to be high in all NRS groups except NCPAP group in the current study. Although ventilator-associated pneumonia and congenital pneumonia may cause increased secretion, pneumonia was not detected in our study cases. Pulmonary findings of our RDS cases regressed after surfactant treatment.

Additionally, the abdominal distension ratio was relatively higher in the NHFOV group than in the other groups herein, but it was not statistically significant.

In recent years, HHHFNC has been used in NICUs as a NRS.[26] Observed in the current study was a lower failure rate in the HHHFNC group when compared with the NCPAP group. Roberts et al.[27] compared NCPAP and HHHFNC as the primary mode of respiratory support and found a higher failure rate in the HHHFNC group. However, in their study, they used this method for early respiratory support without the use of a surfactant. Surfactant treatment was also given to the infants in the current study. Another randomized clinical trial found an increase rate of intubation in the HHHFNC group when compared with the NCPAP group.[28]

However, it was reported that flow levels higher than 4 L/min could solve this problem. In the present study, the initial nasal flow rate was 5 L/min. There were no differences observed in the efficacy and safety between the HHHFNC and NCPAP groups in a study involving 432 preterm babies.[29] Surfactant administration and higher flow rates might explain the success of HHHFNC in the current study. However, efficiency of HHHFNC is still controversial when compared with nasal NCPAP. HHHFNC generates pressure in the nasopharyngeal area, and there is not enough information about how much pressure is reflected into the respiratory tract. High flow rates lead to increase pharyngeal pressure.[30,31] Hence, the amount of pressure formed in the nasopharyngeal space is not exactly known.[32,33] The same failure rate was also found in the HHHFNC group when compared with the NIPPV group. When we evaluate our preterm patients below 28 weeks GA, the failure rate was high in the NCPAP and HFOV groups again.

On the other hand, the NRS methods, in terms of comorbid complications, were also evaluated herein. No significant differences in the sepsis, air leak, hemodynamically significant patent ductus arteriosus, NEC, BPD, or mortality were found. Similar results were found in preterm babies below 28 weeks GA. In the NIPPV group, 4 IVHs were observed, while 2 IVH were observed in the HHHFNC group. However, the IVHs in these patients were grade 1 and there were no neurological symptoms. Another complication investigated was the nasal injury between the groups. Nasal injury was followed-up as granulation tissue, ulceration, necrosis, and deformation of the nasal septum and edge of the nostril. Nasal injuries were higher in the NHFOV and NIPPV groups than in the NCPAP group. This may have occurred due

to viscous secretions and mucosal dryness. Moreover, the least nasal injury was observed in the HHHFNC group, special nasal cannula of HHHFNC might explain this situation. This nasal cannula allows air to leak from the nostril and this reduces nasal injury.[34] Although there was no statistical difference in our study, prolonged NRS duration is a risk factor for nasal injury. The N-PASS score was used to assess the comfort level of the patients, but no differences were found between the NRS groups.

In conclusion, this data suggested that the NIPPV and HHHFNC methods have beneficial effects on preterm infants in NICUs. These methods can be useful but they require more experience to use as a primary mode of respiratory support for RDS. NHFOV is thought to cause less barotrauma and damage to the lungs. However, doctors need to be careful with regards to its complications. Therefore, there is a need for further multicenter randomized controlled trials in a wider population.

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Original Article

Shaken baby syndrome resulting in death: a case series

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ABSTRACT

Background. The aim of this study was to raise the awareness of all healthcare personnel, primarily Emergency Department physicians, forensic physicians, and paediatricians about cases of shaken baby syndrome (SBS), which can be difficult to diagnose, require a high level of suspicion in diagnosis and there is a high likelihood of missed diagnosis.

Methods. A retrospective examination was made of 3400 forensic cases applied with autopsy between 2012 and 2018 to the Forensic Medicine Institution of Malatya Group Directorate.

Results. Of the total cases, 113 were aged <2 years and head trauma was determined in 35. Eight cases were determined as SBS. Of the 8 cases that resulted in death, 75% were male and the mean age was 8.3 months. The trauma had been perpetrated by the father in 50% of cases. There was a history of seizure in 67.5% of the infants and 75% were taken to the Emergency Department with cardiopulmonary arrest. Subdural hematoma and subarachnoid hemorrhage were determined in 37.5% of the cases, subdural hematoma, subarachnoid hemorrhage and intracranial hemorrhage in 50%, and epidural hemorrhage and subarachnoid hemorrhage in 12.5%. In the microscopic examination of the medulla spinalis, focal subdural fresh bleeding was seen in 50% of cases. Bleeding around the optic nerves was determined in 37.5% of cases bilaterally and in 37.5% unilaterally. Retinal hemorrhage was determined in all the cases, which was bilateral in 75%.

Conclusions. The actual worldwide incidence of SBS is not known, and in Turkey, it is thought to be much higher than has been determined. The most important reason for this is thought to be that the diagnosis of child abuse is difficult and physicians do not have a sufficient level of knowledge.

Key words: shaken baby syndrome, autopsy, death.

One of the leading causes of death of physical abuse, which is a type of child abuse, is head trauma.[1] Non-accidental head trauma in children is seen most often in the first year of life and is the most common cause of non-natural death in infancy.[2] The sub-group of "shaken baby syndrome" (SBS) is the most frequently seen non-accidental head trauma in children.[3] SBS is a specific type of child abuse in which the infant is held by the extremities or thoracic region and shaken, resulting primarily in retinal bleeding, bone fractures, diffuse axonal damage, and subdural and subarachnoidal bleeding, through acceleration and deceleration mechanisms.[3,4]

Traumas causing rotation in the brain, as in abuse, usually cause severe and fatal injuries. Unlike adults, the brain is within a thin and elastic skull as ossification has not been completed.[1] In a newborn, the brain constitutes 10%-15% of the body weight, whereas in adults it is 2%-3%. The water content of the infant brain is high and myelin sheaths are thin and immature structures until the age of two years.[1] Therefore, ruptures associated with trauma in immature brain tissue are more common

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than contusions. Particularly below the age of two years, the incomplete myelinisation and the short lengths of axons lay the ground for rupture. In addition, as the neck muscles are still weak, the brain is more exposed to acceleration and deceleration forces.[5]

The aim of this study was to create increased awareness of SBS in all healthcare personnel, but especially emergency department physicians, paediatricians, forensic physicians and forensic pathologist, in difficult-to-diagnose cases and those which are diagnosed and there is serious suspicion, where is a high probability of missing the diagnosis. The current study has the highest number of autopsy case series reported from Turkey.

Material and Methods

The study protocol was approved by the Institutional Ethics Committee of Inonu University and Malatya Group Forensic Medicine Institution (2018/3-19, 30.01.2018). Of the 3400 forensic cases whom autopsy was performed at the Forensic Medicine Institution of the Malatya Group Directorate between 1 January 2012 and 30 June 2018, 113 were identified as autopsies on infants under two years of age. From the evaluation of hospital records, histopathology examination reports, incident reports prepared by the police or armed forces, and witness and defendant statements, 35 cases of head trauma were identified. The cause of death of 8 of these 35 cases was determined as SBS.

These 8 cases were evaluated in respect to sociodemographic data, means of transport to the hospital, admission history, clinical findings, ophthalmic and intracranial injuries, and cause of death with histopathological examination.

Results

The examination was made of 8 cases that presented to the Emergency Department with findings of intracranial bleeding which resulted in death and were diagnosed with SBS following an autopsy. The cases comprised 6 (75%) males and 2 (25%) females. The age range of the cases ranged from 1.5 to 20 months and the mean age was 8.3 months. The trauma was inflicted on the infant by the father in 4 (50%) cases, by the mother in 1 (12.5%) case, and in 3 (37.5%) cases, the perpetrator could not be determined. In 4 (50%) cases, the infant suffered a seizure after the trauma, and there was a history of seizures in 5 (67.5%) cases. It was determined that 6 (75%) infants were brought to the Emergency Department with cardiopulmonary arrest. Of those who responded to resuscitation, 2 (25%) were exitus following follow-up in the paediatric intensive care unit (ICU), 1 (12.5%) in the paediatric surgical ICU, and of those with no history of arrest, 1 (12.5%) was lost in the paediatric ICU and 1 (12.5%) in the brain surgery ICU. With the imaging techniques applied, a linear bone fracture of the skull was determined in 2 (25%) cases. Detailed information of the cases are given in Table I.

Classic autopsy was applied to the 8 cases evaluated. The histopathology samples (brain, cerebellum, medulla spinalis, lung, liver, kidney, heart and bilateral eyeballs with optic nerves) obtained during the autopsy were examined macroscopically and microscopically. The results showed subdural haemorrhage (SDH) and subarachnoid haemorrhage (SAH) in 3 (37.5%) cases intracerebral haemorrhage (ICH) together with SDH and SAH in 4 (50%) cases, and epidural haemorrhage (EDH) and SAH in 1 (12.5%) case. Microscopically focal subdural fresh bleeding was determined in 4 (50%) cases of the medulla spinalis. The eyeballs and optic nerves were examined macroscopically and microscopically. Bleeding was determined around the optic nerve bilaterally in 3 (37.5%) cases and unilaterally in 3 (37.5%) cases, and no bleeding was determined in 2 (25%) cases. Retinal bleeding was determined bilaterally in 6 (75%) cases and unilaterally in 2 (25%) cases (Figs. 1-4).

Table I. Findings of the cases.

Case No	1	2	3	4	5	6	7	8
Age (months)	1.5	4.5	20	1	13	13.5	1.5	11
Gender	M	M	M	M	M	F	M	F
Perpetrator	Father	Father	Father	Unknown	Father	Unknown	Mother	Unknown
Bone fracture	-	-	+	+	-	-	-	-
Arrest at presentation	+	+	-	+	+	-	+	+
Admission to the ICU	+	+	+	-	-	+	-	+
Seizure after trauma	+	-	+	-	-	+	-	+
Intracranial bleeding	ICH SDH SAH	SDH SAH	SDH SAH	SDH SAH	ICH SDH SAH	ICH SDH SAH	EDH SAH	ICH SDH SAH
Bleeding around the optic nerve	+/+	-/+	-/-	+/+	-/+	-/-	+/+	-/+
Retinal haemorrhage	+/+	+/+	+/+	+/+	+/+	-/+	+/+	-/+
Injury in the medulla spinalis	+	+	No sample	No sample	+	+	No sample	No sample

*M: male, F: female, ED: emergency department, ICU: intensive care unit, ICH: intracerebral haemorrhage, SDH: subdural haemorrhage, EDH: epidural haemorrhage, SAH: subarachnoid haemorrhage

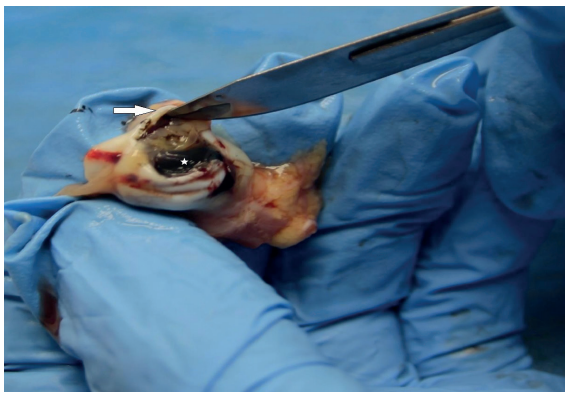

Fig. 1. The asterisk indicates retinal fresh hemorrhage and the arrow indicates the cornea.

Discussion

In the few studies on this subject, the incidence of SBS in infants aged under one year has been reported as 0.14-0.34/1000.[6-10] Although the incidence of SBS is known to be higher, the actual incidence is not fully known because of undiagnosed and misdiagnosed cases.[6] Until 2001, there were no reported cases of SBS in Turkey, which can be attributed to insufficient identification of cases and non-reporting because of cultural reasons.[11] The increase in SBS diagnosis in recent years is associated with greater awareness and sensitivity and the use of modern techniques. Of these, the evaluation of the medical and social history of the child, examination of the scene of the incident, correct evaluation of the macroscopic findings in autopsy and detailed histopathological evaluation are important.[4]

Head trauma in childhood is seen most often before the age of one year, and there is a currently increasing rate of SBS incidence reported as the cause of head trauma.[1] Reports in the literature demonstrate that SBS is generally seen in infants aged under one year. Studies of cases diagnosed with SBS reported the mean age as 9 months in the USA, and as 4.2 months in a 10-year study in Canada.[12-15] In the current study, the mean age was determined to be 8.3 months, which was consistent with findings in the literature.

Previous studies have reported the mortality rate of SBS cases as approximately 25% and death within a few days.[2,3] In a study of 52 infants diagnosed with SBS, mortality was reported in

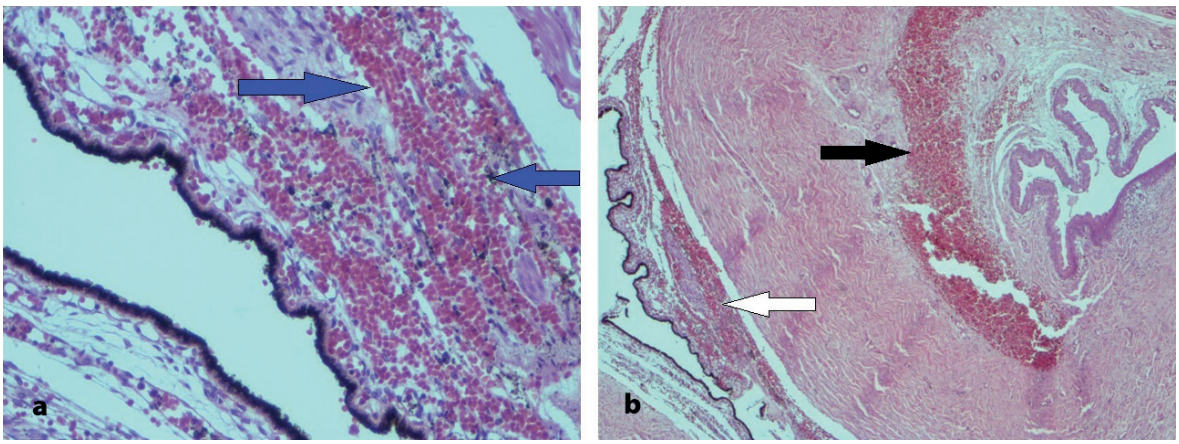

Fig. 2a. Fresh hemorrhage in the retinal layer of the eye (blue arrows). H&E x100. **b.** Fresh hemorrhage area (black arrow) in the optic nerve and fresh hemorrhage focus (white arrow) in the retina adjacent to the optic nerve. H&E x40.

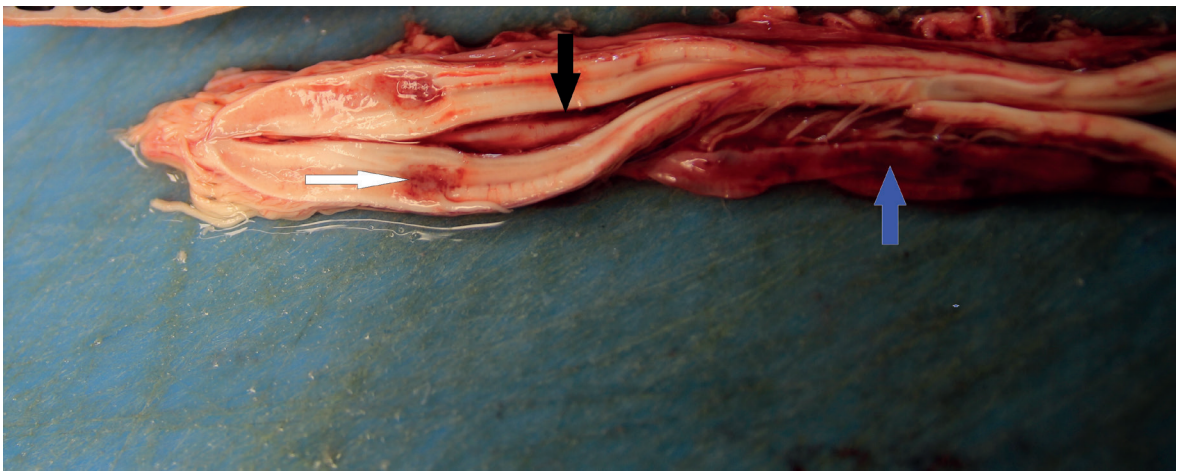

Fig. 3. Focal subdural fresh hemorrhage (blue arrow), focal paranchimal hemorrhage (white arrow) of medulla spinalis and bloody central canal fluid (black arrow).

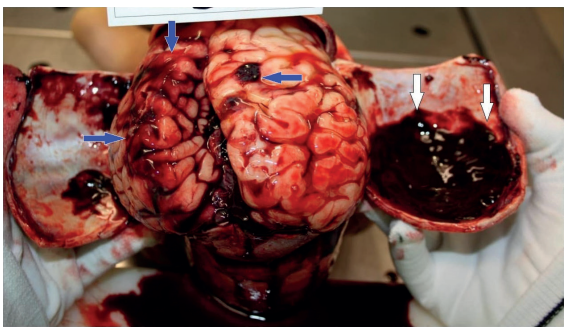

Fig. 4. Subdural fresh hemorrhage (white arrows) and subarachnoid fresh haemorrhage of left and right parietal lobes of brain (blue arrows).

20 (38.5%).[16] It has also been reported that many cases are admitted to the hospital with a coma.[17] In the current study, 6 (75%) cases were brought to the Emergency Department with arrest, and 5 (67.5%) had a history of ICU admission. The infants aged under one year were hospitalised because of cranial trauma, 65% were reported to be victims of child abuse. It was therefore stated that a detailed trauma history must be taken from the family and eyewitnesses.[18]

The parents of five SBS cases initially admitted having shaken the infant[12] and in another study, the parents of five infants admitted shaking them in later interviews.[17] In this study, when the recorded statements were examined, it was determined that 4 (50%) infants had been shaken by the father, and 1 (12.5%) by the mother.

Generally, SBS cases present at a healthcare centre, with no history of trauma, shaking, or falls, and presentation is with complaints of seizure, vomiting, a tendency for sleepiness, coma, respiratory problems, restlessness and poor breast suckling.[12,18] Consistent with the literature, all cases in our study presented with complaints of seizure, vomiting, a tendency for sleepiness, coma, respiratory problems, restlessness or poor breast suckling. Yağmur et al.[11] reported that physical findings were determined in only 1 of 3 SBS cases, and Becker et al.[17] determined physical findings in 2 of 5 infants diagnosed with SBS. Similarly, in the current study, physical findings were determined in 2 (25%) of 8 cases.

Head trauma developing as a result of child abuse is seen up to the age of 4-5 years, and most often in infants below the age of one year.[1] In childhood, the brain tissue is proportionally greater in the body and has a high water content, is located in a thin and elastic skull, the ossification of the skull is not complete, and sutures are not completely closed.[1] SDH is the most frequently seen intracranial bleeding and sometimes SAH may be seen alone or together with SDH in particular.[17] A Canadian study of 364 cases diagnosed with SBS reported that 85% were positive for SDH and in another study that evaluated child abuse cases with head trauma, SDH was determined at the rate of 80-85%.[13] In a study of autopsies performed on children with head trauma as a result of abuse, subdural hematoma was determined in 90%.[14] The histopathological examination of the current study cases showed subarachnoid bleeding in all the cases, SDH in 87.5%, intracerebral haemorhage in 50% and epidural haemorrhage in 12.5%.

In cases of SBS, as the optic disc and distal optic nerve are the regions most affected by tension and rupture forces, samples should be taken from these areas for histopathological examination.[8] Retinal haemorrhage has been seen at the rate of 50%-100% in SBS cases, all layers of the retina are involved in the majority and have been reported as bilateral.[7] Although the presence of subdural and retinal haemorrhage is pathognomic for the diagnosis of SBS[20], only retinal haemorrhage can be determined.[17] In addition, unilateral retinal haemorrhage, although rarely seen, should not be removed from the diagnosis, on the contrary, it greatly supports SBS diagnosis.[15] Similarly in the current study, bleeding around the optic nerve was seen in 75% of the cases, unilateral in 37.5%, and bilateral in 37.5%. Retinal haemorrhage was seen in all the cases, bilaterally in 75% and unilaterally in 25%.

SBS is a preventable problem. If it is not possible to reach the children before they have suffered shaking or other abuse, correct diagnosis and treatment with protective measures become important in the subsequent period. At the stage of diagnosis, it is important that a more detailed anamnesis, the necessary consultations and advanced tests are applied in all suspicious paediatric cases presenting at healthcare institutions, and especially in infants aged under two years. SBS must be considered in the differential diagnosis of infants aged under two years presenting with retinal bleeding, subdural hematoma and non-specific complaints. A diagnosis of abuse will prevent the child being sent home to be abused again and will provide protection for other children in the home.

The true incidence of SBS is still not known. In literature, extremely few SBS cases have been reported from Turkey compared to other developed countries in the world. The most important reasons for this are that healthcare personnel in Turkey do not have a sufficient level of knowledge to identify child abuse, and most cases presenting at the Emergency Department with head trauma or abuse are missed. Therefore, training of healthcare

personnel in the diagnosis of these cases is of great importance. It is also vital in respect of preventative medicine that there is sufficient training and support on the subject of childcare for families of low socioeconomic status, primarily for parents.

It should be remembered that SBS may be present in pediatric patients who come with a history of nausea, vomiting, general condition and seizures. However, a family history that is incompatible with the physical examination findings should bring to mind this syndrome, which is already difficult to diagnose. The exaggerated and inconsistent attitudes of people who take care of the child (mother, father, babysitter) should be a warning for the physicians in terms of SBS. Parents who have exaggerated child love, which is common in our society and welcomed by all of us, should warn the doctor who examines the child of SBS. On the contrary, it should be noted that a SBS can be hidden under this condition. In the autopsies of infants, the diagnosis of SBS must not be forgotten. To be able to make a diagnosis in suspicious cases, correct macroscopic and microscopic evaluations should be supported by sampling.

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Original Article

Evaluation of injury-related under-five mortality in Turkey between 2014-2017

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ABSTRACT

Background. Injury is the most common cause of preventable morbidity and mortality among children. The aim of this study was to determine the epidemiological characteristics and trends of injury-related mortality in children under five and to provide evidence for future preventive strategies.

Methods. Our study was designed as a descriptive cross-sectional study. National under-five mortality data from the Death Notification System database, entered between January 1st, 2014, and December 31th, 2017, was included in the study.

Results. Among all under-five deaths registered in the system, 2,560 injury-related death cases were included in the study. Overall, deaths related to injuries accounted for 4.1% of all deaths in children under five years old. Of all injury deaths, 59.9% of cases were male, 52.7% occurred at home or its close vicinity, and 80.3% were children aged 12–59 months. Injury-related under-five deaths were mainly attributed to traffic injuries (36.5%), falls (12.0%), and suffocation (10.2%). Traffic injuries were the most common cause of injury-related deaths both in infants 0-11 months and children 12-59 months old. The second and third most common causes of injury-related deaths among infants 0-11 months were suffocation and falls, while these were falls and drowning in children aged 12-59 months, respectively. The injury-related under-five mortality rate dropped from 11.3 per hundred thousand in 2014 to 9.1 in 2017. Causes of all unintentional injury-related deaths were associated with season except for other unintentional injuries and exposure to mechanical forces ($p < 0.001$).

Conclusions. The injury-related mortality rate among children under five years declined from 2014 to 2017, however it is still high. To prevent injuries in children under five, it is important to raise awareness and increase the supervision of children by their caregivers. At the national level, multisectorial cooperation with a holistic approach will be of key importance.

Key words: injury, mortality, under-five, children, Turkey.

Injury is the most common cause of preventable morbidity and mortality among children in every country in the world. Injury is defined as intentional or unintentional acute exposure of the human body to thermal, mechanical, electrical, or chemical energy exceeding the limits of physiological tolerance, or deprivation of the human body from vital elements, such as oxygen and heat.[1] Every year more than 5 million people die as a result of injuries, accounting for approximately 9.0% of worldwide deaths.[1,2] It is estimated that, for every child death caused by an injury, there are 129 hospital admissions, 1,635 emergency service visits, and thousands of primary care appointments.[3] Most survivors of injuries are subject to temporary or permanent disability, and injuries are responsible for approximately 6.0% of all disability adjusted life years (DALY).[2] In Turkey, when all age groups are taken into consideration, 4.5% of all deaths,

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5.3% of hospitalizations, and 9.0% of disease burden are due to injuries.[4]

Globally, the child mortality rate has decreased by approximately half over the past 25 years, mainly due to the decline in infectious diseases.[5,6] However, the decline in injury-related mortality rate has been relatively slower. Therefore, deaths due to injuries in children have become increasingly prominent, especially in high income countries.[1,7,8] In 2013, accidental injuries were estimated to result in 324,000 deaths in children aged 1-59 months.[7] According to the World Health Organization's (WHO) 2016 data, injuries account for 7.0% of deaths under the age of five years.[5] Disparities also exist between the child injury mortality rates of developing and developed countries, with 95% of all child injury deaths occurring in developing countries.[1,9]

A detailed evaluation of the epidemiology of injury mortality is important for determining the priorities of preventative public health policies. The "Infant and Child Mortality Surveillance Program" and the "Child Safety Program" are both performed by the Child and Adolescent Health Department (CAHD) of the Ministry of Health (MoH) in Turkey, and have been since 2007 and 2014, respectively. Although the epidemiology of injury-related mortality in children has already been studied in several regional studies[10,11], there is no published study that assesses the injury-related deaths among children under-five at the national level. The aim of this study was to analyze epidemiologic characteristics and trends of injury-related under-five deaths between the years 2014 and 2017 in order to provide evidence for future preventive strategies.

Material and Methods

Our study was planned as a descriptive cross-sectional study. Data on under-five deaths were obtained from the Death Notification System (DNS) database. Since 2013, all deaths are registered in the DNS database. In this database, along with the deceased's personal information, the socio-demographic characteristics, death type, a forensic outcome, and injury information sections are requested to be filled in. Whether the death had a natural cause or is a forensic case, and if the death was due to a communicable or non-communicable disease are asked in the death type section. In instances where forensic case is selected, the forensic outcome area is mandatory. The forensic outcome area includes suicide, homicide, traffic accident, occupational accident, other accidents, and ongoing forensic research options, and helps to determine the intentional status of the death. If an autopsy is performed, the result of the autopsy is recorded in the system by the forensic practitioner. When a death occurred as a result of injury, the injury information section should also be filled in. This section includes information such as the date of injury, place of injury, and whether it was a work accident. All the diseases and conditions contributing to a person's death are also recorded chronologically in the system, using the International Classification of Statistical Disease Classification (ICD-10) codes. In the system, a stepwise causal chain is used in reporting the causes of death. The "main cause" of death is defined as the oldest of the series of diseases that led to the death, and is thus the disease that initiated the death process. The final cause of death is defined as the most recent disease in the series of diseases leading to the death. According to the Infant and Child Mortality Surveillance Program workflows, "Provincial Infant Mortality Monitoring Committees" also review all infant (0-11 months) deaths in detail. The final electronic reports of these committees are sent to DNS by the end of each month.

All child deaths under 60 months (born alive with any gestational week) in DNS between January 1st, 2014, and December 31st, 2017, were included in the study. Among all deaths, the deaths having any of the injury diagnosis codes as their main cause of death were identified by the working group. Injuries due to birth trauma were excluded from the study. A data sheet for evaluating each injury mortality case was filled

out including: 1) date of birth, 2) date of injury, 3) place of injury, 4) date of death, 5) age, 6) gender, 7) province of residence, 8) diagnostic ICD-10 codes, 9) death type, 10) forensic outcome, and 11) autopsy result (if performed).

The causes of injury were classified using the ICD-10 codes determined in the recently published WHO guidelines and used in previous studies by the working group.[12-16] Based on WHO guidelines, the injury-related deaths were divided into intentional and unintentional injury subheadings.[12,13] The intention status of an injury was determined by evaluating the above mentioned death type, forensic outcome, injury information section data, and ICD-10 codes together. The intentional injuries subheading included: self-harm (X60-X84, Y87.0), interpersonal violence (homicide/assault) (X85-Y09, Y87.1), and collective violence and legal intervention (Y35-Y36, Y89, Y89.1). The unintentional injuries subheading included: traffic injuries (V01-V99), poisonings (X40–X49), falls (W00–W19), burns (X00-X19), drownings (W65-74), exposures to mechanical forces (W20-W38, W40-W43, W45, W46, W49-W52), natural disasters (X33-X39), suffocation (W75–W76, W78–W80, W83–W84), and other unintentional injuries (rest of V01–Y98). "Exposures to mechanical forces" included: contact with falling or thrown objects, contact with sharp objects (knives, swords, wedges, glass, etc.), gunshot wounds, contact with machines (agriculture, industry, and electrical appliances), and explosions (gas cylinder, boiler, pressure equipment, bombs, etc.). The subtitle "other unintentional injuries" was used to evaluate exposures to electric current, contact with animals and toxic animals (bite, attack), exposure to heat, cold, or sun, freezing, staying in an oxygen-free environment, food and water deprivation, poverty, accident sequelae, surgical treatment and medical care complications, and cases due to the side effects of exposure to biological substances, drugs, and vaccines.[12,13] In our study, injury-related mortality cases having poorly-defined causes and/or unknown intent due to ongoing forensic research were collected under the third subheading of "other, ungrouped" by the working group.

In calculating cause-specific mortality rates, age-specific population numbers referring to the relevant years were used. The risk of injury varies according to different stages of development, therefore, under-five injury mortality cases were stratified into two groups by age: 0–11 months (infants) and 12-59 months.[17]

The study was approved by the Hacettepe University, Non-Interventional Clinical Research Ethics Committee (2019/13-20) and official permission was obtained from the General Directorate of Public Health (official permission date and number: March 20, 2019; 67414668-020-E.300) for sharing and analysis of the DNS data. Informed consent was obtained from parents before the registration of mortality information on the DNS database.

Data were analyzed using SPSS ver. 23.0 statistical software package. Analyses included arithmetic mean and standard deviation for continuous variables and frequency and percent distributions for categorical variables. Chi-square test was used for group comparisons. Type I error was pre-set at 0.05 for all analyses.

Results

Based on DNS data, between 2014 and 2017, a total of 62,192 under-five deaths occurred, and 2,560 of all under-five deaths were due to injuries. Overall, deaths related to injuries accounted for 4.1% of all under-five deaths in children (Table I). Of all injury deaths, 1,534 (59.9%) cases were male and the female/male ratio was 1/1.5. While 19.7% (n=505) of the cases were in the 0-11 months old age group, 80.3% (n=2055) were in the 12-59 months old age group. In the majority of cases, the injury occurred at home or in the close vicinity (52.7%). When seasonal variations in injury-related deaths were examined, it was found that deaths were highest in the summer (n=963, 37.6%) and lowest in the winter (n=338, 13.2%) (Table I).

Table I. Basic information of injury-related under five mortality in Turkey, 2014-2017 (N=2560).

		2014	2015	2016	2017	Total
Total number of under-five death (N)		16536	16108	15475	14073	62192
Death caused by injury N (%)*		709 (4.3)	632 (3.9)	629 (4.1)	590 (4.2)	2560 (4.1)
Age	0-11 months	144 (20.3)	143 (22.6)	104 (16.5)	114 (19.3)	505 (19.7)
N (%)**	12-59 months	565 (79.7)	489 (77.4)	525 (83.5)	476 (80.7)	2055 (80.3)
Gender	Male	432 (60.9)	374 (59.2)	379 (60.3)	349 (59.2)	1534 (59.9)
N (%)**	Female	277 (39.1)	258 (40.8)	250 (39.7)	241 (40.8)	1026 (40.1)
Place of injury	Home or its close vicinity	363 (51.2)	338 (53.5)	333 (52.9)	316 (53.6)	1350 (52.7)
N (%)**	Areas away from the home	346 (48.8)	294 (46.5)	296 (47.1)	274 (46.4)	1210 (47.3)
	Spring	196 (27.6)	156 (24.7)	171 (27.2)	142 (24.1)	665 (26.0)
Season	Summer	259 (36.5)	238 (37.7)	246 (39.1)	220 (37.3)	963 (37.6)
N (%)**	Autumn	163(23.0)	163 (25.8)	135 (21.5)	133 (22.5)	594 (23.2)
	Winter	91 (12.8)	75 (11.9)	77 (12.2)	95 (16.1)	338 (13.2)

* Proportion of injury-related death numbers to total numbers of under-five deaths of relevant years.
**Column percentage

The injury-related under-five mortality rate dropped from 11.3 per hundred thousand in 2014 to 9.1 per hundred thousand in 2017 (Fig. 1). The largest contributors to this change were the declines in the rates of traffic injuries (from 4.4 to 3.1), drownings (1.1 to 0.7), and falls (1.5 to 1.2) (Fig. 2).

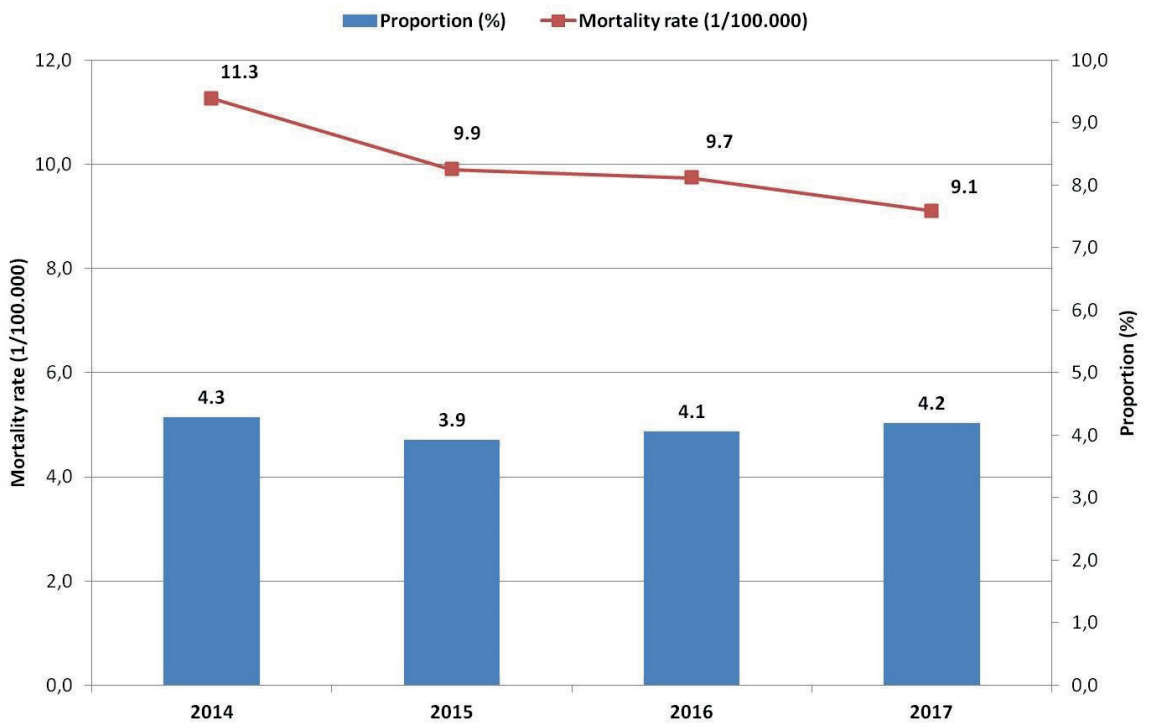

Fig. 1. Injury related mortality rates and the proportion of injury-related deaths to all cause of deaths in under-five children in Turkey, 2014–2017 (N=2560).

*Cause-specific mortality rates were calculated using the number of under-five populations of relevant years.

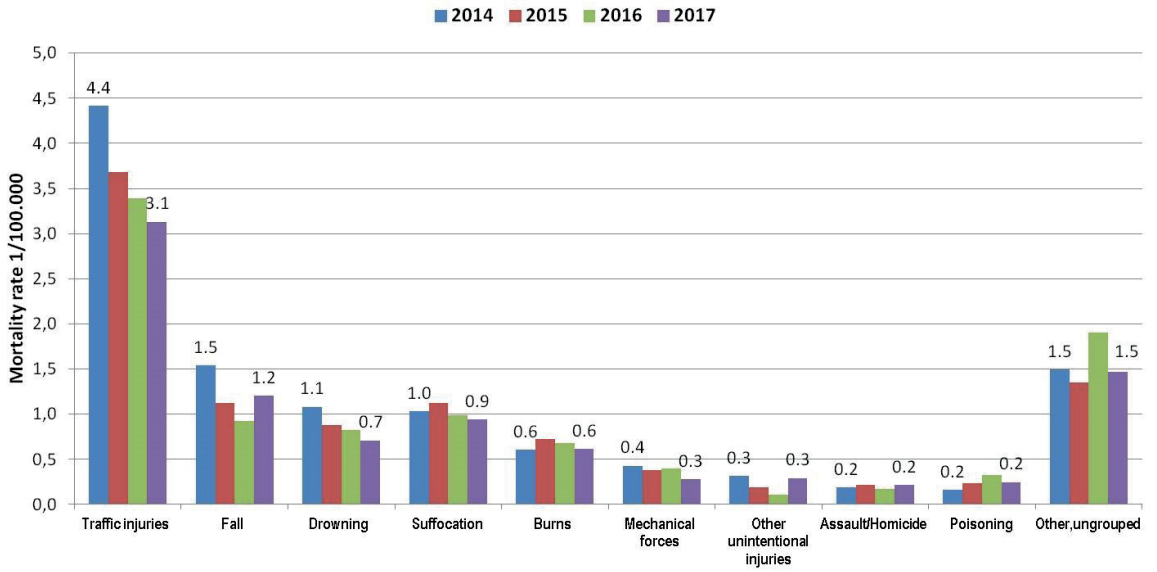

Fig. 2. Trends of mortality rates due to specific types of injury in children aged under-five in Turkey, 2014-2017 (N=2560).

*Cause-specific mortality rates were calculated using the number of under-five populations of relevant years.

Injury-related under-five deaths were mainly attributable to traffic injuries (36.5%), falls (12.0%), and suffocation (10.2%) (Table II). The top cause of injury mortality was traffic injuries, both in infants and among children aged 12-59 months (35.6% in infants; 36.7% in 12-59 months). Among infants, suffocation (27.9%) and falls (10.7%) were the second and third leading causes of injury mortality; among children aged 12-59 months old, falls (12.3%) and drowning (9.6%) were second and third, respectively (Table II). The other ungrouped cases category consisted of 15.5% of all under-five injury mortality cases. During the detailed evaluation, it was found that 96.0% of these cases were trauma cases in which the type of injury could not be determined. All injury-related mortality causes according to age groups are shown in order of frequency in Table II.

The distribution of causes of deaths due to unintentional injuries (N=2110) was further examined by gender, place of injury, and season (Table III). A gender difference was detected only in the case of drownings, which were more common in males (p=0.039). Traffic injuries and drownings were more common in areas away from the home (p <0.001), while the remainder of the unintentional injuries mostly occured at home or in close vicinity to it (p <0.001). All unintentional causes of injury, except for exposures to mechanical forces and other unintentional injuries, showed seasonal differences. Poisoning cases were more common in the winter (p <0.001) (Table III). Detailed analysis of poisoning cases showed that 65.6% of all poisoning-related deaths were due to carbon monoxide intoxication.

Discussion

This is the first nationwide study in Turkey assessing the epidemiology and trends of injury-related mortality in children under five years old. The results of this study show that injury-related under-five mortality rates declined over the past four years. Injuries are important causes of deaths in children worldwide, and significant inequalities have been reported with respect to a country's level of development and income level.[1,8,9,14,18,19] In a study by Peden et al.[1], the death rates due to unintentional injuries in children 1-4 years of age were reported to be 8.5

Table II. Ranking of injury-related death causes by age groups, Turkey, 2014-2017.

Rank	0-11 month (%) (N=505)	12-59 month (%) (N=2055)	Under-five (%) (N=2560)
1	Traffic injuries (35.6)	Traffic injuries (36.7)	Traffic injuries (36.5)
2	Suffocation (27.9)	Falls (12.3)	Falls (12.0)
3	Falls (10.7)	Drowning (9.6)	Suffocation(10.2)
4	Burns (6.5)	Burns (6.6)	Drowning (8.7)
5	Drowning (5.0)	Suffocation (5.9)	Burns (6.6)
6	Assault/Homicide (3.4)	Mechanical forces (4.3)	Mechanical forces (3.7)
7	Poisoning (2.0)	Poisoning (2.5)	Poisoning(2.4)
8	Mechanical forces (1.4)	Other unintentional injury (2.5)	Other unintentional injury (2.3)
9	Other unintentional injury (1.4)	Assault/Homicide (1.7)	Assault/Homicide (2.0)
	Other, ungrouped (6.1)	Other, ungrouped (17.9)	Other, ungrouped (15.5)

Table III. Comparison of unintentional injury related mortality causes by gender, place of injury and season (2014-2017) (N=2110).

Causes	Gender			Place of injury			Season				
	Female (n=845)	Male (n=1265)	p value	Home or its close vicinity (n=983)	Areas away from the home (n=1127)	p value	Spring (n=548)	Summer (n=805)	Autumn (n=479)	Winter (n=278)	p value
Traffic injuries (%)*	46.3	43.0	0.139	2.5	80.7	<0.001	43.1[a]	47.5[a]	46.6[a]	33.8[b]	0.001
Fall (%)*	14.2	14.8	0.711	29.6	1.4	<0.001	14.6[a]	18.4[b]	14.0[a]	4.3[c]	<0.001
Suffocation	12.8	12.2	0.679	25.7	0.8	<0.001	12.2[a]	7.8[b]	13.4[a]	24.5[c]	<0.001
Drownings (%)*	8.9	11.7	0.039	8.2	12.6	0.001	11.7[a]	11.3[a]	11.1[a]	5.4[b]	<0.001
Burns (%)*	8.8	7.4	0.270	16.2	0.8	<0.001	9.1[a]	5.1[b]	6.3[b]	16.9[c]	<0.001
Mechanical forces (%)*	4.3	4.7	0.661	6.8	2.5	<0.001	4.9	4.0	4.6	5.0	0.815
Poisonings (%)*	2.5	3.2	0.314	6.2	0.1	<0.001	2.9[a]	1.7[a]	1.9[a]	8.3[b]	<0.001
Other unintentional injuries (%)*	2.4	3.0	0.380	4.7	1.1	<0.001	1.5	4.2	2.3	1.8	0.010
Total (%)*	100.0	100.0		100.0	100.0		100.0	100.0	100.0	100.0	

* Column percentage
[a,b,c] Different letters are significant in the same row.

per hundred thousand in high-income countries and 49.6 per hundred thousand in low-middle income countries. Similar to our study results, death rates due to unintentional injuries have been reported to decrease in recent years, especially in high and middle-income countries worldwide.[1,8,20] In a recent meta-analysis study by Adeloye et al.[15] including publications between 1990 and 2018, the mortality rate due to injuries for children aged 0-4 years was reported to be 37.7 (95% CI = 32.7- 42.7) per hundred thousand globally.

Causes of injury-related mortality in childhood may vary between countries and regions.[8,9,15,16,18-20] Our study showed that traffic injuries, falls, and suffocation were the top three causes of injury-related deaths among children

younger than 5 years. In a study assessing global trends, traffic injuries, drownings, burns, suffocation, and falls were reported as the leading causes of injury-related death in under-five children.[15] Similar to their results, we found that traffic injuries were the number one cause of mortality in both the 12-59 months old age group and in children under 5. However, in contrast to their results[15], traffic injuries were also found to be the leading cause of injury related deaths in the infant age group in our study. Numerous regional studies in Turkey have examined the causes and characteristics of injuries in children.[10,11,21-27] Although these studies differ in terms of study methodology and the age groups examined, traffic injuries and falls are reported as the top two causes of childhood injury. In a study from Diyarbakır assessing child deaths due to head injuries in 0-17 year olds, 55% of the fatal head traumas in the 0-6 age group resulted from falls and 34% resulted from traffic injuries. Considering all age groups, 81% of traffic injuries occurred outside the vehicle.[10] In a study by Sever et al.[21] evaluating forensic pediatric patients (≤17 years) admitted to the emergency department of a tertiary hospital, the most common reasons for admission in the trauma group were traffic injuries (32.5%) and falls (16.9%). In another hospital based retrospective study assessing 812 patients under the age of 15 suffering a traffic injury and who were admitted to the emergency department of a university hospital found that in-vehicle injuries formed the largest group (59%).[22] In addition, this study emphasized that, in the pediatric age group, traffic injuries involving a pedestrian and vehicle collision led to greater fatalities.[22]

In contrast to global trends and reports from other countries, in our study, falls was found to be the second most common cause of injury-related death among children younger than 5 years and children 12-59 months old.[14-16] An earlier survey from Turkey determined the frequency and risk factors of serious injuries based on parental self-reports of children less than 10 years, admitted to an outpatient clinic of a university hospital. Their study reported the most common type of injury to be falls.[24] Similarly, in an additional study on home injuries among children aged 0-6 years, falls was reported to be the most common cause.[26] However, these studies were performed on injury cases that did not result in mortality, therefore the findings may not be directly comparable.[24,26] These studies do, however, support the fact that falls are among the common causes of childhood injuries in Turkey. In a regional study assessing general characteristics of childhood falls over a period of 8 years in children aged younger than 14 years, the most common types of falls were from balconies (38.5%), stairs (22.5%), and walls (8%).[27] Of the victims, 64% were male and 56% were under 5 years of age. In their study, the most common injury type was found to be head injury (50%) and the overall mortality rate was 3.6%. The authors concluded that, in order to prevent falls from balconies, supervision by parents should be increased, new laws regarding barriers on balconies should be enacted, and campaigns including public education and media attention should be organised.[27] Based on our study results, fall-related mortalities were common in both age groups, mainly occurred at home and/or its close vicinity, and happened in the summer time.

An important result of this study was that, although traffic injuries were the most common cause of injury-related death in children under five, the most important contributor to the decreasing tendency in the under-five injury mortality rate was the reduction in traffic injuries. In Turkey, in order to prevent traffic injuries road safety interventions are in force including the prohibition of speeding and drink-driving, regular traffic controls especially during public holidays, and mandatory use of child restraints for children 0-12 years old as well as the prohibition of children sitting in the front seat. These measures likely explain this decreasing trend in injury mortality rate.[22,28-30] In order to achieve more effective results, existing interventions should be continued more strictly

and audits should be increased.[30]

In our study, both injury-related mortality frequency and causes varied by age. This finding can be explained by differences in the developmental characteristics of different age groups. A general challenge in preventing pediatric injuries is the need to adapt interventions to the child's age and developmental stage.[17] The most important developmental difference occurs during the period around 1 year of age, which is the transition period from infancy to toddlerhood.[9,17] Children from 1 to 4 years of age who are active and curious exhibit higher risk-taking behaviors compared to children under 1 year of age. The risk of injury among infants is mostly due to parental neglect or maltreatment, rather than mobility.[9,14] In our study, the majority of suffocation-related deaths among children younger than 5 years occured in infancy, similar to previous reports.[14,16] Additionally, intentional injuries (assault/homicide) were more common in the infant age group than the 1-4 years group, consistent with the literature.[31] As children grow older, parents tend to reduce their amount of close supervision, leaving the children on their own and tending to believe their child can cope with the injury risks sufficiently.[32] This knowledge may explain why injuries are more common in the 1-4 years age range compared to infants. Previous reports have also indicated that being the firstborn child and living in an overcrowded family both also increase the risk of injury.[24,25,33]

Another important result from our study is that the injury cases resulting in mortality showed seasonal variation. Traffic injuries, falls, and drownings were less common in winter, while suffocation, burns, and poisoning cases were more common in winter. In a recent study in China on the causes of death due to injuries in children under five years of age, seasonal relationships with some types of injuries were similarly reported.[16] Seasonal variation has also been reported in previous regional studies in Turkey[21,22] and, similar to our results, these studies found increased injury cases reported in the summer season. These seasonal relationships can provide some clues in determining preventive activities. The relationship between winter and the high rates of poisoning-related deaths can mainly be explained by carbon monoxide poisoning, pointing to a potential area for effective future interventions. The Ministry of Health initiated a "Carbon Monoxide Poisoning Prevention Program and Action Plan" in 2015.[34] Our findings underline the importance of the need to sustain these programs in our country. Additionally, public announcements on media channels, meteorology warnings in the winter regarding the risk of carbon monoxide poisoning, etc. may all be used as potential interventions to decrease this type of injury.

Our analysis revealed that the majority of injuries leading to fatalities occurred either in the home or its close vicinity. Therefore, to prevent unintentional injuries, interventions should be comprised of arrangements to make these areas safer. Besides providing a safe home and play environment, adequate supervision of children by their caregivers is important to reduce injury risk.[27,35-37] In one study, children who died of unintentional injuries under five years of age were shown to be under parental supervision 3.3 times less than children who survived.[37] Therefore, it is important that parents and caregivers are informed about the causes and occurrence of injuries, as well as how they can be prevented.[35,36] Strong evidence shows that a number of interventions can save lives and mitigate the effects of injuries. Home visits, parent education against child abuse, neglect, and prevention, and counselling by health care professionals are all some of these preventive activities.[29] As an action of our National Child Safety Program, counselling services are provided during the primary care follow-up visits to families with children under five regarding injury prevention. Also, primary health care professionals hold collective awareness activities for the public in Community Health Care Centers.

Some strengths and limitations were present in our study. As we analysed the national under-five mortality data from the DNS database, our results may be generalizable to the community. The major limitation of this study was its secondary analysis nature, thus, all risk factors studied were restricted to those present in our national DNS. DNS, by itself, is prone to self-report bias by parents or eyewitnesses to injury, and could be incomplete or biased due to the nature of the data collected and/or the person entering the data. It is important to increase awareness of physicians diagnosing the mortality and entering death certificates into DNS through in-service training programs, and periodic updating of such information would be of great benefit. Another specific limitation of our data is the high proportion of cases in the "other, ungrouped" subgroup consisting of the cases due to poorly defined causes and unknown intent due to ongoing forensic research. Almost every case in this group consisted of physical trauma. This situation was more frequent in the 12-59 months old age group. Ultimately, this limitation may lead to some causes of injury being under- or over-reported. There is also a possibility of missing cases of injury reflecting child abuse or neglect diagnoses. Inconsistent stories, a previous or subsequent history of child abuse, late presentation for medical care, and inappropriate child care should be warnings for child abuse and neglect cases.[38,39] In the "Provincial Infant Death Monitoring Committees", the deaths occurring during infancy are examined in detail and sent to DNS as a report. A similar reporting system was planned to be launched for the 12-59 months death cases in 2020. This regulation could make positive improvements to the injury-related mortality reporting of under-five children.

The epidemiologic characteristics of fatal childhood injuries vary according to the economic developmental level of a country, the special physical, environmental, and developmental conditions of residential settings, and time.[1,8,9,14,15,18-20] Tailored intervention programs and effective implementation of such programs require robust evidence regarding the epidemiology of the injuries to be managed. Also, continuous monitoring of the impact of such activities is important for ensuring revisions, as needed. For this reason, the current study is crucial for determining the situation and planning future strategies. The decline in injury-related mortality rates among children under-five from 2014 to 2017 is promising. However, the mortality rates in Turkey are still higher than the rates in high-income countries.[8,20] Thus, it should be kept in mind that injury remains among the leading and preventable causes of child mortality. A holistic approach, with well-coordinated multisectorial cooperation will be of key importance when composing effective and comprehensive intervention programs to prevent injuries in children.[40]

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Original Article

Evaluation of micronutrient levels in children and adolescents with obesity and their correlation with the components of metabolic syndrome

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ABSTRACT

Background. Obesity is a significant public health problem worldwide. Vitamin deficiencies, developing due to monotype nutrition, are more likely to be observed in patients than healthy children. The present study evaluates vitamin and micronutrient levels in children and adolescents with obesity and metabolic syndrome compared to healthy controls.

Methods. The study included 73 patients with obesity, 64 patients with metabolic syndrome and 71 healthy children (control group) aged 10 to 16 years. Physical examinations were performed, and waist circumference and systolic blood pressure measurements were recorded. Fasting blood glucose, triglyceride, high-density lipoprotein cholesterol, low-density lipoprotein cholesterol, total cholesterol, insulin, vitamin A, vitamin E, vitamin B1, vitamin B2, vitamin B6, vitamin B12, folic acid and free carnitine levels were analyzed. The homeostatic model of assessment-insulin resistance (HOMA-IR) index was calculated and recorded.

Results. The mean age of all patients was 11.9±2.6 years. The serum insulin level and HOMA-IR index were found to be significantly higher in the obesity and metabolic syndrome groups. No significant difference was found between the groups in terms of vitamin A, vitamin B6 and free carnitine levels. Significantly decreased vitamin E, vitamin B2, vitamin B12 and folic acid and increased vitamin B1 levels were observed in the obesity and metabolic syndrome groups.

Conclusions. Compared to healthy children, children with obesity and metabolic syndrome may have varying degrees of micronutrient and vitamin deficiency due to poor and unbalanced eating habits. These deficiencies should also be considered in the treatment and follow-up of obesity and metabolic syndrome.

Key words: metabolic syndrome, micronutrient, obesity, vitamin.

Obesity is an energy metabolism disorder caused by excessive fat storage in the body, which may also cause physical and mental problems. Obesity is characterized by hypertension, impaired lipid profile and impaired glucose metabolism in addition to the development of insulin resistance, which forms the basis of the metabolic syndrome.[1] The prevalence of obesity is increasing worldwide. Besides, metabolic syndrome constitutes a severe health problem in children and adolescents.[2]

It is assumed that, in exogenous obesity, micronutrient deficiencies can be seen not only due to carbohydrate and protein deficiency but also due to monotype or unbalanced nutrition. The present study hypothesizes that obesity and metabolic syndrome occur as a result of malnutrition and mainly monotype nutrition, and thus, a deficiency in various

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micronutrients. The World Health Organization (WHO) describes this situation as the "double burden of malnutrition", a reference to the coexistence of poor nutrition and excess weight or obesity, and diet-related non-communicable diseases within individuals, households and populations, and over an individual's lifespan.[3] In recent years, based on this idea, new studies have been conducted. The fact that foods, which are rich in calories but nutrient deficient, are cheap and that their accessibility has become easier contributes to the development of obesity.[4-6] Many micronutrients containing vitamins A, C, D and E were consumed in insufficient quantities by most obese adults and at significantly low levels in terms of total diet quality, fruit and micronutrient intake compared to healthy adults.[7]

In a study on obese children, the vitamin A level in the obesity group was significantly lower than that of the healthy control group.[8] In different studies, alpha tocopherol and beta carotene levels were found to be lower in obese children compared to those in the control groups.[9-11] In adults with metabolic syndrome, vitamin B1 levels were found to be lower than those in the control group.[12] An inverse correlation has been reported between thiamine and vitamin B2 levels and body mass index (BMI) in obese children.[13] The vitamin B6 level was found to be lower in patients with metabolic syndrome than that in the healthy population.[12] Vitamin B12 deficiency was higher in obese patients compared to patients with a normal BMI.[14] It has been shown in human and animal studies that carnitine supplementation improves glucose tolerance, especially in insulin-resistant patients.[15] Studies on micronutrients in the obese population have generally been done in adults. A limited number of micronutrient levels were evaluated in a few studies in obese children.

Vitamin A, vitamin E, vitamin B1 (thiamine), vitamin B2 (riboflavin), vitamin B6 (pyridoxine), vitamin B12 (cobalamin), folic acid and free carnitine levels were included in the scope of the study, and it is difficult to comment on them individually in the first stage. The purpose of our research is to evaluate which micronutrient levels are affected in obese children and adolescents or in those with metabolic syndrome and determine their correlation with the components of metabolic syndrome.

Material and Methods

Patients

A written and verbal informed consent was obtained from the parents of all children. The study protocol was approved by the Ethics Committee of Erciyes University, Medical Faculty on 09.01.2015 with the report number 2015/23.

Among patients who were admitted to the Pediatric Metabolism Clinic of the Children's Hospital at Erciyes University Faculty of Medicine (Kayseri Province, Turkey) between April 2016 and April 2018, patients aged 10 to 16 years with a BMI Z-score > 2 were included in the obesity group.[16,17] Most of the children were from Central Turkey.

The patients aged 10 to 16 years meeting at least two international diabetes federation (IDF) criteria (triglyceride level ≥ 150 mg/dl, high-density lipoprotein cholesterol (HDL-C) level ≤ 40 mg/dl, systolic blood pressure ≥ 130 mmHg or diastolic blood pressure ≥ 85 mmHg, and fasting plasma glucose level ≥ 100 mg/dl) in addition to central obesity (waist circumference: ≥90p) were included in the metabolic syndrome group.[18]

The control group consisted of children aged 10 to 16 years, who had no known chronic disease and whose weight and height were within normal limits.

Height and weight measurements were performed with standard devices. BMI was calculated as weight (in kilograms) divided by height (in meters) squared. Waist circumference (WC) was measured at the end of expiration at the level of maximum waist narrowing. They

were evaluated based on normative WC values in Turkish children.[14] Children with a BMI at or above the 95th percentile were classified as obese using Turkish children growth charts based on age and gender.[11] BMI Z-scores were calculated using a software program "WHO AnthroPlusv1.0.4", and following cutoffs for BMI Z-scores defined by the WHO were used: obesity; BMI Z-score > 2, normal-weight; -2< BMI Z-score ≤ 1.[17]

Biochemical Parameters

The blood samples for laboratory tests were taken following a 12-hour fasting period. Fasting blood glucose, triglyceride, HDL-C, low-density lipoprotein cholesterol (LDL-C), total cholesterol (T. Cholesterol), insulin, folic acid and vitamin B12 levels were analyzed and recorded. The biochemical parameters were analyzed using the Cobas 8000 C702 device (Germany) and standard assay kits (Roche, Germany) based on a spectrophotometric method. Insulin, vitamin B12 and folic acid levels were analyzed using the electrochemiluminescence method using Cobas8000 E602 (Germany) device and Roche (Germany) kits.

Serum, plasma and whole blood samples were stored at -80 ° C until the analysis of vitamin A, vitamin E, vitamin B1, vitamin B2, vitamin B6 and free carnitine levels were carried out. Vitamin A and E levels were analyzed using the high-pressure liquid chromatography (HPLC) method with a Thermo HPLC Spectra System (USA) device and Zivak (Turkey) kits. Vitamin B1, vitamin B2, vitamin B6, carnitine levels were analyzed using HPLC methods with an Agilent 1100 series HPLC device (Germany) and Recipe (Germany) kits.

HOMA-IR= Fasting Insulin (μU/mL) x Fasting Blood Glucose*(mmol/L)/22.5

*Blood Glucose was calculated using mg/dl/18=mmol/L formula.

Statistical Analysis

The data were analyzed using the SPSS (Statistical Package for Social Sciences) for Windows 22.0. Descriptive statistics were presented as mean ± standard deviation or median (minimum-maximum) for continuous variables. Nominal variables were presented as several cases and percentages. The percentages between the groups were compared using the Chi-square test. In the comparison of the mean values between the two groups, the t-test was used if the data were normally distributed, and the Mann-Whitney test was used if the data were not normally distributed. Nonparametric data were analyzed using the Chi-square, Kruskal -Wallis or Mann Whitney U tests. The data with normal distribution were analyzed using the Pearson (r) correlation coefficient, while those without normal distribution were analyzed using Kendall's tau correlation coefficient. Also, multiple linear regression analysis, which is a more advanced statistical method, was used to evaluate the correlation between micronutrient values and the parameters of metabolic syndrome obtained in each group [ß regression coefficient was used. R^2 was considered as the coefficient of determination. It was interpreted that the closer the R^2 was to 1, the greater part of the change in the dependent variable could be explained by the independent variable(s)]. For each micronutrient, the forward insertion method was used to identify the most significant clinical markers. The results were evaluated at a significance level of $p<0.05$.

Results

General characteristics of the study population

The clinical characteristics of all patients are presented in Table I.

Biochemical parameters and micronutrient levels

Comparisons of biochemical and micronutrient levels between the groups are shown in Table II. Serum triglycerides were higher in the metabolic syndrome group than in the obesity and control groups (p=0.001, p=0.00, respectively).

Table I. Age, gender and anthropometric characteristics of all groups.

Parameters	Obesity group (n=73)	Metabolic syndrome group (n=64)	Control group (n=71)	p
Age (in years)	12.5 ± 2.3	12.4 ± 2.5	11.2 ± 1.8	0.061
Gender				
Female	36	32	37	0.885
Male	37	32	34	0.451
Body weight (kg)	68.7 ± 17.9[a]	72.8 ± 23.4[b]	36.6 ± 10.2[a,b]	0.001
Height (cm)	152.2 ± 13.3	155.4 ± 13.8	142 ± 11.7	0.052
WC (cm)	84.5 ± 9.7[a]	91.2 ± 3[b]	64.3 ± 5.1[a,b]	0.001
BMI (kg/m^2)	29 ± 4.1[a]	30.5 ± 4.2[b]	17.8 ± 2.7[a,b]	0.001
BMI Z-score	2.46 ± 0.41[a]	2.54 ± 0.47[b]	-0.06 ± 0.79[a,b]	0.001

Data were expressed as the mean ± SD.
[a]. Difference between obesity and control groups as: p <0.05
[b]. Difference between metabolic syndrome and control groups as: p <0.05
WC: Waist circumference, BMI: Body mass index.

Table II. Comparison of biochemical and micronutrient levels between groups.

Parameters	Obesity group (n=73)	Metabolic syndrome group (n=64)	Control group (n=71)	p
Triglycerides (mg/dl)	82.8 ± 29.3[a]	138.7 ± 73.2[a,c]	92.3 ± 39.2[c]	0.001
HDL-C (mg/dl)	49.4 ± 6.3[a]	41.8 ± 10.6[a,c]	49.8 ± 14.3[c]	0.001
T.Cholesterol (mg/dl)	152.6 ± 25.4	168.4 ± 61.3	144.6 ± 30.6	0.063
LDL-C (mg/dl)	87.4 ± 23.6	96.7 ± 56.5[c]	77.4 ± 29.9[c]	0.033
Fasting Glucose(mg/dl)	88.6 ± 7.8	88.4 ± 11	87.8 ± 11.1	0.054
Insulin (μU/mL)	15.4 ± 7[b]	17.6 ± 11.5[c]	12 ± 10.1[b,c]	0.001
HOMA-IR	3.3 ± 1.7[b]	3.7 ± 2.4[c]	2.4 ± 2.3[b,c]	0.001
Vitamin A (0.3-0.7mg/L)	0.7 ± 0.4	0.8 ± 0.4	0.8 ± 0.3	0.790
Vitamin E (5-20 mg/L)	11.5 ± 12.2[a,b]	15.6 ± 12.2[a,c]	29.9 ± 10.7[b,c]	0.001
Vitamin B1 (25-85 μg/L)	46 ± 17.3[b]	45.8 ± 22[c]	36 ± 12.9[b,c]	0.011
Vitamin B2 (40-240 μg/L)	89.1 ± 35[b]	116.7 ± 65.2[c]	174.5 ± 51[b,c]	0.001
Vitamin B6 (3.6-18 μg/L)	11.9 ± 4.8	11.9 ± 5.5	13.6 ± 7.8	0.200
Vitamin B12 (197-771 pg/ml)	311.8 ± 134.8[b]	317.7 ± 138.1[c]	430.7 ± 129.6[b,c]	0.002
Folic acid (3.9-26.8 ng/ml)	11 ± 2.9[b]	10.4 ± 2.8[c]	12.9 ± 3.8[b,c]	0.012
Free carnitine (8.6-85 μmol/L)	27.1 ± 14.8	23.8 ± 11.7	21.4 ± 11.5	0.225

Data were expressed as the mean ± SD.
[a]. Difference between obesity and metabolic syndrome groups as: p <0.05
[b]. Difference between obesity and control groups as: p <0.05
[c]. Difference between metabolic syndrome and control groups as: p <0.05
HDL-C: high-density lipoprotein cholesterol, LDL-C: low-density lipoprotein cholesterol, T.Cholesterol: total cholesterol, HOMA-IR: homeostatic model of assessment-insulin resistance.

HDL-C levels were found to be significantly lower in the metabolic syndrome group than in the obesity and control groups (p= 0.001, p= 0.001, respectively). These two parameters are also metabolic syndrome criteria.

LDL-C levels were higher in the metabolic syndrome group than the control group (p=0.014). Both of the obesity and metabolic syndrome groups' insulin and HOMA-IR levels were higher than the control group (p= 0.001, p= 0.001, p= 0.001, p= 0.001 respectively).

The vitamin E levels were found to be higher in patients with metabolic syndrome than those in obese children (p= 0.007).

Higher vitamin B1 levels were observed in the obesity and metabolic syndrome group than those in the control group (p= 0.06, p= 0.020 respectively).

The vitamin B2 values were lower in the obesity group and the metabolic syndrome group than in the control group (p= 0.001, p= 0.001 respectively).

The vitamin B12 level was lower in the obesity group compared to that in the control group (p=0.001). A significant decrease was found in the vitamin B12 level in the metabolic syndrome group compared to that in the control group (p= 0.011).

Folic acid levels were higher in the control group compared to those in the obesity and the metabolic syndrome groups (p= 0.030, p= 0.006 respectively).

Correlation of micronutrient levels with, total cholesterol, LDL, HDL, HOMA-IR, fasting blood glucose, body weight, WC, and BMI Z-score values in groups are indicated in Tables III, IV, and V.

In the obesity group; the vitamin B12 level was affected negatively by body weight and BMI Z-score (ß=-0.647, p= 0.003, ß= -0.249, p= 0.017 respectively).

In the metabolic syndrome group; the vitamin B2 level was independently affected negatively by triglyceride, HDL-C (two of the metabolic syndrome criteria) and LDL-C, and positively by total cholesterol. (ß= -1.667, p= 0.002; ß=-5.713, p= 0.005, ß=-0.968, p= 0.013, ß=-6.211, p=0.005 respectively) (Fig. 1).

The vitamin B12 level was correlated negatively with body weight (ß= -0.281, p= 0.046).

In the control group; It was found that the vitamin B12 level was affected negatively by body weight in the control group (ß= -0.322, p= 0.014).

Table III. Correlation of micronutrient levels with triglyceride, total cholesterol, LDL, HDL, HOMA-IR, fasting blood glucose, body weight, WC and BMI Z-score values in the obesity group using multiple linear regression analysis.

Obesity	Triglyceride		T.Cholesterol		LDL-C		HDL-C		HOMA-IR		Fasting glucose		Body weight		WC		BMI Z-score	
group	β	p	β	p	β	p	β	p	β	p	β	p	β	p	β	p	β	p
Vitamin A	-	-	-	-	-	-	-	-	0.276	0.042	-	-	0.109	0.052	-	-	--	-
Vitamin E	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Vitamin B1	-	-	-	-	-	-	-	-	-0.310	0.022	-	-	-0.351	0.009	-	-	-	-
Vitamin B2	-	-	-	-	-	-	-	-	-	-	-	-	0.522	0.003	-0.511	0.004	-	-
Vitamin B6	-	-	-	-	-	-	-	-	-	-	-	-	-0.268	0.048	-	-	-	-
Vitamin B12	-	-	-	-	-	-	-	-	-	-	-0.242	0.077	-0.647	0.003	-	-	-0.249	0.017
Folic acid	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Free carnitine	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

HDL-C: high-density lipoprotein cholesterol, LDL-C: low-density lipoprotein cholesterol, T.Cholesterol: total cholesterol, HOMA-IR: homeostatic model of assessment-insulin resistance, BMI: Body mass index, WC: Waist circumference.

Table IV. Correlation of micronutrient levels with triglycerides, total cholesterol, LDL, HDL, HOMA-IR, fasting blood glucose, body weight, WC and BMI Z-score values in the metabolic syndrome group using multiple linear regression analysis.

Metabolic syndrome group	Triglycerides		T.Cholesterol		LDL-C		HDL-C		HOMA-IR		Fasting glucose		Body weight		WC		BMI Z-score	
	β	p	β	p	β	p	β	p	β	p	β	p	β	p	β	p	β	p
Vitamin A	-0.370	0.070	2.470	0.001	-2.236	0.002	-	-	-	-	-	-	-	-	0.379	0.006	-	-
Vitamin E	-	-	-	-	-	-	0.263	0.052	0.358	0.009	-	-	-	-	-	-	-	-
Vitamin B1	-	-	-0.254	0.072	-	-	-	-	-	-	-	-	-0.743	0.008	-	-	-	-
Vitamin B2	-1.667	0.002	6.211	0.005	-5.713	0.005	-0.968	0.013	-	-	-	-	-	-	-	-	-	-
Vitamin B6	-	-	-	-	-	-	0.277	0.051	-	-	-	-	-	-	-	-	-0.341	0.051
Vitamin B12	-	-	-	-	-	-	-	-	-	-	-	-	-0.281	0.046	-	-	-	-
Folic acid	-	-	-	-	-0.298	0.026	-	-	-0.327	0.015	-	-	-0.033	0.053	-	-	-	-
Free carnitine	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

HDL-C: high-density lipoprotein cholesterol, LDL-C: low-density lipoprotein cholesterol, T.Cholesterol: total cholesterol, HOMA-IR: homeostatic model of assessment-insulin resistance, BMI: Body mass index, WC: Waist circumference.

Table V. Correlation of micronutrient levels with triglycerides, total cholesterol, LDL, HDL, HOMA-IR, fasting blood glucose, body weight, WC and BMI Z-score values in the control group using multiple linear regression analysis.

Control group	Triglycerides		T.Cholesterol		LDL-C		HDL-C		HOMA-IR		Fasting glucose		Body weight		WC		BMI Z-score	
	β	p	β	p	β	p	β	p	β	p	β	p	β	p	β	p	β	p
Vitamin A	-	-	0.302	0.017	-	-	0.323	0.011	-	-	-	-	-	-	-	-	-	-
Vitamin E	0.311	0.020	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Vitamin B1	-	-	-	-	0.340	0.010	-	-	-	-	-	-	-	-	-	-	-	-
Vitamin B2	-	-	0.522	0.021	-0.658	0.004	-	-	-	-	-	-	-	-	-	-	-	-
Vitamin B6	0.270	0.062	-0.327	0.025	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Vitamin B12	-	-	-	-	-	-	-	-	-	-	-	-	-0.322	0.014	-	-	-	-
Folic acid	-	-	-	-	-	-	-	-	-0.360	0.006	-	-	-	-	-	-	0.357	0.017
Free carnitine	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

HDL-C: high-density lipoprotein cholesterol, LDL-C: low-density lipoprotein cholesterol, T.Cholesterol: total cholesterol, HOMA-IR: homeostatic model of assessment-insulin resistance, BMI: Body mass index, WC: Waist circumference.

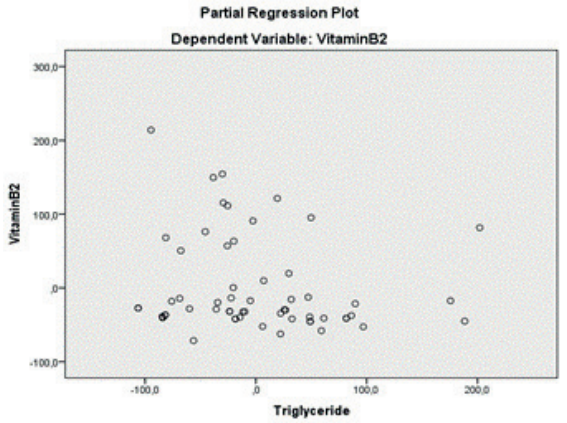

Fig. 1. Correlation graphics by multilinear regression analysis of vitamin B2 and triglyceride levels in the metabolic syndrome group.

Triglyceride affects vitamin B2 levels statistically significantly and negatively. A one unit increase in the triglyceride level decreases the vitamin B2 level by 1.667 units.

The vitamin B12 level was affected negatively by BMI Z-score among total subjects (ß= -0.254, p= 0.004) (Fig. 2), but the vitamin B1 level was positively correlated by BMI Z-score for total children (ß= 0.250, p= 0.005) (Fig. 3).

In the obesity group, acanthosis was observed in five children and stria was observed in 30 patients. Also, in the metabolic syndrome group, 23 children with acanthosis and 36 children with stria were identified. The presence of acanthosis or stria was not associated with the levels of micronutrients or biochemical parameters for all groups or each group individually.

Discussion

In this study, micronutrient levels of children with obesity and metabolic syndrome were compared with each other and the control group. Thus, the relationship between micronutrients with metabolic syndrome criteria and their effects on obesity was analyzed.

According to us, obesity is a kind of malnutrition, which develops unilaterally and as a result of unbalanced nutrition. Hence, certain micronutrient deficiencies may develop in children who have an unbalanced diet. The WHO describes this situation as the "double burden of malnutrition" and points out that obesity may occur more frequently in socio-economically underdeveloped and poorly nourished societies together with malnutrition, and that chronic diseases may develop as a consequence of this situation.[3] In recent years, overweight cases have become more prevalent than underweight cases in underdeveloped and moderately developed countries.[19]

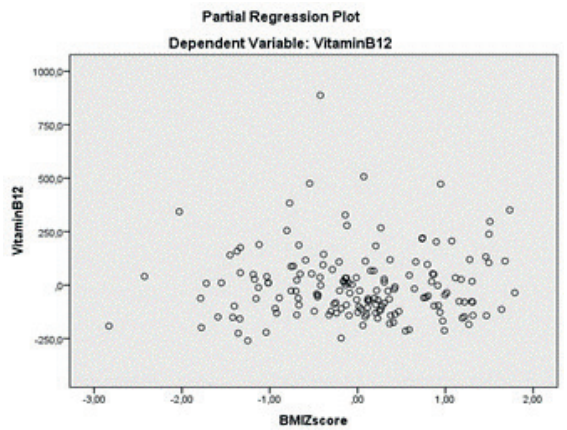

Fig. 2. Correlation graphics by multilinear regression analysis of vitamin B12 and BMI Z-scores in all groups.

BMI Z-score affects vitamin B12 levels statistically significantly and negatively. A one unit increase in the BMI Z-score decreases the vitamin B12 level by 0.254 units.

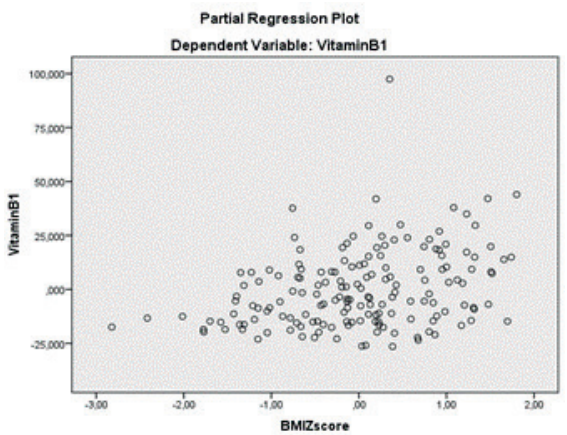

Fig. 3. Correlation graphics by multilinear regression analysis of vitamin B1 and BMI Z-scores in all groups.

BMI Z-score affects vitamin B1 levels statistically significantly and positively. A one unit increase in the BMI Z-score increases the vitamin B1 level by 0.250 units.

In a study conducted in 2001-2008 on a nationally representative level of normal-weight, overweight and obese adults, it was revealed that most micronutrients containing vitamins A, C, D, and E as well as calcium and magnesium were deficient in most of the subjects in the study. This study also emphasized that obese individuals were significantly at low levels in terms of fruit intake, total diet quality and micronutrient intake compared to normals.[7] Apart from the fact that obese patients may consume fewer micronutrients due to the foods they eat, micronutrient deficiencies may result from altered pharmacokinetics involving distribution, metabolism and elimination in obese patients as well.[20] It is known that the pharmacokinetics of drugs are altered due to reasons such as increased adipose tissue, altered blood stream, increase or growth of liver parenchymal cells, and alteration of renal functions in obese people.[21] Micronutrients may also be affected by similar mechanisms.

Previous studies on obese children show that vitamin A levels were significantly lower in obesity groups when compared to levels in healthy control groups, and that the vitamin A levels were directly proportional to BMI and abdominal obesity.[22,23] However, in our study, no difference was found among the groups in terms of vitamin A levels (Table II).

Although recent studies suggest that beta carotene concentrations correlate inversely with HOMA-IR index, the present study showed a direct correlation between the HOMA-IR index and vitamin A level in the obesity group.[24,25] In contrast to this finding, there was no significant correlation between the HOMA-IR index and vitamin A level in the metabolic syndrome group and the control group.

A recent study in overweight and obese patients found that vitamin A positively correlated with HLD-C and negatively correlated with LDL-C and triglycerides. But that study did not include metabolic syndrome.[26] In our study, vitamin A levels positively correlated with LDL-C and total cholesterol in both metabolic syndrome and control groups while it negatively correlated with HDL-C levels in the metabolic syndrome group. The results of a previous study have shown that alphatocopherol levels are low in children with metabolic syndrome and at an average level in obese children, and it was speculated that obese children and children with metabolic syndrome are more prone to oxidative stress.[27] In the comparison of the three groups in our study, vitamin E levels were significantly lower in obese patients and those with metabolic syndrome. The children diagnosed with metabolic syndrome were given a Mediterranean diet, which is rich in fruits and vegetables. It was observed that metabolic syndrome criteria started to improve and vitamin E levels started to increase.[28] Regarding the correlation between vitamin E levels and the parameters of metabolic syndrome, there was a positive correlation with the triglyceride levels in the control group and a statistically insignificant positive correlation with HDL-C in the metabolic syndrome group.

In the control group, there was a positive correlation between the vitamin B1 level and LDL-C. Vitamin B1 levels in adults were found to be lower in the metabolic syndrome group compared to the levels in the control group. Thiamine deficiency was found in children with obesity, and most of those cases were subclinical.[12,29] In contrast to other studies, significantly higher vitamin B1 levels were found in the obesity group and those in the metabolic syndrome group compared to the levels in the control group. Interestingly, vitamin B1 levels were negatively correlated with the HOMA-IR and body weight in the obesity group, and with body weight in the metabolic syndrome group.

The reason for this difference may be explained by the fact that obese children and those with metabolic syndrome typically consume large quantities of cereal products, bread and potatoes, as cereals and potatoes are the main sources of vitamin B1. The association of increased thiamine intake and obesity has been revealed in other reports.[30-32]

An inverse correlation was found between BMI and vitamin B2 levels among obese children, and lower riboflavin levels were found in patients with metabolic syndrome.[13,33] Our study revealed a negative correlation between B2 levels and body weight in the obesity group.

Examination of vitamin B2 levels in our study revealed that these levels were significantly lower in the obesity and metabolic syndrome groups than in the control group. Studies show that riboflavin deficiencies cause functional alterations in adipocytes, and thus have proinflammatory and proinsulin effects, which result from increased adipocytes.[34]

Although no correlation was found between vitamin B2 level and metabolic syndrome criteria in the obesity group, we found a negative correlation with LDL-C in the control group and a negative correlation with triglyceride, LDL-C, and HDL-C and a positive correlation with total cholesterol in the metabolic syndrome group.

It was noted that vitamin B6 supplementation is vital in preventing metabolic syndrome, and pyridoxine levels were lower in patients with metabolic syndrome compared to those in healthy patients.[12,35] Unlike other studies, the vitamin B6 level in this study was normal, and there was no differences among the groups. In the obesity group, an inverse correlation was found between vitamin B6 and body weight. In the literature, there are not many studies that analyze the correlation between vitamin B6 and obesity in children.

In a few studies where obese children are compared with healthy children, vitamin B12 levels were found to be lower in obese children.[14,36-38] In parallel to these findings, our results showed significant lower levels of B12 in the obesity and metabolic syndrome groups than the control group, and B12 levels were negatively correlated with body weight in each group. Also, the vitamin B12 was affected negatively by BMI Z-score among total subjects and in the obesity group.

It is suggested that there is an inverse correlation between folic acid levels and BMI, and that folic acid deficiency can play a role in the etiology of obesity.[13] Our study revealed lower folic acid levels in children with obesity and metabolic syndrome compared to the control group. A lower intake of folic acid was observed in obese children compared to healthy children.[39] It was shown that oral glucose tolerance induced insulin resistance in rats with diet-induced folic acid deficiency.[15] Consistent with this study, we found a negative correlation between folic acid levels and HOMA-IR in the control and metabolic syndrome groups.

In human and animal studies, it has been shown that carnitine supplementation improves glucose tolerance, especially in insulin-resistant patients. However, more precise results can be obtained from animal studies investigating the correlation between carnitine deficiency and glucose intolerance.[15] In our study, no significant difference was found among the groups in terms of the free carnitine level. Besides, there were no correlations in each group, between the biochemical parameters of metabolic syndrome and free carnitine levels in each group. A current meta-analysis shows a significant effect of L-carnitine supplementation as a potential therapeutic agent on weight and BMI in obese and overweight adults.[40]

The weaknesses of our study are that the socio-economic and cultural status of families cannot be evaluated. Likewise, the average daily calorie and vitamin consumption levels of patients cannot be calculated using weekly food lists due to the families' lack of interest.

Considering that studies in the literature on this subject have been performed solely on adults to date, our study will be the first to obtain data findings on this subject, that will involve children with obesity and with metabolic syndrome in Turkey.

In conclusion, none of the micronutrients were measured below the normal range indicating a deficiency. In our study, the

micronutrient content of the diet could not be calculated, because there was no nutritional record. However, measured vitamin levels can indirectly inform us about the vitamin intake of children based on their diets. The fact that higher vitamin B1 levels were found in the children with obesity and metabolic syndrome than healthy children in the control group suggests that these children consume more carbohydrates, which contains grain products, particularly cereal products (pastry etc.). The fact that lower vitamins E, B2, B12 and folic acid levels were found in children with obesity and metabolic syndrome than healthy children in the control group implies that these children do not consume as many green-leaved vegetables, dairies, specialty meats, seafood, citrus fruit and other animal products.

Primary healthcare institutions should ensure to be sensitive towards nutrition training and psychological counseling. The development of health policies for the prevention of obesity should be continued persistently.

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Original Article

Characteristics and outcomes of critically ill children transported by ambulance in a Turkish prehospital system: a multicenter prospective cohort study

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ABSTRACT

Background. The most underdeveloped area in the care of critically-ill-children (CIC) is the prehospital period. Appropriate prehospital assessment and life-saving-interventions (LSI) of this population are challenging and require dedicated resources to ensure the best outcomes. We aimed to determine the characteristics and outcomes of CIC transported to the Turkish Pediatric Emergency Departments (EDs). The frequency and distribution of LSI administered by prehospital providers on route and in the EDs were also investigated.

Methods. This prospective study was conducted at 4 metropolitan cities and 9 tertiary pediatric EDs between August 2014-August 2015. A survey based study evaluated all CIC who were brought by ambulance to the participant EDs. CIC were defined as a patient who requires LSI or needs intensive care admission for any reason. Patient demographics, clinical features, reason for transport, performed procedures in the ambulance or ED were sought. Finally, the short-term outcomes of transported CIC and transport-associated risks were analyzed.

Results. During the study period, a total 2094 children were brought by ambulance to all participant EDs. Only 227 (10.8%) of them were critically-ill. Emergency Medical Services (EMS) providers were less likely to perform procedures in CIC if they were staffed with paramedics (p<0.001). Most procedures were performed on children aged one or older (p<0.001). No procedure was performed in the ambulance for nearly one fourth of patients who received LSI in the EDs. If the EMS did not have a physician, prehospital providers were less likely to provide immediate LSIs (p<0.001). CIC were more likely referred from secondary/tertiary care hospitals. The short-term mortality rate was higher if the ambulance was staffed by only paramedics.

Conclusion. This study demonstrated that Turkish prehospital pediatric emergency care is deficient. We offer a clinical overview of pediatric emergencies to aid EMS directors, policymakers, and ED directors in planning the care of CIC.

Key words: prehospital care, critically ill children, ambulance, emergency medical service, paramedic.

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The concept of emergency medical service (EMS) consists of many areas of emergency care, including the primary evaluation, management, and transport of patients from the field of an injury or illness to their arrival at an emergency care facility (the out-of-hospital or prehospital care), as well as the management within the emergency department (ED) and intensive care unit (ICU).

Significant improvement in EMS systems have been achieved in the United States of America (USA) since the 1970s, especially for the adult population.[1] Early systems have been designed to provide rapid intervention in case of cardiac arrest and rapid transport for motor vehicle crash victims among adults. Since the most significant causes of mortality in the pediatric population are trauma and poisoning, it is important to consider the effects of the 'transport' stage on outcome. Previous studies have reported poor improvement in prognosis among pediatric patients despite the development of a modern EMS.[2,3] This is primarily a result of the higher proportions of adult emergencies and thus the higher rate of adult patients for whom the EMS providers are involved in the early care. Providing medical care for critically ill children (CIC) requires a different skillset from those required for adult providers, including attention to the unique characteristics and needs of the pediatric population.

The implementation of EMS for children significantly reduced the mortality and improved outcomes in pediatric patients with trauma and other medical emergencies in developed countries.[4-6] In Turkey, almost all cities have EMS, which consists of pre-hospital medical care and transport to a medical facility.[7] All EMS demand is made by calls to an emergency number which is 112 in Turkey and similar to some European countries. EMS was primarily developed for adults during the 1990s in our country and this improved the outcome of sudden cardiac arrest. However, there is a significant lack of appropriate investment in EMS infrastructure for CIC. There is a paucity of data concerning pediatric prehospital care outcomes. In our country, information concerning the number of CIC utilizing the EMS system is unknown, thus, could benefit from an optimal system designed to meet their needs.

We aimed to identify the frequency and causes of prehospital pediatric emergencies and important prognostic factors associated with transport by EMS of CIC. The percentage of immediate life-saving interventions (LSI) / non-LSI performed by either EMS providers or ED physicians and their effects on outcomes were also investigated.

Material and Methods

Study design

According to the Address Based Population Registration System (ABPRS) released by the Turkish Statistical Institute in 2014, Turkey had a total population of 77.695 million, with 22.838 million children aged 0-17.

This prospective study was conducted in four metropolitan cities and nine tertiary pediatric EDs in Turkey. Transported CIC to one of the participant ED's (4 training and teaching hospitals and 5 University Hospitals) between 1 August 2014, and 1 August 2015 were enrolled in the study. A data collection form was prepared by the principle author and sent to all nine EDs before patient enrollment began. The local ethical committee of Ege University (13-4.1/14) approved this study.

EMS Present practice

The Ministry of Health Emergency Medical Services General Bureau published the National EMS Scope of Practice Model to provide general recommendations around the scope of practice and licensure.[8] In the most current version of the Scope of Practice Model there are three designations for EMS professionals: Emergency Medical Responders (EMR), Ambulance and emergency care technicians (AEMT), and Paramedics. As stated within the document, their differing roles are;

1. EMR: The primary focus is to initiate immediate life-saving care to critical patients who access the EMS. The scope of EMRs includes the following skills: airway positioning and bag valve mask ventilation, hemorrhage control, automated electronic defibrillator (AED) defibrillation, and cardiopulmonary resuscitation (CPR).

2. AEMT: To provide basic and limited advanced emergency medical care and transportation for critical and emergent patients who access the EMS. AEMTs typically do all an EMR does with the addition of providing medications such as oxygen, sublingual nitro, inhaled medications, oral glucose, EpiPen® administration; taking vital signs; extremity splinting; and spinal immobilization, may perform electrocardiograms (ECGs), endotracheal intubation, nasogastric tube placement, determination of death, defibrillation and pacing, needle thoracotomy, aspiration of newborn meconium, intravenous line placement, and drug administration.

3. Paramedic: To provide advanced emergency medical care for critical and emergent patients who access the emergency medical system. Additionally, they may perform; needle cricothyrotomy, intubation, and 12 lead ECG interpretation.

Study Population and Data Collection

The physician on shift from the participant ED completed the data collection form for each patient. Our age groups were structured according to the Pediatric Advanced Life Support (PALS) guideline for age groups; group 1: 0-28 days, group 2: 28 days-1year, group 3: 1 -10 years old and group 4: > 10 years. The data collection form included information on; demographics, clinical features, triage level, information calls to receiving facility, and reason for transportation. The referring physicians specialties, intervention type provided by EMS or by ED staff were also recorded. CIC was defined as a patient who requires LSI or needs intensive care admission for any reason. Finally, the short-term outcomes of transported CIC and transport-associated risks were analyzed. Informed consent was obtained from the participants families.

Statistical analysis

Statistics Package for the Social Sciences 22.0 software (SPSS Inc.; Chicago, IL, ABD) was used for statistical analysis. Continuous data was represented by mean and standard deviation. Categorical variables were expressed by frequency and cross tables. The chi-square test (or Fisher's exact probability test) was used to compare demographics in terms of age groups, and time and month of presentation. Mann-Whitney U or t-test was performed for two independent groups. p values lower than 0.05 were regarded as statistically significant.

Results

During the study period, a total of 2094 patients were transported to the 9 different EDs of which 227 were CIC (10.8%). Most patients (90%) did not receive LSIs at the ED. Of the participants 53% were boys and the median age was 5.5 years. The higher percentage of CIC was in group 3 (1-10 years) and group 4 (>10 years) 48.9%, and 26.4% respectively. Only a low rate (n=46, 20.3%) of patients were transported by ambulance were accompanied by a physician (Table I). Table I shows the frequency and distribution of EMS transfer sites. Secondary care hospitals served as the main referral site in 57.3% of the cases, and a small proportion of the total number which was 10.6% (n=24) were brought from home. Pediatricians were the most common physicians who gave the interfacility transport decision (48.9%), and the receiving facility did not receive information calls for more than half of the patients (39.2 %) before arrival.

The most common diagnoses were toxicological, traumatic and respiratory emergencies with the rate of 22.5%, 19.4% and 15.8%, respectively (Table II). Children with trauma and toxicity

Table I. Characteristics of critically-Ill-children and EMS crew.

	n (%)
Sex (Male)	121 (53.3)
Age, median (years)	5.5
0-28d	54 (2.6)
28d - 1	328 (15.7)
1-10	1048 (50)
10-18	664 (31.7)
Distance (km), mean (min-max)	63.8 (2.8-800)
Information call	
No	89 (39.2)
Yes	138 (60.8)
Referred by	
Family-care physician	6 (2.6)
Pediatrician	111 (48.9)
Other physician	60 (26.4)
Family or scene	50 (22)
Transport from	
Home	24 (10.6)
Field	26 (11.5)
Primary care hospital	5 (2.2)
Secondary care hospital	130 (57.3)
Tertiary care hospital	32 (14.1)
Other	10(4.4)
EMS Staff	
Physician	46 (20.3)
Paramedic + AEMT	181 (79.7)

EMS: emergency medical service, SD: standard deviation, km: kilometer, min: minimum, max: maximum, AEMT: ambulance and emergency care technician.

were more likely to be older (1-10 years: 43.2% and > 10 years: 71.7%). In contrast, transferred patients who had other medical emergencies were more likely to be younger than one year old (Fig. 1). Most of the intoxications (66.7%) occurred by mouth, and the most common ingested drug was acetaminophen (27.5%), scorpion bite (9.8%) was the second most common non-drug intoxication in CIC (Table II). The median injury severity score (ISS) for trauma patients (n=44) was 6, of which only 9.1% (n=4) had a high ISS score (ISS>11) (Table II). Three of those patients died at the ED, all of the remaining trauma patients were admitted to the pediatric intensive care unit (PICU).

Table II. Diagnosis of children who arrived by EMS.

Toxicity	51 (22.5)
Ingestion	34 (66.7)
Acetaminophen	14 (27.5)
NSAI	6 (11.8)
Antidepressant	8 (15.6)
Multidrug	6 (11.8)
Organophosphates	4 (7.8)
Rodenticide poisoning	3 (5.9)
Carbon monoxide poisoning	2 (3.9)
Scorpion bite	5 (9.8)
Snake bite	3 (5.9)
Trauma	44 (19.4)
ISS>11	4 (9.1)
ISS≤11	40 (90.9)
Medical emergencies	132 (58.1)
Respiratory	36 (15.8)
Bronchopneumonia	19
Acute bronchiolitis	7
Asthma attack	4
Tracheostomy patient	4
Foreign body in respiratory tract	2
Infectious diseases	25 (11)
Sepsis / septic shock	11
CNS infection (meningitis/ encephalitis)	13(9/4)
Hepatitis A	1
Neurological	23 (10.1)
Status epilepticus	18
Febrile status epilepticus	2
Intracranial hemorrhage	2
Guillain-Barré syndrome	1
Cardiac	10 (4.3)
Cardiomyopathy / Heart failure	6
Cardiac arrest	2
SVT	2
Endocrinological	6 (1.1)
Diabetic ketoacidosis	5
Hypoglycemia	1
Gastrointestinal	4 (3.8)
Bleeding	3
Esophagus atresia	1
Non-traumatic surgical	4 (4.1)
Splenic rupture	1
Duodenum perforation	1
Corrosive substance ingestion/ inhalation	2
Other	24

NSAI: non-steroid anti-inflammatory, SVT: supraventricular tachycardia,
CNS: central nervous system, VP: ventriculo-peritoneal

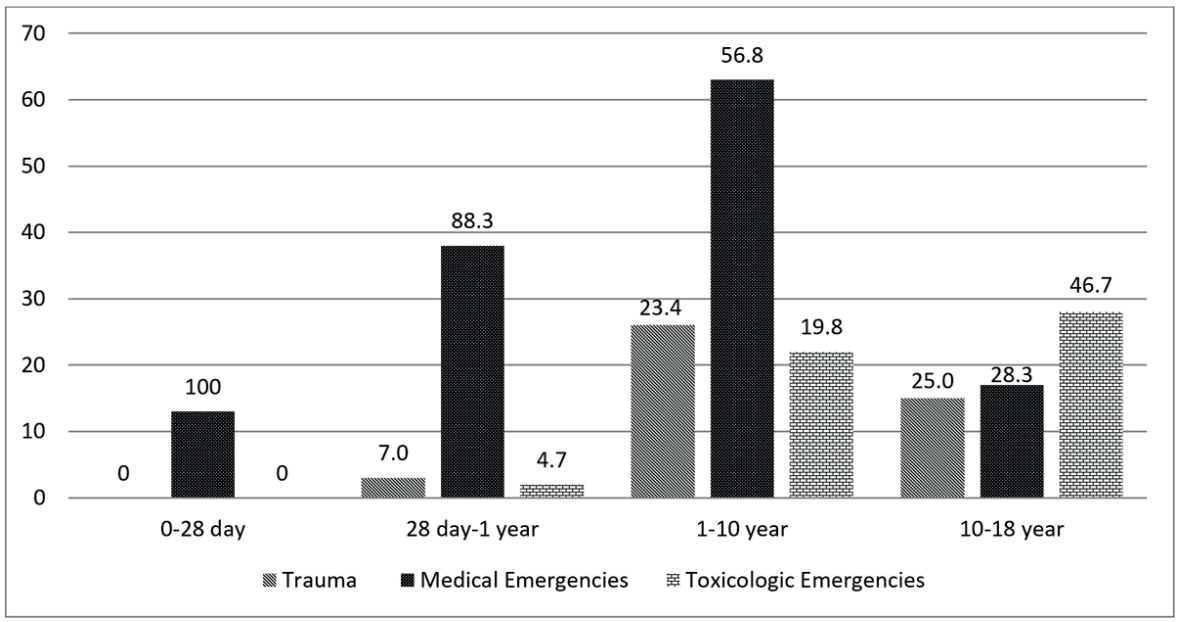

Fig. 1. Percentage (%) of diagnosis distrubutions by age groups.

For the secondary analysis, we collected information on pre-hospital procedures for all patients arriving by EMS. Although 86 % (n=195) of children underwent procedures during transport by EMS providers, no procedure was performed for 32 (Table III). Of those 32 patients, 12 had respiratory failure, 5 had sepsis/septic shock, 5 had intoxication, 4 had status epilepticus, 2 had trauma (one of them had epidural hematoma), 2 had a scorpion bite, one had supraventricular tachycardia and other one had cardiopulmonary arrest. Most procedures were performed on children aged one or older (Table III) ($p<0.001$). Results showed that if the EMS did not have a physician, prehospital providers were less likely to provide immediate LSIs (Table IV) ($p<0.001$). No procedure was performed in the ambulance for nearly one-third of patients (7/25) who received immediate LSIs such as CPR and intubation in the ED (Table III).

The survival rate did not differ when the referral site and performed LSI was compared with short-term outcome (Table V). Table VI shows the clinical features of nine patients who were brought by ambulance and subsequently died (three of these died on route). There were severe trauma in 3, cardiogenic shock with congenital cardiac disease in 2, respiratory failure in 2 and septic shock in 2 non-survived patients. Four of 9 patients who died were not informed by telephone before arrival to the receiving facility. A vascular line was present in 8/9 (no intraosseous lines), intubation was performed in 5 and CPR in one patient.

Table III. EMS procedures (%) of critically-Ill-children.

Age group	Vascular access	Blood glucose monitoring	Given medication	BVM ventilation	Endotracheal intubation	CPR	No procedures on EMS	Total patient (n)
0-28d	53.8	38.5	0	7.7	7.7	0	30.8	13
28d-1y	58.1	16.3	7.0	2.3	2.3	2.3	30.2	43
1-10y	81.1	8.1	3.6	4.5	2.7	0.9	8.1	111
10-18y	80	13.3	10	1.7	1.7	0	10	60

*d: day, BVM: bag-valve-mask, CPR: cardiopulmonary resuscitation, EMS: emergency medical service.

Table IV. Comparison of transported patients who received life-saving interventions based on referral sites.

	Life-saving-interventions					*p*
	Physician		Paramedic + AEMT		Total (n)	
Referred from	Yes	No	Yes	No		
Home	3	0	5	16	24	
Scene	3	1	9	13	26	
Primary care hospital	1	1	0	3	5	*< 0.001*
Secondary care hospital	17	6	22	85	130	
Tertiary care hospital	4	6	4	18	32	
Private hospital	3	1	2	4	10	
Total patients (n)	31	15	42	139	227	

*AEMT: ambulance and emergency care technician. § p values were determined with Student's t-test for continuous variables. Chi square tests or Fischer's exact tests for categorical data where appropriate.

Table V. The relationship between life-saving-interventions and staff on the EMS with mortality in critically-Ill-children.

	Life-saving interventions on the EMS [n (%)]		EMS Staff [n (%)]		Total	*p*
	Yes	No	Physician	Paramedic + AEMT		
Non-survived	7 (9.6)	2 (1.3)	3 (6.5)	6 (3.3)	9	< 0.001
Alive	66 (90.4)	152 (98.7)	43 (93.5)	175 (96.7)	218	
Total	73	154	46	181	227	

EMS: emergency medical service, AEMT: ambulance and emergency care technician. * p values were determined with Fischer's exact tests.

Discussion

One of the most selected forms of misuse involving EDs is the misuse of EMS. Research has shown that in several areas of the United States as well as in Canada, Sweden, and England, the rate of inappropriate ambulance use is 40% to 50%.[9,10,11,12] Excess uses of EMS among pediatric patients with a variety of low acuity conditions have been demonstrated. Our study showed that around 90% of patients brought by ambulance did not have high acuity conditions upon arrival to the EDs. The potential adverse consequences of non-urgent EMS use include increased ED crowding, and limits rapid ambulance response for patients whose condition requires immediate care and LSI. However, critically ill patients who would likely benefit from rapid prehospital care, are under-utilizing these services like our results showed.[13-15]

The referral system in Turkey plays a major role in managing the flow of patients from primary to secondary and tertiary care hospitals (as the health care system structure is based on those three levels). All secondary care hospitals in Turkey have at least two pediatricians and provide medical care upon referral by a primary care physician and that requires more specialized knowledge, skill, or equipment than the primary care physician can provide. Since those hospitals do not have well-structured pediatric intensive care unit (PICU) we believe that CIC deserve to be referred. The explanation for referral from tertiary ED to another tertiary facility may the inadequate number of PICU and limited bed capacity. Although, guidelines exist concerning the timing of transfer, for certain groups of patients and the decision to transfer should be made by consultants after full assessment and discussion between referring and receiving EDs. The current study showed that 40% of CIC were brought to EDs without

Table VI. Characteristics of non-survived patients.

No	Sex	Age (mo)	Distance by EMS (km)	Referral sites	EMS staff	Procedures in EMS					Information call	Diagnosis
						Oxygen by mask	Vascular access	BVM	Endotracheal intubation	CPR		
1	F	72	5	Tertiary	Paramedic	Yes	Yes	No	No	No	No	Respiratory failure
2	F	36	110	Secondary	Physician	Yes	Yes	No	No	No	Yes	Septic shock
3	F	13	3	Home	Paramedic	No	No	Yes	No	No	No	*Respiratory failure
4	F	84	95	Secondary	Paramedic	No	Yes	Yes	No	No	No	*Trauma
5	F	19	4	Primary	Physician	No	Yes	Yes	Yes	Yes	No	*Congenital cardiac anomaly
6	F	14 days	100	Secondary	Paramedic	No	Yes	Yes	Yes	No	Yes	Congenital cardiac anomaly
7	F	24	30	Secondary	Paramedic	No	Yes	Yes	Yes	No	Yes	Septic shock
8	M	3	20	Tertiary	Physician	No	Yes	Yes	Yes	No	Yes	Trauma
9	M	42	20	Field	Paramedic	No	Yes	Yes	Yes	No	Yes	Trauma

mo: month, EMS: emergency medical service, BVM: bag-valve-mask, CPR: cardiopulmonary resuscitation
*Died during transport

information calls. This improper referral leads to poor outcome of high acuity medical conditions.[16] Local policies should be prepared to show referral patterns, available expertise, and clinical circumstances.

A previously published report indicated that children older than 7 years old were more likely to be transported by EMS due to trauma and intoxication, although infants transported by ambulances were predominantly for other medical emergencies.[17] Similarly, in a Canadian study medical emergencies represented the majority of cases of EMS under two years of age, trauma being the most common reason in children over nine.[18] The majority of EMS transports in our groups were traumatic and toxicological emergencies (80%) rather than medical illness (20%) in older age groups.

The most common pre-hospital pediatric procedures are cervical spine immobilization, vascular line, and basic and advanced airway management.[8] Although the Turkish Ministry of Health authorizes paramedics and AEMT to perform all the procedures listed above, in our study many CIC who needed immediate LSI did not receive any procedure. Some patients who required immediate vascular line (Supraventricular tachycardia, status epilepticus, altered mental status, respiratory failure) were brought to the ED without prehospital procedure. We can explain this by the difficulty of establishing the vascular line in a pre-hospital setting especially in younger, less cooperative children. Paramedics and AEMT receive very limited training compared to physicians, and most rarely have to manage seriously ill or injured children.[19] Although EMS personnel and paramedics are responsible for performing all vital interventions in CIC, previous studies have shown that paramedics were required to provide advanced life support, such as endotracheal intubation in trauma patients, in less than 1% of cases a year.[20-22]

The timing of emergency procedures is of critical importance during transport, and early management can be beneficial in improving

outcomes.[23] Recent studies emphasize that the "golden hours" in pediatric transport is not important, and that misapplication of this concept can result in early and goal-directed interventions being delayed.[24] EMS personnel reported feeling comfortable performing any critical procedure if they are well trained and experienced.[25] Requirements for pre-hospital pediatric resuscitation in children with medical emergencies and trauma were similarly rare in our study, and usually in children younger than 10.

Similar with previous studies the level of LSIs performed by EMS staff in this study was quite low.[8,26] We believe that paramedics possessed insufficient knowledge and experience to carry out these procedures, and that they preferred to transport patients to the ED without stabilization, even in the event of respiratory or cardiac arrest. This is the most likely explanation for obviously non-survived patients, or subjects who have been pronounced dead, being transported via the EMS. In such cases, mortality cannot be prevented (since it has already occurred), and transporting such cases places the public and the EMS crew at risk of a vehicular accident in the process. In addition, there can be no benefit to the patient in transporting a dead body. Unnecessary transport of the non-survived patients restricts EMS resources for other patients who may truly benefit from them.

Our study has several limitations, including a lack of physiological data for patients at the time of pick-up (blood pressure, pulse, Glasgow coma scale, body temperature, respiratory rate, oxygen saturation, general condition, etc.), and a lack of detailed information concerning physical examination, accurate reasons for referral, some transport times, costs, and patient characteristics in the referral hospital. We also did not analyze and compare patients outcomes who received LSIs in the ED but who were not transported by ambulance.

In conclusion, this study presents comprehensive epidemiological and outcome data for CIC transferred by EMS system in Turkey. Patients transported by ambulance and referred from secondary or tertiary hospitals were more severely ill than those brought from the field or primary care facilities. When pre-hospital procedures for CIC were not performed higher mortality occurred. This study indicates a deficiency in Turkish pre-hospital pediatric emergency care, and we hope that our findings can assist with the development and improvement of the pediatric EMS system in Turkey. Information concerning the pre-hospital transport of pediatric cases can help improve care and prevent unnecessary resource use. Our study offers a clinical overview of pediatric emergencies that should be of assistance to EMS administrators, policymakers, and ED directors in planning for the care of acutely ill and injured children in Turkey.

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Original Article

Practice, self-confidence and understanding of pediatric obstructive sleep apnea survey among pediatricians

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ABSTRACT

Background. Pediatricians play an important role in the screening, diagnosis and management of childhood obstructive sleep apnea (OSA). This study used a questionnaire to explore the knowledge, self-confidence and general practices of childhood OSA among Thai pediatricians.

Methods. This was a descriptive cross-sectional survey study, using a newly developed questionnaire; including: 21 knowledge items, 4 self-confidence items, questions regarding OSA screening, number of OSA cases per month and OSA management.

Results. A total of 307, convenient pediatricians; from different types of hospitals across all regions of Thailand, participated in this study. The median, total knowledge score was 19 (range 14–21). Two-thirds of the respondents felt confident/extremely confident in their ability to identify and manage children with OSA. The average number of OSA cases reported by pediatricians was 5.9 cases per month. During a general medical check-up, 86.6% of the respondents did not routinely ask about OSA symptoms. Significant odds ratios (ORs) for the use of montelukast, as the first-line drug for OSA in young children, were observed in pediatric allergists and pulmonologists (adjusted OR 2.58, 95% CI 1.11–6.01 and adjusted OR 2.20, 95% CI 1.2–4.02) (P = 0.008), respectively, compared to general pediatricians and other sub-specialties.

Conclusions. Pediatricians had a high level of overall OSA knowledge, and good self-confidence in identifying and managing children with OSA. However, a low recognition rate and unawareness of OSA screening were observed.

Key words: knowledge, pediatricians, practice, obstructive sleep apnea, self-confidence.

Obstructive sleep apnea (OSA) is the most common form of sleep-disordered breathing (SDB) in children. It is characterized by prolonged and repetitive partial or complete upper airway obstruction during the sleep period that results in hypoxemia and hypercapnia, which affects sleep quality.[1-3] Undiagnosed or untreated childhood OSA may lead to a significant negative effect on health-related quality of life and cause serious cardiovascular complications, metabolic abnormalities, neurocognitive and behavioral problems and a failure to thrive. Early recognition and treatment of childhood OSA is crucial to prevent morbidity and to also provide better quality of life for both children and their families.

Routine screening for SDB in primary pediatric care settings has been recommended by the American Academy of Pediatrics (AAP) since 2012.[4] However, many previous studies showed unawareness, low recognition rate, and under management of children with OSA among community-based primary care physicians and academic settings.[5,6] Some studies reported improvement of care in childhood OSA was associated with better knowledge, positive attitudes and formal education in childhood OSA.[7,8]

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Pediatricians are the key people, who play an important role in the screening, diagnosis, and management of childhood OSA; however, currently there is no information regarding knowledge, self-confidence and practices related to childhood OSA in Thailand. The Thai guidelines for childhood obstructive sleep apnea was first published in 2015, to provide a national standard practice guideline for the diagnosis and management of OSA among the pediatric population.[9] In spite of the available guideline, a knowledge gap still persists, and the practices of OSA treatment continue to be heterogeneous. Therefore, this study used a questionnaire to explore the knowledge, self-confidence, and general practices of childhood OSA among Thai pediatricians. The outcome measurement of this study may contribute to a better understanding of the importance of OSA, and the confidence of pediatricians in their ability to screen, diagnose and manage childhood OSA.

Material and Methods

Study design and population

This study was a descriptive cross-sectional survey study, conducted from January to March, 2019; using the questionnaire to assess the knowledge, self-confidence and general practices of childhood OSA among Thai pediatricians. The study was approved by the Human Research Ethics Committee of the Faculty of Medicine, Prince of Songkla University at 7th February 2019 (REC.62-001-1-1). The study participants consisted of convenient pediatricians who were currently working in Thailand.

Sample size calculation

The sample size was calculated based on an estimate of the finite population proportion equation.[10,11] It was estimated that 80% of Thai pediatricians had good knowledge and self-confidence scores (higher than 80%). It also was determined that a sample size of 257 pediatricians was required to represent the population of Thai pediatricians, with a sampling error of 5% at a 95% confidence level and 10% allowed for non-respondents.

Developing the questionnaire

A newly developed questionnaire was used to evaluate the knowledge, self-confidence and practices of Thai pediatricians in concerns to OSA. The questionnaire consisted of 3 parts.

1. OSA knowledge part

The OSA knowledge part consisted of 21 items, which were presented in a true or false format.

Validity testing of the knowledge items used the individual content validity index (I-CVI) method, by four pediatric pulmonologists. If at least 3/4 of the expert members gave the individual items a score of relevant or extremely relevant, then the items were considered for inclusion in the final questionnaire.

For internal consistency, a pilot test, conducted by 20 pediatric staff doctors, was used to assess the questionnaire's reliability. Cronbach's alpha by SPSS software was 0.572.

2. Self-confidence part

The self-confidence part consisted of 4 items, used to evaluate the confidence of pediatricians in their ability to identify children at risk of OSA, initiate treatment, and follow-up of the children with OSA as well as their confidence to give information.

3. Practices part

The practices part consisted of 3 items, used to evaluate the number of OSA cases each month, frequencies of performing history taking for OSA symptoms and therapies for OSA management.

Study Procedure

The questionnaire was created on a Google form, and submitted to convenient pediatrician participants, using the Line application and E-mail. There was a consent paragraph in the

participant's information sheet, and an informed consent process was done in active voluntary action to complete the survey online. The questionnaires were completed anonymously.

Data management and analysis

Data were collected from the Google forms, and the analysis used R program version 3.5.1. For the knowledge parts, the total scores were presented as percentages, median and interquartile range (IQR).

The chi-square test, Kruskal-Wallis test, rank sum test, and the logistic regression model were used to assess the differences between the knowledge score and associated factors. Spearman's rank correlation was used to evaluate the relationship between the total knowledge score and self-confidence score. A P-value < 0.05 was considered statistically significant.

For practice items, the number of children with OSA in general practice is presented as mean. The frequency of history taking of OSA and OSA treatment are reported as percentages.

Results

The convenient respondents totaled 307 pediatricians, ranging from 28 to 60 years of age. Characteristics of the respondents are shown in Table I. Most of the respondents were female (82.7%), and nearly half were general pediatricians (40.4%). More than half of the respondents had less than 10 years of experience, since pediatric board graduation. There were 128 community-based pediatricians and 147 pediatricians who worked in teaching hospitals. Most of the respondents worked in either central Thailand (45.3%) or southern Thailand (27.7%).

Table I. Baseline characteristics of respondents (N = 307).

Characteristic	N (%)
Female	253 (82.7)
Age (years) (median; range)	37 (28-60)
Specialty training	
General pediatrician	124 (40.4)
Pediatric allergist	27 (8.8)
Pediatric pulmonologist	60 (19.5)
Other pediatric sub-specialist	96 (31.3)
Years since pediatric board graduation	
< 5	98 (31.9)
5–10	102 (33.2)
11–20	72 (23.5)
> 20	35 (11.4)
Working place	
Community-based hospital	128 (41.7)
Primary care hospital	20 (7.3)
Secondary care hospital	33 (12.0)
Private hospital/private clinic	59 (21.5)
Others	16 (5.2)
Teaching hospital	147 (47.9)
University hospital	66 (24.0)
Tertiary care hospital	81 (29.5)
Unknown	32 (10.4)
Location in Thailand	
Northern	26 (8.5)
Eastern	27 (8.8)
Northeastern	27 (8.8)
Western	3 (1.0)
Southern	85 (27.7)
Central	139 (45.3)
Total OSA knowledge score ≥ 80%	282 (91.9)
General knowledge score ≥ 80%	288 (93.8)
Identification & Evaluation knowledge score ≥ 80%	299 (97.4)
Management knowledge score ≥ 80%	176 (57.0)

OSA knowledge part

From the 21 knowledge items, the mean, total knowledge score was 18.5; with the median being 19 (range 14–21). All sub-categorical knowledge domain scores are shown in Table I. The responses in each individual knowledge item are listed in Table II. Most of the knowledge items were answered correctly (range 63.8–99.7%).

Table II. Percentage of respondents answering each knowledge item correctly.

Question item	Correct response	N (%)
General knowledge of OSA		
1. Prevalence of childhood OSA in Thailand is 1–5%.	True	273 (88.9)
2. Children with OSA have attention deficit hyperactivity disorder as a complication.	True	306 (99.7)
3. Severe childhood OSA causes right side heart failure.	True	299 (97.4)
4. Obese children have a higher prevalence of OSA than the general childhood population.	True	293 (95.4)
5. Childhood OSA can be found in children with failure to thrive.	True	289 (94.4)
6. Severe degree of snoring is correlated with severe OSA in children.	False	235 (76.5)
Identification & evaluation of OSA		
7. The most common cause of childhood OSA is adenotonsillar hypertrophy.	True	299 (97.4)
8. Snoring ≥ 3 nights/week is the chief complaint of childhood OSA.	True	227 (73.9)
9. Evaluation of tonsil size, facial deformity, signs and symptoms of allergic rhinitis should be done to find the cause of childhood OSA.	True	291 (94.8)
10. Children with suspected OSA should have a lateral soft tissue neck film taken.	True	265 (86.3)
11. Overnight pulse oximetry monitoring is a benefit for the diagnosis and severity assessment of OSA.	True	279 (90.9)
12. Polysomnography is the gold standard for the diagnosis of OSA.	True	302 (98.4)
Management knowledge of OSA		
13. Children with suspected OSA from history and physical examination should start treatment with an OSA medication. There is no need to wait for a confirmed diagnosis.	True	279 (90.9)
14. Montelukast is the first line drug for young children who are suspected of having OSA.	False	196 (63.8)
15. Systemic steroids are beneficial for the treatment of severe OSA.	False	210 (68.4)
16. Intranasal steroids help reduce the size of the tonsils and the adenoid gland.	True	268 (87.3)
17. Adenotonsillectomy has a good outcome for treatment of OSA > 85%.	True	251 (81.8)
18. OSA symptoms may be worse in the acute period after adenotonsillectomy.	True	266 (86.6)
19. Residual OSA after adenotonsillectomy can be managed by overnight CPAP.	True	303 (98.7)
20. Weight reduction for obese children can improve OSA symptoms.	True	305 (99.3)
21. Treatment of OSA in children is the same as in adults.	False	251 (81.8)

Factors associated with knowledge score

We compared the total knowledge score and knowledge score in sub-categorical knowledge domains between specialty training using the Kruskal-Wallis test. Pediatric pulmonologists had a significantly higher, total knowledge score (P = 0.045) as well as identification & evaluation score (P = 0.047) than non-pulmonologist pediatricians. No difference in total knowledge score and sub-categorical knowledge domains were observed between pediatricians who work in community or teaching hospitals, nor between ≤ 10 and > 10 years of pediatrics practice experience (Table III).

Factors associated with incorrect answers in focus items

The overall percentage of correct responses in the 4 knowledge items (items 6, 8, 14, and 15) was lower than 80%. Linear regression analysis was performed to evaluate the factor determinants of the incorrect answers in these focus items.

Table III. Factors associated with knowledge and sub-categorical knowledge score (N = 307).

Factors	N (307)	Total knowledge score	General knowledge score	Identification & Evaluation score	Management score
		median (IQR)	median (IQR)	median (IQR)	median (IQR)
Specialty					
Pediatric allergist	27	19 (17,20)	6 (5.5,6)	6 (5,6)	7 (6,9)
Pediatric pulmonologist	60	19 (18,20)	6 (5,6)	6 (5,6)	8 (7,8)
General pediatric & other specialties	220	19 (17.8,20)	6 (5,6)	6 (5,6)	8 (7,8)
		P = 0.045*	P = 0.06	P = 0.047*	P = 0.57
Practice experiences (years)					
≤ 10	200	19 (18,20)	6 (5,6)	6 (5,6)	8 (7,8)
> 10	107	19 (18,20)	6 (5,6)	6 (5,6)	8 (7,8.5)
		P = 0.90	P = 0.54	P = 0.29	P = 0.87
Working place					
Community-based hospital	128	18 (17,20)	6 (5,6)	6 (5,6)	7 (7,8)
Teaching hospital	147	19 (18,20)	6 (5,6)	6 (5,6)	8 (7,8)
Unknown	32	19 (18,20)	6 (5,6)	6 (5,6)	8 (7,9)
		P = 0.11	P = 0.27	P = 0.93	P = 0.06

For item 14, significant odds ratios (ORs) for the answer of montelukast being used as the first-line drug for young children suspected of OSA were observed in pediatric allergists (adjusted OR 2.89, 95% CI 1.27–6.58) and pulmonologists (adjusted OR 2.29, 95% CI 1.25–4.17) (P = 0.003), respectively; compared to general pediatricians and other pediatric sub-specialties. For item 15, we found a significant ORs for pediatric allergists to respond incorrectly to the question of systemic steroids having a benefit for the treatment of severe OSA (adjusted OR 2.5, 95% CI 1.1–5.69; P = 0.04); compared to general pediatricians and other sub-specialties. Item 8 asked about snoring more than 3 nights/weeks as the chief complaint of childhood OSA. Both general pediatricians and pediatric allergists had significant ORs of incorrect answers (adjusted OR 3.3, 95% CI 1.4–7.79) and 6.36 (95% CI 2.04–19.8) (P = 0.002), respectively, compared to the pulmonologists.

Self-Confidence part

According to the self-confidence assessment, the median score of self-confidence was 12 (IQR 10,15) (range 4–16). Most of the respondents felt confident/extremely confident in their ability to identify and manage children with OSA in all items (Table IV). However, the Spearman's rank correlation did not find a significant correlation between total knowledge score and self-confidence score (rho = 0.095, P = 0.09).

Practice part

Overall, Thai pediatricians in their practices saw an average of 5.9 cases per month (range 1–60) of childhood OSA, while the pediatric pulmonologists saw 15 cases per month of children with OSA. Seventy-one percent of Thai pediatricians "*always*" screened for OSA in obese children, but only 13.4% of Thai pediatricians "*always*" asked about OSA symptoms in general medical check-ups.

Figure 1 shows the percentage of therapies prescribed by Thai pediatricians for OSA management. Intranasal corticosteroids (INS) were "*often/always*" prescribed in 44.6% and 30.9%, respectively. Thirty-point seven percent of respondents reported "often" and 14.7% reported "*always*" as prescribing montelukast. Systemic corticosteroids (for example; prednisolone/dexamethasone) and oxygen

Table IV. Responses to self-confidence items (N = 307).

Self-confidence items	1 Not confident	2 Slightly confident	3 Confident	4 Extremely confident
	N (%)	N (%)	N (%)	N (%)
1. I feel confident identifying children at risk for OSA.	4 (1.3)	67 (21.8)	151 (49.2)	85 (27.7)
2. I am confident in my ability to start treatment for OSA.	4 (1.3)	65 (21.2)	137 (44.6)	101 (32.9)
3. I am confident in my ability to manage and follow-up children with OSA.	8 (2.6)	90 (29.3)	138 (45.0)	71 (23.1)
4. I am confident in my ability to give the patient information on OSA.	2 (0.7)	59 (19.3)	147 (47.9)	99 (32.2)

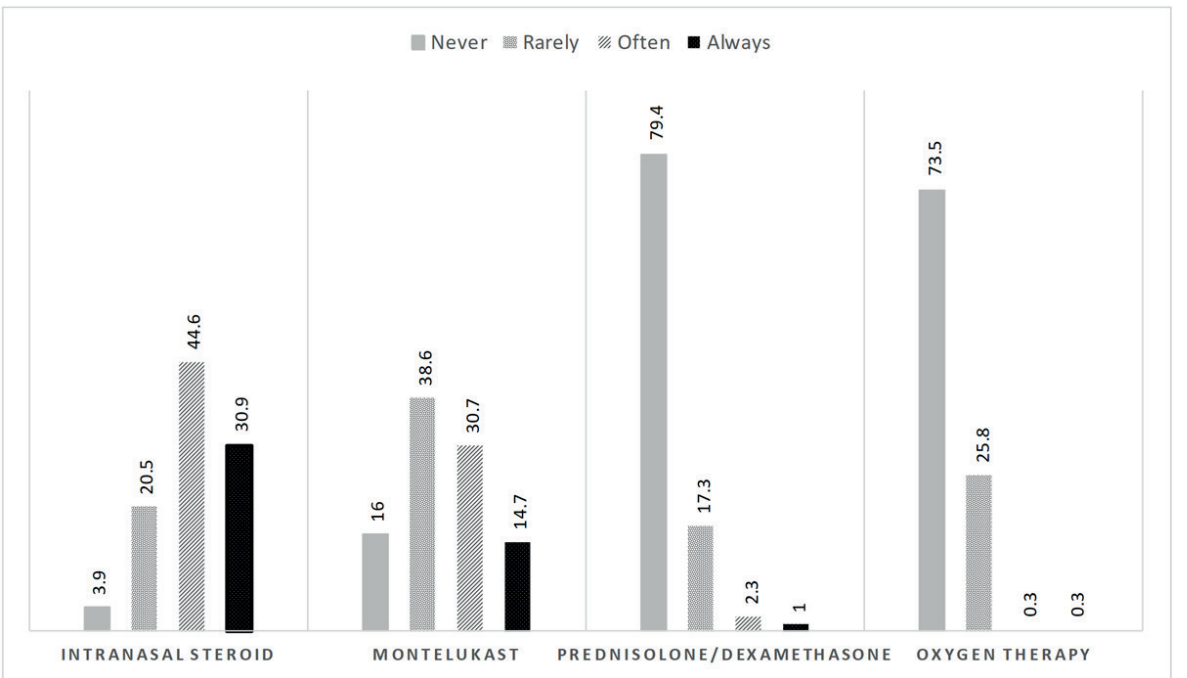

Fig. 1. Percentages of therapies prescribed by Thai pediatricians for obstructive sleep apnea management.

therapy were rarely used for management of children with OSA.

Discussion

This PSU-OSA Survey aimed to explore Practice, Self-confidence, and Understanding of pediatric OSA among Thai pediatricians. This is the first childhood OSA survey in Thailand, since the Thai Guidelines for Childhood Obstructive Sleep Apnea was first published in 2015. Overall, the study found that Thai pediatricians had a high self-confidence score, which indicated that they were confident in their ability to identify, their management, and follow-up of children with OSA. We found that among Thai pediatricians, 91.9% had a total OSA knowledge score ≥ 80%. Compared to a previous study in the United States; Uong et al.[8], found that the mean knowledge score in pediatric OSA was 69.6%. Moreover, the results of surveys concerning adult OSA also had similar findings, where the overall knowledge scores ranged from 66% to 76%.[12-14] The results of this study had higher knowledge scores, because the population in this study included

only pediatricians; whereas the previous studies included primary physicians and pediatricians, additionally the question items were also different.

However, 4 of the 21 knowledge items were problematic. There was a discrepancy in the answers of item 14. The study results from the practice part showed that almost half of the responders prescribed montelukast to treat OSA in general practice. From a linear regression analysis we found significantly higher ORs in pediatric allergists (adjusted OR 2.89) and pulmonologists (adjusted OR 2.29) who answered that montelukast was used as the first line OSA therapy in young children, compared to the general pediatricians and other sub-specialties. This was possibly caused by young patients, particularly under the age of 3 years who were referred to the specialist, according to the recommendation of the Thai Guidelines for OSA. Furthermore, montelukast is approved for patients aged 6 months or older, that was younger than the age-approval of intranasal corticosteroids (INS), which is older than 2 years of age.[15]

When we focused on other items, the associated factors with incorrect answers were subspecialty, general pediatricians and pediatric allergists, who had significant ORs of incorrect answers. Different levels of training possibly had an effect on knowledge. Unlike a previous study, we didn't find significant differences in the incorrect answers in terms of years of practice or place of work. This may have implied that the national recommendations, which are accessible to all physicians, caused overall knowledge homogeneity.

We found that 87.3% of the responders knew that INS help reduced the size of the tonsils and the adenoid gland (item 16). Adenotonsillar hypertrophy is a common etiology of childhood OSA, but up to 24.4% of responders never or rarely used INS for the management of OSA. This finding exemplified a barrier of knowledge. Bridgeman MB. reported several barriers that can impede the use of INS, including concerns about safety and steroid side effects; especially growth suppression, a child's resistance towards intranasal medication, undesirable sensations associated with intranasal administration, and misperceptions regarding the loss of response from frequent use.[16] The true barriers of INS among Thai pediatricians need to be explored.

Overall, in general practice of OSA, we found that Thai pediatricians saw 5.9 cases per month of children who were suspected of having OSA. However, in the sub-specialty analysis, general pediatricians reported only 2 cases per month, while pediatric pulmonologists reported an average of 15 cases per month. These findings reflect the fact that most childhood OSA patients in Thailand were seen by OSA specialists. Despite, the high level of OSA knowledge, and good self-confidence in OSA practice observed among general pediatricians, they reported a low number of patients in clinical practice. Interventions to encourage general pediatricians to participate in OSA practice may be needed.

Our study found that 86.6% of the responders did not routinely ask about OSA symptoms in general medical check-ups, in spite of the recommendations of AAP and the Thai guidelines for childhood OSA. These findings were consistent with a previous study from Erichsen and Rosen that offered evidence of a low OSA recognition rate and unawareness of pediatricians; particularly general pediatricians concerning the screening of OSA.[6,7] Therefore, interventions to increase OSA awareness and encouragement of pediatricians to perform history taking for OSA symptoms are needed to find children who are at risk of OSA. This would provide for early detection and optimize OSA management outcomes.

The strength of this study is the information on OSA practice in Thailand, based on an adequate sample size and the demographic data, which included: age, specialty training, years of pediatric experience and type of hospital. Although, our study discovered problems in the general practice of OSA, it may not explain the cause of the problems; particularly

in medications used to manage OSA and the barriers from knowledge to practice. More focus is needed on education and intervention, so as to identify and overcome the barriers for the use of INS.

This study has limitations. First, this study possibly had self-selection bias.[17] Additionally, the respondents were those who had access to Line application and Google form; so the calculation for non-respondents was added, and the final participant numbers were met. In addition, we could not calculate the number of non-respondents, because we could not access the list of Thai pediatrician emails due to confidentiality concerns.

Good knowledge and self-confidence in the management of childhood OSA was observed among Thai pediatricians; whereas, a low recognition rate and unawareness of OSA screening is still problematic. Misunderstandings in some knowledge points were identified; especially concerning medications, including INS and montelukast.

More research needs to focus on practice points; especially the use of INS and montelukast, which may provide a better understanding and overcome the barriers of OSA treatment. Intervention to encourage pediatricians to move from good knowledge to practice in OSA screening would provide early case detection and improve OSA management outcomes.

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Original Article

Oral health status of asthmatic children using inhaled corticosteroids

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ABSTRACT

Background. Due to the increase in the prevalence of asthma, especially in childhood, oral health problems arising from the use of asthma medications have become a major health concern. Inhaled corticosteroids are widely used since they are the cornerstone of asthma management. This study aimed to investigate the effects of inhaled corticosteroids on oral health and various factors regarding active ingredients in inhalers, inhaler-spacer device use, rinsing the mouth after administration of inhaler, oral hygiene practices and parents' education on dental caries susceptibility in young children with asthma.

Methods. Children with asthma who were treated with inhaled corticosteroids for at least 6 months were included in the study. A questionnaire related to demographic characteristics and oral health practices of children was conducted with parents and the children were examined using a dental mirror and probe under dental unit lighting after air-drying the teeth.

Results. Oral health status was evaluated in 115 children, 54 (42-66) months old, 61% male and 39% female with determination of dmft-dmfs, DMFT-DMFS, ICDAS II, plaque index and gingival index. No statistically significant difference was found between sugar-containing and sugar-free inhalers, duration of inhaled corticosteroid use, inhaler-spacer device use, rinsing the mouth after administration of inhaler and dmft-dmfs, DMFT-DMFS, plaque and gingival index ($p>0.05$). Long-term inhaled corticosteroid use was significantly associated with oral candidiasis ($p=0.029$). Also, the results showed that mothers' educational level had a significant effect on children's oral health ($p_{dmft/dmfs}<0.001$; $p_{gingival\ index}=0.049$; $p_{plaque\ index}=0.005$).

Conclusion. Due to the effect of immunosuppression, long-term use of inhaled corticosteroids enhances the risk of development of the opportunistic pathogen candida. Regular dental visits and preventive dental treatments are needed in these patients for healthy oral status.

Key words: asthma, children, inhalers, corticosteroid, oral health.

Asthma is a chronic inflammatory disorder of the airways characterized by hyper-responsiveness and episodic, reversible symptoms of airflow obstruction, either spontaneously or with treatment. It is a serious health problem throughout the world. Asthma has a higher incidence among preschool children.[1]

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Inhaled corticosteroids, through their anti-inflammatory effects have been the cornerstone of asthma management for decades. However, use of inhaled corticosteroids creates concerns due to fear of systemic side effects such as suppression of adrenal axis and longitudinal growth, or decrease in bone mineral density. The administration route of these drugs is inhalation so they act directly on airways, nevertheless this may lead to local adverse effects regarding oral health. The association of asthma with oral health status such as dental caries, periodontal disease, dental erosion and

oral mucosal changes have been the subject of discussion among dental practitioners.[2] Asthma medications, especially inhalers, may cause mouth dryness, which makes the person vulnerable to caries and predisposes the child to fungal infections. Epidemiological studies investigating the effect of asthma on dental caries are conflicting. The majority of the studies conclude that asthmatic children have higher dental caries prevalence, whereas some oppose such conclusions.[3-6] The increased susceptibility to dental caries may also be due to the anti-asthmatic medications containing fermentable carbohydrates. The most common is lactose monohydrate, and although it is one of the least cariogenic sugars, this still can lead to increased dental caries risk.[7]

Studies assessing the periodontal status of children with asthma have revealed different results.[3,4,8] Corticosteroids are thought to play an important role in the development of candida infection owing to immunosuppression.[9]

In this study, we aimed to evaluate the oral health of young children with asthma and also possible effects of some variables on oral health such as type of inhaled corticosteroids, inhaler-spacer device use, rinsing the mouth after administration of inhaler, sex, oral hygiene practices and parents' education.

Material and Methods

Institutional Hacettepe University Ethics Committee approved the study (2016, GO 16/108-08) and written informed consent was obtained from parents. 115 children with asthma, 54 (42-66) months old, 61% male and 39% female who were admitted to Hacettepe University, Department of Pediatric Allergy and treated with inhaled corticosteroids for at least 6 months were included in the study. The exclusion criterion was the presence of any systemic illness other than asthma.

After a face to face questionnaire covering demographic data including age, sex, education level of parents, medical and dental history, oral health habits, and complaints of participants related to oral health (halitosis, toothache, etc.) were completed by the parents, all of the children were examined by the same pediatric dentist with a dental mirror and ball-ended explorer (WHO 973/80-Martin, Solingen, Germany) according to World Health Organization (WHO) criteria.[10] The dental caries diagnosis was checked for intra-examiner agreement by employing two separate evaluations in 10 children with 1-week interval between examinations. (Kappa score for ICDAS II = 0.88). The caries status was diagnosed according to dmft/dmfs-DMFT/DMFS indices in which decayed (D/d), missing (M/m) and filled (F/f) teeth are appraised and reported according to number of teeth (DMFT/dmft) or surfaces (DMFS/dmfs) involved with codes and criteria determined by WHO.[10] Total number of decayed, missing and filled deciduous teeth were recorded as children's dmft/dmfs; permanent teeth were recorded as children's DMFT/DMFS. This index is the most commonly used index in research because of its advantages including being simple, easy to apply and being statistically useful. However, in this scale, obvious cavity lesions extending into the dentine have been scored. In addition, diagnosis of dental caries was performed according to International Caries Detection and Assessment System-II (ICDAS II) index which was improved to include early enamel caries lesions. Stage of lesions were also used by means of visual/tactile examination without radiographs.[11]

The gingival health and the amount of plaque were assessed using the plaque and gingival index of Silness and Löe.[12,13] The grade of plaque evaluation was performed in four regions (mesial, distal, lingual, labial) for each tooth. The plaque accumulation was evaluated and coded as follows:

0- No plaque

1- A film of plaque adhering to the free gingival margin and adjacent area of the tooth, which cannot be seen by the naked eye, but only by using a disclosing solution or by using a probe.

2- Moderate accumulation of deposits within the gingival pocket, on the gingival margin and/or adjacent tooth surface, which can be seen by the naked eye.

3- Abundance of soft matter within the gingival pocket and/or on the tooth and gingival margin.

Scores for each surface were added and divided by the total number of surfaces of examined teeth in order to obtain the individual plaque score. The mean plaque index was classified as follows: no < 0.1, minimal 0.1–1.0, moderate 1.1–2.0, and heavy 2.1–3.0. The gingival health status was determined similar to plaque index on four regions (mesial, distal, lingual, labial). The ball-ended explorer was slightly inserted into the gingival pocket between gingiva and teeth with a probing force not exceeding 20g.[10] The gingival health was evaluated and coded as follows:

0- Normal gingiva: No inflammation

1- Mild inflammation: Slight changes in color, slight edema. No bleeding on probing.

2- Moderate inflammation: Redness, edema and glazing. Bleeding upon probing.

3- Severe inflammation: Marked redness and edema/ulceration/tendency to bleed spontaneously.

The individual gingival score was calculated as the plaque score. Finally, the mean score for each index was calculated by adding the individual scores and dividing by the total number of patients.

Also, the soft tissues including the tongue were examined intraorally and the differences were registered. The control levels of asthma were categorized as 'well-controlled', 'partly controlled' and 'uncontrolled' based on symptom frequency and reliever medication need within the last 4 weeks according to the Global Initiative for Asthma (GINA).[14] The children were grouped by the active ingredient of inhaled corticosteroids (fluticasone propionate and budesonide+lactose) that they used. The status of oral thrush development, which shows candida infection, during or immediately after inhaler use was questioned to the parents. The oral health indices in deciduous dentition and permanent teeth were compared by the parental education level.

The results were analyzed with SPSS 22.0 (SPSS, Inc, Chicago, IL, USA). Number, percentage, mean, standard deviation, median, 1st and 3rd quartiles, minimum and maximum values were estimated for descriptive statistics. Shapiro–Wilk test, tested the normality of the distributions; Mann Whitney-U test was used for comparing two independent groups. Chi-square was used to assess the significance of the differences between categorical variables. The significance level was considered as 0.05 in all analyses.

Results

115 children 54 (42-66) months old, 61% male and 39% female were involved in the study. The children's major complaints were halitosis, dental caries, oral candidiasis and toothache. According to parental report, mouth breathing was present in one-third of children (n=40). The frequency of brushing teeth was determined as less than once a day (54.8%), once a day (17.4%), twice a day (16.5%), three times a day (0.9%) and 10.4% of children did not brush their teeth. More than half of the children (67%) had not visited a dentist previously

The duration of use of inhaled corticosteroids was 24.0 (12-24) [median (1st and 3rd quartiles] months. Asthma levels of the children were well controlled (53.9%), partly controlled (26.9%) and uncontrolled (19.2%). The children had been using Flixotide propionate (74.8%) and budesonide+lactose (25.2%) (Table I).

Among parents; 33.0% of the mothers, 26.1 % of the fathers had graduated from high school and 27.8 % of the mothers, 29.6% of fathers had graduated from university. The remaining parents were educated until secondary school.

Table I. Characteristics of patients according to self-reports of parents (Ankara-Turkey, 2016).

Characteristics (n=115)		
Age [median(interquartile range)]	54 (42-66) months	
Sex	n	%
Male	70	61
Female	45	39
Frequency of tooth brushing	n	%
No brushing	12	10.4
Less than once/day	63	54.8
Once/day	20	17.4
Twice/day	19	16.5
3 times/day	1	0.9
Complaints related to oral health n	n	%
Yes	85	73.9
Halitosis*	53	62.4
Dental caries*	42	49.4
Oral candidiasis*	32	37.6
Toothache*	24	28.2
Previous dental visits	n	%
No	77	67
Yes	38	33
Asthma control level	n	%
Well controlled	62	53.9
Partly controlled	31	26.9
Uncontrolled	22	19.2
Type of inhaler	n	%
Flixotide propionate	86	74.8
Budesonide+lactose	29	25.2

There is more than one answer; the percentages calculated over the 85 patients reporting any complaint.

The patients who had experienced oral candidiasis had a significantly longer duration of inhaled corticosteroid use compared to the ones who had not experienced oral candidiasis (p=0.029) (Table II). With regard to oral care habits (children with no brushing & less than once a day or brushing) compared to oral candidiasis; the complaint of oral candidiasis was less in children who brushed their teeth regularly (n=13, 40.6%) but this difference was not statistically significant (p>0.05).

The mean dmf/DMF scores for teeth and surfaces were dmft=2.69±3.58, dmfs=4.97±8.27, DMFT=0.28±0.58 and DMFS=0.28±0.58 (Table III). It was found that 58.3% of the children had shown tooth decay on their primary and/or permanent teeth by visual inspection. The mean plaque indices were 0.69±0.43 and gingival indices were 0.24±0.27 for the whole study group (Table III). The reported gingival inflammation was mild in 70.4% and moderate in 2.6% of the study group. In addition, 27% of the study group had no gingival inflammation. 8 of 115 children had a geographic tongue appearance.

In the intraoral examination, all the children's existing teeth were given codes according to the ICDAS II index, and the highest individual ICDAS codes taken were recorded (Table IV). It was determined that 4.3% of the whole children

Table II. Duration of inhaled corticosteroid use by patients with complaint of oral candidiasis (Ankara-Turkey, 2016).

	Oral candidiasis		z	p*
	Yes	No		
	Median (Min-Max)	Median (Min-Max)		
Duration of inhaled corticosteroid use (month)	24 (6-72)	16 (6-60)	-2.186	0.029

*Mann Whitney U Test, p<0.05

Table III. Distributions of dmft(S)/DMFT(S), gingival and plaque indices scores (Ankara-Turkey, 2016).

Distributions	dmft* (n=115)	dmfs* (n=115)	DMFT* (n=18)**	DMFS* (n=18)**	Plaque index (n=115)	Gingival index (n=115)
X±SD	2.69±3.58	4.97±8.27	0.28±0.58	0.28±0.58	0.69±0.43	0.24±0.27
Median	1.0	1.0	0.0	0.0	0.57	0.18
1.Quartile	0.0	0.0	0.0	0.0	0.32	0.07
3.Quartile	4.0	6.0	0.25	0.25	0.96	0.33
Min-Max	0-15	0-43	0-2	0-2	0.0-2.01	0.0-2.0

*Total number of decayed, missing and filled teeth (DMFT/ dmft) and surfaces (DMFS/dmfs) were recorded. Dmft/s for primary teeth, DMFT/S codes for permanent teeth are involved in.
**DMFT/DMFS values could only be evaluated in 18 patients due to mixed dentition.

Table IV. International caries detection and assessment system-II (ICDAS II) scoring criteria.[11]

Score	Clinical criteria description
0	Sound tooth: no evidence of caries after prolonged air-drying (5 s), surfaces with developmental defects (enamel hypoplasia, fluorosis), tooth wear (attrition, abrasion and erosion), and extrinsic or intrinsic stains will be recorded as sound.
1	First visual change in enamel: opacity or discoloration is visible after prolonged air-drying, which is not or hardly seen on a wet surface.
2	Distinct visual change in enamel: opacity or discoloration distinctly visible when wet, lesion must still be visible when dry.
3	Localized enamel breakdown owing to caries with no visible dentin or underlying shadow.
4	Underlying dark shadow from dentin with or without localized enamel breakdown.
5	Distinct cavity with visible dentin: visual evidence of demineralization and dentin exposed.
6	Extensive distinct cavity with visible dentin with more than half of the tooth surface involved or possibly reaching the pulp.

in the study had completely healthy teeth, 31.3% had initial caries (ICDAS=1 or 2), 6.1% had moderate (ICDAS=3 or 4), and 58.2% had advanced caries (ICDAS=5 or 6). There was no significant difference by sex (p=0.394) (Table V).

No significant differences were found in terms of dental caries, plaque indices, gingival indices and inhaler content between sugar-containing (budesonide+lactose) and sugar-free (flixotide propionate) inhaler groups.

The score of caries in deciduous dentition, gingival and plaque indices were significantly lower in children of mothers with high education level. ($p_{dmft/dmfs}<0.001$; $p_{gingival\ index}=0.049$; $p_{plaque\ index}=0.005$). The score of caries in permanent teeth was lower in children of mothers with high education level, but this difference was not statistically significant. No statistically significant difference was found when comparing the education levels of the fathers.

Table V. Distribution of ICDAS II codes classification by sex (Ankara-Turkey, 2016).

ICDAS	Sex				
	Female		Male		
Codes	n	%*	n	%*	P**
0 (sound)	2	4.4	3	4.3	0.394
1-2 (initial)	18	40.0	18	25.7	
3-4 (moderate)	3	6.7	4	5.7	
5-6 (severe)	22	48.9	45	64.3	
Total	45	100	70	100	

*Column percentage
**p>0.05

No significant differences were found between oral health outcome variables (dmft/dmfs, DFT/DMFS, gingival and plaque indices) and asthma control levels (well-controlled, partly controlled and uncontrolled) (p>0.005).

In this study, the mean duration of inhaled corticosteroid use in 115 asthmatic children was 19.84±11.46 months. As the duration of inhaled corticosteroid use prolonged, dmft/dmfs and DMFT/DMFS scores, gingival and plaque indices were found to increase. But we did not find a statistically significant association between duration of inhaled corticosteroid use, inhaler-spacer device use, rinsing the mouth after administration of inhaler and dmft-dmfs, DMFT-DMFS, plaque and gingival index (p>0.05).

Discussion

Asthma and dental caries are the most common chronic diseases of childhood and two major causes of school absenteeism.[15,16] Studies regarding dental caries in the primary and permanent dentition, oral hygiene and diet, duration of asthmatic symptoms, exposure time to medication and severity of asthma, the association between asthma and caries have been prominent issues in the literature. Further studies are required due to inconsistent consequences and there is no study regarding the severity of dental caries in asthmatic children as far as we know. In the present study, dental caries were assessed according to WHO criteria and recorded according to the DMFT/DMFS, dmft/dmfs index and ICDAS II index. Although oral health status in children with asthma have been reported in some studies, there is no study, which evaluated the severity of caries. The ICDAS index system allows the evaluation of initial caries and has codes according to the stages of caries.[11,17] DMF index, has been used worldwide in dental research but is unable to provide accurate data about initial lesions and severity of lesions.

In previous studies, researchers usually have studied case-control groups consisting of healthy and asthmatic children. It was reported that healthy children had higher DMFT scores than asthmatics.[5,6] Shulman[18] and Meldrum[19] found no association between dental caries and asthma. Mazzoleni et al.[20] noted that DMFT scores were statistically significantly higher in asthmatic children, but there was no significant difference in dmft scores between groups. Stensson et al.[3] reported that 29% of 3-year-old children and 49% of 6-year-old children with asthma had dental caries and preschool children with asthma have higher caries prevalence than healthy children. The factors discriminating for caries in asthmatic children had been considered as higher intake of sugary drinks, mouth breathing, and immigrant background. In the present study, 67 (58.3%) of the children had shown caries in terms of dmf index. However, according to the ICDAS II index, only 5 (4.3%) had healthy teeth, 36 (31.3%) had initial, 7 (6.1%) had moderate and 67 (58.2%) had advanced caries lesions. Regarding

the severity of caries, similar to this study, no healthy children were present in a study conducted on 150 children with adenotonsillar hypertrophy in which mouth breathing and dry mouth are also common predisposing factors to caries in asthmatic children.[21] The differences in the dental caries prevalence might be sourced from the concomitant factors aggravating dental caries as diet, hygiene, the severity of caries, and mouth breathing.

The results in the present study showed that mothers' educational level had a significant effect on the oral health of children with deciduous dentition but the fathers' educational level had no significant effect. Khalilzadeh et al.[22] reported that the DMFT scores of children with high economic income and high education level of their mothers were lower. In another study, it was reported that individuals with high socioeconomic status had better oral health than those with low socioeconomic status.[23]

McDerra et al.[24] reported that the presence of calculus, gingival inflammation and dental plaque in asthmatic children was significantly higher than the control group. Laurikainen et al.[25] noticed that asthmatic children had higher periodontal disease and lower salivary flow rate. Studies showed that more dental calculus was seen in asthmatic patients due to increased calcium and phosphate in the saliva. The children in these studies were older than the present study. There are a few studies in preschool children with asthma. Stensson et al.[3] reported gingivitis in 14.9 % of preschool children with asthma. In present study, only 2.6% of the children had moderate gingival inflammation showing bleeding by probing and dense calculus accumulation was seen in only 1 patient. Differences in oral care habits, mouth breathing prevalence, the consumption of sugar-containing drinks and the period of medication exposure of children could be the reason for these variations in studies.

Ersin et al.[26] found that the duration of drug use and the severity of asthma increased the risk of caries development. The increased caries prevalence according to the severity of asthma may be due to increased dosage and frequency of medications used in asthma treatment.[27] Milano at al.[28] reported that duration of use and frequency of medications used in asthma treatment were associated with increased dental caries, and deciduous dentition was more affected. The duration of inhaled corticosteroid use was higher in asthmatic children with dental caries in present study, but this difference was not statistically significant. Eloot et al.[4] examined the effects of asthma severity and duration of drug use on caries prevalence, gingival and plaque indices and found no statistically significant differences. The results of this study are consistent with the results obtained in our study.

Tootla et al.[7] showed that inhaled corticosteroids caused a significant decrease in plaque pH. The study of Shashikiran et al.[15] was conducted with 105, six to fourteen-year-old asthmatic children to determine the condition of their dental caries and their periodontal status before and after taking anti-asthmatic medication, for a period of 1 year and these were matched with their controls. The results showed that the salbutamol inhaler had an increased caries rate with high significance over other groups, which was followed by salbutamol tablets and the beclomethasone inhaler, respectively. In the group using an inhaler with beclomethasone, there was an increase in the caries rate compared to healthy individuals, but this was not statistically significant. In our study, patients used sugar-containing and sugar-free inhaled corticosteroids. The salbutamol inhaler was not routinely used, only when asthmatic children had an episode. Therefore, the aim of our study was to determine the effects of inhaled corticosteroids rather than the effects of β2-agonist drugs. According to the data obtained from this study, the caries prevalence and gingival indices were not significantly different in the sugar-containing group and the sugar-free group.

Use of the inhaler spacer and rinsing the mouth with water immediately after use of an

inhaler was described as a protective factor for development of caries but no statistically significant difference was found in our study.

Inhaled corticosteroids constitute the most important group of drugs for bronchial asthma treatment due to anti-inflammatory effects.[29] Immunosuppression effects of steroids provide a selective growth advantage to the opportunistic pathogen c. albicans.[30] Knight and Fletcher[31] reported that patients receiving corticosteroid therapy had higher levels of glucose in their saliva than in the control group. This supports growth, proliferation and adherence of C. albicans to oral mucosa cells.[32] Kuna et al.[33] reported that oral candidiasis occurred in 10-30% of patients using inhaled corticosteroids. Similarly, we also learned that oral candidiasis had developed in 32 (37.6%) children during the use of inhaled corticosteroids in the present study. In addition, oral candidiasis increased statistically as duration of inhaled corticosteroid usage increased.

In asthmatic patients, the presence of geographic tongue is based on an underlying allergic condition.[34] In our study 8 of 115 children had a geographic tongue appearance.

According to the results of the present study, the halitosis complaint reported by parents was 62.4%. It was reported in previous studies that halitosis could be seen due to oropharyngeal candidiasis or mouth breathing which are possible symptoms in asthma patients.[3,35,36] In this study, mouth breathing or oral candidiasis was present only in one-third of children. The method of determination of halitosis; (in the present study, it was evaluated via a questionnaire completed by parents) or differences in oral care habits and the mouth breathing prevalence of the study group-children with asthma could affect the halitosis prevalence. As far as we have reviewed the literature, no study regarding the prevalence of halitosis in asthma children is present. More studies with a high sample size should be conducted to reveal the possible correlations with halitosis in asthmatic children.

The results of this study should be considered in light of its limitations. As this was a non-representative descriptive study of the asthmatic children who were treated with inhaled corticosteroids only, the results cannot be generalized to the whole population. Prospective cohort studies with larger sample sizes including both asthmatic children using inhalers and children without asthma are strongly suggested to fully clarify the mechanism between asthma, inhalers and oral health by performing multivariate analyses like logistic regression.

In conclusion; oral health is an essential part of general bodily health. Poor oral hygiene adversely affects both physical health and quality of life. Patients should be referred to dentists by their physicians before they take inhaled corticosteroids. Oral health can be preserved through a multidisciplinary approach, periodical dental check-ups, close monitoring and preventive management.

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Original Article

Investigating the risk factors for antibiotic lock therapy failure in pediatric cancer: a single center retrospective analysis

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ABSTRACT

Background. Catheter-related bloodstream infection (CRBSI) is one of the most common complications of central lines. Data concerning the effectiveness and safety of antibiotic lock therapy (ALT), especially in pediatric hematology and oncology patients, have not yet reached sufficient levels of evidence. We aimed to share our center's experience on ALT in pediatric cancer and to investigate the causes of ALT failure.

Methods. All cases with CRBSI and treated with ALT administiration in children with cancer between January 2015 and May 2019 were reviewed. Patients characteristics, laboratory and clinical findings, treatments, outcome of ALT, recurrences and reinfections were recorded. Patients with successful and unsuccessful ALT outcomes were compared in order to identify the risk factors for ALT failure.

Results. Sixteen eligible CRBSI treated with adjunctive ALT were identified. The most common pathogens were coagulase negative staphylococci (8/16, 50%). Treatment failure was observed in 31.2% (5/16). Younger age alone was an independent risk factor for treatment failure (0.9 vs 6.8 years, p = 0.038). Recurrence and reinfection rates were 23.1% and 16.7%. Mild bleeding occured in two cases (12.5%) and occlusion causing catheter removal was seen in one (6.3%).

Conclusions. ALT was found to be a safe modality with a success rate of 68.8% in children with cancer at our center and younger age was an independent risk factor for treatment failure. Future studies with larger sample sizes are needed to determine the factors affecting the ALT outcome, especially in childhood malignancies.

Key words: antibiotic lock therapy, catheter-related bloodstream infections, cancer, childhood malignancy.

Central venous catheters (CVCs) are critical for children with cancer owing to difficulties in intravenous access, the necessity of safe administration of chemotherapeutics, frequent need for treatments such as parenteral nutrition, blood products and antibiotics. Catheter-related bloodstream infections (CRBSI) are the most common complication of central lines and can affect up to one-fourth of children with cancer during their treatment course.[1-3] Besides serious complications like endocarditis, septic thrombophlebitis and metastatic infection by hematogenous spread, CRBSI can also cause prolonged hospital stay, increased risk of mortality and hospital costs.[2,4,5]

When CRBSI is related to a long term CVC, catheter salvage can be attempted by administering both systemic antibiotics and antibiotic lock. The objective of antibiotic lock therapy (ALT) is to eliminate the microbial biofilm from long term CVC by locking a high-concentration antibiotic solution into CVCs. Although recommendations related to ALT seem clear, data concerning the effectiveness and safety of ALT -especially in children with

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cancer - have not yet reached sufficient levels of evidence.[6,7]

With this study we aimed to share our center's experience on ALT in pediatric cancer patients with long term catheters and to investigate the causes of treatment failure.

Material and Methods

Patient selection

Patients with cancer who have long-term central venous or port catheters, aged 0-18 years, received ALT for CRBSI from January 2015 to May 2019 at Dokuz Eylul University Hospital were enrolled. All data were collected retrospectively through patient files and medical records. The research was reviewed and approved by the Ethics Committee of Dokuz Eylul University Medical Faculty (No: 2020/04-53, date: 17.02.2020) and an informed consent was required for the participants.

Definitions

CRBSI was defined as isolation of a recognized pathogen (other than a common skin contaminant) from blood culture or of a common skin contaminant from at least two separate blood cultures in a patient with clinical findings and with no other source of infection other than the central catheter. Patients with only one blood culture positivity for common skin concomitants (*Corynebacterium spp., Bacillus spp., Propionibacterium spp.*, coagulase-negative staphylococci, viridans group streptococci, *Aerococcus spp. and Micrococcus spp...*) or with another infection source or with a short-term central catheter were excluded from the study.

Recurrence was defined as occurrence of another CRBSI with the same organism and re-infection was defined as occurrence of another CRBSI with a different organism after both blood cultures from catheter hub and peripheral vein were cleared and clinical improvement was achieved.[8,9]

ALT success was defined as defervescence, clinical improvement and clearance of cultures in 72 hours and no recurrence or re-infection within 30 days after discontinuation of ALT.

ALT failure was considered in the presence of one of the following situations:

- Persistent bacteremia or persistence of clinical findings despite 72 hours of antibiotic therapy to which the infecting organism is susceptible or,
- Catheter removal due to infection, mortality potentially attributable to sepsis, recurrence or re-infection within 30 days after discontinuation of ALT.

Data collection and follow-up period

Data were collected retrospectively on participants' demographics, underlying diagnosis, inpatient unit, length of hospital stay until the CRBSI diagnosis, laboratory data, catheter type, date of catheter placement and removal, microbiologic data, total parenteral nutrition, antimicrobial treatment (systemic and lock therapies, duration, agent, concentration, anticoagulants) and anti-cancer therapies on a structured proforma.

A patient was included in the study only once with the first CRBSI episode treated with ALT. All patients were followed up for recurrence or re-infection until catheter removal, death or the end of the study period whichever came first.

Concerning the complications of ALT, medical records were also investigated for bleeding or occlusion of the catheter.

Analysis

Patients were divided into two groups according to ALT outcome and compared with each other in terms of age, gender, underlying disease, absolute neutrophil count (ANC), lymphocyte and monocyte count, CRP values, length of hospital stay until CRBSI, catheter day, catheter type, time to ALT, heparin concentration in the lock solution and dwell time.

Statistical analysis was performed with the "IBM Statistical Social Sciences SPSS 22.0" software. Variables with nonnormal distribution were indicated with medians and interquartile range (IQR). The categorical data were analyzed using a chi-square test or the two-sided Fisher's exact test. The Mann-Whitney U test was used to test the nonnormal distribution of data. P values less than 0.05 were considered statistically significant.

Results

Between October 2015 and May 2019, 16 patients aged 0-18 years who had been followed up for childhood malignancies, had a permanent catheter and underwent ALT were included in the study. The median age was 3.8 (IQR: 0.9-11.5) years. Male to female ratio was 1.28/1. Among nine hematologic malignancies (% 56,3) only one was acute myeloblastic leukemia and the rest were acute lymphoblastic leukemia (ALL). The other diagnoses were neuroblastoma in four, brain tumor in two and hepatoblastoma in one patient (Table I).

Nine patients were receiving intensive chemotheraphy (induction phase for leukemias and active chemotheraphy for solid tumors); two patients with leukemia were in the consolidation phase and one was in reinduction phase of the treatment protocol. Two patients with leukemia were on immunosuppressive therapy in post-transplant period (matched sibling donor allogenic hematopoietic stem cell transplantation). Of the two remaining patients, one was receiving radiation therapy for her brain tumor, and the other had ALL and developed necrotizing pancreatitis.

The incidence of CRBSI was 5.64/1000 catheter-days among the cases included. The most prevalent pathogens identified in the study were coagulase negative staphylococci (CoNS) (50%) and gram negative bacteria (31.3%) followed by polymicrobial (12.5%) and fungal (6.2%) infections (Table II).

All patients recieved ALT and systemic antibiotics according to the sensitivity of the microorganism. Antibiotic concentrations and the duration of therapy were in accordance with

Table I. Demographic and clinical features of all 16 children.

Age [years (IQR)]	3.8 (0.9-11.5)
Gender [n (%)]	
Male	9 (56.3)
Female	7 (43.8)
Underlying diagnosis [n (%)]	
Leukemia	9 (56.3)
Solid tumor	7 (43.8)
HSCT	2 (12.5)
Inpatient unit [n (%)]	
Hematology/oncology unit	15 (93.8)
ICU	1 (6.2)
Lenght of stay until CRBSI [days (IQR)]	3,5 (1-17.8)
Neutropenia (ANC≤500/mm^3)	10 (62.5)
Catheter type [n (%)]	
Tunneled double-lumen catheter	2 (12.5)
Port catheter	14 (87.5)
Catheter-day [days (IQR)]	150 (20-204)

≤500/mm^3 within the period of 3 days before and after the culture day.
IQR: Interquertile range, HSCT: hematopoietic stem cell transplantation, ICU: intensive care unit, CRBSI: catheter-related bloodstreaminfection, ANC: absolute neutrophil count.

Table II. Microorganisms in catheter-related bloodstream infections.

Organisms [n (%)]		
Gram positive	8 (50)	
Coagulase-negative staphylococci		8 (50)
Gram negative	5 (31.3)	
Klebsiella oxytoca		1 (6.2)
Pseudomonas aeruginosa		1 (6.2)
Escherichia coli		1 (6.2)
Acinetobacter baumannii		1 (6.2)
Enterobacter cloacae		1 (6.2)
Polymicrobial	2 (12.5)	
Stenotrophomonas maltophila, Bacillus cereus		1 (6.2)
Acinetobacter lwoffii, Enterococcus faecium		1 (6.2)
Fungus	1 (6.2)	
Candida pelliculusa		1 (6.2)

the guideline of Infectious Diseases Society of America: vancomycin 5 mg/ml, ceftazidime 0.5 mg/ml and ciprofloxacin 0.2 mg/ml.[5] caspofungin -the only antifungal lock- was administered as 10 mg caspofungin with 200 IU heparin in a 3 ml solution based on former experience in the literature.[10] Dwell time was 24 hours for all but one in which the patient received 12-hour-vancomycin and 12-hour-ceftazidime lock. Time interval from the first positive culture to ALT was 2.5 (IQR: 2-3,7) days. In terms of complications, bleeding developed in two cases and occlusion in only one patient. Bleeding was not severe in both events but the occluded catheter had to be removed within 14 days after the occlusion. Heparin concentration in lock solutions was 100 IU/ml in both patients with bleeding but they were two and four months old. Occlusion occured in a 7 month-old patient who had a lock solution containing 2500 IU/ml heparin (Table III).

Frequency of ALT failure was 31.2% (5/16) in the present study. When successful and unsuccessful ALT outcome groups were compared with each other, a statistically significant difference was observed only for age (Table IV). Median age of unsuccessful group was notably lower than the successful group (0.9 vs 6.82 years. *p= 0.038*). The catheter was removed from two patients due to catheter dysfunction within 14 days in the first case and due to persistant clinical findings such as fever despite 72 hours of appropriate therapy in the second. Other reasons for treatment failure were recurrences in two cases and re-infection in the other one within 30 days after discontinuation of ALT. The pathogens in the unsuccessful group were coagulase negative staphylococci (CoNS) in three, *Enterobacter cloacae* in one and polimicrobial agents (*Acinetobacter lwoffi* and *Enterococcus faecium*) in the last.

All patients were followed up for recurrence or re-infection until catheter removal, death or the end of the study period. Median of surveillance period was 9.2 months (2.5-16.3) in the successful treatment group and 3.6 months (1.1-12.8) in all cases. Recurrence was observed in about one-fourth of the patients and re-infection occurred in 16.7% during surveillance period. On the 30th day, all children included in the study were alive. Causative agents in the first and second episodes of both recurrences and reinfections were all CoNS except for one in whom *E. cloacae* was isolated in the first episode but reinfection was observed with a CoNS again. With an additional statistical analysis we found younger age to be a significant risk factor not only for treatment failure but also for recurrence and/or reinfection. The median age was 0.89 years in patients with recurrence or reinfection and 11.4 years in others (p= 0.011).

Table III. Characteristics of antibiotic lock therapies.

		n	(%)
Antibiotic locks	Vancomycin (5 mg/ml)	8	(50)
	Ceftazidime (0.5 mg/ml)	3	(18.6)
	Ciprofloxacin (0.2 mg/ml)	2	(12.5)
	Caspofungin (3.3 mg/ml)	1	(6.3)
	Vancomycin (5 mg/ml)-Ciprofloxacin (0.2 mg/ml)*	1	(6.3)
	Vancomycin (5 mg/ml)-Ceftazidime (0.5 mg/ml)**	1	(6.3)
Heparin concentration (IU/mL)	100	7	(46.7)
	2500	3	(20.0)
	5000	5	(33.3)
		Median	(Range)
Others	Dwell time, h	24	(12-24)
	Duration, d	10,5	(7-17)
	Time to ALT, d	2,5	(1-8)
		n	(%)
Complications during ALT	Occlusion	1	(6.3)
	Bleeding	2	(12.5)

*consecutively.
**simultaneous.
H: hour, d: days, Time to ALT: number of days from collection of first positive blood culter to initiation of antibiotic lock therapy, TPA: tissue plasminogen activator.

Discussion

The most common adverse effects of central venous access devices are catheter-related bloodstream infections.[11] According to the 2017 summary report of the national health service-related infections surveillance network, the overall CRBSI rate in all pediatric intensive care units in our country is 4.8/1000 catheter-days and 5.6 in pediatric intensive care units in university hospitals.[12] CRBSI rate in a single pediatric hematology-oncology unit in Turkey was found to be 7.4/1000 catheter-days.[8] In our research CRBSI rate was calculated in patients who underwent ALT and found to be 5.64/1000 catheter-days. Our results are consistent with the data in our country.

The most common infectious agents in surgically inserted catheters are known to be coagulase negative staphylococci, followed by enteric gram negative bacilli, *S. aureus* and *P. aeruginosa*.[5] In a study by Wolf et al.;[3] gram-positive, gram-negative, polymicrobial agents and *Candida* species were found to be the most prevelant cause of CRBSIs among 94 pediatric cancer patients. Our data are similar to previous studies in the pediatric patient group and especially in childhood malignancies in terms of the distribution of CRBSIs agents.[8,13-15]

When CLABSI is related to a long term CVC catheter salvage can be attempted by administering both systemic antibiotics and antibiotic lock according to current guidelines. Yet, catheter salvage is not recommended in the following circumstances: complicated CRBSI (suppurative thrombophlebitis, endocarditis, osteomyelitis, or other metastatic infection), severe sepsis or hemodynamic instability, persistent bacteremia despite 72 hours of antibiotic therapy to which the infecting organism is susceptible, infections caused by *Staphylococcus aureus*, *Pseudomonas aeruginosa*, fungi, mycobacteria or *Bacillus* species and in case of tunnel infections, port abscesses or exit site infections.[5] In our study, antibiotic lock therapies were administered with respect to recommendations of the latest guidelines. However, some conditions that ALT is not

Table IV. Comparison of patient groups with antibiotic lock therapy success and failure.

	Success (n= 11)		Failure (n= 5)		*p*
Age, y (IQR)	6.82	(1.2-12.2)	0.9	(0.5-2.3)	0.038
Gender [n (%)]					0.596
Male	4	(36.4)	3	(60)	
Female	7	(63.6)	2	(40)	
Underlying disease [n (%)]					0.596
Leukemia	7	(63.6)	2	(40)	
Solid tumor	4	(36.4)	3	(60)	
Allogenic HSCT [n (%)]	1	(9.1)	1	(20)	>0.99
ANC, /mm^3 (IQR)	300	(0-1325)	500	(0-2400)	0.953
Neutropenia [n (%)]	6	(54.5)	4	(80)	0.580
Duration of neutropenia, d (IQR)	1	(1-9.5)	3.5	(1.3-23.8)	0.476
Lymphocyte count, /mm^3 (IQR)	300	(300-1150)	1250	(375-1600)	0.503
Monocyte count , /mm^3 (IQR)	100	(0-250)	200	(200-)	0.209
CRP, mg/L (IQR)	36	(7-69)	27	(11.5-70)	>0.99
LOS until CRBSI, d (IQR)	3	(1-10)	11	(1.5-46.5)	0.320
Catheter-day, (IQR)	141	(18-196)	168	(31-307)	0.743
Catheter type [n (%)]					>0.99
Tunneled double-lumen catheter	1	(9.1)	1	(20)	
Port catheter	10	(90.9)	4	(80)	
Time to ALT, d (IQR)	2	(1-3)	3	(2.5-4)	0.18
Heparin concentration, IU/ml (IQR)	1300	(100-5000)	2500	(100-3750)	0.953
Dwell time, h (IQR)	24	(24-24)	24	(18-24)	0.583

y: year, IQR: interquertile range, HSCT: hematopoietic stem cell transplantation, ANC: absolute neutrophil count, d: day, CRP: C-reactive protein, LOS: length of stay, CRBSI: catheter-related blood stream infection, ALT: antibiotic lock therapy, h: hour.

recommended but yet performed can come to one's attention. These were the cases who have really high risks concerning the complications of the catheter removal procedure and a crutial need for intravenous access. *Candida pelluculosa, P. aeruginosa* and polimicrobial agents involving *Bacillus spp.* were isolated from those three patients. Treatment was carried out with close monitoring of clinical and laboratory findings and eventually, ALT outcome was favourable in all. After the publication of recent guidelines, there have been many research findings indicating that ALT may also be beneficial for the pathogens mentioned above.[16-18]

ALT has its own risks. The potential for occlusion or systemic exposure of anticoagulants and antibiotics exists. We observed occlusion in one (6.3%) and bleeding in two (12.5%) patients. However, it is not possible to attribute those to ALT since there was no control group. In the study by Wolf et al.,[19] there were no difference regarding ALT complications between the two groups, "ALT plus systemic antibiotic" and "systemic antibiotics only".

There have been different definitions and results in the literature concerning the success and effectiveness of antibiotic lock therapy. In a single randomized placebo-controlled trial, treatment failure was found to be 33% with adjunctive ALT (and 57% in systemic treatment only, p=0.10) on day 180.[20] However, this trial does not reflect our target population as it was performed among 44 CRBSI episodes including various populations such as children, adults, hematology/oncology, hemodialysis and gastroenterology patients. In another study,

success rate of ALT in adult cancer patients was reported to be 75.9% and was not very different from our results.[21] In our study the failure rate of lock therapy was 31.2%. Similar results despite different populations are also noteworthy.

In a case control study conducted by Wolf et al.,[19] to determine the benefit of ALT in pediatric cancer patients, failure rate was not different statistically in "systemic antibiotic plus ALT" (50%) and "systemic therapy alone" groups (38.4%, p=0.24). Treatment failure was defined as catheter withdrawal due to infection within 14 days of the CRBSI onset, death due to infection or relapse with the same microorganism within 252 days. The fact that our failure rate is relatively low compared to Wolf et al. can be explained by differences in definitions such as the time period for relapse.

Factors for ALT failure have been investigated poorly in the literature. In a study within pediatric hemodialysis patients by Onder et al.[22] younger age and higher serum phosphorus levels were associated with both treatment failure and reinfection. In a study conducted by Chen et al.[23] in pediatric cancer patients whose catheter was removed due to CRBSI, catheter withdrawal was observed in 11.9%, and the majority (9 of 17 patients, 52%) were under two years of age. When we evaluated the factors leading to failure in ALT (which was observed as 31.2%), the main risk factor was found to be younger age alone (0.9 vs 6.8 years, p= 0.038). Despite the differences in definitions and research groups our data are consistent with the existing literature.

In a systematic review and meta-analysis of various patient groups on anti-infective lock therapies, 20% relapse was reported in patients receiving ALT.[24] Adler et al.[9] observed that 45 (51%) of 87 catheters rescued in children with cancer had reinfection or recurrence. The pathogen was CoNS in all but one of the 12 patients with recurrence, and multivariate analysis revealed previous CRBSI due to CoNS and older age as significant risk factors for recurrence. Of note, the recurrence and reinfection rates were 23.1% and 16.7% in our study, and all recurrences and reinfections were also with CoNS. Results of our study was similar to Adler's in the standpoint of pathogens of recurrences or reinfections. In contrast to Adler's, we found younger age to be a significant risk factor for recurrence or reinfection (median 0.89 vs 11.4 years, p= 0.011).

The current study has some limitations. First of all, the sample size is not sufficiently large to perform multivariate risk factor analysis. Secondly, its retrospective and single-center design could allow confounders to affect the results and cannot be generalized to all populations. We believe that our center's experience contributes to the literature in spite of its limitations, as it is the first report which evaluates ALT outcome in pediatric cancer patiens from Turkey.

Although recommended in the last guidelines for diagnosis of CRBSIs, differencial time to positivity and quantitative blood cultures could not be used in the present study, as these techniques have recently been applied in laboratories of our hospital. But, we consider this not to be a serious limitation. Firstly, many previous clinical studies did not use those criteria in a similar way.[6,19,25] Secondly, in a study by Wolf et al,[19] analysis of episodes meeting the more stringent definition of "proven CRBSI" using differencial time to positivity showed similar rates of treatment failure.

Antibiotic lock therapy was found to be a safe modality with a success rate of 68.8 % in pediatric cancer patients at our center and younger age was an independent risk factor for treatment failure. Future studies with larger sample sizes are needed to determine the factors affecting the ALT outcome, especially in childhood cancers. Identifying groups with low treatment success may lead to changes in the population in which ALT was recommended and may increase treatment success. Our study is important since it is the first report which evaluates ALT outcome in pediatric hematology/oncology patients from Turkey.

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Original Article

Screening traumatic life events in preschool aged children: cultural adaptation of Child and Adolescent Trauma Screen (CATS) Caregiver-report 3-6 years version

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ABSTRACT

Background. Given the high prevalence of potentially traumatic events (PTEs), pediatric providers are in a novel position in early identification and referral of the children with PTEs. Yet paucity of culturally adapted instruments to screen PTEs and related symptoms in preschool-aged children limits pediatric providers. This multicenter study aimed to screen the traumatic life events of preschool-aged children admitted to pediatric outpatient clinics at four different cities of Turkey and to determine the socio-demographic risk factors associated with PTEs. Moreover, it was also intended to develop the cultural adaptation of the Child and Adolescent Trauma Screen (CATS) Caregiver-report 3-6 Years version to Turkish and to analyze its reliability.

Methods. Participants of this multicenter study were collected from four different hospitals in three different regions (Central Anatolia, Black Sea, and Southeastern Anatolia regions) of Turkey. All parents of children at the age of 3-6 years who were admitted to the hospital for a clinical visit (sick-child or well-child visits) were invited to the study. In total, 188 preschool-aged children were included in the study. Socio-demographic characteristics of the child and the family were questioned and parents were asked to fill out the Turkish version of the CATS Caregiver-report.

Results. Internal consistency (Cronbach's alpha) of the Turkish version of the CATS Caregiver-report 3-6 Years was found as 0.86. This study revealed that half of the participating children had experienced at least one PTE and more than one fourth experienced more than one event. Strikingly, no association between socio-demographic risk factors and PTE exposure was identified suggesting that PTE exposure is indeed widespread in our study population.

Conclusion. The findings of this study manifest the importance of routine screening of PTEs and related symptoms in children.

Key words: Potentially traumatic events, trauma screening, early childhood, cultural adaptation.

Children may encounter various kinds of possible traumatic events throughout their lives. Based on World Health Organization surveys, childhood adversities were reported by more than one-third of the population.[1] These adverse experiences can be perceived as positive, tolerable, or toxic stress depending on the intensity, duration, timing of the event, and the support given by caregivers.[2] Sustained adversities perceived as toxic stressors were reported to yield various long-term consequences on child's health and development.[3,4] However, some of these events

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are preventable and their unfavorable effects can be improved by activating supporting systems and early interventions.

Given the high prevalence of potentially traumatic events (PTEs) and its impact on health, the concept of trauma-informed care has gained importance. Trauma-informed care involves understanding, recognizing, and responding to the impacts of all kinds of trauma. Routinely screening for trauma exposure and related symptoms is the first step of trauma-informed care.[5] Moreover, pediatric providers are in a novel position to identify the children and families with at risk of adverse experiences.[6] American Academy of Pediatrics (AAP) issued a technical report including recommendations for pediatricians to screen children and their families who are at risk for toxic stress.[7]

Screening with standardized tools is needed for better recognition of traumatic experiences in the lives of children and the impact of events on their functioning. In the literature, there are many available instruments for screening and assessment of trauma in children and adolescents.[8] However, there are few instruments to screen PTEs and posttraumatic stress symptoms (PTSS) of preschool-aged children.[9]

Considering the recommendations of AAP, this study intended to screen the traumatic life events of preschool-aged children admitted to pediatric outpatient clinics at four different cities of Turkey in three different regions. In addition, it was aimed to define the socio-demographic risk factors associated with potentially traumatic events. Due to the paucity of validated screening instruments in the Turkish language for preschool-aged children, it was also intended to develop the cultural adaptation of the Child and Adolescent Trauma Screen (CATS) Caregiver-report 3-6 Years version to Turkish and to determine its reliability. The CATS questionnaire, which is a screening instrument based on the DSM-5 criteria for Posttraumatic Stress Disorder, is developed to measure PTEs and PTSS. The CATS Caregiver-report 3-6 Years version is used in preschool-aged children based on caregiver report.

Material and Methods

Study population

This multicenter study was designed by the Department of Developmental Pediatrics at Hacettepe University Faculty of Medicine. Participants of this study were collected from four different hospitals (Hacettepe University Hospital, Sivas Cumhuriyet University Hospital, Tokat State Hospital, and Diyarbakir Gazi Yasargil Training and Research Hospital) in three different regions (Central Anatolia, Black Sea, and Southeastern Anatolia regions) of Turkey. The sample size for the reliability study was determined to be at least 150 (10 for each item of CATS) participants. Therefore, it was planned for each four investigators from each hospital to include 50 parents to the study. The data was collected between April-November 2019. All parents of children at the age of 3-6 years who were admitted to the hospital for a clinical visit (sick-child or well-child visits) with various reasons were invited to the study. Parents who accepted to participate were included in the study. Approval for the research project was obtained from the Hacettepe University Ethics Committee (GO 19/388). Informed consent was obtained from all individual participants included in the study.

Procedures

In the first part of the interview, demographic characteristics of the child and the family (age, gender of the child, additional chronic illnesses of the child, educational level, occupation, and socioeconomic status of the family, determined by the Hollingshead-Redich index[10]) were questioned. Based on the Hollingshead-Redich index, parents were divided into three groups as low, middle, and high.

In the second part, parents were asked to fill out the Turkish version of the CATS Caregiver-

report. The CATS is a questionnaire to screen for exposure to PTEs and PTSS.[11] The CATS Caregiver-report 3-6 Years has a 15-item structured PTEs checklist and if at least one PTE is evident, parents are asked to fill out the 16-item PTSS checklist with a rating scale of 0=never, 1=once in a while, 2=half the time, and 3=almost always. The PTEs checklist includes items on natural disasters (serious natural disaster like a flood, tornado, hurricane, earthquake, or fire), accidents (serious accident or injury like a car/bike crash, dog bite, sports injury), experiencing or seeing violence (to be slapped, punched, or beat up/seeing someone get slapped, punched, or beat up), sexual abuse (to be touched private parts), traumatic loss (sudden or violent death of someone close to child), medical procedures (stressful or scary medical procedure) and wars (being exposed to wars). The CATS is based on the DSM-5 criteria and items map onto criteria B: intrusions (having bad dreams related to a stressful event, etc.), C: avoidance (avoiding anything that is a reminder of a stressful event, etc.) D: negative alterations in cognitions and mood (increase in negative emotional states, etc.) and E: hyperarousal (being overly alert or on guard, etc.). Lastly, CATS involves questions on the interference of PTSS in five key areas of functioning (getting along with others, school, hobbies, family relationships, and general happiness).[11] It is worth noting that since the CATS questionnaire is developed as a screening instrument, the clinical symptoms should be diagnosed by the clinical interviews of mental health professionals. In United States sample, Cronbach's alpha coefficient of preschool version was reported as 0.92.[11]

CATS is a license-free open access instrument.[12] Permission was granted from the first author of the international CATS study[11] for the translation and cultural adaptation of the original Caregiver-report 3-6 Years. The CATS Caregiver-report 3-6 Years was first translated from English to Turkish by two of the authors, resulting in a single version after the consensus meeting. Then an independent native speaker back translated the scale into English. After a pilot study with five patients, minor adaptations were made for cultural suitability and the final Turkish version of the CATS Caregiver-report 3-6 Years was obtained.

The researchers and clinicians can reach the Turkish version of the CATS Caregiver-report 3-6 Years at this link[12] and cite the current article.

Statistical analysis

Statistical analyses were performed utilizing the IBM SPSS for Windows Version 22.0. Numerical variables were summarized as mean ± standard deviation or median [25th-75th percentile]. Categorical variables were given as frequencies and percentages. Categorical variables were compared by chi square test. Normality of the continuous variables was evaluated by Kolmogorov-Smirnov test. Homogeneity of variances was tested by Levene test. Differences between the groups according to continuous variables were determined by independent samples t test or Mann-Whitney U test as appropriate. One-way ANOVA or Kruskal-Wallis test was used to compare more than two independent groups. *Post hoc* comparisons were done by Tukey HSD or Dunn test. Relation between continuous variables was determined by Spearman correlation coefficient. Internal consistency of scale was given by Cronbach's alpha coefficient. A p value less than 0.05 was considered as significant.

Results

Parents of 188 children were enrolled. The numbers of the participants from four hospitals were 53, 50, 35, and 50 respectively. Demographic features of the participants are presented in Table I.

Internal consistency (Cronbach's alpha) of the Turkish version of the CATS Caregiver-report 3-6 Years was found as 0.86. Internal consistencies of four clusters are given in Table II.

According to the CATS questionnaire, 49.5% of the study population experienced at least

Table I. Demographic features of participants.

Demographic features	Results (N: 188)
Male/ female, n/n	103/85
Age of child (years)[a]	4.5 [3 – 6]
Chronic health condition of child, n (%)	
None	143 (76.1)
Neurodevelopmental disorder	35 (18.6)
Other chronic diseases	10 (5.3)
Age of mother (years)[b]	33.2 ± 5.7
Age of father (years)[b]	36.9 ± 6.1
Total education duration of mother (years)[b]	9.3 ± 4.8
Total education duration of father (years)[b]	10.5 ± 4.5
Socioeconomic status (Hollingshead index), n (%)	
High	49 (26.1)
Middle	35 (18.6)
Low	104 (55.3)
Lower income (<2000 Turkish Lira/per month), n (%)	55 (29.3)
Preschool enrollment, n (%)	91 (48.4)

[a]: Median value and minimum –maximum values are presented.
[b]: Mean value and standard deviation are presented.

Table II. Internal consistency of the Turkish version of CATS Caregiver-report 3-6 years.

	Cronbach's alpha
CATS total score	0.86
Cluster B: Re-experiencing	0.73
Cluster C: Avoidance	0.62
Cluster D: Negative alterations in cognitions and mood	0.62
Cluster E: Hyperarousal	0.74

one of the potentially traumatic events. While 20.7% of participants were exposed to one PTE, 28.7% were exposed to more than one PTE. The percentages of the participants who experienced at least one PTE were similar in Hacettepe University Hospital (56.6%), Sivas Cumhuriyet University Hospital (57.1%), and Tokat Satate Hospital (56.0%). However, it was 30.0% in Diyarbakır Gazi Yaşargil Training and Research Hospital and this difference was statistically significant (p= 0.016).

Among 15 PTEs, the most common events experienced by the children in the study population were: i) stressful or scary medical procedure (23%), ii) slapped, punched, or beat up in their family (16%), iii) seeing someone in the community get slapped, punched (14%), and iv) seeing someone in the family get slapped, punched, or beat up (13%) and another stressful or scary event (13%) in the study population. Among 93 participants who experienced at least one PTE, 20 had scores of PTSS higher than the clinical cut-off (≥16). The children with high scores of PTSS (10.6%) in the study group were referred to mental health professionals.

Participants were divided into two groups: i) children without any PTEs and ii) children with at least one PTE. The comparisons of the demographic features of these two groups are presented in Table III. Between the two groups, gender, age of the child, diseases of the child, age of the parents, education durations of the

Table III. Comparison of demographic features between children with and without PTEs.

Demographic features	Children without PTEs (N: 95)	Children with ≥1 PTEs (N: 93)	p value
Child characteristics			
Male gender, n (%)	51 (53.7)	52 (55.9)	0.759
Age of child (years)[a]	4.4 ± 1.0	4.5 ± 1.1	0.879
Presence of chronic disease, n (%)			0.145
Developmental problems	12 (12.6)	6 (6.5)	
Other chronic disease	10 (10.5)	17 (18.2)	
Preschool enrollment, n (%)	41 (43.1)	50 (53.7)	0.146
Family characteristics			
Age of mother[a]	32.7 ± 5.9	33.6 (5.4)	0.267
Age of father[a]	36.4 ± 6.2	37.5 (6.0)	0.216
Education of mother (years)[a]	9.0 ± 4.9	9.5 (4.6)	0.342
Education of father (years)[a]	10.3 ± 4.9	10.7 (4.2)	0.44
Number of children[b]	2 [1 – 6]	2 [1 – 9]	0.827
Family size[b]	4 [1 – 5]	4 [1 – 5]	0.673
Socioeconomic status[b]	4 [3 – 10]	4 [3 – 10]	0.365
Household income[b]	2 [1 – 4]	2 [1 – 4]	0.398

[a]: Mean value and standard deviation are presented
[b]: Median value and minimum-maximum values are presented.

parents, socioeconomic status and income of families, family size, and number of children were not statistically significantly different. Therefore, models to explore the factors to predict the presence of PTEs were not significant.

Discussion

Assessments of child trauma exposure and posttraumatic symptoms were reported to be challenging especially in preschool-aged children.[13,14] Moreover, paucity of linguistically and culturally adapted screening instruments for young children could be a limiting factor for international health care providers. The general Cronbach's alpha coefficient of the Turkish version of the CATS Caregiver-report 3-6 Years was quite similar to that of the original study. Although, Cronbach's alpha coefficients of clusters of CATS Caregiver-report 3-6 Years were not provided in the original study, in the current study, they were found similar to the values of CATS Caregiver-report 7-17 Years.[11] Since the results of this study demonstrated a good internal consistency of the Turkish version of the CATS Caregiver-report 3-6 Years, we recommend health care providers to utilize this instrument as a screening tool for PTEs and related PTSS in preschool-aged children.

This study revealed that almost half of the participating children have experienced at least one of the PTEs and more than one-fourth of the study population experienced more than one event. The proportion of PTEs exposure can be affected by the type of screening instrument and the type of setting that participants were recruited from. Due to the lack of studies using the same instrument to screen PTEs in hospital settings, we could not directly compare our findings with the literature. The proportion of one PTE exposure was higher than that of a previous study where the percentage of any childhood adversity was reported to be 38.9% in high-middle income countries.[1] This dissimilarity could be linked to the abundance of PTEs type of 'stressful or scary medical procedure' experienced by our participants, which is understandable since we recruited them from hospital settings. However, there

exist other studies reporting higher percentages of preschool-aged children with a history of at least one traumatic event.[15,16] Moreover, a study reported that half of the preschool-aged children living in poverty have been exposed to one or more PTEs.[17] Likewise, the majority of our participants belong to low socioeconomic status and we found half of the participants have experienced at least one of the PTEs. However, we did not find an association of socioeconomic status with either the presence or the number of traumatic events, in contrast to several previous reports.[17,18]

There have been inconsistent reports on the gender differences in PTE exposure.[17,19] We found no differences between female and male participants in terms of exposure to PTEs. On the other hand, in the literature, there have been few studies with controversial findings[20-22] on the association between adverse childhood experiences and chronic conditions or developmental problems of preschool-aged children. In this study, PTEs exposure was not found to be more prevalent in children with developmental problems compared to typically developing children. Moreover, the prevalence of chronic conditions was not statistically different between children without PTEs, with one PTE and more than one PTE. In addition, we did not identify any other socio-demographic risk factor associated with PTEs exposure suggesting that experiencing PTEs was, in fact, widespread in our study population.

During the interpretation of the current results, limitations should not be disregarded. Since we recruited participants who were admitted to hospitals for various reasons, our PTEs rates may not be generalized to community settings. Another limitation is that the present study did not focus on coping mechanisms or resilience factors of children. On the other hand, due to the sample size, the factors underlying the lower PTEs percentage in one of the centers compared to the other three centers could not be identified. Finally, we culturally adapted and analyzed the reliability of the Turkish version of the CATS Caregiver-report 3-6 Years but we did not conduct a standardization study of the instrument. On the other hand, one may suspect the risk of underreporting of PTE exposure by caregivers due to desirability effects. However, we believe that this was not a limitation of the current study since the rates of PTEs were already higher than the previous reports.[1]

To the best of authors' knowledge, this multicenter study with a diverse population from four different hospitals in Turkey, is the first national and one of the several international studies screening the possible traumatic life events in preschool-aged children. This study revealed that a considerable proportion of children were exposed to PTEs and approximately 20% of them had high scores of PTSS. Given the high prevalence of PTEs, the findings manifested the importance of routine screening of PTEs and related symptoms in children.

The current study that screened PTE exposure and related symptoms among children who were admitted to hospitals with various reasons revealed a high prevalence of exposure to PTEs. Despite its high prevalence, pediatric providers do not routinely screen children for PTE exposure and possibly overlook the impact of exposure. Mental health professionals utilize multiple methods for the comprehensive assessment of trauma and diagnosis of post-traumatic stress disorders.[14] However, our findings highlighted the need for screening PTE exposure and related symptoms by pediatricians who would have an important role in early identification of trauma-exposed children and their referral to mental health professionals, thereby interventions can be developed to reduce exposure and its impact on children.

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Original Article

Fecal elastase levels in children diagnosed with functional abdominal pain-not otherwise specified

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ABSTRACT

Background. Although the exact pathophysiology of functional gastrointestinal diseases remains unclear, numerous etiologies have been blamed, including visceral hypersensitivity, gastrointestinal motility disorders, psychological factors, intestinal mucosal inflammation, intestinal microbiota, and post-infectious syndromes. In the present study, we aimed to evaluate pancreatic insufficient patients diagnosed with *functional abdominal pain-not otherwise specified* (FAP-NOS) according to Rome IV criteria.

Methods. The study included a total of 110 patients aged 4-17 years who were diagnosed with FAP-NOS according to Rome IV criteria. The control group consisted of 80 patients with no gastrointestinal disorders and chronic diseases. Glucose, amylase, lipase, pancreatic amylase, immunoreactive trypsinogen (IRT) and fecal elastase (FE-1) levels were examined for each patient.

Results. No significant difference was found between the two groups with regard to lipase, pancreatic amylase, IRT, and serum glucose levels. However, the amylase levels were significantly higher and the FE-1 levels were significantly lower in the study group compared to the control group (p=0.007 and p<0.001). The cut-off value detected in in ROC analysis for the diagnostic value of FE-1 in predicting FAP-NOS was found to be 140.107 μg/g. Based on this value, the sensitivity, specificity, PPV, and NPV of FE-1 were 82.1%, 66.2%, 77%, 73%, respectively. Accordingly, the likelihood of FE-1 in providing a positive value in patients with FAP-NOS was almost 9 times higher than in individuals without FAP-NOS.

Conclusions. FE-1 levels were significantly lower in children diagnosed with FAP-NOS and we consider that this difference could be attributed to malabsorption secondary to dysbiosis as there is not enough data.

Key words: FAP-NOS, fecal elastase, pancreas insufficiency.

Almost 35-38% of elementary school children are admitted to hospital once a week due to abdominal pain.[1,2] Of these admissions, only one-third are diagnosed as functional abdominal pain disorders (FAPD).[3] Although the exact pathophysiology of functional gastrointestinal diseases (FGIDs) remains unclear, numerous etiologies have been blamed, including visceral hypersensitivity, gastrointestinal motility disorders, psychological factors, intestinal mucosal inflammation, intestinal microbiota, and post-infectious syndromes.[4]

Chronic pancreatitis (CP) is considered an extremely rare condition in the general population. However, postmortem studies have indicated that the disease is not as rare as expected and that it has a prevalence of 6-12% in the general population and many patients remain undiagnosed.[5] Patients with CP and those with FAP, particularly those with diarrhea-predominant irritable bowel syndrome (IBS-D), often present to hospital with similar complaints such as abdominal pain and diarrhea. In such patients, however, routine pancreatic function tests and pancreatic

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imaging techniques are not recommended as per 2016 Rome IV criteria.[6]

Leeds et al.[7] evaluated patients with IBS-D and found that 19 (6.1%) of them had low fecal elastase-1 (FE-1) levels. Talley et al.[8] reported that one in 50 patients that had otherwise unexplained abdominal pain and/or diarrhea or IBS-D were diagnosed with pancreatic exocrine insufficiency (PEI).[8] In both of these studies, most of the patients were at advanced ages and alcohol abuse was blamed as the most common etiology. Nevertheless, to our knowledge, there have been no such studies conducted on children.

According to Rome IV criteria, abdominal pain that does not otherwise fit a specific disease such as irritable bowel syndrome, functional dyspepsia, and abdominal migraine is termed as *functional abdominal pain-not otherwise specified* (FAP-NOS).[3] In the present study, we aimed to evaluate pancreatic function test results in patients diagnosed with FAP-NOS according to Rome IV criteria.

Material and Methods

The study included a total of 110 patients aged 4-17 years who were admitted to our pediatric outpatient clinic and were diagnosed with FAP-NOS according to Rome IV criteria between April 1 and September 1, 2019. The control group consisted of 80 patients with no gastrointestinal disorders and chronic diseases. However, two patients in the study group and three patients in the control group were excluded from the study due to technical difficulties. As a result, a total of 185 patients comprising 108 patients with FAP-NOS and 77 control subjects were included in the study.

Age, gender, and body weight and height were recorded for each patient. The standard deviation score (SDS) for body weight was also calculated.[9]

Serum samples were obtained from each patient and were analyzed for glucose, amylase, lipase, and pancreatic amylase levels. The measurements were performed spectrophotometrically, using a Beckman Coulter AU5800 autoanalyzer. Additionally, 3 cc of blood samples were taken for the measurement of immunoreactive trypsinogen (IRT) levels and were collected into biochemical tubes containing no anticoagulants. Within the first hour after the collection of blood samples, the tubes were centrifuged at 3,000 rpm for 20 min +4 °C and the supernatants were placed in Eppendorf tubes and then were stored at -80 °C until analysis. The samples were analyzed concurrently by Ylbiont ELISA Kit (range, 2-600 ng/L; sensitivity, 1.01 ng/L).

A single stool sample was obtained from each patient and was stored at -20 °C. Prior to the analysis, the samples were transferred to the laboratory and were thawed at 2-8 °C and then kept at room temperature for one hour. After achieving room temperature, the samples were analyzed concurrently by Ylbiont ELISA Kit (range, 20-6000 ng/L; sensitivity, 10.15 ng/L).

Statistical analysis

Data were analyzed using IBM SPSS for Windows version 22.0 (IBM Corp. Released 2013. IBM SPSS Statistics for Windows, Version 22.0. Armonk, NY: IBM Corp.). Descriptive statistics were expressed as mean ± standard deviation (SD) for parametric data, as median (minimum-maximum) for nonparametric data, and as frequencies (n) and percentages (%) for categorical data. Variables were compared using Student's t-test for parametric data (t-test for independent variables), Mann-Whitney U test for nonparametric data, and Pearson's chi-squared test for categorical data. The diagnostic performance of FE-1 in predicting FAP-NOS was analyzed using Receiver Operating Characteristic (ROC) analysis and its accuracy was analyzed using the area under curve (AUC) obtained from ROC analysis. The optimal cut-off value of FE-1 was calculated according to the Youden Index. Based on the optimal cut-off value, sensitivity, specificity, negative predictive value (NPV), positive predictive

value (PPV), positive prediction rate, negative prediction rate, likelihood ratio (LR), positive likelihood ratio (LR+), negative likelihood ratio (LR+), and odds ratio were calculated. A *p* value of <0.05 was considered significant.

The study was approved by Institutional Ethics Committee of Yüzüncü Yıl University (Approval Date: March 19, 2019; No. 2019/07). A written informed consent was obtained from each parent/guardian.

Results

The study included a total of 185 patients, comprising 95 (51.4%) boys and 90 (48.6%) girls with a mean age of 8.92 ± 3.41 (median, 8) years. No significant difference was found between the study and control groups with regard to age and gender (*p*=0.524 and *p*=0.302, respectively) (Table I). Similarly, no significant difference was found between the two groups with regard to lipase, pancreatic amylase, IRT, and serum glucose levels (*p*= 0.672, *p*= 0.432, *p*= 0.110, *p*= 0.801, respectively) (Table I). However, the amylase levels were significantly higher and the FE-1 levels were significantly lower in the study group compared to the control group (*p*= 0.007 and *p* <0.001, respectively) (Table I, Figs 1-2).

The cut-off value detected in in ROC analysis for the diagnostic value of FE-1 in predicting FAP-NOS was found to be 140.107 μg/g (Fig. 3). Based on this value, the sensitivity, specificity, PPV, and NPV of FE-1 were 82.1%, 66.2%, 77%, and 73%, respectively. Moreover, the false positive rate was 0.179 and the LR+ and LR- values were 2.43 and 0.278, respectively. Accordingly, the likelihood of FE-1 in providing a positive value in patients with FAP-NOS was almost 9 times higher than in individuals without FAP-NOS. On the other hand, a significant odds ratio was obtained for the diagnostic value of FE-1 in predicting FAP-NOS (95% Confidence Interval [CI]: 4.52-17.82).

Nevertheless, no significant correlation was found between FE-1 and amylase, lipase, pancreatic amylase, and IRT levels in the study group (r= 0.49, r= 0.147, r= 0.044, r= 0.036, respectively) (*p* >0.05 for all).

Discussion

Clinical manifestations of PEI may vary according to underlying etiologies, stage of disease, nutritional status, and numerous other factors.[10,11] However, patients with PEI often present with typical symptoms such as steatorrhea and weight loss, most of which can be observed in the late stage of the disease.[12] In early stages, however, patients may present with nonspecific symptoms such as abdominal distension and pain.[13] In line with this data,

Table I. Demographic and biochemical characteristics.

	Study group (n=108)	Control group (n=77)	*p*
Age (years)	8 (4-17)	9 (4-17)	0.347[1]
Gender (female. %)	56 (51.9%)	34 (44.2%)	0.302[2]
Body weight SDS	0.205 (-0.6-1.8)	0.36 (-0.4-2.1)	0.064[1]
Lipase (U/L)	17 (5-77)	16 (7-36)	0.672[1]
Amylase (U/L)	75 (34-144)	64 (14-109)	0.007[1]
Pancreatic amylase (U/L)	20 (3-50)	20 (10-41)	0.432[1]
IRT (μg/L)	73.42 (20.2-338)	56.4 (12.05-338.52)	0.110[1]
Fecal elastase (μg/g)	122.1 (22.49-216.59)	147.85 (24.75-240.25)	<0.001[1]
Glucose (mg/dl)	88.2 ± 8.447	87.9 ± 9.725	0.801[3]

[1]: Mann-Whitney U test, median (minimum-maximum), [2]: Pearson's Chi-squared test [3]: Student's t-test
IRT: immunoreactive trypsinogen

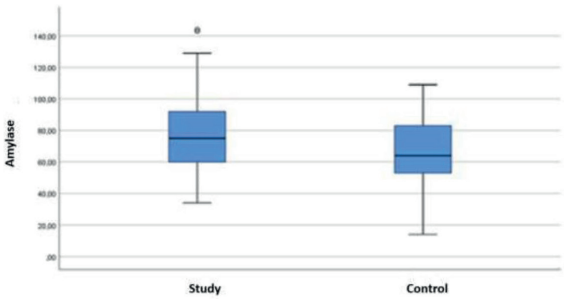

Fig. 1. Amylase levels in both groups.

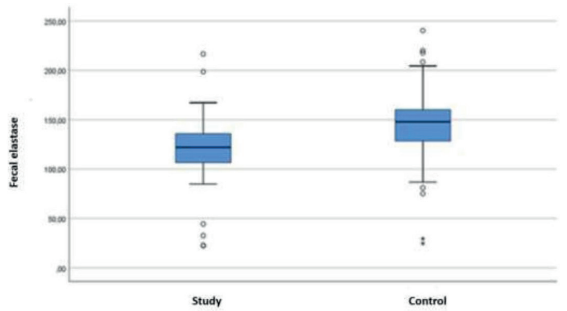

Fig. 2. Fecal elastase levels in both groups.

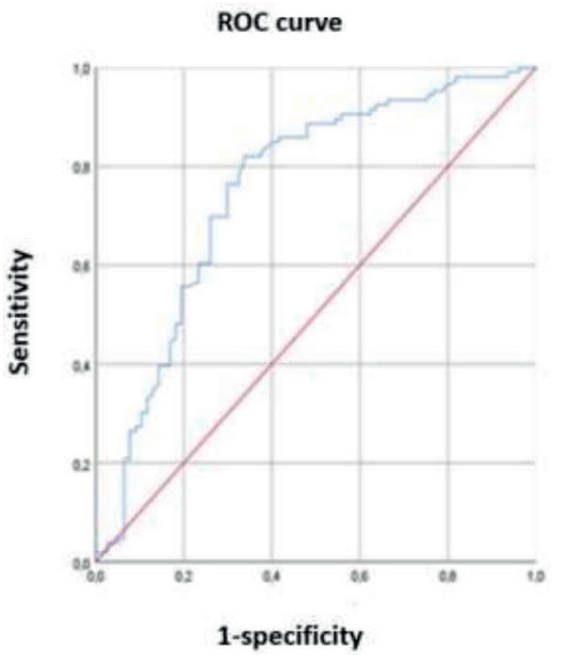

Fig. 3. Diagnostic value of fecal elastase in predicting FAP-NOS (AUC: 0.753, 95% CI: 0.678-0.827, p<0.001).

we designed the present study based on the hypothesis that PEI could be a cause of FAP-NOS.

Pancreatic exocrine functions are commonly assessed based on the levels of enzymes including lipase, pancreatic amylase, fecal elastase, and trypsinogen. In the present study, we measured the levels of these enzymes and found no significant difference between the study and control groups with regard to lipase, pancreatic amylase, and trypsinogen levels while serum amylase levels were significantly higher in the study group compared to the control group. Similarly, Feng et al.[14] evaluated patients undergoing oral double-balloon enteroscopy and reported that serum amylase levels were significantly increased in all the patients. The authors also noted that hyperamylasemia correlated well with increased intestinal permeability associated with bowel inflammation. Meaningfully, clinical conditions including increased concentrations of mucosal proinflammatory cytokines, gut microbiota alterations, increased intestinal permeability, and impaired intestinal motility have been blamed in the etiopathogenesis of FAP, all of which ultimately result in chronic abdominal inflammation.[15] Accordingly, we consider that the increased amylase levels detected in our study group could not have a pancreatic origin and could be associated with bowel wall inflammation, a condition which is also included the pathophysiology of FGID. Moreover, this hypothesis can be supported by the normal levels of pancreatic amylase and lipase detected in both groups.

Fecal elastase-1 (FE-1) test could be a viable first-step test in the evaluation of pancreatic functions due to its reliability, practicality, and cost-effectiveness. In the present study, FE-1 test was performed to assess pancreatic insufficiency and it was revealed that the FE-1 levels were significantly lower in the study group compared to the control group. On the other hand, it is commonly known that patients with chronic pancreatic insufficiency and low FE-1 levels typically have dysbiosis as well.[16] Additionally, Salvatore et al.[17], detected low FE-1 levels in 28% of patients with infectious enteritis which demonstrated that low FE-1 levels could

also be seen in conditions characterized by impaired gut microbiota such as infectious enteropathies. In a similar way, Stein et al.[18] also detected low FE-1 levels in patients with malabsorption symptoms such as diarrhea and weight loss. Accumulating evidence suggests that PEI may also develop secondary to gastric surgery and other malabsorption symptoms such as celiac disease, Zollinger-Ellison syndrome, and human immunodeficiency virus (HIV) infection.[19-21] In such patients, pancreatic enzyme replacement therapy (PERT) has been shown to reduce malabsorption by altering intestinal microbiota, inhibiting bacterial overgrowth in the duodenum and jejunum, and increasing the intestinal absorptive capacity.[21] Pezzilli et al.[22] evaluated patients with pancreatic diseases and found increased fecal calprotectin levels and decreased FE-1 levels in the patients. The authors concluded that the impaired intestinal microbiota in patients with pancreatic insufficiency could be associated with intestinal inflammation. On the other hand, dysbiosis is also blamed in the pathophysiology of FGID.[23,24] Based on these findings, we consider that the decreased FE-1 levels detected in our study group might be associated with malabsorption and inflammation secondary to dysbiosis. Moreover, the absence of a significant correlation between FE-1 and pancreatic amylase, lipase, and IRT levels implicates that PEI could be a secondary condition rather than a primary condition.

In our study, the cut-off value detected in ROC analysis for the diagnostic value of FE-1 in predicting FAP-NOS was found to be 140.107 μg/g. According to this value, the sensitivity, specificity, PPV, and NPV of FE-1 were 82.1%, 66.2%, 77%, and 73%, respectively. In the literature, there are numerous sensitivity and specificity values reported for FE-1. Löser et al.[25] reported that the sensitivity of FE-1 in predicting PEI was 63% for mild PEI and 100% for moderate and severe PEI and its specificity was 93% for all forms of PEI. Siegmund et al.[26] reported that the sensitivity of FE-1 was 54% for mild PEI, 75% for moderate PEI, and 79% for severe PEI. Wali et al.[27] reported that the sensitivity and specificity of FE-1 were 41.7% and 49.2%, respectively. On the other hand, some other studies showed that the sensitivity and specificity of FE-1 at a cut-off value of 200 μg/gr ranged between 63.77%-89.5% and 93%-99%, respectively.[28,29] Although all these values have been reported for PEI, to our knowledge, there have been no studies reporting on the cut-off, sensitivity, and specificity values of FE-1 in predicting FAP-NOS. In our study, for the first time in the literature, the cut-off, sensitivity, and specificity values of FE-1 in predicting FAP-NOS were calculated and the analyses indicated that the likelihood of FE-1 in providing a positive value in patients with FAP-NOS was almost 9 times higher than in individuals without FAP-NOS, which was a remarkably high value.

In conclusion, the underlying causes of FAP in children remain unclear although a number of factors including increased concentrations of mucosal proinflammatory cytokines, gut microbiota alterations, increased intestinal permeability, and impaired intestinal motility have been blamed in the etiopathogenesis of FAP. In the present study, FE-1 levels were significantly lower in the study group compared to the control group and we consider that this difference could be attributed to malabsorption secondary to dysbiosis as there is not enough data about this. Considering that there is paucity in similar studies evaluating FAP in children, we suggest that further studies are needed to substantiate our findings.

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Original Article

Over restrictive elimination of foods in children with food allergy

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ABSTRACT

Background. Previous studies demonstrated critical deficits in diagnosis and management of childhood food allergy (FA), and recent developments in FA research support adopting a proactive approach in FA management. Our objective was to describe FA knowledge and management patterns of pediatricians.

Method. We applied a 24-item survey to 170 general pediatricians, pediatric allergists and pediatric gastroenterologists practicing in Turkey.

Results. Some IgE-mediated symptoms of FA such as cough, urticaria, wheezing and anaphylaxis were falsely recognized as symptoms of non-IgE-mediated FA by 30%, 29%, 25% and 19% of the participants, respectively. By contrast, 50% of the participants falsely recognized bloody stool, a finding of IgE-mediated FA. Most frequently and least frequently used diagnostic tools were specific IgE (30.5%) and oral food challenge test (1.7%), respectively. Maternal diet restrictions and infant diet restrictions were advised by 82% and 82%, respectively. Percentages of physicians eliminating only 1 food were 21%, 19%; 2 foods were 15%, 11%; 3 foods were 7%, 8%; 4-5 foods were 8%, 11%; 5 to 10 foods were 21%, 26%; and >10 foods were 28%, 25% from the maternal and infant diet, respectively. Cow's milk, cheese, butter, yoghurt, baked milk products and hen's egg were the most commonly restricted items.

Conclusion. Overall, FA knowledge of pediatricians was fair. Pediatricians utilize an overly restrictive approach when advising diet eliminations in FA. Recent developments favor a more proactive approach to induce immune tolerance and need to be encouraged in pediatric clinical practice. Future educational efforts should focus on emphasizing the deleterious effects of injudicious and extensive eliminations.

Key words: diet elimination, food allergy, knowledge, survey, nutrition.

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The prevalence of clinical food allergy (FA) is estimated to be 10% among preschool children globally and has been showing a steadily increasing trend.[1-3] The rise in the number of admissions of infants with FA complaints have compelled physicians to learn about and adapt to different types of FA such as IgE-mediated and non-IgE mediated FA.[4] The differences between the two types include disparate ways of presentation. While some of infants with FA may present with chronic atopic dermatitis

symptoms, others may be admitted with anaphylaxis.

The mainstay of FA treatment is avoidance of triggering foods.[4] Arguably, prescribing dietary interventions, especially to children with allergic disease may often times be a challenging task. Half of the children with FA suffer from severe, acute allergic reactions and one-third have multiple food allergies.[2] Therefore, it is essential to employ appropriate diagnostic tools to identify the exact offending products and educate parents and patients about strict adherence. On the other hand, the latest studies have repeatedly shown that some children with FA benefit from more liberal diets.[5] In terms of prevention of FA in at-risk infants, several studies found that early introduction of food allergens significantly reduced the prevalence of FA when food allergens were included in the diet earlier on in life.[6,7] Moreover, any interference with nutrition in a growing child should be well-planned due to the serious consequences of malnutrition.[8,9]

In accordance with the challenges that the physicians face, being treated for FA may be a challenging period for patients and their families as well. The elimination of major foods, the risk of anaphylaxis and chronic symptoms such as eczema or failure to thrive perturb the social and psychological wellbeing of affected children. Accordingly, there is strong evidence that FA and food hypersensitivity has an impact on psychological distress and on the quality of life of children and their families.[10,11]

Currently only few data exist that describe practices in the diagnosis and management of FA among pediatricians in Turkey.[12] Similar studies that evaluated the FA-related practices of primary care physicians (PCP) and/or pediatricians in the US, Finland, Kuwait, Australia and Croatia have consistently demonstrated significant deficits.[13-17] Given the developing need for the physicians to adapt themselves to recent changes in FA management, the wide range of different clinical presentations of IgE and non-IgE mediated FA, and the impacts of FA and diet restrictions on the patient's quality of life we prepared this survey to describe the FA knowledge and practices of general pediatricians, pediatric allergists and pediatric gastroenterologists.

Material and Methods

Questionnaire

A self-administered questionnaire about childhood FA was designed and administered to general pediatricians, pediatric allergists and pediatric gastroenterologists practicing in Turkey. The 24-item questionnaire was composed of 10 questions about the demographic characteristics of the participants and 14 questions about their clinical approach to FA. Of the 14 questions that were on FA; 8 were multiple-choice, 5 were dichotomous and 1 was an open-ended question (Supplementary data).

The survey included questions about the frequency of encountering patients with FA, symptoms of IgE and non-IgE mediated FA, symptoms of FA in patients with atopic dermatitis (AD), diagnosis of FA in AD, and the practices regarding dietary management of FA.

In this study, the term FA is used to refer to both IgE and non-IgE FA, with relevant specifications being used if either one is referred to. The study was approved by the Koc University ethical committee on 13/07/2016 (report number: 2016.165.IRB2.092), and written informed consent was obtained from all participants.

Statistical analysis

Statistical analyses were conducted using SPSS statistics version 18 (SPSS Inc, Chicago, IL, USA). Data were manually transferred into Excel and then into SPSS version 18 for analysis. Categorical variables were presented as frequency and percentiles. Normally distributed values were expressed as means.

Table I. Demographic characteristics of the participants.

Characteristic	Pediatricians, n (%)
Gender	
Female	114 (67.1)
Male	56 (32.9)
Age	
25-30 years	54 (31.8)
30-40 years	64 (37.9)
40-50 years	38 (22.5)
>50 years	13 (7.7)
Specialty	
General pediatrics	117 (69)
Pediatric allergy[a]	29 (17)
Pediatric gastroenterology[a]	24 (14)
Specialty education center	
University hospital	80 (50)
Research and training hospital	80 (50)
Practicing center	
Secondary Care Facility[b]	24 (14.8)
Tertiary Care Facility[c]	138 (85.2)
Current working status	
Fellow in training	63 (38.4)
Specialist	78 (47.6)
Faculty member	23 (14)

[a] Specialist or fellow in training
[b] Government hospital, Private hospital
[c] University hospital, Research and Training Hospital

Results

A total of 170 physicians completed the survey. The characteristics of the participants are shown in Table I. Physicians who cared for patients with FA constituted 90% of the cohort.

Knowledge on symptoms of FA

Physicians were asked about the symptoms that would prompt suspicion of FA in the context of atopic dermatitis (AD) specifically, since work-up in AD involves screening for general FA symptoms. Blood in stool (91%), urticaria (84%), appearance of AD lesions (81%), abdominal symptoms (72%), perioral erythema (68%), diarrhea (68%) and vomiting (63%) were among the most common symptoms that made the physicians suspect FA in an infant diagnosed with AD. Other commonly identified symptoms of FA are outlined in Figure 1A. Competence of the physicians with regards to differentiating IgE-mediated and non-IgE mediated symptoms of FA are shown in Figure 1B.

Diagnostic approach to FA

In the diagnosis of FA, the following approaches were utilized in order of most-to-least commonly preferred: specific IgE (sIgE) (30.5%), clinical findings (25.8%), skin prick tests (25.3%), response to diagnostic elimination diets (5.3%), atopy patch testing (5%), oral food challenge test (OFC) (1.7%). The participants were allowed to select more than one answer.

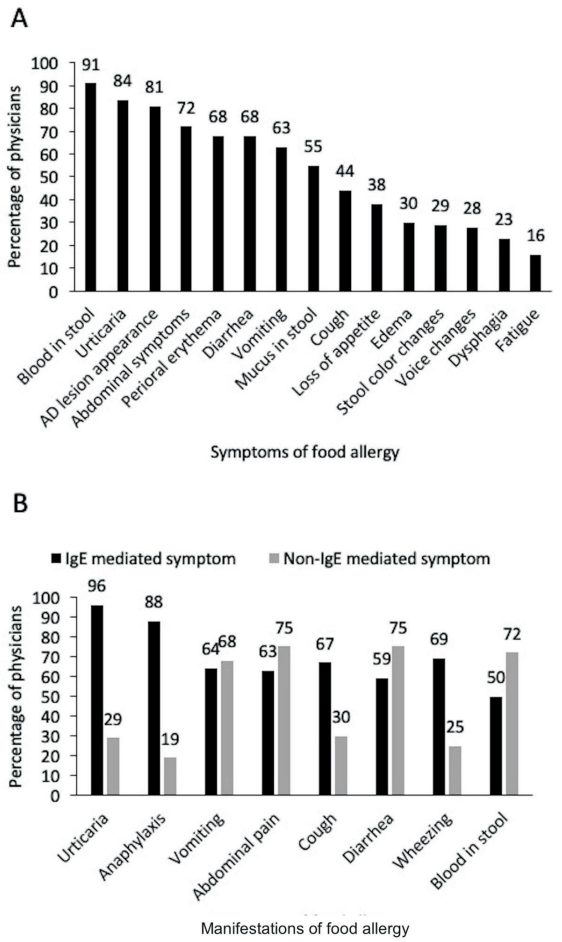

Fig. 1. Symptoms and manifestations of food allergy according to the physicians. A. Symptoms suggestive of food allergy in infants diagnosed with atopic dermatitis. B. Manifestations of IgE- and non-IgE-mediated food allergy.

Recommendation of maternal diet restriction (MDR) in FA

Eighty-two percent of the physicians responded that they advise MDRs in the management of infants diagnosed with FA. Regarding the improvement of FA symptoms following a period of MDR, 72%, 19% and 5% selected complete clinical improvement, partial clinical improvement and no clinical improvement, respectively. (Fig. 2).

Characteristics of MDRs advised in FA

Majority of the physicians routinely restricted greater than five food products from the

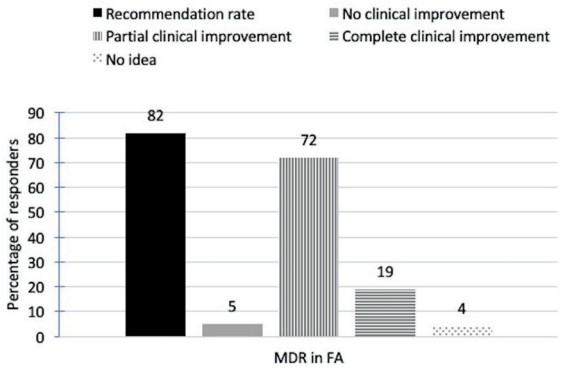

Fig. 2. Dietary management practices and perceived outcomes; MDR: maternal diet restriction, FA: food allergy.

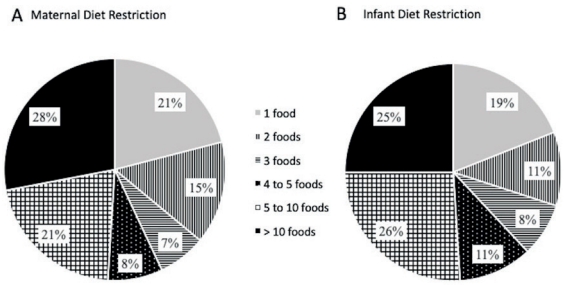

Fig. 3. Percentage of physicians eliminating different numbers of food products. A. from maternal diet in the management of FA, B. from infant's diet in the management of FA.

maternal diet: 21% restrict 5 to 10 foods, 28% restrict >10 foods (Fig. 3A). Cow's milk (86%), cheese (70%), yoghurt (56%), butter (56%), baked milk products (51%), hen's egg white (50%), goat's milk (46%) and peanut (43%) were the most commonly restricted products. Relatively uncommon allergens in infancy such as strawberry (21%), tomato (18%), cacao (14%), orange (12%) and blackberry (9%) were restricted more commonly than wheat (7%) and lentils (5%) (Fig. 4).

Calcium supplementation was offered by 83% of the physicians to the breastfeeding mothers who were on a cow's milk (CM) restricted diet.

Recommendation of infant diet restriction (IDR) in FA

Eighty-two percent of physicians responded that they use infant diet restriction in the

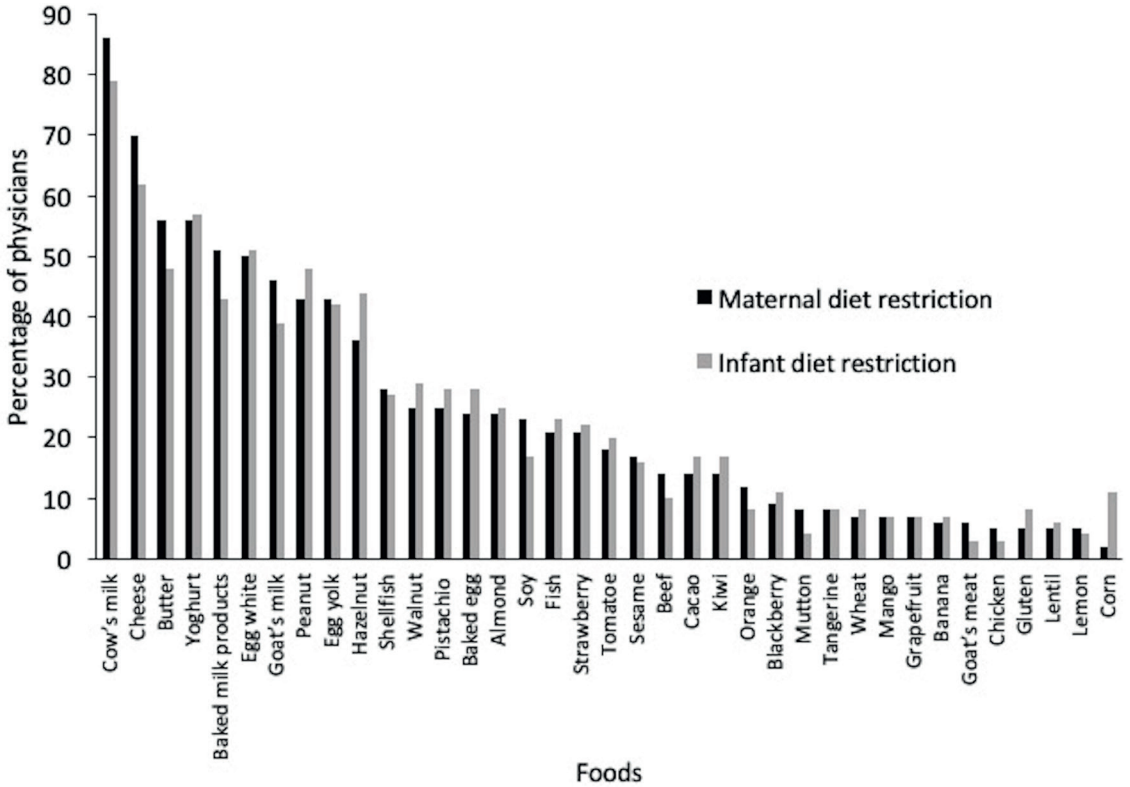

Fig. 4. Percentage of physicians eliminating different food products from the maternal and infant's diet in the management of food allergy.

management of FA. It should be noted that the IDRs employed in the management of breastfed infants with FA were noted to be empirical and not directed by allergen identification, since only a negligible percentage of the physicians declared that they use diagnostic elimination and oral food challenge (**Diagnostic approach to FA**).

Half of the physicians restricted 5-10 foods (26%), or >10 foods (25%) from the infant's diet (Fig. 3B). Among the blanket restrictions employed by the physicians, the most commonly restricted categories of foods were dairy products (cow's milk 79%, cheese 62%, yoghurt 57%, butter 48%, baked milk products 43% and goat's milk 39%), peanut (48%), tree nuts (hazelnut 44%, walnut 29%, pistachio 28% and almond 25%), egg (hen's egg white 51%, hen's egg yolk 42% and baked egg products 28%), seafood (fish 28% and shellfish 27%) and other foods (strawberry 22%, tomato 20%, soy 17%, cacao 17%, kiwi 17% and sesame 16%). (Figs 4, 5). In addition, 23% of physicians added probiotics to the infant's diet.

Introduction of complementary foods

Physicians were asked the following two-part question regarding the time for introduction of complementary foods to the diets of infants with FA:

- Do you delay introduction of complementary foods for your patient? 23% reported that they delay the introduction of complementary foods.
- When do you recommend the introduction of complementary foods to your patients' diet? 94% reported that they introduce complementary foods by 6 months of age. 5% of physicians reported that they wait until 1 year of age to introduce complementary foods.

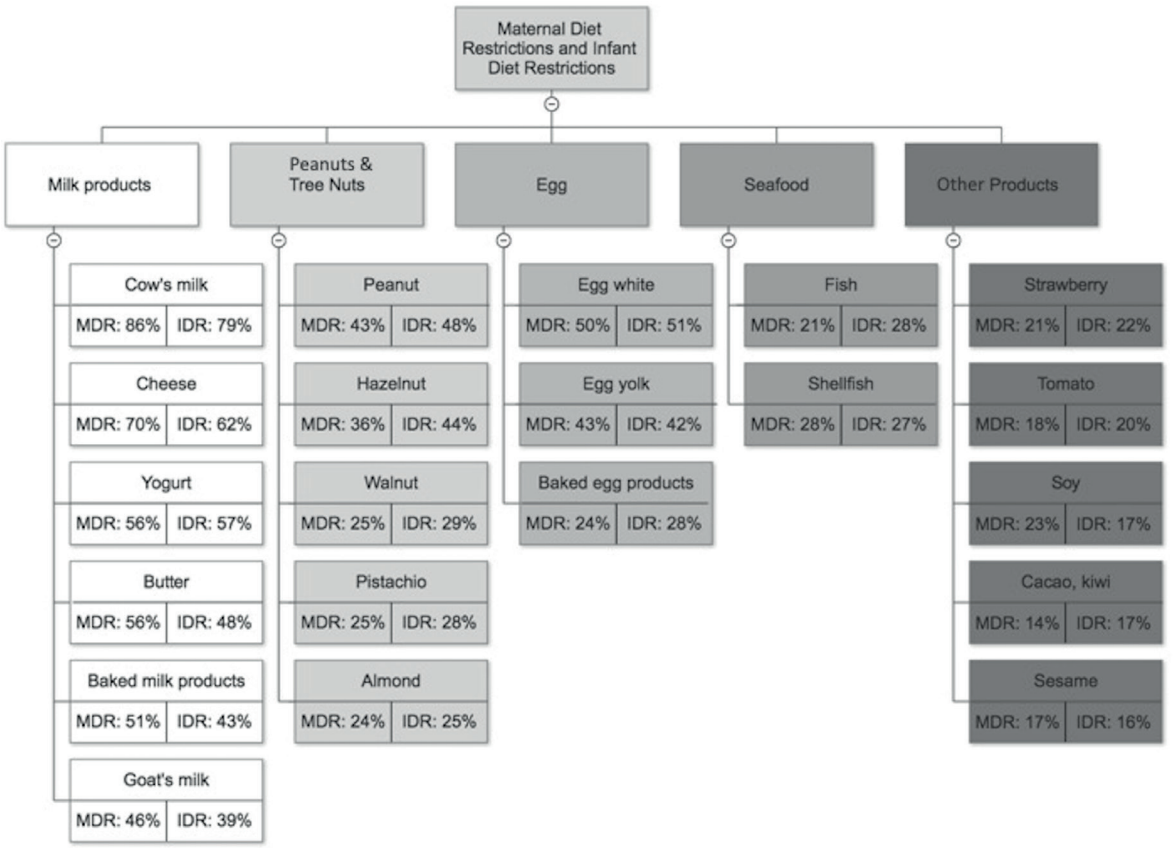

Fig. 5. Percentage of physicians restricting different food products from the maternal diet and infant's diet based on food categories.

The top 3 most common foods for introduction were vegetables (55%), fruits (26%) and yoghurt (12%) in FA, respectively. Both questions in essence inquired the timing of introducing complementary foods. The contradictory results of the two questions probably means that some of the physicians introduce a small number of complementary foods.

Discussion

This study provides insights into the role and extent of nutritional management in childhood FA. The paramount finding is that pediatricians advise highly extensive and strict diet eliminations both to nursing mothers and to the infants with food allergy. This practice is incongruent with the latest clinical studies that have consistently demonstrated the benefits of more proactive therapeutic strategies. Recently, early introduction of allergenic foods to infants was shown to be effective in preventing development of FA.[6,7] Furthermore, proactive therapeutic interventions for the treatment of FA such as introduction of baked milk and baked egg products, preference of extensively hydrolyzed formula with (or without) probiotics, and use of oral immunotherapy have gained important roles.[18-20] This study describes the fearful and reserved approach of the physicians to FA.

Participants' knowledge on symptoms and signs of FA was fair. However, the distinction between manifestations of IgE and non-IgE-mediated FA was somehow unclear. A non-negligible fraction falsely responded that non-IgE-mediated FA causes urticaria (30%) and anaphylaxis (20%). Additionally, the acute respiratory symptoms of FA were under-

recognized by many. Such knowledge gaps in the distinction between acute reactions from chronic conditions could hinder parental education and delay the administration of life-saving treatments, which was also noted as a significant problem among Finnish primary care physicians in the care for FA.[21]

Clear shortcomings in the diagnosis of FA were appreciated. The critical findings are that only one-third of the physicians take into account the clinical manifestations of the infants with suspected FA and only 1.7% use diagnostic diet elimination followed by OFC in the diagnosis of FA. Studies investigating the FA practices in the US, Australia, Croatia, Finland and our study share the common finding that clinical diagnosis of FA is a major weakness of the PCPs and/or pediatricians.[13-15,17] Internationally, physicians have a tendency to overlook clinical findings, skip OFCs and use sIgE as their only diagnostic method. Physicians should be reminded that sensitization and clinical allergy are disparate entities and that all of the current guidelines are consistent in their recommendation to perform diagnostic diet eliminations and/or OFCs as confirmatory steps in FA work-up.[22,23]

Moving on to the diet eliminations themselves, it was found that more than half of the physicians restrict at least 5 food products in any diet restriction they recommend to the nursing mothers and to the infants with FA. Especially regarding maternal diet restrictions for FA, this is a questionable approach for several reasons. Food antigens pass into breastmilk in minimal concentrations, hence only a small percentage of infants benefit from MDRs.[24] Although cases of anaphylactic reactions upon breastfeeding have been described previously, diagnostic diet eliminations followed by OFC should be advised to identify the smaller percentage of infants that are really under risk.[23]

By restricting high number of food products without making patient-based modifications, Turkish pediatricians are potentially paving the way for increases in allergies. Evidence suggests that early exposure to allergenic foods reduces the risk of atopic disease through promoting regulatory T-cell pathways, especially in infants with AD who have impaired skin barrier.[25] Observational prospective studies and randomized clinical trials investigating the effects of early exposure to CM, cooked eggs and peanut found significantly lower levels of FA with early exposure.[6,26,27] Studies showing correlation of egg protein concentrations in breastmilk and in the maternal diet with IgG4 levels in infants which indicate development of immune-tolerance further support this.[28] Another important risk is low intake of protein, carbohydrate, vitamins and especially calcium which may result in stunting and growth failure.[29,30]

Despite the observed theoretical and practical weaknesses, physicians were adequately knowledgeable in many areas. First of all, the categories of food products that are most commonly restricted; i.e. dairy, peanut, tree nuts, egg, seafood and legumes, fruits are most common allergens that have been shown to cause symptoms in Turkish children.[31] Secondly, the practice of restriction was observed to be more liberal in the categories of dairy and tree nuts. Only a fraction of those restricting CM also restrict the products that contain similar proteins as yoghurt, cheese, butter and the baked forms. Similarly, peanut is restricted more commonly than other tree nuts (Fig. 5). Such an approach is favorable since, of the patients with cow's milk protein allergy (CMPA), 70-80% tolerate baked milk products and 50% tolerate cheese, yoghurt and butter.[18] It is argued that inclusion of these products also facilitate the development of tolerance, and improve anxiety and social withdrawal.[5,18]

In addition, majority of the physicians were aware of the common symptoms of both IgE and non-IgE mediated FA. Most pediatricians prescribed calcium supplementation to mothers with CM restrictions in accordance with relevant guidelines.[4,32] Finally, the practice of weaning was noted to be appropriate with only 10% of physicians delaying complementary feeding beyond 6 months.[31] However, although

most physicians introduced complementary foods by 6 months, they regarded themselves as delaying introduction in a separate question. A confusion regarding the definition of delayed introduction was noted herein which may mean an introduction of a smaller number of foods at 6 months or later.

The limitations of this study include the small sample size of this study that can hardly reflect all practices in the country. Most participants practiced in bigger cities of Turkey and their resources may be different from the clinical settings in rural areas. Most of the pediatricians were younger than 50 years old. While this suggests our sample had less clinical experience, the impacts of current residency training were reflected more effectively. In addition, our analysis included fewer pediatric allergists and gastroenterologists than general pediatricians. Analyzing them as a single group could have resulted in higher knowledge levels.

In conclusion, FA practices had both strengths and weaknesses. Our results demonstrate a significant concern towards FA, which was reflected in clinical practice as excessive and extensive diet restrictions which were not guided by clinical information and confirmation. The two most important endpoints that should be raised to the physicians are the importance of using appropriate diagnostic methods as well as the recent advances in treatment and prevention of FA.

Supplementary data is available at:

http://www.turkishjournalpediatrics.org/uploads/turkjped.2021.01.013.S1.pdf

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Original Article

The HLA groups and their relationship with clinical features in Turkish children and adolescents with celiac disease

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ABSTRACT

Background. We aimed to investigate the relationship between human leukocyte antigens (HLA)-groups and clinical features, and degree of intestinal injury in children with celiac disease (CD).

Methods. Study group included 73 (50 females, 68.5%) children with CD. Demographic and clinical features, accompanying autoimmune diseases, family history for CD and degree of damage in small intestinal mucosa (according to Marsh classification) at the time of diagnosis were determined. Twenty-two siblings of celiac patients without CD (15 females, 65.2%) consisted control group 1, and 66 (40 females, 60.6%) people from the normal population consisted control group 2.

Results. The allele frequencies of HLA B8, B50, C6, C7, DR3, DR7, DQ2, and DR3 homozygosity were higher in the patient group. HLA DQ2 positivity was 89% in the patient group, 73.9 and 45.5% in control groups 1 and 2, respectively ($p < 0.0001$). HLA A30, C14, DR11, DQ3 frequency were lower in patients compared to both control groups. HLA-DR15 alleles in patient and control group 1 was significantly lower compared to the general population ($p < 0.05$). Thirty (41.1%) patients had typical, 43 (58.9%) patients had atypical presentation. Thirteen (17.8%) patients had other autoimmune diseases. There was no association between coexisting autoimmune diseases and the HLA antigens. Fifteen patients (20.5%) had a positive family history for CD; patients with HLA A69, B41 and C12 alleles had a higher positive family history ($p < 0.05$). Intestinal mucosal damage was as follows: 5 patients (6.8%) had Marsh 2, 25 (34.3%) Marsh 3a, 28 (38.4%) Marsh 3b, 15 (20.5%) Marsh 3c. Patients with HLA-DR15 alleles had more frequent Marsh 3a lesions ($p < 0.05$).

Conclusions. B8, B50, C6, C7, DR3, DR7, DR3/DR3, DQ2 alleles were risk factors for CD in the Turkish population. HLA C14, DR11, DR15, and DQ3 alleles were found to have a protective role in the same population.

Key words: celiac disease, histopathology, HLA typing, pediatric, Turkish.

Celiac disease is an autoimmune disease that is triggered by gluten (found in cereals like wheat and barley) intake in gluten sensitive individuals, eventually resulting in small intestinal impairment.

Celiac disease can present with various clinical manifestations from asymptomatic disease to celiac crisis.[1] Genetic and environmental factors together play a role in disease development. HLA are known as the most important genetic factors. Celiac disease's physiopathologic mechanism is similar to other autoimmune diseases like type-1 diabetes mellitus (DM), autoimmune

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thyroiditis and Sjögren's syndrome. Celiac co-existence with these diseases is common.[2,3]

The purpose of this study was to determine Turkish children's HLA subgroups and to investigate the relationship between disease presentation pattern, association with other autoimmune diseases, family history of CD, severity of histopathological involvement and HLA antigen groups.

Material and Methods

This study was carried out at Hacettepe University, Faculty of Medicine, Department of Pediatric Gastroenterology Hepatology and Nutrition between January 2009 and March 2010.

We determined 73 celiac patients (study group) on follow-up diagnosed according to modified European Society for Paediatric Gastroenterology, Hepatology and Nutrition (ESPGHAN) criteria.[4] Records of the patients were reviewed and complaints, demographic features, family history, presence of any comorbidities, presence of any chromosomal diseases such as Down syndrome and Turner syndrome, antibody titers, and histopathological findings (Marsh classification) of small intestine were recorded.

Additionally, two control groups were created and their HLA groups were also determined; these were CD patients' siblings without any health problems together with normal serum levels of IgA and negative celiac antibodies (control group 1) and 66 healthy individuals from the general population (control group 2).

Patients were divided into three groups according to their clinical characteristics at time of diagnosis: typical, atypical and silent celiac disease.[4] Antiendomysial antibody (EMA) levels were measured by indirect immunofluorescence technique (monkey liver tissue, European Gmbh, Lübeck, Germany). Levels of tissue transglutaminase antibody (TTG) IgA and IgG were measured by ELISA (Euroimmune, Lübeck, Germany).

All patients underwent esophagogastroduodenoscopy and six biopsy samples were obtained from duodenum and at least two of them were from the bulbus. Severity of small intestine involvement was scaled as Marsh 1, 2, 3a, 3b, 3c based on modified Marsh classification.[5]

Four ml of venous blood samples were taken into EDTA tubes from all patients and those in control groups after obtaining the children's and/or their parents' consent. DNA samples were obtained from blood samples by using Qiagen EZ 1 DNA Blood Kit (Qiagen Pty Ltd, Australia) and were kept at -80 °C until HLA group study was performed. DNA samples were amplified by Olerup SSPTM DQ-DR-SSP Combi Tray (Olerup SSP AB, Sweden) and Corbett Research SSKT 011 Thermal Cycler. After agarose gel electrophoresis, bands with positive PCR products were selected and analyzed with SCORE 97 3-301 T program.

HLA A allele from one patient, HLA B allele from three patients and HLA A allele from one child in the control group couldn't be obtained because of technical problems.

This study was approved by Hacettepe University Non-interventional Clinical Researches Ethics Board (16.07.2009/ HEK 09/179).

Statistical analysis

Study data were analyzed using SPSS v.11.5 software package. Descriptive data were expressed as the mean (± standard deviation) or percentage of the study group. Differences between the frequencies among groups were analyzed by chi-square or Fisher's exact test, where appropriate. Odds ratios was calculated to determine the association between the development of celiac disease and HLA types. A two-tailed p-value less than 0.05 was considered statistically significant.

Results

The study group included 73 celiac patients [mean age 7.29±4.50 years (range 0.71-17.40); 50 females (68.5%)]. Control group 1 included 23 patients [mean age 8.91±3.70 years (range 3-17); 15 females (65.2%)], and control group 2 was composed of 66 individuals [mean age 24.07±1.50 years (range 2-61); 40 females (60.6%)].

Rate of HLA DQ2 positivity was 89% in the patient group while it was 45.5% in control group 2 (p <0.0001). Positive rate of DQ2 was higher among patient's relatives (73.9%) in comparison to the general population, too (p=0.018). Although HLA DQ2 positivity rate was higher in patients compared to control group 1, it was not statistically significant (p=0.073). We did not detect DQ8 allele in either patient or control groups. It was determined that frequency of HLA B8, B50, C6, C7, DR3 and DR7 alleles was significantly higher among patients in comparison to the control groups. However, the percentages of DQ2, C6, C7, and DR3 were not statistically different between patients and control group1. The frequency of HLA B27, C14, DR11, and DQ3 alleles were significantly different among groups and HLA A30 frequency nearly reached significance. Although it was not statistically significant, frequency of HLA DR15 allele in patients and control group 1 was lower in comparison to control group 2 (Table I).

Table II shows the frequencies of the combination of risky alleles (DQ2, DR3, B8) associated with CD. HLA B8/DQ2 and HLA B8/DR3 haplotypes were significantly more common in patients compared not only to control group 2 but also

Table I. Frequency of different HLA alleles in patient and control groups.

HLA	Patients N (%)	Control group 1 N (%)	Control group 2 N (%)	p
Alleles more common in patients				
DQ2	65 (89.0)*	17 (73.9)*	30 (45.5)	<0.001
B8	27 (38.6)[a#]	3 (13.0)[#]	8 (12.1)	0.001
B50	16 (22.9)[a†]	1 (4.3)[†]	2 (3.0)	0.001
C6	26 (35.6)[‡]	4 (17.4)[‡]	11 (16.7)	0.024
C7	36 (49.3)[¶]	7 (30.4)[¶]	18 (27.3)	0.021
DR3	58 (79.5)[β]	17 (73.9)[β]	23 (34.8)	<0.001
DR7	19 (26.0)[γ]	1 (4.3)[γ]	10 (15.2)	0.04
Alleles less common in patients				
A30	2 (2.8)[b]	3 (13.6) [b]	9 (13.8)	0.053
B27	0	4 (17.4)	3 (4.5)	0.002
C14	1 (1.4)	7 (30.4)	12 (18.2)	<0.001
DR11	8 (11.0)	7 (30.4)	22 (33.3)	0.005
DQ3	28 (38.9)	13 (56.5)	41 (62.1)	0.017
Alleles less common both in patients and control group 1				
DR15	4 (5.5)	2 (8.7)	12 (18.2)	0.054

[a] Data missing for 3 patients
[b] Data missing for 1 patient
* p = 0.073 (patients vs. control group 1)
[#] p = 0.023 (patients vs. control group 1)
[†] p = 0.046 (patients vs. control group 1)
[‡] p = 0.10 (patients vs. control group 1)
[¶] p = 0.11 (patients vs. control group 1)
[β] p = 0.57 (patients vs. control group 1)
[γ] p = 0.025 (patients vs. control group 1)

Table II. HLA B8/DQ2, DR3/DQ2, B8/DR3 haplotype frequencies in patients and control groups.

HLA groups	Patients N (%)	Control group 1 N (%)	Control group 2 N (%)	p
HLA B8/ DQ2	25 (35.7)[a*]	3 (13.0)*	7 (10.6)	0.001
HLA DR3/DQ2	53 (72.6)	15 (65.2)[#]	19 (28.8)[#]	<0.001
HLA B8/DR3	26 (37.1)[a†]	3 (13.0)[*†]	8 (12.1)	0.001

[a] Data missing for 3 patients
* p = 0.039 (patients vs. control group 1)
[#] p = 0.002 (control group 1 vs. control group 2)
[†] p=0.03 (patients vs. control group 1)

Table III. HLA antigens and risk of development of celiac disease.

HLA antigen	Odds ratio	95% Confidence interval	p
B8	4.5	1.88-10.99	0.001
B50	9.4	2.08-43.08	0.004
C6	2.8	1.23-6.18	0.013
C7	2.6	1.27-5.27	0.008
DQ2	9.8	4.05-23.5	<0.001
DR3	7.2	3.38-15.47	<0.001

control group 1. While there was no difference regarding HLA B8/DQ2 and HLA B8/DR3 haplotypes between 2 control groups, HLA DR3/DQ2 haplotype was significantly more common in control group 1 than control group 2.

Table III shows odds ratios for CD in different HLA types. The presence of DQ2 allele increased the risk of CD 9.8 times.

Thirty (41.1%) of the patients presented with typical findings and 43 (58.9%) with atypical findings. No silent disease was detected. When the relationship between disease presentation and HLA groups was evaluated, it was found that only HLA DQ6 allele was related with typical presentation.

Totally 13 (17.8%) of the patients had another coexisting autoimmune disease; seven had type1 DM, six had autoimmune thyroiditis. When the relationship between HLA types and having another autoimmune disease was evaluated, no association was found between HLA type and having more than one autoimmune disease.

In the patient group, 15 families (20.5%) had two or more celiac disease patients. It was determined that the individuals who had HLA A69, B41 and C12 alleles had significantly higher chance of having a relative suffering from CD (p <0.05).

Histologic examination revealed that 5 (6.8%) patients had Marsh 2, 25 (34.3%) patients Marsh 3a, 28 (38.4%) patients Marsh 3b, and 15 (20.5%) patients had Marsh 3c changes. When the relationship between the severity of histopathologic changes with HLA groups was investigated, a positive correlation of HLA 15 allele with Marsh 3a lesion was found (p <0.047). No relation between histopathological impairment and any other allele was found.

In our study, we did not detect any relation between co-existence of CD and any other autoimmune disease and HLA antigens. Although, a higher risk of co-existence of other autoimmune diseases and CD with HLA DR4 antigen was detected, it did not reach statistical significance (p=0.06). HLA DR7 tended to be more common among CD patients without other autoimmune diseases (p=0.08).

Discussion

Genetic, environmental and immunological factors together play a role in the development of CD. HLA DQ2 and DQ8 are known as the most important genetic factors.[1] Beside HLA DQ2/DQ8, the most common celiac disease related alleles are HLA DR3 and HLA B8.[6-9] Our results show that in Turkey HLA DR3 and HLA B8 alleles are also frequent among celiac patients.

The studies investigating the relationship between CD and HLA alleles in the Turkish population are limited.[10-14] When the results of these studies are examined, it is seen that HLA DQ2, DQ8, B8 alleles are risky alleles in the emerge of CD in all studies. Apart from these alleles, some alleles may also be risky for the development of CD. Some alleles may have preventative roles. These results are summarized in Table IV.

Our findings also confirmed that the most important genetic risk factor for CD is positivity of DQ2 (89%). Considering that B8 and B50 are significantly higher in the patient group than the control group 1, these alleles may be factors that facilitate the occurrence of the disease. However, it is still controversial as to whether these alleles predispose toward CD. Considering the studies performed in our country B8 antigen was reported to be highly positive among celiac patients. In some studies performed in Turkey and Europe DR3 was frequent and considered a disease-related allele among CD.[10,15,16]

Although there was a statistically significant difference regarding DR3 and C6 between the three groups, the difference between the patient group and control group was not significant in our study. There is no other study reporting HLA C6 allele frequency among Turkish celiac patients. Two studies performed in Turkey reported carrying HLA C7 allele as a risk factor for CD, similar to our study.[10,13]

Although there are no other studies supporting the relationship of this allele with CD, there is one study reporting that the presence of C7 may be related to the late presentation of CD.[17] It was reported that DR7 was also frequent among celiac patients similar to our study.[10,13] Our results generally supported other studies performed either in Turkey or other countries. However, distinctly from other studies HLA B50 allele was detected more frequently among our patient group in comparison to control groups and HLA A25 and A2, which were considered as risk factors in other studies, were found to be similar in both groups (data not given). With these results we suggest that HLA B8, B50, and DR7 may be factors that increase the effect of the presence of DQ2 on the development of CD among Turkish people.

There are only a few studies about the protective role of some alleles against CD and there is no consensus on this subject. Ruiz del Prado et al.[18] noted that B8, C7, DR3, DR7 and DQ2 are more prevalent among patients while C4 and DQ1 are prevalent in the general population and these can be protective from the disease. A study from our country reported that HLA A29, B51, C5, DQ1, DR14, DR15, and DR16 alleles to be more prevalent in the general population compared to patient groups.[13] In this study HLA B27, C14, DR11 and DQ3 alleles were less frequent among patients, compared to control groups, and HLA A30 tended to be lower, although statistically non-significant. The HLA DR15 allele was rare

Table IV. Risky and protective HLA alleles in Turkish children with celiac disease.

Authors	Year	Number (N)	Risky alleles (HLA)	Protective alleles (HLA)
Tümer et al.[10]	2000	33	DQ2, B8, A2, C7, DR3, DR7, DR3/DR4, DR3/DQ2, DR7/DQ2	
Erkan et al.[12]	1999	30	DQ2, B8, A25, DR18	B4
Kuloğlu et al.[13]	2008	75	DQ2, B8, B13, C7, DR7, DR17	
Present study		73	DQ2, B8, B50, C6, C7, DR3, DR7	DQ3, A30, B27, C14, DR11, DR15

in patients and their relatives compared to the general population. The frequencies of alleles detected in the control group in our study were not compatible with this literature except DR15 that might be protective.

Genetic factors affecting the clinical presentation of the disease has been claimed by some researchers. It was stated that individuals with HLA B8 and C7 presented with the disease in elder ages.[17] The relation of disease presentation with HLA B8 and DR3 was noted as well.[19] Majorana et al.[20] reported that patients with tooth enamel disorder carried HLA DR3 genotype with a higher incidence. There is only one study stating the correlation between HLA and disease presentation in the Turkish population. In this study consisting of 75 patients (40 with typical and 35 with atypical presentation), it was reported that HLA B35, DR11, and DQ7 antigens were related to typical and HLA B8 antigen with atypical presentation.[13] In this study only HLA DQ6 related to typical presentation was found; but no relation with the others was obtained.

Genetic factors have been suggested to play a role in the development autoimmune diseases such as type 1 DM, autoimmune thyroiditis and Addison disease and this relation has been basically ascribed to major HLA antigens.[2,3] Recent studies have shown some HLA antigens to be related to the tendency for these disorders while some others having protective effects.[13,21] Britten etal.[22] investigated the relation of HLA DQ gene expression and susceptibility to diseases especially to type1 DM. They reported that DQ2 and DQ8 antigens to be related to increase in disease risk. HLA DR3 positivity was found to be higher among patients who have type 1 DM and CD together compared to the normal population and patients with only type 1 DM.[23] Baker et al.[24] noted that HLA DQ2 and DQ8 alleles were the genetic reasons for autoimmune disease as well as CD. In another study, in patients with type1 DM, T-cell activity against wheat antigens causing intestinal inflammation were not related to DQ2 allele but to DR4 allele.[25] It was also reported that DR4 haplotypes such as DR4/DQ8 could be associated with CD susceptibility.[26] In our study we did not detect any relation between co-existence of celiac disease and other autoimmune diseases and HLA antigens. However, a higher risk of co-existence of other autoimmune diseases and celiac disease with HLA DR4 antigen was detected, not reaching statistical significance (p=0.06). HLA DR7 was prevalent among CD patients without other autoimmune diseases again not reaching statistical significance (p=0.08). It will be easier to comment on these antigens' roles in studies with higher patient numbers.

It was shown that the incidence of CD among European populations was similar to that of immigrants with European ancestry in USA and Australia.[27] It was previously reported that having a relative with CD, especially having a sibling, was the most important risk factor for the disease, raising the risk up to 20%. Besides, the risk was found to be the highest in HLA DQ2 positive male patients in a family having two affected siblings. It was also reported that the asymptomatic relatives of patients with CD could have either intestinal mucosal structural deterioration or functional alterations of permeability. It was also stated that the risk of other autoimmune diseases and intestinal lymphoma was increased in these asymptomatic relatives.[27] A study from an Asian population indicated the incidence of CD in relatives of celiac disease patients to be 4.4% and the rate of relatives negative for HLA DQ2/DQ8 was found as 15%.[28] In our study we observed the frequency of alleles (DQ2/DR3) causing predisposition to celiac disease in healthy siblings of patients was higher than the normal population. However, no correlation of these alleles and celiac disease occurrence in more than one patient in the same family was detected. There was an increased frequency of HLA A69, B41 and C12 alleles in patients with relatives having CD in their families. There are no previous studies investigating CD and HLA antigen relation in families with high number of patients. In this respect we believe our study

is contributing to this new area. The results should be tested in studies with larger numbers of subjects.

The factors related to the damage of small intestinal mucosa in CD has not been well clarified. There are studies pointing to the relation between the degree of mucosal damage and HLA antigens. Jores et al.[29] stated that the histo-pathological damage was more severe in patients homozygous for HLA DQB1*0201 allele. Karinen et al.[30] reported that HLA DQB1*0201 allele was related to more severe disease, late histological amelioration and early onset of disease. Another study indicated that CD patients homozygous for HLA DQ2 alleles should be diagnosed at early stages of the disease because of higher rates of intestinal T cell lymphoma development.[31] A total of 23 patients in our study group was homozygous for DQ2 allele but no relevance to the degree of histopathological damage was detected. Simone et al.[32] emphasized the strong relation between soluble cytotoxic T lymphocyte related protein-4 (sCTLA-4) gene and autoimmune disease development and also stated that sCTLA-4 was higher in CD patients without treatment and those levels correlated to anti-tissue transglutaminase antibody levels and the degree of intestinal mucosal damage. No relationship between HLA antigens and sCTLA-4 levels were detected. We detected that histological damage was significantly severe in CD patients with HLA B8 antigen. We could not find any study investigating the relation between HLA B8 antigen and the severity of damage in the small bowel. However, HLA B8 antigen was reported to be more frequent in Europe and in our country.[10,13,19] There are studies claiming that this antigen has a relationship with disease tendency, disease presentation and age of disease onset.[7-10,17,19] In our study, it was also found that HLA DR3 and HLA B8 alleles were common besides HLA DQ2 allele. However, no relationship was found between HLA DQ2 and DR3 alleles and the severity of intestinal mucosal damage. In patients with DR15 allele the mucosal damage was lower. There is no data disclosing the relationship between mucosal damage and DR15 in the literature.

In conclusion, HLA DQ2, B8, B50, C6, C7, DR3 and DR7 were identified to be risky alleles for the development of CD in the Turkish population while HLA B27, C14, DR11, DR15, DQ3 alleles, and partly HLA A30 could be protective. DQ8 allele was not found in either the celiac or control groups. HLA DQ allele was found to be related to typical presentation, and HLA DR15 allele was predictive of mild mucosal damage.

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Original Article

Clinic and demographic characteristics of pediatric patients with Lichen sclerosus

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ABSTRACT

Background. Lichen sclerosus (LS) is a chronic disease of the skin, for which the pathogenesis is not known. It can lead to various changes of the skin and the genital area, potentially leading to both functional as well as cosmetic problems for the patient, thus disrupting the quality of life. In this study; the purpose was to review the clinical characteristics and the treatments of the 15 pediatric patients under the age of 18 followed up in our out-patient clinic with a diagnosis of LS and to compare the findings with literature data.

Methods. Between 2011 and 2017, the files of 15 patients diagnosed clinically and/or histologically with LS in our clinic were retrospectively examined. The demographic characteristics, clinic and laboratory findings, treatment options of the patients are reported.

Results. Of the patients included in the study 14 were girls and one was a boy. The average age was 11.6 years (5-17 years), the average age for the initial disease was 7.8 years (2-13 years). The average duration of the disease at the diagnosis was 3.9 years. The most common form was genital vulvar type (8/14 girls) without anal and cutaneous involvement, and each of them suffered from itching. One of the cases had genital LS as well as extragenital morphea lesions. Two of the 15 patients were ANA positive. The other antibodies were negative. In two cases with extragenital involvement, lesions were widespread and they were in blachkoid form.

Conclusion. LS is a chronic disease that progresses with recurrences and regressions. In our study, the most common LS type was genital type (60%). There was extragenital involvement in 6 patients (40%). Extragenital involvement was the most common on the trunk. Diagnosis, treatment and follow-up during childhood is highly important to prevent any possible future anatomical or psychological damage and genital malignancies.

Key words: child, epidemiology, extragenital, genital, lichen sclerosus.

Lichen sclerosus (LS) is a chronic inflammatory skin disease with genital and extragenital involvement and its cause is unknown.[1] It was defined for the first time by Halopeau[2] in 1887. The International Vulvovaginal Diseases Study Group named the disease "Lichen sclerosus" in 1976.[1,3] As patients are followed by many specialties such as dermatology, gynecology, urology and pediatrics, the incidence of LS is not known precisely.[4] It is mostly seen in caucasians and the rate in women is 6-10 times higher.[5] It has a bimodal starting age as in prepubertal and postmenopausal.[5] The prevalence in dermatology clinics is estimated to be 1/300-1/1000.[5,7,8] Of the patients diagnosed with LS 10-20 % are children[7,9] and of these children 7-15% are prepubertal and 90% of these are between 3-13 years old.[3,10] In the prepubertal phase, lesions appear at around 5 years of age (average 5.4 years), but usually a diagnosis is only made at around the age of 6 to 7 years.[8,11] The disease usually takes a chronic course with phases of exacerbations and remissions. The cause of LS is unknown, however, studies are focusing on autoimmunity, genetics, hormonal factors, various infections, local irritations and trauma.[12-15] The most frequent site involved is on the genital area.

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Anogenital area involvement ranges between 85-98%.[1,9,14,16,17] Clinically, it starts in the genital area as an erythematous macule and papular lesion. White, atrophic, bright plaques develop overtime. Lesions resemble the shape of the number 8 around the vulva and anus. Due to the itching of the lesions, ecchymosis and petechial bleeding may occur on the genital area. Sclerosis, hyperkeratosis, erosion, fissure, ulceration and bulla may be observed. During the healing process, these lesions may cause postinflammatory hyperpigmentation.[3,13,15] Girls suffer anogenital itching, dysuria and painful defecation, while boys suffer from phimosis. Extragenital lesions occur in around 6-20% and clinically, they display similar characteristics to genital lesions.[6,7,18] It is mostly seen in the neck, shoulders, upper body, wrist flexor surfaces. It is rarely seen on the palms, soles, hairy skin and face.[19-21] Annular, blaschkoid, keratotic and bullous forms of this type have been reported.[19-21] Koebnerization is frequent and LS with koebnerization may be accompanied by plaque type morphea.[22,23] Some authors claim that LS and morphea have a common pathogenesis.[11,22,24] Involvement of the genital area might lead to functional problems and skin involvement may cause cosmetic problems.[4,6] Also, rarely, oral involvement has been reported.[25]

Even though there are retrospective studies, examining various LS parameters in the literature. The studies from Turkey on this topic are mostly in the form of case reports.[26-28]

The purpose of this study was to examine the clinic and demographic characteristics of 15 pediatric patients diagnosed with LS whom we have monitored in our outpatient clinic, to assess laboratory findings, to examine the treatment options provided and compare them with literature data.

Material and Methods

The study was approved by Yıldırım Beyazıt University, Yenimahalle Training and Research Hospital Ethics Committee (Number 2017/09/03; Date: 24/10/2017). The medical charts of patients diagnosed clinically and/or histopathologically for LS during 2011-2017 were retrospectively examined. The criteria to be involved in the study was set as being under 18 years of age at the time of diagnosis. The demographic characteristics of the patients, the starting age of the disease, its duration, clinic type, anatomic area, accompanying symptoms, triggering factors, laboratory findings, and applied treatment options were recorded. The existence of additional diseases that could accompany LS was also investigated. The data is presented in average and ranges, categorical data has been provided in percentage, in figures.

Results

There were 15 pediatric LS patients diagnosed and followed at our outpatient clinic during the 2011- 2017 period. A biopsy had been taken from all cases with extragenital lesions and histopathologic assessments were made (Figs 1-3). A biopsy had been taken from a case with genital involvement who relapsed. The total number of patients to receive a biopsy was 8. Diagnosis of others were made by history and clinical examination.

The demographic and clinic data, laboratory findings and treatments the subjects received are given in Table I. The age range was between 5 and 17 years (11.6 years on average). The duration of the disease was 3 months to 13 years (3.9 years on average). The starting age of the disease was 7.8 years. Of the patients 93.3 % were girls (n=14) and there was one boy (n=1, 6.7%). Girl to boy ratio was 14/1. The most common LS type was genital type by 60% (n=9). This was followed by extragenital involvement in 6 patients (40%). These patients had only extragenital involvement. Genital and extragenital involvement were not seen together. Skin lesions were mostly located on the trunk and lower extremity. In one girl, segmental lesions were observed on the lower extremity, while in another segmental lesions

Table I. The characteristic features of the patients.

No	Age	Gender	Age disease started	Disease duration	Itching	Area involved	Accompanying disease	ANA (+)	Biopsy	Therapy
1	11	F	-	1 Year	Yes	Genital	Neuro fibromatosis	-	-	TS, T
2	17	F	7	11 Years	-	Left Leg		-	+	K+T+C+P
3	12	F	2	10 Years	Yes	Genital	Vit D and Vit B12 deficiency Thyroid diseases	-	+	TS+T relapse
4	11	F	9	2 Years	-	Bilateral leg front sides	--	+	+	TS
5	12	F	10	2 Years	Yes	Back, trunk	-	-	+	TS+T
6	7	F	6	1 Year	Yes	Genital	Morphea		+	TS+T
7	14	F	10	4 Years	-	Genital				TS
8	13	F	7	6 Years	Yes	Genital			-	TS+K
9	16	F	13	3 Years	Yes	Genital	-		-	TS
10	14	F	11	3 Years	-	Trunk	-	-	+	TS
11	6	F	4	2 Years	Yes	Genital	-			TS
12	10	M	7	3 Years	Yes	Genital -penis	-	-	-	Circumcision TS
13	17	F	7	10 Years	-	Blasckoid and plaque in trunk arm	Iron, VitB12, Vit D deficiency	+	+	MTX+T
14	5	F	5	3 Months	Yes	Trunk, legs	-	-	+	TS
15	10	F	9	9 Months	Yes	Genital	-	-	-	TS

K= Calcipotriol, TS= Topical Steroid, T= Tacrolimus, MTX= Methotrexat, P= Pimecrolimus, C= Colchicine, ANA= Antinuclear antibody, F= female, M= male

were observed on the leg and also on the abdomen.

One girl had LS on the genital area and also morphea lesions on the trunk, the diagnosis of which had been proved histopathologically. None of the patients had oral involvement. All patients with genital involvement (also including the boy) suffered from intense itching. Those with extragenital lesions did not have any itching and were only complaining about the appearance.

In terms of accompanying diseases, there was a case with thyroid disease and another with vitamin B12 deficiency, two patients had vitamin D deficiency and another one with iron deficiency. One patient has neurofibromatosis. No family history of LS or autoimmune disease (rheumatologic disease, thyroid disease, vitiligo, diabetes etc.) history in the families had been observed in any of the cases. One of the subjects with extragenital involvement had a severe sunburn history on his back. Following the examinations made for this study, no infectious agent and/or disease, that could have triggered the disease, was detected. Only 2 girls had ANA positivity (at 1/100 titer,13.3%). AntidsDNA, anti-histon antibody, anti- scleroderma 70, Borrelia burgdorferi antibodies were negative.

As a first option, topical potent steroid (mometasone furoate) was given to the patients. Subjects who did not respond within 3 months were given pimecrolimus and/ or tacrolimus (for about 3 months). A patient with segmental extragenital lesions spreading to the leg and trunk was given methotrexate (MTX) and

topical tacrolimus treatment for 6 months. The patient's lesions improved. Another patient with segmental spread blaschkoid involvement on the leg was given colchicine and tacrolimus treatment for 9 months. The male patient developed balanitis that led to phimosis and was circumcised. Relapse was observed in two patients, one with genital the other with extragenital involvement. One of these patients had been given colchicine, the other had been given topical steroid. The relapse was observed 10 years after the end of treatment. After relapse, the first patient was treated with colchicine and topical tacrolimus, the second patient was treated only with topical tacrolimus.

Discussion

This study focused on 15 children diagnosed with LS and the factors possibly related to its etiology, in addition demographic characteristics and treatment options have been assessed. Findings such as starting age of the disease, gender ratio, morphologic characteristics of lesions and disease duration were similar to the literature, but the sites of involvement regarding extragenital involvement were different (Table II). Literature indicates that girls are affected 4-10 times more often than boys.[3,9,14] This ratio was much higher in this study, 14/1. LS appears during childhood at around 7-15% of the cases and it is mostly between the ages of 3-14.[15] In

Table II. The comparison of our study with the literature.

Studies Time (Year) Number of Patients	Age range (Years)	Area involved	Accompanying Disease	Therapy	Complications, Relapse, improvement
Current study 15	5-17	9 Genital/ 6 Extra genital	Vitamin B12 deficiency Thyroid diseases Morphea	Topical potent steroid Calcipotriol, Tacrolimus, Methotrexat, Pimecrolimus, Colchicine,	- 2 Relapses (13.4%) 86.6% clearance
Nerantzoulis et al. 2017 15	4-19	Genital	-	Topical potent steroid	Phimoses Labial adhesion 5 Relapses
Anderson et al. 2016 14	2-10	13 - Genital/ 1-Extra genital	-	Topical potent steroid Tacrolimus	93 % clearance
Casey et al. 2015 72	3-14	Genital	-	Topical potent steroid	72.6% clearance
Jensen et al. 2012 36	1-18	Genital	-	Topical potent steroid Tacrolimus	-
Cooper et al. 2004 74	0-16	Genital	-	Topical potent steroid	22% clearance 67 % partial
Helm et al. 1991 33	<18	Genital	Hypothyroidism Diabetes mellitus Alopecia areata	Topical potent steroid	44% clearance
Meyric Thomas et al. 1988 47	0-20	Genital	Thyroid diseases Diabetes mellitus Anemia	-	-

some studies, the average age when symptoms appeared in girls with vulvar involvement was 5-5.4 years, age of diagnosis was 6.7-7.6, and delay in diagnosis was 1-1.6 years.[11,14,17,29,30] Nerantzoulis et al.[31] reported the average age for vulvar involvement in children as 8.8 and a diagnosis delay of 7 months. The age range of subjects in this current study was 5-17. Even though the average age was 11.6, the diagnosis duration was 3.9 years. The reason for delayed diagnosis in our study could be related to the fact that patients were followed by different disciplines of medicine which may have caused a delay for them to reach dermatologists.

Previous studies reported 10-17% ratio of family history in LS patients, which indicates a genetic susceptibility.[11,15,17,32] However, none of the subjects involved in this study had any family history which was asked to the parents. This could be due to having a low number of patients and a lack of information on the medical charts.

In LS etiology, various triggering factors such as trauma, severe sunburns and surgical operations have been blamed.[3,10,15,33] One of the subjects with extragenital involvement had a severe sunburn history in his back. This was thought to have triggered the disease with koebnerization effect.

Some studies reported a connection between LS and various infectious agents such as Borrelia, Ebstein Bar Virus, Hepatitis C, Human Papilloma Viruses.[3,34-37] However, findings of studies concerning these viruses are contradictory.[7,13,38] While some studies found them to be meaningful, others could not establish any connection.[7] In the current study we were not able to detect any infectious agents and/or disease, that could have triggered the disease. In LS, immunity associated autoimmune diseases are reported in 4-24%, autoantibody positivity is reported to range between 42% and 80% in all age group.[8,10,12,14,33,39] The clinical and prognostic importance of these antibodies is not known. They may be important for studying the accompanying disease. In our study, 13% of the children had positive ANA positivity (at 1/100titer), and 6% of the children had an autoimmune disease.[12,33] Kreuter et al.[40] found ANA positivity as 9.6% for females and 0.7% for males, but they did not specify the child rate. The female patients with LS more often had autoimmune diseases when compared to males in their study (respectively,18.9%, 5.1%). We also observed that autoimmunity was more common in the girls with LS although we only had one male patient. Circulating antibodies against IgG extracellular matrix 1 (ECM1) proteins were detected in 74% in the literature.[1,18,41] We did not evaluate anti-ECM1 antibody levels in the 15 children in our study.

Clinically, there are two types of involvements in LS; genital and extragenital. Genital involvement occurs in around 80-95%. In this study, genital type LS was also more common, by a ratio of 60%. Genital LS diagnosis can be made simply by clinic data and history. Vulvar involvement is very frequent in LS. Erosion, ecchymosis and similar findings can be observed due to severe itching (Fig. 1). Sexual abuse should also be part of the differential diagnosis as ecchymosis and petechial focuses are seen in the genital area. Referring these patients to dermatology clinics

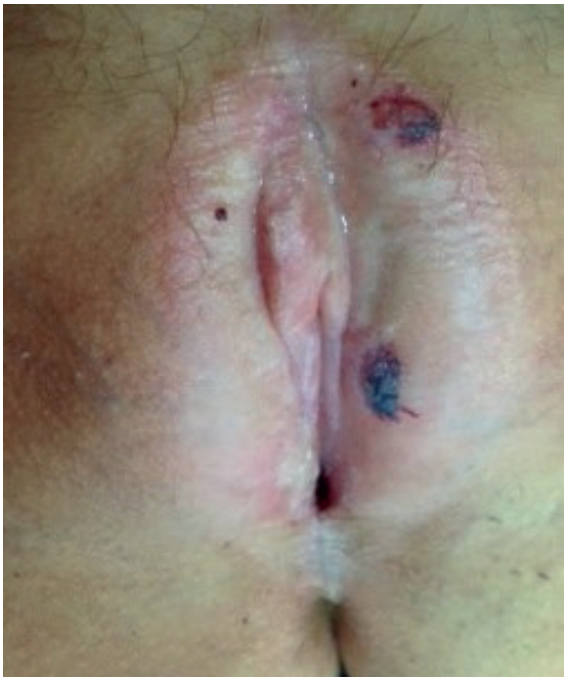

Fig. 1. The ulcers on genital lichen sclerosus lesions.

is important to ensure a correct diagnosis.[17] The patients in the current study also had itching. However, petechia and ecchymosis were observed in only 2 subjects. The possibility of sexual abuse was investigated for these children with ecchymosis and petechial focuses but none could be determined.[13]

Ulceration, dysuria and painful defecation may also occur in these patients. The existence of genital itching simplifies the lesions to be more widespread and permanent due to the koebnerization characteristic of LS. Due to scar formation over time, the anatomic structure of the genital area may be distorted, leading to vaginal tightness in girls, and phimosis in boys, along with other issues for both genders.[17,30,39,42] Smith et al.[16] observed in 25% healing in LS, also reporting that 75% of the symptoms continued and that the change in vaginal appearance is 50%. Despite reacting to treatment in the study, 2 relapses developed.

In some studies, LS has been detected in 9-52 % of the boys with phimosis.[11,29] In another study, 57% which is localized to the foreskin, 4% with meatal involvement and 20% with urethral involvement have been reported.[43] The age of observing balanitis in boys has been detected as 28 months- 18years (8 years in average).[29,43] The only boy in the study with penis involvement was 10 years old and he had been suffering from phimosis for 3 years. Extragenital lesions are seen in 15-20 % of the patients. These lesions may be seen along with anogenital lesions[3,9] Isolated skin involvement is around 6%. In general, the upper trunk, axilla, femur, hips are more frequently involved while the face, hairy skin, hands and feet are more rarely involved.[7,8,14] (Figs 2 and 3). Another interesting finding of the study was that extragenital involvement were in the form of isolated involvement. Some authors have detected 4% of extragenital involvement[11-15], but this rate was higher in this study (40%).[12]

For patients with extragenital involvement to be able to diagnose LS, diseases such as atrophic lichen planus, cicatricial pemphigoid, lichen simplex chronicus, psoriasis, contact dermatitis, morphea, lichen niditus, porokeratosis, vitiligo, idiopathic guttate hypomelanosis, discoid lupus eritematosus have to be considered in the differential diagnosis and a biopsy should be taken.[16]

In the histopathology of LS, there is band type lymphocytic infiltration at the surface dermis and vacuolar degeneration at the basal layer during the early stages and thickening due to lower infiltration, sclerosis in dermis and fibrosis and also atrophy in the epidermis during the latter stages.[9,24]

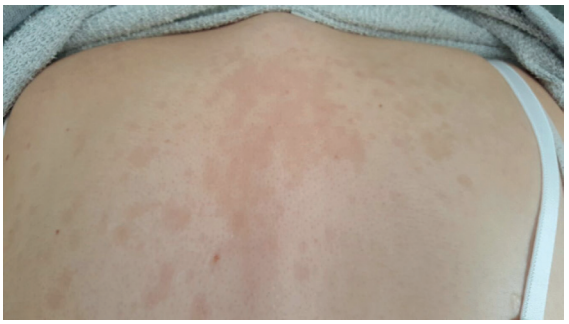

Fig. 2. Lichen sclerosus lesions on the back.

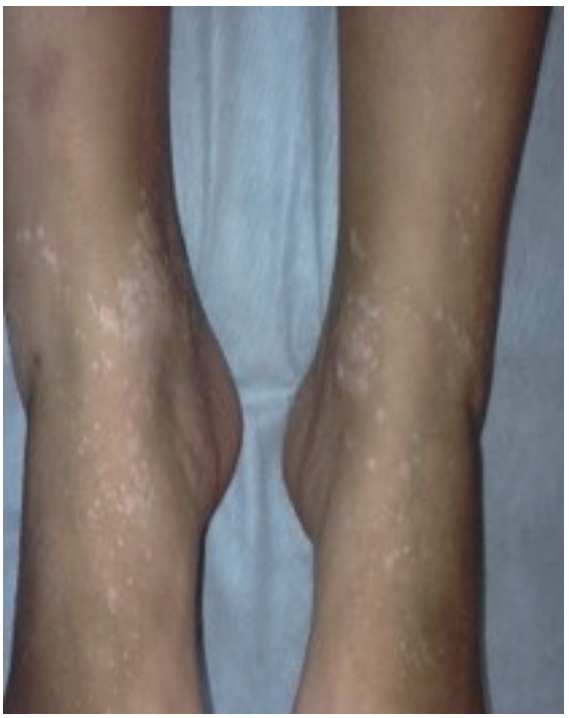

Fig. 3. Lichen sclerosus lesions on the dorsum of the foot.

In this study, the definitive diagnosis of patients with extragenital involvement have been given by assessment under the light of these histopathologic findings. Lesions were mostly placed in the trunk and lower extremity. Lesions in 2 cases on the lower extremity were spread and blaschkoid type (Fig. 3). This is also a rare event and only a few cases have been reported in literature.[19-21]

The literature also contains oral lesion involvement.[25] However, no oral involvement was observed in this study. The existence of autoimmune disease has been observed in 12-42% of LS patients.[10-12,17,22,33,39] The most frequent among these are thyroid disease, vitiligo, irritable intestine disease, alopecia areata, rheumatoid arthritis, pernicious anemia, morphea, psoriasis, atopic dermatitis and diabetes mellitus coexistence.[7,12,15,33,45] Murphy et al.[44] observed 12% of thyroid pathology and 2% of pernicious anemia. It has been reported that autoimmunity is more common in girls with genital involvement.[7,42] In this study, thyroid disease was observed in 6% (n= 1), and Vitamin B12 deficiency anemia in 13%. In addition, with regards to accompanying diseases, two patients had vitamin D deficiency and one case had iron deficiency anemia.

Extragenital LS accompanied by a plaque type morphea is a frequent event and some authors claimed that they have a common pathogenesis.[22,45] In this study, morphea coexistence was detected in one subject with genital involvement. As the reason for lichen sclerosis is not fully known, there is no effective treatment for it. The purpose of the treatment is to minimize the development of the disease during the early stages to prevent functional and cosmetic complications.[18,46,47] Therefore, early diagnosis, treatment and follow-up are thought to be significant. For the treatment of LS with genital involvement, use of potent local steroids is recommended. This type of treatment is thought to be reliable and effective.[3,13,31,46,47,51-53]

As a first option, we preferred potent steroid creams for our subjects. Due to the atrophy risk in topical corticosteroids, use of topical calcineurin inhibitors has been considered for treatment in recent years.[13,18,30,46,51,52] Patients not benefiting from the treatment were given topical calcineurin inhibitors, topical calsipotriol and betametazon. In limited lesions phototherapy (UVA1, narrowbant UVB, UVA) is among the other treatment options for both genital and extragenital type LS.[7,47] However, none of our patients were given phototherapy. Lesions in the extragenital areas do not respond to topical steroid treatment as much as genital disease. In the literature, different treatments such as topical calcineurin inhibitors, systemic retinoids UVB, PUVA, UVA, oral stanozolol, topical oxatomide, topical calcipotriol, anti-malarials and various antibiotics have been tried.[46,47] In this study, we administered topic tacrolimus and pimecrolimus therapy as well as topical steroids in patients with extragenital involvement.

Methotrexate (MTX) may be given to patients with LS.[48] In our study, we administered MTX therapy to a patient with multiple extragenital lesions for 6 months. Lesions improved leaving post-inflammatory hyperpigmentation.

A study conducted in Turkey by Parlak et al.[49] on patients with morphea, reported that giving colchicine stopped the formation of new lesions and that the side effect profile was low, thus it could be an effective and reliable option. Considering the joint pathogenesis of LS and morphea, a subject with blaschkoid type spread lesions was given colchicine treatment for 9 months, due to the fibroblast inhibition, effect on elastic fibers and anti-inflammatory aspects of this drug.[50] The lesions improved with post-inflammatory hyperpigmentation, however, relapse developed in the lesions after a few years.

There is no need for surgery in non-complicated LS, surgical methods should only be used in the presence of malignity when correcting cicatrix.[46] In the genital area of boys the most frequent involvement is seen in the foreskin, therefore burning, difficulty in pulling back the foreskin,

micturition difficulty can be experienced and phimosis could develop.[46] Accordingly in this study, a male subject had to be circumcised due to developing phimosis. Clinic progress of LS is not clear as it changes. There is a belief that healing occurs in most patients by puberty.[17,42] In some studies, it has been reported that lesions fully heal up to 25%, while there is symptomatic healing in 75-93 %, and this could recur.[54] Studies have concluded that vulvar anatomy deforms by 20-50% despite treatment.[13,16,30,53,54] In addition, it may lead to functional deformities such as micturition disorder and constipation. Cooper et al.[39] reported a 60% recurrence in girls that they have monitored for 15 years. In this study, lesions in most of the girls with vulvar involvement were improved through treatment.

Only a subject with thyroid pathology had recurring lesions recurred after puberty. This could indicate that there is a possibility of recurrence in patients with autoimmunity. Some studies concluded that the disease does not regress by puberty, emphasizing the need for careful monitoring of the patients for malignity development risk.[16,42,54] In adults, malignity development ranges between 4-5%.[8,12] Malignity in childhood is considered to be non-existent, however, malignity has been reported in the literature in a subject with genital involvement that started during childhood and continued in adulthood.[42] Therefore, early diagnosis and treatment are thought to be important for these patients. The purpose is to control symptoms, prevent and treat complications and detect malignity in the early stages. A multidisciplinary approach is required. Following proper treatment in girls with the genital lesion, no permanent deformation developed in any of them. Extragenital lesions do not have any malign change risk. No malignity development was observed during the follow-up of any of our patients with genital involvement. By affecting deeper tissues, extragenital lesions can lead to deformation. No deformation has been observed in our patients.

The most common form of childhood LS is genital type, as observed in our study. Genital itching was the most frequent symptom. Therefore, LS must be considered in the differential diagnosis of genital itching in children. Patients should be carefully questioned in terms of symptoms and examined. Extragenital involvement could be at a considerable degree in LS. In genital involvement that continues for a long time and resists treatment, a biopsy must be done to exclude malignity.

LS is a chronic disease that progresses with recurrences and regressions. A self-regression may be observed in girls after menarche.[54] However, the risk of recurrence during adulthood is not exactly known. Diagnosis and treatment at early stages is important in LS, due to cosmetic and functional deformities that may progress severely. Therefore, family physicians, pediatricians, gynecologists, urologists and dermatologists should work in collaboration. The limiting factors of this study were its retrospective nature, few number of patients and the lack of detailed family history. In order to achieve more definite data, more comprehensive, prospective multicenter studies are needed.

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Original Article

Endonasal choanal atresia repair; evaluating the surgical results of 58 cases

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ABSTRACT

Background. Choanal atresia is the most common congenital nasal anomaly, with an incidence of 1:5000–1:8000 live births. Atresia can be seen as membraneous, bony or mixed type. When it is bilateral, it is accepted life-threatening, therefore bilateral atresia necessitates immediate intervention. Diagnosis is confirmed by endoscopic examination and computed tomography. The absolute treatment is surgical, and different approaches have been proposed.

Methods. Herein, we describe our 15-year experience in the treatment of 58 patients of congenital choanal atresia with transnasal endoscopic approach, and we compare the efficacy of placement of an intranasal stent and applying mitomycin while endoscopic microsurgical repair.

Results. The study included 41 female patients (71%) and 17 male patients (29%) with congenital CA. The mean age was 3 years ranging from 10 days to 16 years. The atretic plate was bilateral in 24 patients (41%) and unilateral in 34 (59%). The most common atresia type was the mixed type with 29 patients (50%). A total of 17 patients (29%) required postoperative revision(s). Postoperative revisions were more frequent among patients with bilateral CA (50%), and with mixed CA (31%). Stenting was used additionally by surgical correction for 10 patients. After stenting, fibrosis and restenosis was seen in 7 patients (79%). Mitomycin C was applied peroperatively in 8 patients. Restenosis after mitomycin application was seen in 4 patients (50%).

Conclusions. By our experience, endoscopic microsurgical repair of atresia proved to be an effective and safe procedure, results compared with adjuvant treatment modalities like stent or mitomycin C use, was not better. Restenosis was the major problem seen after surgical correction.

Key words: choanal atresia, stent, transnasal endoscopic approach, mitomycin C.

Choanal atresia (CA) is the most common congenital nasal anomaly, and is encountered in approximately 1:5000–8000 live births and it is slightly more prevalent among females.[1,2] The failure of the embryologic nasobuccal membrane of Hochstetter to rupture gives rise to CA.[3] As a result of the unsuccessful membranous rupture, the nasal cavity is narrowed by a bony obstruction of the choanae which is caused by the pterygoid plate laterally and vomer medially.[3] Most cases also have a mucosal membranous component to the obstruction. More than half of the patients (70%) have a mixed bony-membranous atresia.[3] CA may be unilateral (more common, 60% of all cases) or bilateral.[4] Owing to the necessity of nasal breathing in newborns, bilateral cases present as an airway emergency existing with respiratory distress since the moment of birth. Bilateral atresia patients characteristically present with cyclic cyanotic spells that improve with crying (paradoxical cyanosis).[5] Initial therapy of this airway obstruction, paradoxical

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cyanosis, and stridor; should concern immediate maintenance of an adequate airway by oral airway or intubation.[5] Unilateral CA presents usually between 5 and 24 months, with a snoring cry, unilateral rhinorrhea, mouth breathing, respiratory failure and may go unnoticed.[5,6] When there is a suspicion of CA, it can be evaluated by advancing a nasogastric tube through the nostrils or searching the presence/absence of airflow out of the nostrils, and it can be directly confirmed by nasal endoscopic examination and computed tomography scan (CT).[6] When nasal endoscopy is performed suboptimally, the definitive diagnosis is established with a CT scan of the paranasal sinuses and skull base. The narrowing of the posterior nasal cavity will be demonstrated by CT scan, such as medial bowing of the lateral wall of the nasal cavity, and the impingement at the level of the anterior aspect of the pterygoid plates and a widening of the posterior portion of the vomer.[7] Patients with unilateral or bilateral CA should be screened for associated congenital disorders. Associated congenital anomalies may be identified in patients with CA, such as developmental delay, hormonal disorders, maxillofacial anomalies, cardiac disorders cerebral abnormalities.[8]

Almost all cases are now repaired via an endoscopic approach with the aid of powered instruments.[3] Despite that, revision surgery may even be required, because the major problem encountered in the management of CA is re-stenosis after surgical correction, and it occurs because of scar and/or granulation tissue formation. Adjunct therapies and techniques have been employed to avoid such problems. Inserting nasal stents and applying topical mitomycin (an aminoglycoside antibiotic isolated from Streptomyces caespitosus) intra-operatively to the choanae can be performed to decrease the risk of re-stenosis, but the reported efficacy of these measures vary widely with some studies showing counterproductive effects.[9]

Herein, we describe our experience in the transnasal treatment of congenital CA with endoscopic approach efficacy of placement of an intranasal stent, and applying mitomycin in our tertiary care center.

Material and Methods

Institutional review board approval was obtained from Hacettepe University (GO 14-474, 5.11. 2014). Patient records, undergoing surgery for congenital CA at the Hacettepe University Faculty of Medicine's Otorhinolaryngology clinic over a period of 15 years (January 2000-January 2015) were evaluated retrospectively. Data collection was based on reviewing case histories. Eight patients who had correction surgery previously for CA at other institutions were excluded from the study. A total of 58 patients were analyzed in our study.

The variables analyzed; Gender, type of atretic plate, side of atresia, details of surgeries performed (transnasal endoscopic repair only or application of nasal stent or applying topical mitomycin C), the need of revision surgery were evaluated.

Preoperative evaluation; We performed a thorough prior assessment of all patients by nasal endoscopic examination, with a flexible fiberscope (2.2 mm) or a rigid endoscope (2.7 mm), and for 13 patients we performed an additional CT scan of the craniofacial complex.

Surgical approach; All cases were performed by a transnasal approach using endoscopy under general anesthesia. We did not use navigation systems in any of the cases. In all cases, cottonoids impregnated in local anesthetic (lidocain) and a vasoconstrictor (adrenaline 1%) were applied in both nasal fossa. Nasal endoscopy was then carried out using a nasal endoscope (2,8 mm, Karl Storz). Following the bottom of the nasal fossa, the choana was opened medially and inferiorly. The incision of the mucosa was carried out with sicle knife. Once the nasal mucosa covering the atretic plate was removed, the plate and the posterior part of the vomer were extracted with microsurgical

instrumentation (ear curette or drill). The edges of the neo-choana were smoothed and the bone surface was covered with mucosa. All patients were treated with anti-reflux therapy postoperatively, and also antibiotics for 5 to 7 days (ampicillin+sulbactam 50 mg/kg/day). For 7 days, patients were fed through an orogastric probe. In addition, local postoperative treatment with nasal humidification and Daily suction was performed.

Results

The study included 58 patient with congenital CA. Patient characteristics are detailed in Table I. All cases were repaired via endoscopic approach, and the mean follow-up time after surgery was 51 months (ranging from 6 months to 8 years). As a support for the intranasal neo-choana, 10 (17%) patients underwent placement of a silicone stent (intubation tube, number of 3-4) for 1 to 4 weeks. Topical mitomycin C (0.4 mg/ml, for all rough surfaces, without using a stent) was used in 8 (14%) patients at the time of when neo-choana was created. Of participants Transnasal endoscopic repair only was performed on 69% (40), Transnasal endoscopic repair and Stenting was performed on 17% (10) and Transnasal endoscopic repair using Mitomycin C was performed on 14% (8). The effectivity for surgery was determined by the absence of respiratory symptoms and patent choanae on endoscopic examination and no need for revision. A total of 17 (29%) patients required postoperative revision, from 1 to 6 times. Revision surgery needs according to CA type and surgical correction type are detailed in Table II and III.

Discussion

CA is the most common congenital nasal anomaly. It is characterised by the obliteration of the posterior nasal opening, due to the imperforation of the oronasal membrane. The safety and efficacy of endoscopic repair of this imperforation has been well demonstrated.[3,4,10] Postoperative formation of scar tissue, synechia and granulation tissue are commonly reported and all may be reasons for recurrent nasal obstruction. The use of adjuvant stenting and mitomycin C has been adopted over the years in attempts to minimize these problems. Despite that, there is a controversy regarding the usefulness of placing stents, application of adjuvant mitomycin C.[4,9,11]

Various possible techniques and surgical approaches were used to treat CA but currently two surgical approaches are mainly used: transnasal and transpalatal. The complications that can be seen as a result of the transpalatal approach are unusual but include, palatal flap breakdown or fistula, high arched palate deformity and need for orthodontic treatment, blood loss requiring transfusion.[12] The main goal of surgery include establishing a normal nasal passage, and additionally it is essential to prevent further damage to the growing structures.[13] By using transnasal endoscopic surgery approach, these goals can be achieved. Surgical repair with transnasal endoscopic technique provides an excellent visualisation of the posterior nasal defect and because of its safety and effectivity, transnasal endoscopic surgery has currently become the procedure of choice.[14] Teissier et al.[10] described the transnasal endoscopic repair as a well-tolerated technique, and despite of its learning curve, they concluded that it was a successfull treatment technique.

Table I. Patient characteristics.

Patient characteristics	n (%)
Gender	
Male	17 (29%)
Female	41 (71%)
Unilateral CA	34 (59%)
Right side	14 (24%)
Left side	20 (35%)
Bilateral CA	24 (41%)
Type of atresia	
Mixed	29 (50%)
Membranous	16 (28%)
Bony	13 (22%)

CA: choanal atresia

Table II. Classification of revision surgery needs.

Revision surgery performed for	Number of revision surgery/Total number of defined patients (%)
Patients with unilateral CA	5/34 (15%)
Patients with bilateral CA	12/24 (50%)
Patients with bony CA	4/13 (30%)
Patients with membranous CA	4/16 (25%)
Patients with mixed CA	9/29 (31%)
Total patients	17/58 (29%)

CA: choanal atresia

Table III. Revision need, after surgery classified according to type of management.

Surgical details for revision surgery	Number of revision surgery/Total number of defined patients (%)
Revision needed after transnasal endoscopic repair+ stenting	7/10 (70%)
Revision needed after transnasal endoscopic repair+ using Mitomycin C	4/8 (50%)
Revision needed after transnasal endoscopic repair only	6/40 (15%)
Total revision patients	17/58 (29%)

José Luis Llorente et al.[4] published their surgical results of 10 patients. Seven cases (70%) with a bilateral CA and, 3 cases (30%) with unilateral CA had been operated. They used transnasal endoscopic surgical repair in all cases. Neither stents nor topical mitomycin were used in any case. After a mean follow-up of 27 months, no restenosis were detected in any of the cases and no further surgery or dilatations were required, so they gave a 100% success rate with endoscopic repair.

Traditionally, the use of stents has been a part of the postoperative management of CA repair for maintaining the patency for newly formed choana. The use of stents for CA repair is very controversial in the relating literature. Some reports have found that stenting is associated with an increased incidence of restenosis up to 50-70%.[15] Stents may induce a foreign body reaction, and be an infectious nidus, which will trigger an inflammatory response. As a result of inflammation, it may lead to granulation tissue formation, and eventually restenosis.[9] In our study, patients whom we inserted stents had higher incidence of restenosis 7/10 (70%) than those who had no stent with a restenosis rate of 6/40 (15%). More than half of the patients that used a stent needed revision, therefore stent usage was not seen successful for the correction of CA, and that was attributed to continuous inflammation caused by stents, the greater formation of granulation tissue and chronic inflammation resulting in fibrosis and restenosis.

Mitomycin C is a topical aminoglycoside antibiotic that is thought to reduce scar and granulation tissue formation by the inhibition of fibroblast growth and migration.[3] The use of mitomycin C, proposed reducing the scar formation and improvement in healing, and it had been used as an additional therapy for CA repair. The use of mitomycin in the larynx and trachea, improves the outcomes, but its efficacy in patients with CA, in preventing restenosis has been variable.[11,16,17] Holland et al.[17] found an improvement in the outcome and increase in re-stenosis with the use of mitomycin, with a small number of patients. Following Holland et al.[17], Prasad et al.[18] also proposed of a lower incidence of re-stenosis, following these reports the use of mitomycin C gained an increasing acceptance. Kubba et al.[5] and Al-Ammar et

al.[19] did not find any significant difference in the outcome between patients who received mitomycin C treatment and those who did not. Likewise, when assesing the long term success of mitomycin, Teissier et al.[10] showed non persuasive effect. In our study, 50% (n:4/8) of those who had mitomycin C at primary surgery had at least one restenosis requiring revision surgery. Comparison between the efficacy of adjacent modality addited to surgery showed that Mitomiycin C was more effective than stent usage in our experience, but the role of topical mitomycin C was not associated with any significant improvment either.

By our experience, endoscopic microsurgical repair of atresia proved to be an effective and safe procedure, results compared with adjuvant treatment modalities like stent or mitomycin C use, was not better. Restenosis was the major problem seen after surgical correction.

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Case Report

Acute hemorrhagic edema of infancy – is it really a mild, benign disease?

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ABSTRACT

Background. Acute hemorrhagic edema of infancy (AHEI) is a rare vasculitis, which presents dramatically as palpable purpuric skin lesions on the limb, face and auricles, with swelling of these parts and low-grade fever, in children up to 2 years of age. To date, approximately 400 cases have been described in the literature. The etiology remains mostly unknown. With or without treatment, AHEI goes to spontaneous recovery within 1-3 weeks, usually without any complications. To our knowledge, compartment syndrome as complication of AHEI has only been reported in one case. We present an unusual case of AHEI with serious complications due to compartment syndrome of the right-hand fingers.

Case. A 16–month–old male child presented with fever and sudden appearance and rapid spread of palpable, painless, non–itching ecchymotic hematomas on the thigh, cheeks, earlobes, forearms, dorsum of hands and feet, with mild edema of these regions. Complete systemic examination and all vital parameters were normal for age. There was no history of bleeding disorders in the family. Except low hemoglobin on complete blood count and increased D-dimer values, all other laboratory investigations were in the normal range.

Changes on the right forearm and hand expanded on almost the entire dorsal side and all surfaces of the fingers, with pronounced swelling and formation of bullous lesions, which were spreading and cracking. Skin biopsy confirmed nonspecific small–vessel vasculitis. That required the use of Methylprednisolone, low-molecular-weight heparin, antibiotics and debridement of necrotic eschar, with necrectomy of the affected fingers.

Conclusions. Early recognition of AHEI is important to avoid unnecessary investigation and therapy. On the other hand, our reported case warns that unexpected complications may occur.

Key words: acute hemorrhagic edema, infancy, compartment syndrome.

Acute hemorrhagic edema of infancy (AHEI) is a rare form of hypersensitivity reaction which occurs in children up to 2 years of age.[1,2] It presents as sudden non–itching, painless localized palpable purpuric targetoid skin lesions of the extremities, face and auricles, followed by limb and facial swelling and low–grade fever. Children are usually minimally affected.[3] AHEI is vasculitis of small dermal vessels, characterized by inflammation with fibrinoid necrosis and extravasation of red blood cells.[2,4] The etiology is mostly unknown.[4-6] In the majority of patients, laboratory analyses are within normal values.[4,7] Treatment with antihistamines and/or corticosteroids have been reported, but is still controversial.[3-5,7-9] According to most authors, this is unnecessary due to its spontaneous recovery within 1-3 weeks, usually without any complications.[4,7,8,10] Less than 10% of patients have extra cutaneous manifestations.[7,10-15]

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Our case of AHEI had serious complications due to compartment syndrome of the right-hand and fingers which formed necrotic eschar on affected parts of the right forearm. To the best of our knowledge, so far, only one case of AHEI and compartment syndrome has been described.[16] Our patient required application of the low-molecular-weight heparin and debridement of necrotic parts. Unfortunately, necrotomy of the affected fingers had to be performed.

Case Report

A 16-month-old male child was admitted to the Pediatric Clinic on the second day of his complaints, with a history of fever (38.2°C) and sudden appearance of ecchymotic purpura on both sides of the thighs and tiny petechial changes on the earlobes. Described lesions spread rapidly, with the appearance of new similar changes on the cheeks, lower legs and forearms, as well as on the dorsum of the hands and feet, with mild edema of these regions and all fingers. Some changes appeared as hematomas, with dimension between 0.5 and 10 cm in diameter, and individual changes as palpable, painless, non–itching, clearly limited lesions, of similar dimensions, with necrotic parts in the center and purple periphery (Fig. 1 and 2). Swelling of the left earlobe was also noticed, as well as tiny petechial changes on buccal mucosa, which were not extensive and did not disturb feeding. In the primary care, the patient was treated only with Paracetamol.

On admission, except for the described symptoms, complete systemic examination was normal and all vital parameters were stable. Otherwise, the child was delivered from regular full term pregnancy, with

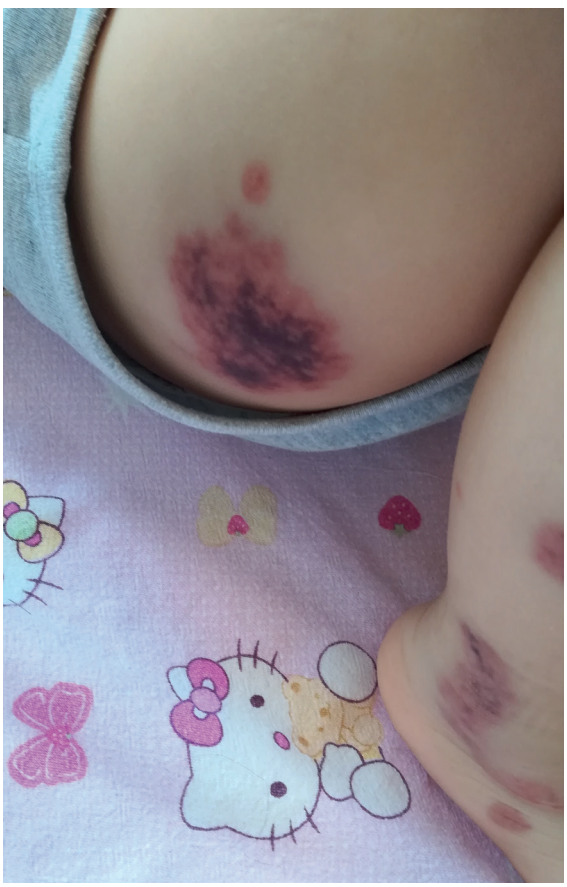

Fig. 1 and 2. Hematomas and ecchymotic purpuric skin lesions on different parts of the body.

normal psychomotor development, and was age appropriately vaccinated (the last vaccine against Measles, Mumps, and Rubella was three months prior). He was circumcised two months before admission. He had also suffered from viral gingivostomatitis 20 days before the onset of the current disease. Buccal mucosa was treated with symptomatic local therapy only and fully recovered within 10 days. For the past four months, anemia had been treated with iron supplementation. There was no history of bleeding disorders in the family.

Initial laboratory investigations showed anemia on complete blood count (CBC) (hemoglobin 88 g/L, ferritin 6 g/L, serum iron 2.0 μmol/L), with normal number of erythrocytes, platelets and leucocytes. The patient had normal blood coagulation screening (activated partial thromboplastin time (aPTT), prothrombin time (PT) and fibrinogen), markers of inflammation (C–reactive protein and procalcitonin), erythrocyte sedimentation rate, liver and kidney function tests, electrolytes, serum proteins and albumins, values of immunoglobulins and complements and urine analysis. The only parameter that was beyond the reference values was D–dimer (7.21 ng/ml) (Table I). Also, urine and blood cultures, virology analyses (Epstein Barr, Cytomegalovirus, Herpes viruses, Adenovirus, Parvo B19, Hepatitis B and C, HIV) and Immunoglobin M (IgM) on Mycoplasma pneumonia were all negative. Tests for congenital (protein C and S, antithrombin III (AT III), activated protein C resistance V (APCR-V) assay, homocysteine – Table I) and acquired thrombophilia (lupus anticoagulant, anticardiolipin and beta-2-glycoprotein I antibodies), hormones and antibodies of the thyroid gland, antinuclear antibodies, anti-streptolysin O, anti-myeloperoxidase, anti-proteinase 3 antibodies and rheumatoid factor were within the normal range. Ultrasound examinations of the abdomen excluded visceral involvement.

After rapid assessment and exclusion of other clinical conditions, we started treatment with Methylprednisolone 1mg/kg intravenous (iv) once daily. However, on the second day of hospitalization, changes on the right forearm expanded and merged with each other, making palpable, hemorrhagic, edematous plaques in different phases, on almost two thirds of the dorsal side, with pronounced swelling of dorsum of the right hand and fingers (Fig. 3). Doppler examination showed that there were regular flows through the blood vessels of the right forearm. There was no intravenous access in that arm.

In the next following days, these changes spread along the entire dorsal side of the forearm and hand, predominantly along the radial side, giving the impression of necrotic tissue, with more pronounced swelling, and with changes on the whole surface of fingers. Also, small bullous changes appeared on necrotic parts (Fig. 4). Repeated Doppler tests discovered weaker to absent vessels` flow in apical parts from the second to fourth finger, without any evidence of thrombus. All laboratory tests were repeated. CBC showed anemia (hemoglobin 58 g/L), with a decreased number of erythrocytes ($2.51x10^{12}$/L) and hematocrit (21%), which required packed red blood cell transfusion. The value of D–dimer increased up to 16.34 ng/ml. All other repeated laboratory tests were within normal range.

Low-molecular-weight heparin was initiated at a dose of 100 IU/kg iv two times daily and the

Table I. Most significant laboratory parameters and comparison of patient's values along with normal values.

Laboratory parameters	aPTT (s)	PT (s)	Fibrinogen (g/L)	D-dimer (ng/ml)	Protein C (U/mL)	Protein S (U/mL)	AT III (%)	(APCR-V)
Normal values	25–35	11–13	2.0–4.0	≥ 0.5	0.72–1.23	0.60–1.60	80–120	≥ 2.3
Patients values (first / repeated)	31.6 / 32.1	12.4 / 11.9	4.237 / 3.926	7.21 / 16.34 / 2.89 / 0.97	1.09 / 1.13	1.26 / 1.04	94.7 / 112.1	2.7 / 3.1

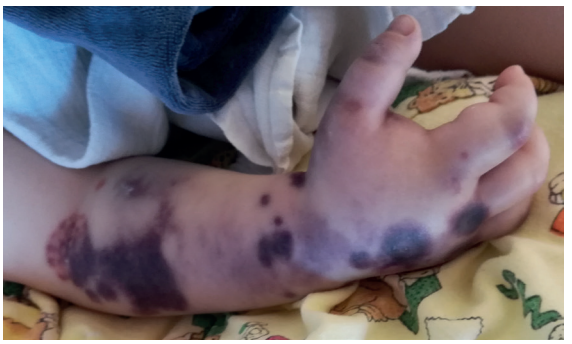

Fig. 3. Palpable, hemorrhagic, edematous changes in different phases on dorsal side of the right forearm and swelling of dorsum of the right hand and fingers.

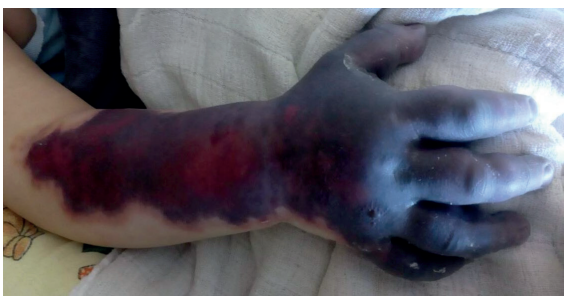

Fig. 4. Hemorrhagic changes on entire dorsal side of the right forearm, hand and whole surface of fingers, with pronounced swelling, and small bullas and necrotic parts.

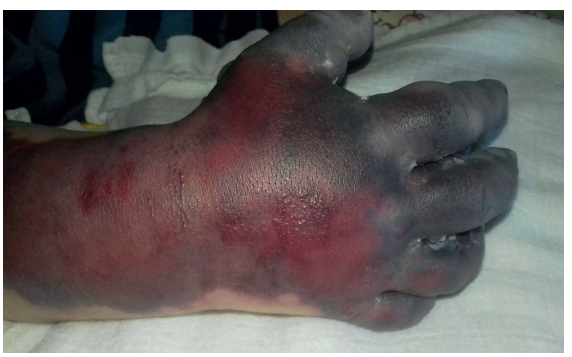

Fig. 5. Macerated bullous changes between fingers, with compartment syndrome and suspect necrosis of the proximal parts of the second to fourth finger.

dose of Methylprednisolone was increased to 1mg/kg iv three times daily. Therapeutic option of pulsed doses of Methylprednisolone 20mg/kg daily for 4 days was presented to the parents, which they refused. During hospitalization, the anti-factor Xa assays were in the therapeutic range. Also, a skin biopsy was done, and later finding confirmed nonspecific small-vessel vasculitis, with fibrin deposits in the vascular wall and perivascular infiltrates composed of neutrophils and eosinophils, with erythrocyte extravasation, again without any evidence of thrombus. On direct immunofluorescence testing, nonspecific vascular deposits of different immunoglobulins were found.

During the illness, the child was in a good general condition, afebrile, with normal laboratory analyses, after transfusion maintaining hemoglobin values around 100 g/L, with gradual decline of D–dimer values. All other skin changes regressed, as well as changes in the oral cavity, but swelling of the right hand and fingers increased, with rising and cracking of necrotic bullous lesions. We added Ceftriaxone 70 mg/kg iv once daily to his therapy. Bullous changes between fingers occasionally macerated in close contact, movements of fingers were difficult, and that lead to the compartment syndrome with the consequent suspect necrosis of the proximal parts of the second to fourth finger (Fig. 5). On the tenth day of hospitalization, incision of bullous changes and decompression was performed, along with the necrectomy of the surface parts of the skin. All swabs taken from the wounds were sterile.

The ongoing therapy was continued, Methylprednisolone 1mg/kg iv three times daily and low–molecular–weight heparin 100 IU/kg iv two times daily, with the daily wound dressing. Distal parts of the second and third finger remained necrotic and dried eschar was formed on the dorsal side of right forearm and hand (Fig. 6). Therefore, on the 15th day of hospitalization debridement of necrotic tissue and amputation of the distal phalanges of the second and third finger was performed. After that, the child had two more operations with debridement of the remaining necrotic tissue from the forearm and hand and amputation of the middle phalanges of the second and third fingers. Afterwards, skin was transplanted from the right thigh and covered all vulnerable surfaces (Fig. 7). On regular controls, the child

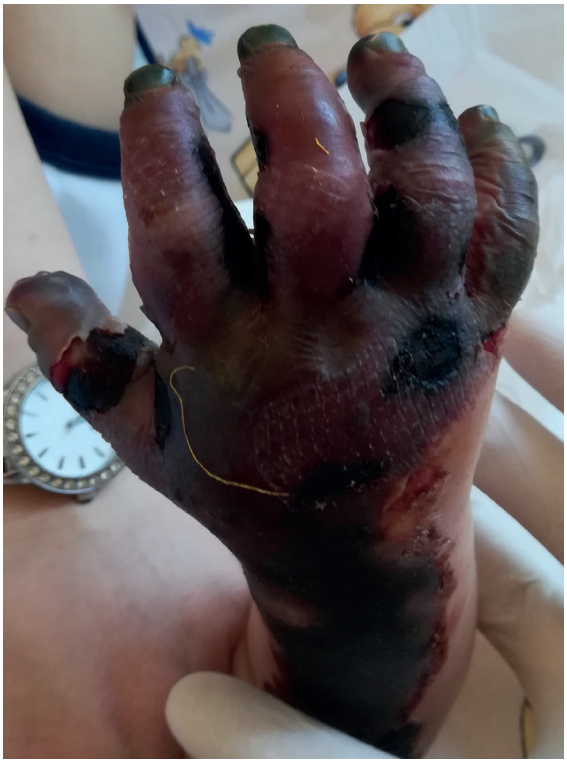

Fig. 6. Necrotic distal parts of the second and third finger and dried eschar on the dorsal side of right forearm and hand.

had no other skin changes, with adequate healing of the wound and the acceptance of the transplant after the first six months follow–up period.

Written informed consent was obtained from the parents of the children.

Discussion

AHEI was first described in 1913 by Snow[17], later also named Finkelstein–Seidlmayer disease. Initially it was considered a variant of Henoch-Schönlein purpura, later however it was established that it represents a separate clinical entity.[1,2] There are no reported data on the incidence, probably because of its rarity and confusion with other more common conditions. In 2008, a systematic review by Fiore et al.[8] reported approximately 300 patients with AHEI, with male predominance (2:1 ratio). A review of literature by authors showed that the number of described cases increased to approximately 400

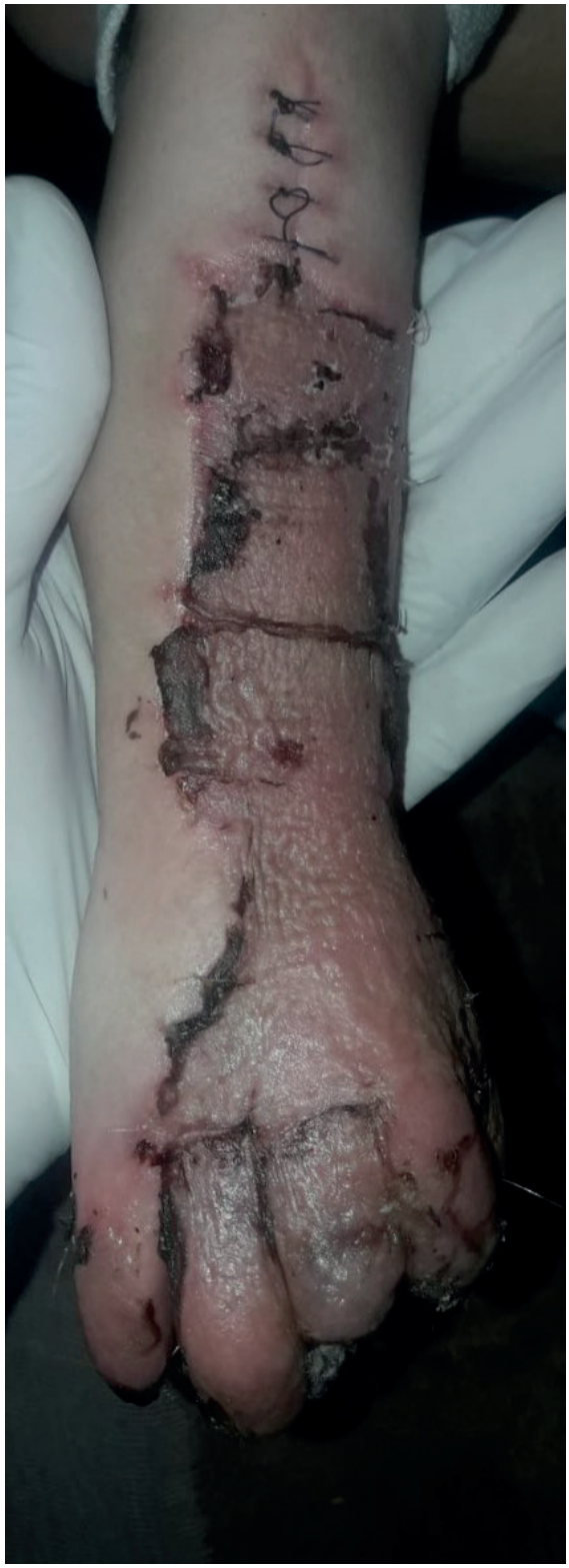

Fig. 7. Amputated distal and middle phalanges of the second and third finger and transplanted skin on the all vulnerable surfaces of the right forearm and hand.

in the following years, probably due to better knowledge of the disease.

All authors recognized AHEI as a condition with very dramatic and impressive clinical presentation.[2,4,7,8,10] Children have a prodromal illness, which is followed by the onset of palpable ecchymotic skin lesions (cockade purpura), followed by swelling.[1-5,7,8] Lesions have a symmetrical distribution. Edema is classically asymmetrical, affecting the auricles, face and extremities (frequently dorsum of hands and feet).[1-4,7-10] Some areas may even become necrotic or bullous.[18] All described cases were in good general condition. Compared with our patient, the onset of the disease clinically corresponded completely to the classic form.

AHEI has been associated with viral or bacterial infections, vaccination and drug intake.[5-8,19] It occurs more during winter, which supports infectious agents as a triggering factor, according to Chesser et al.[19] in 75% of cases. Another 10% of cases were connected with immunization.[6] We assume that the virus that caused gingivostomatitis was responsible for initiating the immune process in our patient, but all virology examinations were negative.

The diagnosis of AHEI is initially made on clinical basis and can be made without a skin biopsy.[1,2] However, a lot of diseases with similar dermatologic manifestations with potentially severe clinical presentations must be promptly differentiated (meningococcemia, skin lesions in septicemia, drug eruptions, Henoch-Schönlein purpura, Coxsackie disease, Sweet syndrome, erythema multiforme, angioedema, child abuse, idiopathic thrombocytopenia and allergies).[1-3] The onset of our case was like AHEI, but in the following days, the patient's clinical picture was very similar to purpura fulminans. Unlike purpure fulminans, our patient did not develop disseminated intravascular coagulopathy, given normal fibrinogen and platelet counts over the course of the disease, also protein C and S values were normal, and skin biopsy did not establish the presence of thrombus in the tiny blood vessels.[20]

If performed skin biopsy shows leukocytoclastic vasculitis of small dermal blood vessels.[1,2,5] According to the majority of authors, biopsy of the skin should be performed only in case of an unclear diagnosis, progression of the disease or appearance of complications.[1,2,7,8] Similar opinion is also accepted for the introduction of therapy, because of the benign, self–limiting course of the disease.[3-10] Risikesan et al.[9] considered that rapid progression could nevertheless be an indication for steroid therapy, as we did in our case, but cited authors gave pulsed doses of Methylprednisolone 20mg/kg daily for 4 days. With regards to our case, this was presented to the parents as a therapeutic option, which they refused, so we had to continue with 1mg/kg 3 times daily, which was obviously a mistake and did not gave a satisfactory result. According to the medical practices in Serbia, the parents, whose child is hospitalized, sign the consent to implement diagnostic procedures and they are informed about the initial therapeutic protocols. However, according to the law, they are also must be informed about any changes in the therapy during hospitalization and possible side effects. In doing so, they have the legal right to simply reject the proposed therapeutic procedure. In aforementioned case, the parents and both assigned doctors, hematologists and plastic surgeons frequently discussed the case, but parents thought that pulsed doses of Methylprednisolone would be too harmful, because they educated themselves about the benign nature of the disease. In cases with extensive bullous lesions, secondary infection of ruptured bullas must be treated locally and with systemic antibiotics. However, Lai-Cheong et al.[18] advise that systemic antibiotics should nonetheless be given to combat the infection and to stop further antigenic stimulation.

In the majority of described cases, laboratory tests are generally within normal limits. We noticed that the value of D-dimer increases with the progression of AHEI, but within normal values of blood coagulation screening, fibrinogen and platelets. High activity of coagulation and fibrinolysis is associated with

active disease of Henoch-Schönlein purpura and/or ANCA-Associated Vasculitis.[21,22] The underlying mechanism is not fully clear yet. The production of the proinflammatory cytokines in active vasculitis, such as Tumor necrosis factor (TNFα), interleukin-1 or C5a, may trigger thrombotic processes by increased expression of tissue factor on endothelial cell and/or neutrophils. Furthermore, markers of endothelial cell damage and apoptotic endothelial cells have been shown to become procoagulant.[21,22] In the mentioned studies, the D–dimer values in the active phase of vasculitis did not increase as much as in our case. On the other hand, a study by Chao-Hung[23] showed that even a very high D–dimer value was not necessarily the exclusive marker of thromboembolic disease. As there was no evidence of thrombosis in our patient, the question remains why D–dimer values were high during disease progression and whether it could serve as some kind of prognostic parameter.

The fact is that the pathophysiological mechanism of AHEI is a release of erythrocytes through damaged blood vessels and that our patient was anemic at the onset of AHEI, however we were surprised by the drastic drop in values of hemoglobin and number of erythrocytes during deterioration of local findings on the right hand.

Very rare complications have been reported including intussusception, hematuria, proteinuria, hypocomplementemia and scarring.[11-15] Other systemic symptoms such as abdominal pain, gastrointestinal bleeding, arthritis and nephritis were rarely reported as well. Severe articular involvement was described in one case, as well as testicular torsion.[7,8,10] Mucosal involvement is rare, conjunctiva, palate, buccal or/and nasal mucosa were mostly affected.[11] Only one case of compartment syndrome of the left foot has been described so far. Following the fasciotomy, there was marked clinical improvement in the limb perfusion.[16] In our case, disease progression on the right forearm and hand was surprisingly rapid, despite the therapy initially given. Bullous changes between fingers made the compartment syndrome with the consequent necrosis of the proximal parts. Apart from the application of low-molecular-weight heparin, incision of bullas for decompression, debridement of necrotic parts and amputation of the affected fingers had to be performed. After skin transplantation and clinical improvement, both active and passive movements of the right arm and fingers were intact.

Although AHEI is a rare entity, pediatricians should be familiar with its presentation. Early recognition of AHEI is important in order to avoid unnecessary or invasive medical investigation and therapy, because of its benign nature. On the other hand, our reported case warns that unexpected complications may occur. The treatment with the corticosteroids, low–molecular-weight heparin and antibiotics in the case of extensive lesions should be considered. The D-dimer values may be used to monitor disease progression.

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Case Report

Complicated pneumonia due to exogenous toxic substances in children

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ABSTRACT

Background. Toxic substances may be inhaled or aspirated not always by patients themselves but sometimes by healthcare providers unintentionally by nasogastric administrations or medications. Aspirated toxic particles may cause injury in the pulmonary epithelium at various levels of the respiratory tract, leading to a spectrum varying from simple symptoms to severe chronic disease. Imaging features of substances are different depending on their structure and are not well known. Moreover, on follow-up, radiologic findings are not well correlated with clinical findings which progress or recover more rapidly.

Case. In this case series, we report three different chemical pneumonitis due to aspiration of exogenous substances of paint thinner, activated charcoal and liquid paraffin. We present three cases with chest radiographs, distinctive computed tomography findings and follow-up images.

Conclusions. Aspiration of exogenous materials reveals typical imaging findings such as the metallic density of charcoals, fat attenuation of liquid paraffin and necrotic areas within consolidation in hydrocarbon poisoning. Recognition of imaging findings is very important for the diagnosis and characterization of toxic substances.

Key words: chemical pneumonia, lipoid pneumonia, activated charcoal aspiration, computed tomography, bronchoscopy.

Chemical pneumonia occurs due to the aspiration of exogenous toxic substances such as volatile hydrocarbons, milk or different kinds of oils that are toxic for the lungs.[1] Clinical suspicion, detailed information from the family and comprehensive evaluation of the history of the patient are crucial for the diagnosis in order to find out the agent responsible for chemical pneumonia in children.[2] Like the other forms of aspiration pneumonia, lower lobe infiltrates on chest x-ray are important findings of chemical pneumonia along with a sudden onset of dyspnea and coughing.[2] Iatrogenic reasons, such as prescription of mineral oil or liquid paraffin, administration of activated charcoal for drug intoxication may also induce chemical pneumonia.[3] Herein, we present three pediatric patients with aspiration of different exogenous toxic agents and distinctive imaging features along with follow-up and the review of the literature.

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Case 1

A 12-year-old girl with a known diagnosis of mucopolysaccharidosis type 3 was admitted to the emergency department due to ingestion of paint thinner that was noticed by her mother. Gastric lavage was performed immediately, control laboratory findings were normal and she was discharged with a prescription for proton pump inhibitors. Four days later, she presented to the emergency department with fever and malaise. Physical examination revealed bilateral coarse crackles and decreased breath sounds on the left side. Her fever was 39°C and her oxygen

saturation was 80%. There was an infiltration of the left lower lobe and pleural effusion on her chest x-ray (Fig. 1A). The clavicles were short and broad; the ribs were tapered proximally and widened distally consistent with mucopolysaccharidosis. There was also thoracic dextroscoliosis. Her pleural effusion was also confirmed by ultrasound. Sulbactam ampicillin treatment was started with the diagnosis of chemical pneumonitis. However, her clinical findings did not resolve as expected and CT examination was performed. The contrast enhanced CT revealed consolidation of the left lower lobe. Consolidation including multiple nodular hypodense areas were subtle on the parenchymal window of chest CT (Fig. 1B) but more conspicuous on the mediastinal window (Fig. 1C) that did not enhance probably due to necrosis. Pleural effusion was also noted. CT findings were consistent with necrotizing pneumonia due to aspiration of paint thinner. Her treatment was shifted to vancomycin, cefotaxime, and salbutamol, thereafter oxygen saturation normalized, clinical findings resolved and chest x-ray findings regressed in comparison with the initial x-ray (Fig. 1D).

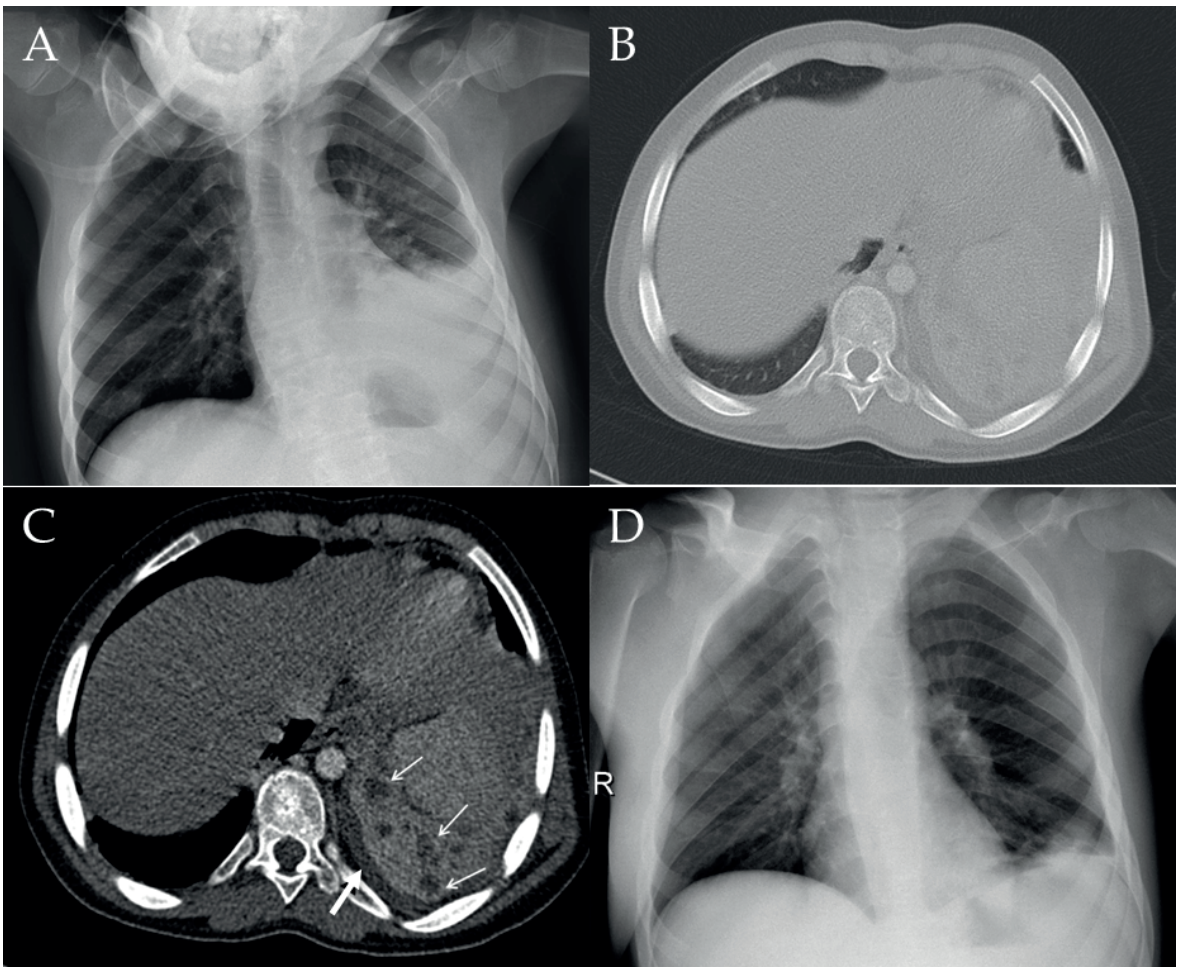

Fig. 1. (A) Chest x-ray represents left lower lobe infiltration and left pleural effusion. The clavicles are short and broad. The ribs are tapered proximally and widened distally consistent with mucopolysaccharidosis. There is also thoracic dextroscoliosis. Axial contrast enhanced CT images on parenchymal window **(B)** and mediastinal window **(C)** demonstrate left lower lobe consolidation along with left pleural effusion (thick arrow). Hypodense nodular areas within consolidation are more conspicuous (arrows) on mediastinal window. **(D)** Follow-up chest x-ray 11 days following treatment reveals regression of the effusion and parenchymal findings.

After a course of two weeks treatment, she was discharged without any complications.

Case 2

A 3-years and 8-month-old boy was admitted to another institution due to ingested overdose of an unknown drug. Activated charcoal was given by nasogastric tube. The distal tip of the nasogastric tube was placed into the stomach; however, he aspirated a small amount of the activated charcoal due to gastroesophageal reflux. His status deteriorated suddenly and he subsequently required intubation and hospitalized in the intensive care unit for 15 days with the diagnosis of charcoal aspiration. Bronchoalveolar lavage was performed in order to remove the charcoal particles. He was then referred to our pediatric pulmonology clinic for bronchoscopic evaluation. Upon his admission to our hospital, he had a dry cough and his sputum contained charcoal particles. Chest x-ray revealed patchy infiltration of the right lower lobe consistent with charcoal aspiration (Fig. 2A). Chest CT showed multiple tiny nodules forming tree-in-bud appearance at the upper and middle lobes of the right lung and the lingula of the left lung (Fig. 2B). Hyperdense nodules in the right lower lobe revealed significant metallic artifacts due to charcoal

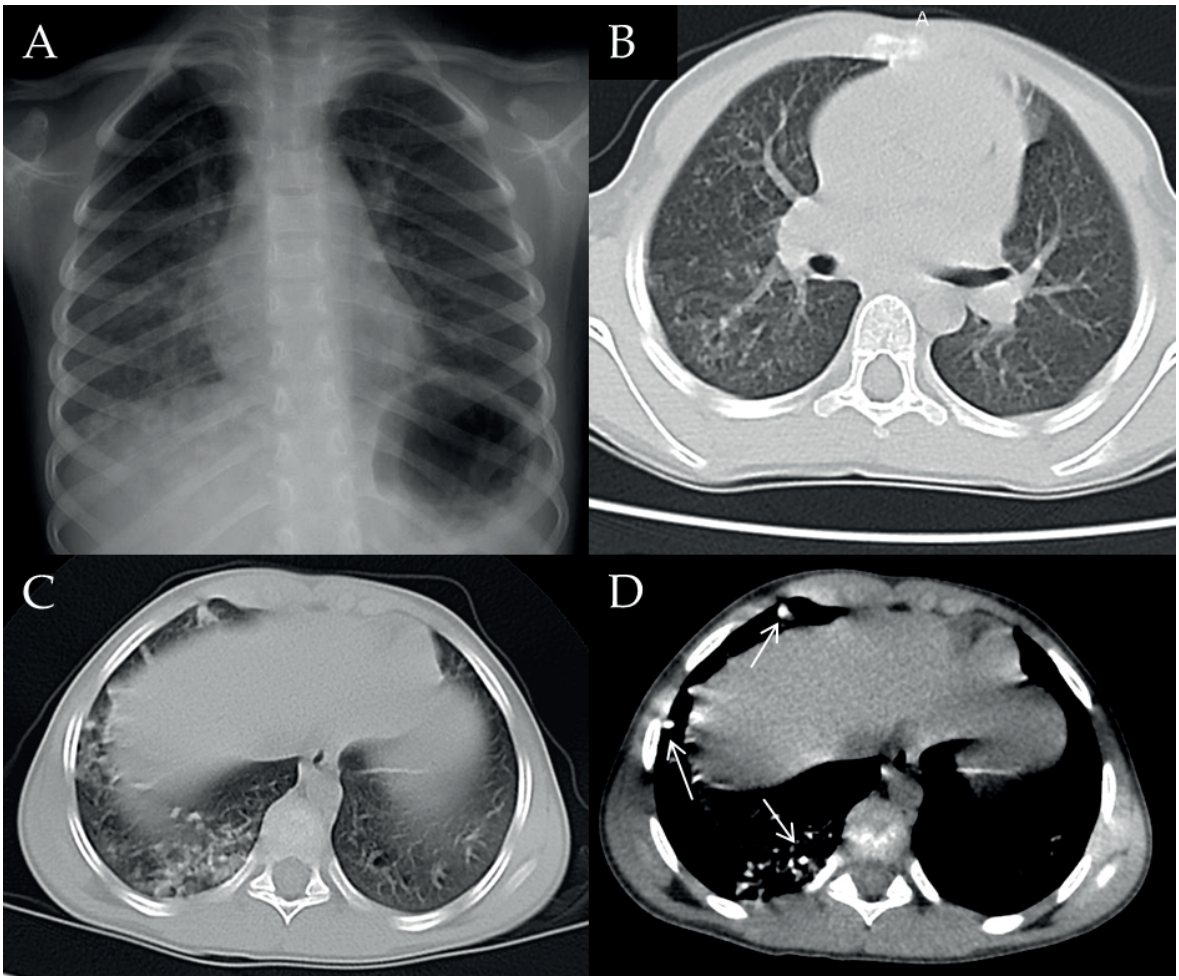

Fig. 2. (A) Chest x-ray shows patchy nodular infiltration of the right lower zone. **(B)** Axial CT with lung parenchymal window shows `tree in bud` pattern nodular opacities in right upper and middle lobes. Parenchymal **(C)** and mediastinal **(D)** window of chest CT at the more caudal section demonstrates metallic densities (arrows) that are consistent with activated charcoal particles at the lower lobe.

within the nodules that were more prominent on the mediastinal window (Fig. 2C, D). Bronchoalveolar lavage was performed twice at our center to remove remaining charcoal particles with 200 cc saline for each time. After the second lung lavage, charcoal particles were not observed in the sputum anymore. His follow-up chest x-ray performed 3 months later demonstrated nodular opacities at the right lower zone similar to previous imaging with a mild decrease in size and number.

Case 3

A 7-year-old girl with a history of developmental delay and a chromosomal unstable translocation (46XX der (12) t(3:12) (q26.3;q24.3)) had a persistent cough for about 3 years and had recurrent lower respiratory tract infections when she presented to the pediatric pulmonology division. She was operated on twice firstly due to anal atresia during the newborn period and secondly due to an inguinal hernia at 5 years of age. She was hospitalized two times for lower respiratory tract infections and used oral antibiotics and inhaled salbutamol for several months. Chest x-ray revealed right middle lobe, right lower lobe, and left lower lobe consolidations (Fig. 3A). She received antibiotic therapy. Chest CT examination revealed that the left lower lobe consolidation and patchy hypodense areas in the consolidation (Fig. 3B,C). There was also patchy consolidation of the right lower lobe.

In the meantime, her mother expressed that for a long time she had been giving her daughter liquid paraffin for chronic constipation. Patients history, excluded infectious and other lung pathologies and CT findings were consistent with lipoid pneumonia due to chronic aspiration of the liquid paraffin. Her bronchoscopy evaluation resulted 70% lipid-laden macrophage which supported the diagnosis.

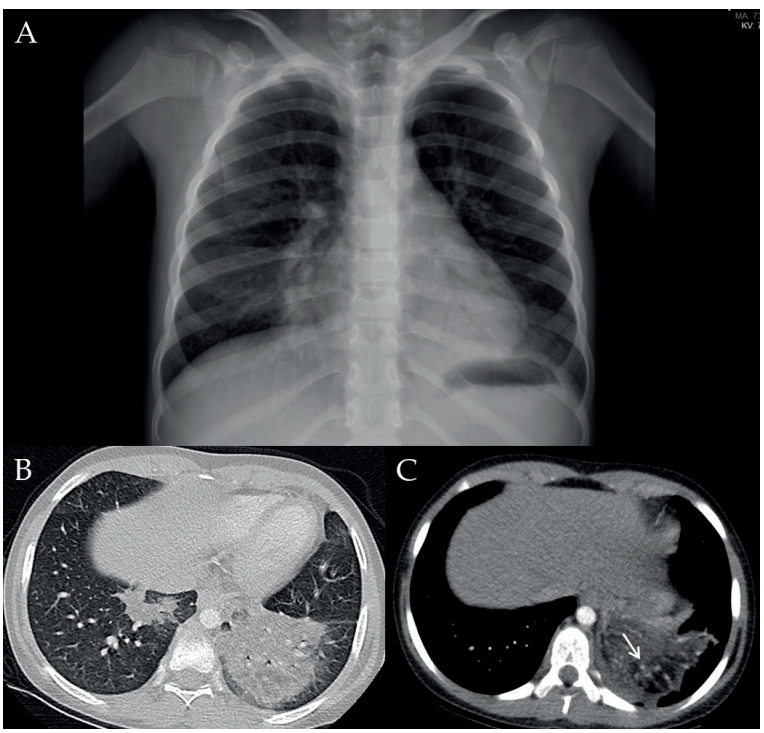

Fig. 3. (A) Chest x-ray demonstrates consolidation of left lower lobe, right lower lobe and right middle lobe. **(B)** Axial chest CT image on parenchymal window demonstrates right lower lobe and left lower lobe consolidation. **(C)** On mediastinal window; fat attenuated areas (arrows) within consolidation are more conspicuous consistent with lipoid pneumonia.

Informed consent and permission were obtained from the parents regarding the publication of these cases.

Discussion

Paint thinner is a widely available and cheap hydrocarbon product that was commonly abused by homeless children.[2] On the other hand, careless usage of toxic substances at home, keeping them in the bottle of fruit juice or beverages may facilitate accidental drinking by children.[1] The most common complication of hydrocarbon intoxication is chemical pneumonia.[1] However, the prevalence of severe chemical pneumonia after hydrocarbon poisoning is less than 2%.[2] Destruction of bronchial and alveolar cells results in interstitial inflammation, intraalveolar hemorrhage and edema, hyperemia, bronchial necrosis, and vascular necrosis.[4] Imaging features of hydrocarbon aspiration include unilateral or bilateral lung consolidation, well-defined nodules, pneumatoceles (well-defined cavitary nodules), pleural effusion, and spontaneous pneumothorax.[1] Chemical pneumonia due to inhalation of hydrocarbon products is described as "fire eater`s pneumonia" in various articles in the literature.[5] Apart from pleural effusion and lower lobe consolidation, low attenuating nodular areas, probably consistent with necrosis, is seen in some cases of the adult population similar to our case.[5] Radiological regression is slower than clinical recovery that may persist up to 8 months.[2] The main objective of the treatment of hydrocarbon poisoning is to reduce the symptoms.[2] Medical therapy with antibiotics and steroids related to hydrocarbon poisoning is still controversial.[1] Since the majority of patients have a benign clinical course, steroids do not seem to offer any additional benefit.[6] Likewise, in our first patient, we used only antibiotics without steroid treatment. In that patient, control CT was not performed in order to avoid radiation exposure; on her follow-up chest radiographs, there was regression of nodular opacities and pleural effusion.

Activated charcoal is frequently used in pediatric patients with toxic ingestion and the most common complication is vomiting.[7] Pulmonary aspiration of charcoal is a rare complication with a few case reports in the literature.[7] A majority of the aspiration mechanism is due to aspiration of gastric content including charcoal particles while vomiting, rather than from direct aspiration which may occur by the insertion of the nasogastric tube into the trachea mistakenly.[8] The incidence of the nasogastric tube placement into the trachea or the distal airways ranges from 0.3% to 15%.[9] Thus, the position of the tube should be verified with an x-ray before the administration of activated charcoal. Graff et al.[7] reported a case with `tree in bud` nodular opacities in the lower lobes in a patient with direct activated charcoal aspiration. The reason for this appearance is severe bronchiolar impaction with obliteration of the distal bronchioles and contiguous branches by charcoal particles.[8] In our second case, the same pattern was revealed on the chest CT in the right lower and middle lobe. Furthermore, chest CT demonstrated metallic artifacts occurred by punctate hyperdense charcoal particles within the nodules. Another complication related to the aspiration of activated charcoal into the airways is pleural involvement. Pleural effusion containing charcoal particles and pneumothorax associated with bronchopleural fistula may be seen if the nasogastric tube is introduced into the distal airways.[9] Treatment of aspiration is a common problem encountered in pediatric emergency rooms and requires repeated lung lavage and long-term follow-up.[7]

Exogenous lipoid pneumonia occurs in patients with a history of chronic laxative ingestion, and use of oil-based nose drops. The presentation of the clinical scenario could be either acute or chronic.[10] Acute exogenous lipoid pneumonia is uncommon and a majority of is caused by aspiration of a large quantity of a petroleum-based product or in performers (fire-eaters) who use liquid hydrocarbons for flame blowing. Chronic exogenous lipoid pneumonia is more common and usually results from repeated

episodes of aspiration or inhalation of oils over an extended period. Although it typically occurs in older patients, it may be seen in children, especially those with a predisposition to aspiration, including intellectual disability and cleft palate.[10] The severity of the parenchymal disease in lipoid pneumonia depends on the type, amount, frequency, and length of time of aspirated or inhaled oils. Free fatty acids trigger a severe inflammatory reaction that results in focal edema and intraalveolar hemorrhage.[10] The excessive inflammation can destroy the alveolar walls and the interstitial tissue, and the resultant fibrosis may progress to end-stage lung disease. In the acute phase of lipoid pneumonia, CT may reveal ground glass opacities or consolidation segmental or lobar in distribution and predominantly involves the middle and lower lobes.[10] Also, CT can demonstrate areas of fat density as low as −30 HU within the consolidation and nodules, a finding diagnostic of lipoid pneumonia like in our third case.

In conclusion, the presented cases emphasize the need for increased awareness among caregivers and pediatricians about the potential hazards of the usage of toxic substances and the importance of comprehensive patient history. Aspiration of exogenous materials may reveal typical imaging findings within consolidations or nodules such as metallic artifact of charcoals, fat attenuation of liquid paraffin and necrotic areas within consolidation in hydrocarbon poisoning. Recognition of radiological signatures is crucial not only for the diagnosis and characterization of the toxic substance but also for management, follow-up and prognosis.

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Case Report

Nasopharyngeal carcinoma in a child with Kartagener's syndrome

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ABSTRACT

Background. Kartagener's syndrome, a subgroup of primary ciliary dyskinesia, is characterized by situs inversus totalis, chronic sinusitis and bronchiectasis. To date, the association of malignant diseases and Kartagener's syndrome has been reported and all cases except angioimmunoblastic T cell lymphoma in a child have been seen in adulthood.

Case. A 10-year-old boy who was followed with the diagnosis of Katagener's syndrome, presented with a progressive mass in the cervical region for 6 months. Physical examination revealed mental retardation, multiple lymphadenopathies, the largest in the left cervical region (4x4 cm), and pectus carinatum. Also, on cardiovascular examination, apex beat was felt on the right fifth intercostal space along midclavicular line. Magnetic resonance imaging of nasopharynx showed narrowing of the nasopharyngeal airway with an increase in wall thickness up to 2.5 cm on the posterior wall of the nasopharynx. Also, bilateral multiple cervical lymphadenopathies were noted. The pathological examination of the biopsy from cervical lymphadenopathy revealed a diagnosis of undifferentiated nasopharyngeal carcinoma. Chemotherapy was started for nasopharyngeal carcinoma chemotherapy regimen including cisplatin, docetaxel, and 5-fluorouracil. After four cycles of chemotherapy there was a significant regression in nasopharyngeal mass and lymphadenopathies. The patient underwent radiotherapy to the nasopharynx and bilaterally cervical regions. The patient has been in follow-up for 6 years well and tumor free. However, he is still under the supervision of the pediatric immunology and allergy departments due to recurrent respiratory infections and sinusitis.

Conclusion. We present a case of nasopharyngeal carcinoma which developed in a child with Kartagener's syndrome. To our knowledge, this is the first report of nasopharyngeal carcinoma in a child with Kartagener's Syndrome.

Key words: Kartagener's syndrome, nasopharyngeal carcinoma, children.

Kartagener's syndrome (KS), is a rare and autosomal recessive primary ciliary dyskinesia. In KS the motility of the cilia is affected which leads to impaired mucociliary clearance. As a result, it causes recurrent chronic infections of the respiratory system. Generally, symptoms begin shortly after birth. Kartagener's syndrome is characterized by a clinical triad including situs inversus, chronic sinusitis and bronchiectasis.[1-3] Non-respiratory symptoms or signs such as chronic otitis media, conductive hearing loss, infertility in women and men, hydrocephalus and retinitis pigmentosa can be observed.[2]

In KS, a few malignancies have also been reported. These are renal cell carcinoma[4-7], testicular germ cell tumor[8,9], lung cancer[10,11], carcinoma of the ethmoid labyrinth[12], colon

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carcinoma[13], angioimmunoblastic T cell lymphoma[14], adenosquamous carcinoma of the paranasal sinuses[15], adenocarcinoma of the cervix uteri and endometrium[16], adrenal adenocarcinoma[17], and papillary thyroid carcinoma.[18] Only one of these publications is a pediatric patient with the association of Kartagener syndrome and malignant disease.[14]

Herein, we present a case of nasopharyngeal carcinoma in a child with KS. To our knowledge, this is the first report of nasopharyngeal carcinoma in a child with KS.

Case Report

A 10-year-old boy presented with progressive mass in the cervical region for 6 months. With these complaints, antibiotic treatment was applied to the patient in another center and because his complaints were not resolved, subsequently, he was referred to our hospital. From his past medical reports we learned that he was treated in the neonatal intensive care unit for respiratory distress and birth asphyxia in neonatal period and he was operated for Morgagni hernia at 1 month of age. Situs inversus totalis was diagnosed during this period. Later, he was treated at the local center many times due to nasal congestion and frequent recurrent respiratory infections. He was the second child of consanguineous parents. His cousin who had situs inversus totalis also had recurrent respiratory infections. The investigations for recurrent respiratory infections including the immunoglobulin levels, alpha-1-antitripsin level and sweat chloride test were normal. The saccharin test is the only screening test that is unreliable in children aged <12 years but could not be done because of his mental retardation. In this patient, the diagnosis of KS was made based on clinical history of chronic upper and lower airway diseases, the presence of situs inversus totalis and positive family history (Fig. 1).

The genetic analysis is complicated by multiple existing phenotypes. Although genetic testing for some cases of PCD is possible, the Task Force Committee does not recommend it as part of initial diagnostic testing.

On admission, physical examination revealed mental retardation, multiple lymphadenopathies, the largest in the left cervical region (4x4 cm), and pectus carinatum. Also, on cardiovascular examination, apex beat was felt on the right fifth intercostal space along midclavicular line. The remaining system examinations of the patient were unremarkable.

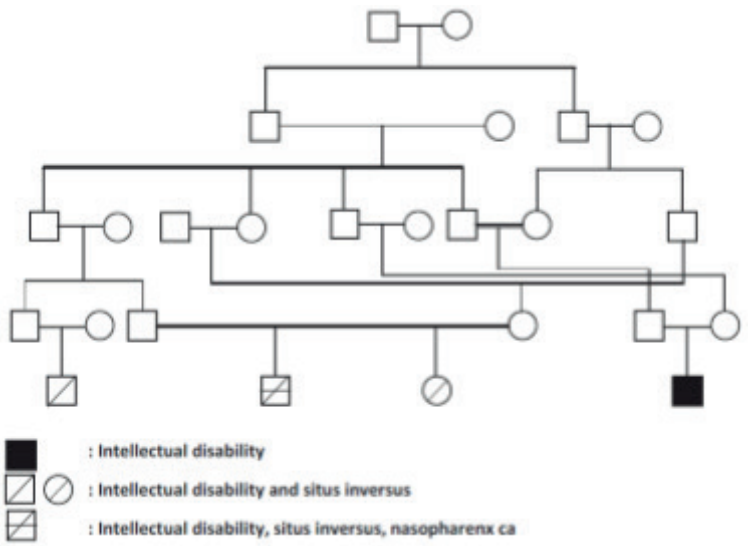

Fig. 1. The pedigree of the patient.

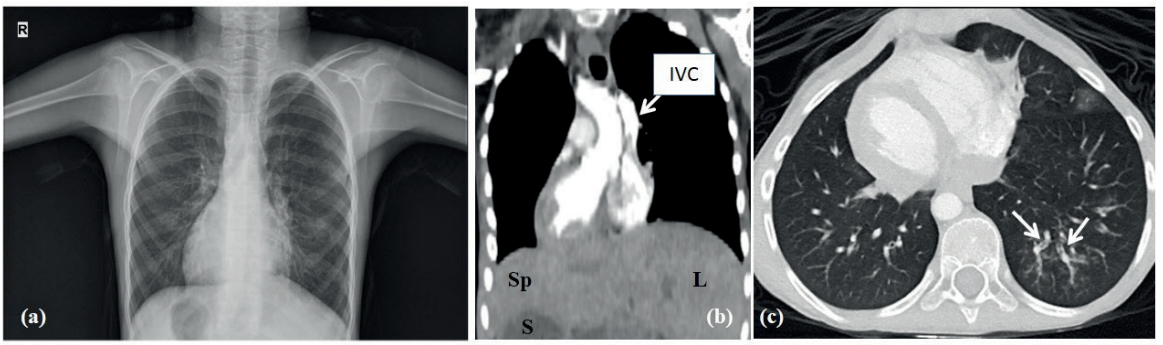

Fig. 2. (a) Posteroanterior chest X-ray showing dextrocardia; **(b)** Radiological findings of situs inversus totalis, IVC: inferior vena cava, Sp: spleen, S: stomach, and L: liver; **(c)** Minimal dilatation and thickening of bronchi in the left lung are shown.

Complete blood count, peripheral blood smear and biochemical examinations were normal. The posteroanterior chest X-ray showed dextrocardia (Fig. 2). Magnetic resonance imaging of nasopharynx showed narrowing of the nasopharyngeal airway with an increase in wall thickness up to 2.5 cm on the posterior wall of the nasopharynx (Fig. 3). Also, bilateral multiple cervical lymphadenopathies were seen.

The pathological examination of the biopsy from cervical lymphadenopathy revealed a diagnosis of undifferentiated nasopharyngeal carcinoma (Fig. 4). The patient was staged as III (T2N2M0) according to American Joint Committee on Cancer NPC staging classification, after a metastatic work-up had been completed. EBV serology was consistent with previous EBV infection. Nasopharyngeal carcinoma chemotherapy regimen including cisplatin (75 mg/m^2, on day 1), docetaxel (75 mg/m^2, on day 1) and 5-fluorouracil (750 mg/m^2/day, on days 1 to 4) was started. After four cycles of chemotherapy, there was significant regression in nasopharyngeal mass and lymphadenopathies. The radiotherapy of 60 Gy was given to nasopharynx and 54 Gy was delivered to the neck by intensity modulated radiation therapy with a simultaneous integrated boost. The patient has been in follow-up for 6 years without any recurrence. However, he is still under the supervision of the pediatric immunology and allergy department due to recurrent respiratory infections and sinusitis.

Written consent for publication of this case report and accompanying images was obtained from the parents of the patient.

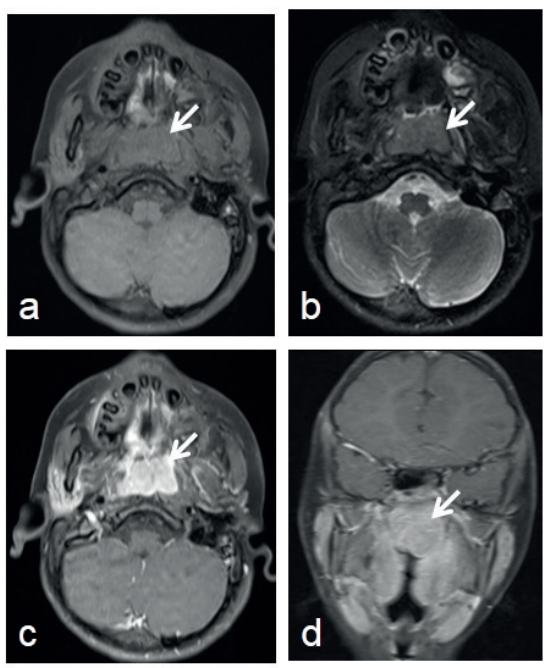

Fig. 3. Axial fat-saturated T1 **(a)** axial T2 **(b)** weighted images show a solid mass lesion (arrow) in the nasopharynx. Axial **(c)** and coronal **(d)** fat-saturated contrast enhancement T1-weigted images show marked contrast enhancement in the mass lesion (arrow).

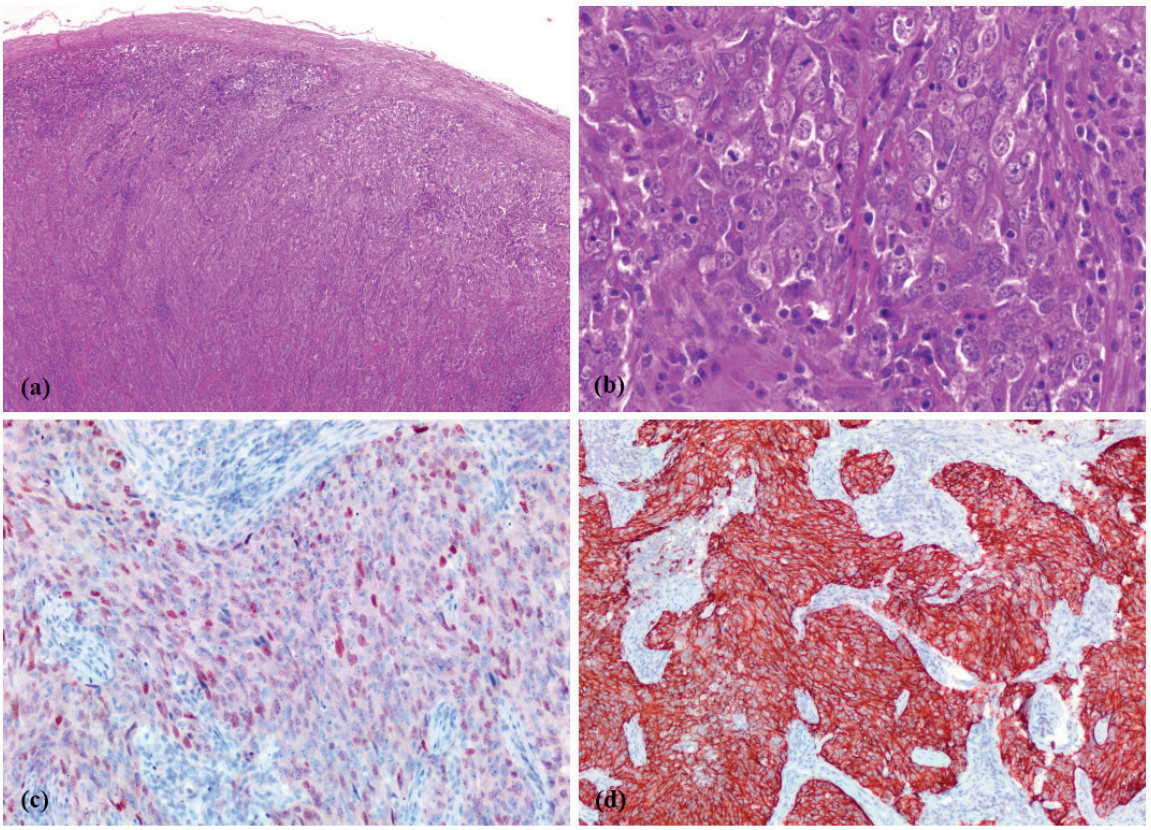

Fig. 4. (a): HE shows the tumor infiltrating the lymph node, (HEx50), **(b)**: High power view of the tumor: A tumoral lesion composed of pleomorphic cells with eosinophilic cytoplasm, large vesicular nuclei and prominent nucleoli in syncytial pattern. Numerous mitosis is noted. (HEx400), **(c)**: Nuclear positivity in tumor cells with immunohistochemical p63, (p63x200), and **(d)**: Strong cytoplasmic staining in tumor cells with immunohistochemical HMW-CK, (HMW-CKx100).

Discussion

Kartagener's syndrome, also called immotile cilia syndrome, is a rare clinical entity inherited as an autosomal recessive trait. It is characterized by the triad of the sinusitis, bronchiectasis and situs inversus. It is a genetically heterogeneous disease with primary ciliary dyskinesia. Many genes may be affected. However, the main problem is the dysfunction of the cilia and impaired mucociliary clearance.[1-3] Clinical findings are usually respiratory findings including unexplained respiratory distress in term neonate, daily productive cough since early infancy, daily nonseasonal rhinosinusitis since early infancy, chronic otitis media and persistent middle ear effusions, digital clubbing (rare in children), atypical asthma unresponsive to therapy, recurrent pneumonias and bronchiectasis, left-right laterality defects, situs inversus totalis or heterotaxy with or without complex congenital heart disease, and miscellaneous findings such as male infertility (immotile sperm), female subinfertility, ectopic pregnancy and hydrocephalus.[1-3] The analysis of ciliate epithelial cells in order to assess function and ultrastructure may aid diagnosis. There is no single gold standard test and diagnosis should be made in a specialized center following review of clinical history. The genetic analysis is complicated by multiple existing phenotypes. Although, genetic testing was not recommended as part of initial diagnostic testing. Unfortunately, biopsy analysis and genetic test could not be done in this case.[21]

A few cases of malignant diseases have been reported in primary ciliar dyskinesia or KS. These reported cancers to date are renal cell carcinoma, testicular germ cell tumor, lung cancer, carcinoma of the ethmoid labyrinth, colon carcinoma, angioimmunoblastic T cell lymphoma, adenosquamous carcinoma of the paranasal sinuses, adenocarcinoma of the cervix uteri and endometrium, adrenal adenocarcinoma and papillary thyroid carcinoma.[4-18] It is seen that most of these tumors are tissue or organ cancers with ciliated epithelium and all of the cancers have occurred in adulthood or elderly, except one pediatric patient. The majority of these tumors can be explained by impaired mucociliary clearance and exposure to chronic irritations.

Angioimmunoblastic T cell lymphoma, is a rare tumor, particularly diagnosed in pediatric patients. The case was reported 6 years after a heart transplant in a pediatric patient with primary ciliary dyskinesia. Immunosuppressive drugs used after the heart transplant are usual suspected agents but it is not possible to completely rule out the role of primary ciliary dyskinesia.[14] Another interesting case is adenosquamous carcinoma of paranasal sinuses. It is mostly seen in the 5th and 6th decades of the life. However, the presentation of this tumor at the age of 34 years in this patient with KS suggests that it may be associated with disruption of mucociliary clearance.[15]

Nasopharyngeal carcinoma is a rare tumor of childhood which originates from the epithelium of the nasopharynx. The association of Epstein-Barr virus with undifferentiated nasopharyngeal carcinoma is well known in children.[19,20] Herein, we present a case of nasopharyngeal carcinoma in a child with KS. To our knowledge, this is the first report of nasopharyngeal carcinoma in a child with KS. In our patient, the presence of carcinoma originating from nasopharynx with ciliated epithelium suggests the contribution of dysfunction of the cilia and impaired mucociliary clearance to the carcinogenesis. In our patient, the most important limitation for the diagnosis of KS was the lack of ciliary ultrastructure on electron micrographs or genetic examination.

In conclusion, malignant diseases of tissue or organs with ciliated epithelium should be kept in mind in the follow-up of patients with KS or primary ciliary dyskinesia.

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Case Report

Brachial plexopathy as a consequence of nerve root swelling after shoulder trauma in a patient following an acute seizure

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ABSTRACT

Background. Epileptic seizures might be associated with an increased risk of fractures, either as a result of trauma after a fall or as a result of excessive muscle contraction. In the pediatric population, excessive muscle contraction is a more significant risk factor for fractures, due to the lack of maturity in the musculoskeletal system, while antiepileptic therapy itself can lead to a reduction of bone density. Proximal humeral fractures in the pediatric population are not frequent but both proximal humeral fractures and shoulder dislocation increase the chance of brachial plexus injuries and peripheral nerve lesions.

Case. In this case report, we present a patient who suffered both avulsive greater tuberosity humeral fracture and anterior shoulder dislocation, initially diagnosed by radiography, with consequent brachial plexus injury of the left arm after an epileptic seizure followed by excessive muscle contraction. Electromyoneurography initially showed amplitudes' reduction in tested nerves along with signs of muscle denervation as well as clinical examination signs of the left arm muscular hypotrophy and hypoesthesia, especially in the left humero-scapular region. Electrotherapy and kinesitherapy as well as intramuscular dexamethasone injections administered three weeks after the injury finally improved the clinical examination findings in the patient.

Conclusion. The early detection of swelling compression, accompanied with appropriate therapy may prevent the progression of axonal damage and preserve the functional status of the affected limb.

Key words: seizure, shoulder trauma, brachial plexopathy, children.

Seizures might be associated with an increased risk of fractures, where individuals with non-epileptic seizures have a lower risk for fractures versus those with epileptic form.[1] Furthermore, seizures particularly of epileptic form might cause shoulder dislocation and recurrent instability as well as arthritis.[2-4] The incidence of shoulder dislocation occurring during seizures is around 0.6%. A majority of these dislocations are posterior but might also be anterior, although very rarely reported.[5-7] The trauma of the shoulder could be the consequence of a fall during the seizure episode, as well as the seizure itself, due to the excessive muscle contraction.[8] Additionally, drug-induced reduction in bone mineral density could increase the risk for fractures in these patients.[9]

Fractures and dislocations in the shoulder region could cause lesions of the brachial plexus to a various degree.[7] Fernandez-Torre et al.[10] reported a rare brachial plexopathy in an adult patient as a consequence of a generalized tonic-clonic seizure. Early recognition of brachial plexus lesions is important for the

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on-time inclusion of the proper treatment and rehabilitation. These plexopathies if not recognized and treated timely, might lead to a chronic disability with additional complications[11], affecting the patients' quality of life.

In this case report, we present a patient who developed shoulder trauma with both anterior shoulder dislocation, proximal humeral fracture and injury of the brachial plexus of the left arm during the acute episode of a seizure.

Case Report

A sixteen-year old adolescent was admitted to the University Children's Hospital. He was conscious with pain, paresthesia and muscle weakness in the upper left limb, had a tongue wound and had an antalgic body position. The symptoms such as disorientation and generalized seizure in a supine position on the left flank began on the day of admission. The seizure which was not followed by involuntary urination and defecation but with hypersalivation lasted around ninety seconds and ceased after oral midazolam administration. Cefixime antibiotic therapy was initiated five days before the seizure due to a diagnosed sinusitis, all the time without fever. Clinical and diagnostic monitoring of the patient had started four years before the seizure, after having the first of his four afebrile crises of consciousness, when oral clobazam and benzodiazepine therapy was initiated despite the fact that all computed tomography and electroencephalography results were within the reference range. On the admission day, the patient took zinc instead of clobazam by mistake. Heteroanamnestic data revealed no evidence of allergies to any food or medicine. The patient was in the normal state of consciousness, properly responsive, afebrile, eupneic, acyanotic and anicteric. Neurological findings included an antalgic position of both body and left arm, where weakness of the muscle, pain and paresthesia were present. Only a minimal ability to move his fingers was present, while muscle strength, tone, mobility and sensibility were all significantly impaired compared to the right arm. Neurological exam including cranial nerves was normal and there were no meningeal irritation signs. There were no signs of neurovascular compromise of the left arm: distal pulses were palpable and there was neither swelling nor skin discoloration.

Family history anamnesis revealed no specific information. Neurological development of the patient was compatible with his age and gender, excluding above mentioned afebrile crises of consciousness. All of his immunizations were on schedule with no recent immunization.

All laboratory blood tests showed no significant deviance, as well as biochemical tests, excluding minimally increased levels of glucose – 8.6 mmol/l and C-reactive protein – 5.9 mg/l. Initial radiography demonstrated the presence of the left shoulder dislocation.

On admission to the hospital, initial radiography imaging was performed (Fig. 1). The proximal humerus was in an internal rotation position. The fractured fragment of the proximal humerus included greater tuberosity and was dislocated anteriorly and caudally around five millimeters. Orthopedic

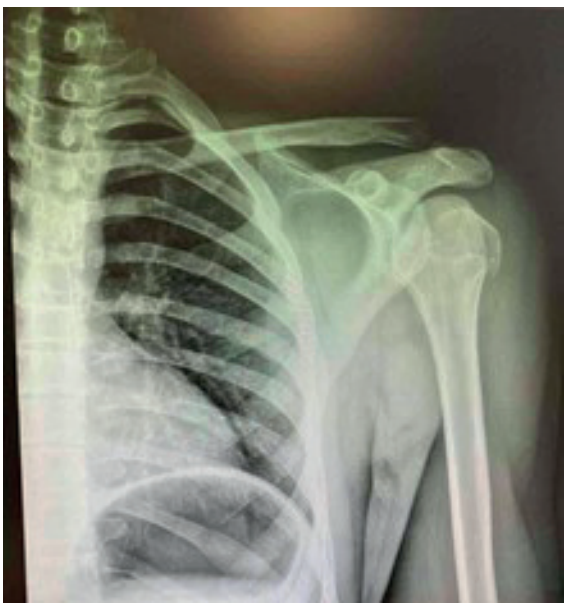

Fig. 1. Plain radiography of the left shoulder.

reposition was performed and the patient's left arm was immobilized with a plaster splint in the position of elbow semiflexion as well as internally rotated in the shoulder. The patient was referred for further physical therapy and rehabilitation treatment.

Electroneurography diagnostics were performed on the left upper limb both 2 weeks and 1 month after the trauma. On the first exam, the axillar nerve of the left arm was unresponsive to supramaximal stimuli, while motor amplitudes for ulnar, median and radial nerve on the left side were reduced, with the radial nerve being most severely affected (Table I). Sensory nerves (median, ulnar and radial) were affected as well, with reduced sensory amplitude and slightly reduced sensory conduction velocities (SCV) for median nerve and border SCV for ulnar and radial nerve (Table I). In all tested muscles of the left upper limb on the first exam, pathological spontaneous activity was detected, with no activity for voluntary muscle contraction of the deltoid muscle, and severely reduced trace for all other tested muscles (Table II). Manual Muscular Test (MMT) on the left hand showed the presence of reduced strength two weeks after trauma. For fingers and hand extensors the grade was 0 out of 5 (the patient could neither extend left hand nor fingers); for fingers abductors the grade was 1/2 out of 5 (there was slight abduction of fingers on the left arm with low amplitude in active motion range), while for fingers flexors the grade was 3 out of 5 (the flexion of left hand in the wrist was possible, but the patient could not hold the subjects). Sensory disturbances were present as well in the areas of tested nerves innervation of left hand and fingers.

On the second exam, for motor nerves, there was an improvement noticed in amplitudes and motor conduction velocities for the median nerve (Table I). For sensory nerves, improvements in amplitudes were noticed for ulnar and radial nerve and sensory conduction velocities for all tested sensory branches (Table I). Despite the present of spontaneous

Table I. Nerve conduction studies values.

Nerves		Left arm			Right arm		
Motor nerves							
Nerve	Exam	Distal latency (ms)	Amplitude (mV)	MCV (m/s)	Distal latency (ms)	Amplitude (mV)	MCV (m/s)
Median (APB)	First	3.7	3.7	47.5	4.0	10.0	55.1
	Second	3.7	6.6	54.2	-	-	-
Ulnar (ADM)	First	2.9	2.9	60.5	3.2	10.0	64.7
	Second	2.5	5.0	51.3	-	-	-
Radial (EIP)	First	5.8	0.3	-	2.7	2.5	-
	Second	2.6	2.0		-	-	-
Sensory nerves							
Nerve	Exam	Latency (ms)	Amplitude (μV)	SCV (m/s)	Latency (ms)	Amplitude (μV)	SCV (m/s)
Median (Ring)	First	3.0	4.6	47.5	-	-	-
	Second	2.8	4.1	50.0	-	-	-
Ulnar (Ring)	First	2.8	2.9	50.0	-	-	-
	Second	2.3	6.3	60.9	-	-	-
Radial (Thumb)	First	2.9	4.2	49.1	-	-	-
	Second	2.2	5.2	54.5	-	-	-

APB: adductor pollicis brevis muscle, ADM: abductor digiti minimi muscle, EIP: extensor indicis proprius muscle, Ring: ring finger, Thumb: thumb finger.

Table II. Electromyographic findings.

Muscles	First exam			Second exam		
	Spontaneous activity		Recruitment	Spontaneous activity		Recruitment
Left arm	FP	PSW	pattern	FP	PSW	pattern
Biceps	+	+	1-2 MU	+/++	+	--/---
Deltoid	+	+	No activity	+/++	+	2 MU
Triceps	+	/	1-2 MU	++	/	2 MU
EDC	+	+	1-2 MU	+/++	+/++	---
FDI	+	+	2 MU	+/++	/	---
APB	+	+	--/---	NP	NP	NP

FP: fibrillation potentials, PSW: positive sharp waves, AMP: amplitude, DUR: duration, PPP: polyphasic, EDC: extensor digitorum communis muscle, FDI: first dorsal interosseus muscle, APB: adductor pollicis brevis muscle, MU: motor units, NP: not performed.

activity in all tested muscles, signs of voluntary muscle action were improved on the second examination (Table II). Further, MMT grade for fingers extensors was 1 out of 5 and for hand extensors was 1/2 out of 5 (there was an initial active extension of the left hand and extension of fingers); for fingers abductors the grade was 2 out of 5 (increase in amplitudes of active fingers abduction of the left hand was observed), while for fingers flexors the grade was 3/4 out of 5 (the patient could form the wrist on left hand and hold the subjects in). Improvements of sensory disturbances was noticed particularly for the median nerve.

The overall electroneurographic findings pointed to the presence of severe, predominantly axonal lesion of motor branches of the radial and axillar nerve and moderate to moderately severe degree of a predominantly axonal lesion for motor branches of the median and ulnar nerve of the left hand. Furthermore, findings pointed out the presence of sensory neuropathy for tested nerves of the left hand.

Magnetic resonance imaging revealed the presence of posttraumatic signs of the plexus traction, in the form of edema of the lower and middle trunk and posterior cord as well as in radial and ulnar nerve (Fig. 2 – a, b, c).

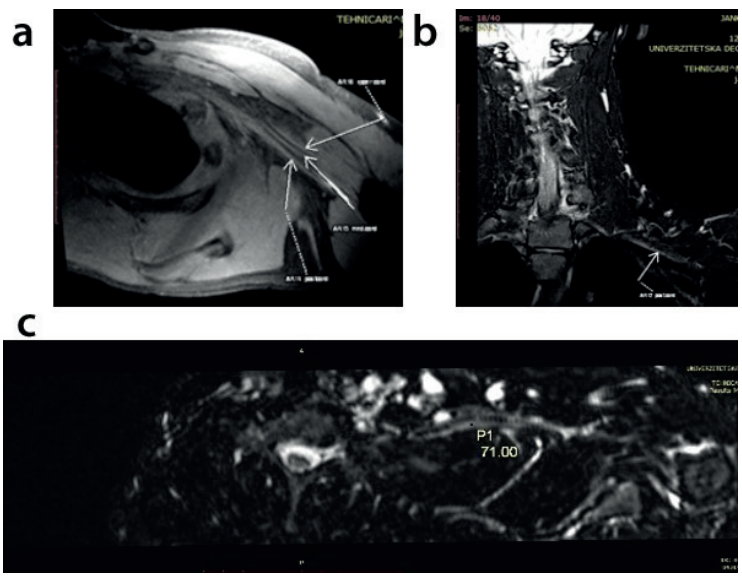

Fig. 2. a) MRI projection of three brachial plexus cords; **b)** MRI projection of brachial plexus posterior cord and **c)** MRI projection of brachial plexus posterior cord volume.

Physical therapy was administered two weeks after the injury, five days a week, including electrotherapy (electrostimulation for fingers abductors and extensors and hand extensors), as well as kinesitherapy (passive range of motion of affected distal parts of hand and fingers, particularly extension, and active flexion of fingers) during three weeks. Clinical examination revealed muscular hypotrophy in the left humero-scapular region (Fig. 3).

Three weeks after the injury and followed by MRI findings, dexamethasone intramuscular therapy was administered in the dosage of four milligrams for three days.

The parents and patient were informed about the purpose of the case report presentation and informed consent was obtained.

Discussion

Proximal humeral fractures in the pediatric population are not frequent, comprising around 2% of all fracture subtypes in children.[12] Shoulder dislocation as well as proximal humeral fractures increase the chance of brachial plexus injury as well as isolated peripheral nerve lesions. However, traumatic brachial plexus injuries are rare in children, and their prevalence is reported to be ten times lower than in adults with multitrauma.[13] Aside orthopedic trauma, vascular events could be additional factors that influence not only the onset of brachial plexus injury but its severity degree. Furthermore, for patients with seizures, excessive muscle contractions may contribute to a certain degree of brachial plexus or various isolated peripheral nerve damage as well. Since there was no data of trauma or fall, the possible explanation for the mechanism of proximal humeral fracture in this patient was thought to be excessive muscle contractions in this area during the seizure event.

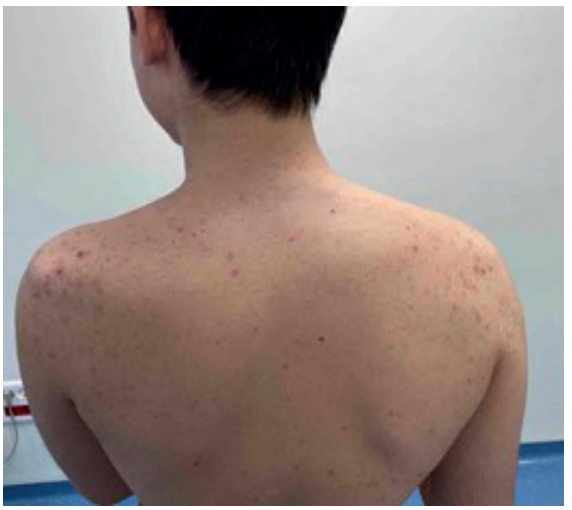

Fig. 3. Muscular hypotrophy of the left humero-scapular region.

Brachial plexus injuries can be divided according to the level of injury as supraclavicular and infraclavicular injuries.[14] Hems and Mahmood stated in their study that for patients with a shoulder dislocation and infraclavicular type of brachial plexus injury most cases do not require surgical intervention, but it should be noticed that the need for surgical intervention might be predicted by the pattern of nerve and skeletal injury.[14] However, functional reduction of the affected upper limb should be assessed and these patients should be included in physical therapy and rehabilitation program. The administration of dexamethasone along with physical therapy of the affected upper limb in our patient accelerated functional status improvement, possibly by the mechanism of nerves swelling reduction, consequently reducing mechanical pressure on axons and by implementing electrotherapy for muscle activity and vascular circulation improvement.

Early detection of nerve lesions presence in pediatric patients is the major cornerstone for proper treatment planning and optimal functional recovery. Aside from displaced fragments, the pain in the acute phase might be one of the limiting factors that influence the motoric assessment of affected upper extremity movements in affected joints. Therefore, neurophysiological evaluations (electromyoneurography – EMNG) are vital in the process of detection of peripheral nerve damage and its severity degree, with an optimal time of performance between 2-3 weeks for

assessment of axonal lesions.[15] If there is axonal damage, 10-21 days after the injury, the spontaneous pathological activity (fibrillation potentials and positive sharp waves) will appear during the muscle evaluation at rest by needle electrode.[15] Electromyoneurography is also an important diagnostic tool in the follow-up of these patients, where signs of reinnervation particularly in severe cases can be detected prior to the clinical signs of improvement.

Clinical symptoms of brachial plexus lesions are not just a consequence of the direct trauma but may be a consequence of the swelling compression of nerves. Having this in mind, swelling might be a potential cause of the functional deterioration in the pediatric population with seizure events. Additionally, a significant risk factor in the pediatric population is musculoskeletal immaturity, thus insufficient biomechanical joint stability that could be infringed by an acute seizure episodes. Therefore, early detection of the swelling compression, accompanied with proper therapy may prevent the progression of axonal damage and preserve the functional status of the affected upper limb.

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Case Report

The general movements assessment and effects of an early intervention in an infant with Cri du chat syndrome: a case report

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ABSTRACT

Background. Cri du chat syndrome (CdCS) is a rare orphan genetic disorder. Infants with CdCS have a neurodevelopmental dysfunction, but there are limited studies on their spontaneous movements or effect of the early interventions in children with CdCS. This study aimed to describe early spontaneous movements and investigate the effects of an early intervention in an infant with the CdCS.

Case. We analyzed the detailed general movements assessment (GMA) of an infant with CdCS at 14 weeks, and the Bayley Scales of Infant and Toddler Development-third edition (Bayley-III) were used for the determining and the follow-up of developmental functioning at 14 weeks, 6 months and 12 months. The infant was included in an early intervention beginning from 14 weeks. Fidgety movements were absent. The motor repertoire appeared significantly reduced, and the movement character was monotonous at 14 weeks. Although the infant achieved some developmental milestones with the early intervention program, the improvements were not reflected in the Bayley-III composite score.

Conclusions. As a consequence, abnormal GMA results, including fidgety movements and concurrent movement patterns, seen in CdCS can be associated with early signs of neurodevelopmental dysfunction. Early intervention programs in infants with genetic disorders could help enable the early achievement of motor milestones.

Key words: cri du chat syndrome, early intervention, general movements, motor development, physiotherapy.

Cri du chat syndrome (CdCS) is a rare orphan genetic disorder occurring as a result of deletions of variable size in the short arm of chromosome 5 (5p–).[1] The deletion size has been reported to vary between 5 and 40 Mb, and severe phenotypic and cognitive impairment is associated with larger deletions.[2,3] The incidence of CdCS is estimated between 1:15.000 and 1:50.000 live births.[4,5]

The most marked characteristics are facial dysmorphism, high-pitched monotonous crying, low birth weight, microcephaly, hypotonia, poor growth, developmental delay, delayed motor skill acquisition, clumsiness and intellectual disability.[6] Asphyxia, hypotonia and feeding difficulties have been reported beginning from the newborn period, and their growth and development are remarkably slow.[5] As the child gets older, the muscle hypotonia observed in the neonatal period, is replaced with hypertonia, and the microcephaly becomes more prominent.[5,6] Honjo et al.[7] reported that neurodevelopmental delay was detected in all of the patients with CdCS. Most commonly associated neuroimaging features include pontine and median cerebellar peduncle hypoplasia, cerebellar vermian atrophy, with additional findings such as; corpus callosum

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dysplasia, mega siterna magna, fourth ventricle enlargement, cerebellar vermian atrophy and delayed myelination.[8]

Of the children with CdCS 72.2% acquired independent walking at ages ranging from 19 months to 12 years, and in which the same study reported that 20.85% of the patients, who were older than 2 years old, were not able to walk.[7] However, it has been reported that these children can achieve many skills despite the delay in various developmental areas.[9] Although the clinical features and characteristics, and phenotype of CdCS have been reported in several studies, the general movements assessment (GMA) and the effect of an early intervention have not been previously examined. This first case report aimed to; (i) describe the age-specific detailed GMA which includes the fidgety movements and concurrent motor repertoire in a 3 to 5-month-old infant with CdCS, and (ii) investigate the effect of an early intervention on developmental functioning in an infant with CdCS.

Case Report

The female infant was born at 40 gestational weeks with a birth weight of 2920 g by cesarean delivery. She was the first child of a 26-year-old mother. CdCS was suspected from the crying pattern at birth and the family was referred to the Department of Genetics. She stayed in the neonatal intensive care unit for 2 days due to lack of sucking. Karyotype analysis at the age of 3 months revealed CdCS together with 46,XX,del(5)(p14). The patient then was referred to the Department of Gastroenterology for feeding difficulties. She had no hearing or vision problems. The pediatric neurologist referred the patient for physical therapy and rehabilitation when she was 3 months old. She was not able to achieve head control and had hypotonia including reduced antigravity movements, decreased resistance to passive movements, an excessive range of joint mobility in her first evaluation at Developmental and Early Physiotherapy Unit, Faculty of Physical Therapy and Rehabilitation. Her movements were observed to be quite monotone, slow and repetitive.

The infant was included in an early-individualized physiotherapy program that is routinely applied in our unit due to the delay in motor development observed with the initial evaluation (14 weeks). The early intervention was applied for 2 sessions per week for 45 to 60 minutes each time for a total of 9 months in line with the individual motor developmental needs of the infant by an experienced physiotherapist. The objectives of the treatment were identified as postural support, facilitation of movement, developing symmetrical posture, providing muscle balance, transferring weight, and proximal stabilization to support the daily activities of the infant. Positions such as supine, prone and side-lying together with handling techniques and additional sensory stimulation were used in the physiotherapy program in the early period. These were followed by the sitting position, rolling from the supine to the prone position, rolling from the prone to the supine position, and supported standing with the growth of the infant and motor progression. The family was also given a home-program by the physiotherapist on a weekly basis.

When she was 14 weeks old, five-minute video recording was made for the Prechtl GMA which is based on visual gestalt perception and is widely used in the prediction of neurological dysfunction in infants in the earliest period of life.[10,11] Age-specific detailed GMA which includes fidgety movements and concurrent motor repertoire and postural repertoire were assessed via the video recording. Using the score sheet for the Motor Optimality Score for 3- to 5-Month-Old-Infants–Revised,[12] we defined a motor optimality score (MOS) with a maximum value of 28 (for the best possible performance) and a minimum value of 5.[13] The score sheet contains the following five sub-sections: (i) temporal organization and quality of fidgety movements, (ii) observed movement patterns other than fidgety movements, (iii) age-adequate movement repertoire, (iv) observed

postural patterns, and (v) movement character.[12] The video recording was evaluated by two certified raters. Our patient displayed no fidgety movements, and a detailed assessment showed an age-inadequate movement repertoire at this time. Kicking, mouth movement, foot-foot contact were abnormal. The infant showed head and body asymmetry, and abnormal variability of finger postures in the postural patterns. The movement character was monotonous. The MOS was 6/28.

At 14 weeks, 6 months and 12 months old, we applied the Bayley Scales of Infant and Toddler Development–third edition (Bayley-III) that is widely used to measure the developmental functioning of children aged 1 to 42 months.[14] At 14 weeks the Bayley-III composite score for cognition was 65 (95% CI: 60-76), for language 79 (95% CI: 73-88), and 73 (95% CI: 68-83) for the motor domain. At 6 months and 12 months, the infant achieved several more items in all domains but the age-specific composite scores were still lower than average. At 6 months, the result of her Bayley-III composite score for cognitive domain was 65 (95% CI: 60-76), for language domain 79 (95% CI: 73-88), and motor domain 49 (95% CI: 45-61). According to Bayley-III composite score result at 12 months, cognitive domain was 55 (95% CI: 51-67), language 62 (95% CI: 57-72), and 46 (95% CI: 43-58) for the motor domain (Table I). Hence, she scored below 2 SD in all domains. The parents gave their informed consent for this publication.

Discussion

To the best of our knowledge, this is the first case report that describes the fidgety movement and concurrent motor repertoire in an infant with CdCS at the early period and shows the effects of an early intervention on developmental functioning results. Clinical assessments are crucial for predicting later neurodevelopmental outcomes and following functional outcome in long-term development, because neuroimaging techniques such as magnetic resonance imaging (MRI) are not always available for such young infants. Studies showed that brain development continues postnatally, driven by motor cortex activity,[15,16] therefore, early intervention might play an important role in the improvement in the development of children who have a neurodevelopmental disorder.

Many diseases that are subsumed under the umbrella term of genetic disorders have quite different symptoms, findings or complaints. Several studies in infants with genetic disorders demonstrated that by using the detailed GMA for the assessment of motor repertoire, that these infants might have a wide range MOS result.[17-19] As in previous studies, MOS was associated with gross motor functional abilities and activity limitations in children with cerebral palsy;[20,21] fine and gross motor performance in children born with very low birth weight;[22] cognitive development in children born preterm;[23] and, with language performance in typically developing children,[24] hence these differences in MOS results in infants with genetic disorders might be an early indicator of their later neurodevelopmental level. According to our findings, GMA results were abnormal at 14 weeks, which revealed also significantly reduced MOS like the other genetic disorders such as Smith Magenis syndrome,[17] some infants with Down syndrome[18] and Prader Willi Syndrome.[19] Lacking variable and complex quality of general movements indicate an abnormality in the nervous system.[25] Underlying mechanisms of fidgety movements and the resulting low MOS are unclear in infants with CdCS, but our case report is a contribution to the literature.

Mainardi et al.[9] reported that all their patients with CdCS had motor delay, and the severity of motor delay was related to the size of the deletion. Furthermore, 81.9% of the patients were diagnosed in the first year of life, and it was reported that 71.6% of the patients started physiotherapy before one year-old in the same study.[9] Although patients who started physiotherapy before one year-old are indicated to have better results in the achievement of developmental skills, there was no statistical

Table I. Items that child was able to do initially at 14 weeks, and items that the infant achieved at 6 and 9 months according to the Bayley-III scale.

	The Bayley Scales of Infant and Toddler Development–third edition (Bayley-III)				
	Cognitive scale	Language scale		Motor scale	
		Receptive communication	Expressive communication	Fine motor	Gross motor
14 weeks	Item 1: Calms when picked up Item 2: Responds to surroundings series: inspects Item 3: Regards object for 3 seconds Item 4: Habituates to rattle Item 5: Discriminates between objects Item 6: Recognizes caregiver Item 7: Becomes excited in anticipation Item 8: Regards object for 5 seconds	Item 1: Regards person momentarily Item 2: Tolerates attention Item 3: Calms when spoken to Item 4: Reacts to sounds in the environment Item 5: Responds to a person's voice	Item 1: Undifferentiated throaty sounds Item 2: Social smile Item 3: Vocalizes mood Item 4: Undifferentiated nasal sounds	Item 1: Hands are fisted Item 2: Eyes follow moving person Item 3: Eyes follow ring (horizontal) Item 4: Eyes follow ring (vertical) Item 5: Attempts to bring hand to mouth Item 6: Retains ring Item 7: Eyes follow ring (circular) Item 8: Head follows ring	Item 1: Thrusts legs in play Item 2: Thrusts arms in play Item 3: Controls head while upright series: lifts head Item 4: Controls head while upright series: 3 seconds Item 5: Turns head to sides Item 6: Makes crawling movements

Table I. Continued.

	The Bayley Scales of Infant and Toddler Development–third edition (Bayley-III)				
	Cognitive scale	Language scale		Motor scale	
		Receptive communication	Expressive communication	Fine motor	Gross motor
6 months	Item 9: Reacts to disappearance of face Item 10: Shifts attention Item 11: Shows visual preference Item 12: Habituates to object Item 13: Prefers novel object Item 14: Habituates to picture Item 15: Prefers novel picture Item 16: Explores object Item 17: Carries object to mouth Item 18: Inspects own hand Item 19: Mirror image series: approaches Item 20: Responds to surroundings series: awareness of novelty	Item 6: Searches with head turn Item 7: Discriminates sounds Item 8: Sustained play with objects	Item 5: Social vocalizing or laughing Item 6: 2 vowel sounds	Item 9: Eyes follow rolling ball Item 10: Keeps hands open Item 11: Rotates wrist	Item 7: Controls head in dorsal suspension Item 8: Controls head in ventral suspension Item 9: Controls head while upright series: 15 seconds Item 10: Holds head in midline Item 11: Holds head upright while carried Item 12: Controls head while prone series: 45° Item 13: Rights head Item 14: Rolls from side to back Item 15: Elevates trunk while prone series: elbows and forearms Item 16: Sits with support series: briefly Item 17: Controls head while prone series: 90°
12 months	Item 21: Persistent reach Item 22: Mirror image series: responds positively Item 23: Plays with string Item 24: Bangs in play	-	Item 7: Gets attention Item 8: 2 consonant sounds Item 9: Uses gestures	Item 12: Grasps suspended ring Item 13: Block series: reaches for block Item 14: Block series: touches block Item 15: Block series: whole hand grasp Item 16: Reaches unilaterally Item 17: Food pellet series: raking grasp	Item 18: Elevates trunk while prone series: shifts weight Item 19: Sits with support series: 30 seconds Item 20: Rolls from back to sides Item 21: Elevates trunk while prone series: extended arms

significance.[9] Guala et al.[26] who carried out another study, investigated the effect of two rehabilitation programs on the development and reported that no significant difference was found. There are several findings concerning physiotherapy,[9,26] but some information in these studies is unclear, such as specific and detailed information about the physiotherapy, the child's initial functional levels, and age of onset of physiotherapy. Our findings contribute to the literature that early-individualized physiotherapy programs might be effective in achieving developmental milestones.

Furthermore, the improvement of our infant's development, especially in the motor and cognitive domains, in the first months of life was also more than the subsequent periods with physiotherapy, for this reason, we point out that starting rehabilitation in the first 6 months is very important. According to our findings, although our infant achieved some developmental milestones, these achievements were not reflected in the Bayley-III composite score results. The improvements in the Bayley-III raw score results were not enough to catch up with her peers.

We recognize several limitations to this case report. Firstly, we did not obtain a cranial magnetic resonance imaging study, because of the age of the infant and the need for anesthesia. Secondly, our findings cannot be generalized because they are based on only one infant.

In conclusion, apart from the clinical features and characteristics and phenotype of CdCS, our findings demonstrated that abnormal GMA results, including fidgety movements and concurrent movement and postural patterns, might be associated with malformations of the central nervous system development at the earliest age in infants with CdCS. Additionally, according to our findings, early-individualized intervention might be important to reduce the neurodevelopmental delays of these children and to achieve the developmental milestones earlier. Whether these improvements in our infant are effects of the early intervention or just a result of the normal growth and development process is controversial as in the whole pediatric group. Our findings need to be verified in a large cohort.

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Case Report

A rare intrauterine onset growth retardation syndrome caused by mosaic 19p13.3 microduplication: evaluation of GH/IGF- 1 axis and GH therapy response

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ABSTRACT

Background. 19p13.3 microduplication syndrome is a newly defined intrauterine onset growth retardation syndrome characterized by microcephaly, moderate intellectual disability, speech delay, and mild dysmorphic features. The *PIAS4* gene located in this region plays a crucial role as a transcriptional co-regulator in various cellular pathways including STAT, p53/TP53 and growth hormone (GH) signaling and mutations in this gene are thought to be responsible for clinical features.

Case. We present a 10 year-old girl with intrauterine onset growth retardation, microcephaly, and mild facial dysmorphic features. Treatment with GH was started at 4 years and 9 months of age targeting the severe short stature (-3.65 standard deviation score, SDS) since she had significant IGF-1 response to exogenous GH. Microarray study demonstrated a 19p13.3 microduplication of 4.4 Mb. FISH analyses revealed mosaic extra signals (27.5% on blood lymphocytes, and 47% on buccal epithelium) of 19p13.3 region. At the age of 10, her height was at -2.37 SDS, and she had mild intellectual disability which has been described in 19p13.3 microduplication syndrome.

Conclusion. We present here a patient with typical findings of 19p13.3 microduplication syndrome and also with a prominent response to GH treatment, which has not been reported previously in this syndrome.

Key words: 19p13.3 microduplication, intrauterine growth retardation, microcephaly, *PIAS4*, growth hormone.

The p13.3 locus on chromosome 19 is 6.9 Mb in length and contains 307 genes. Duplications and deletions of this region were reported in about 50 patients, the majority of which were deletions.[1-6] Siggberg et al.[3] reported microcephaly, intrauterine onset growth retardation and 0.8 Mb-long duplication on 19p13.3 (chr:19: 1952590_6908729) in three patients from a family with similar clinical features (microcephaly, moderate to severe intellectual disability, speech delay and dysmorphic findings) and defined the 19p13.3 microduplication syndrome. Andries et al.[7] also earlier reported a case that has similar characteristics of the syndrome with pure terminal duplication of the short arm of chromosome 19. Novikova et al.[5] tried to identify the genetic mechanisms of the syndrome and suggested that *PIAS4* (Protein Inhibitor of Activated STAT Protein 4), *ZBTB7A* and *MAP2K2* genes of this region are responsible for the characteristic findings of this syndrome. It was hypothesized that *PIAS4* gene duplication has the essential role for microcephaly and intrauterine growth retardation.

Herein, we report on a patient with both 19p13.3 microduplication and a positive growth response to growth hormone (GH) treatment, which were not associated previously.

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Case Report

A 12 month-old girl was admitted due to her atypical facial appearance and microcephaly (Fig.1). Her parents were first cousins once removed. Due to oligohydramnios and fetal intrauterine growth restriction, fetal karyotype analysis was performed and revealed normal at 25 weeks of gestation. The pregnancy was complicated by gestational diabetes mellitus. The patient was born at 36 weeks and 4 days of gestation. Her birth height, weight, and head circumference (HC) were 38 cm (-3 standard deviation score, SDS), 1370 gr (-2.6 SDS), and 27.8 cm (-3 SDS), respectively.

Physical examination at age 1 showed dysmorphic features including distinctive facial appearance with round facies, brachycephaly, sparse hair, bitemporal narrowing, highly arched sparse eyebrows, upslanted short palpebral fissures, low set large ears, and micrognathia with chin dimple (Fig. 1a,b). Her height, weight, and HC were 67cm (-2.29 SDS), 6400 gr (-3.4 SDS), and 38.5 cm (-5.7 SDS), respectively. Biochemical tests of blood and urine, abdominal ultrasonography (USG), echocardiography and cranial MRI were normal. She gained head control at 3 months of age, sat without support when 11 months-old and walked when 2.5 years-old. At 13 months of age, the Denver-II test result was 73%, compatible developmental delay.

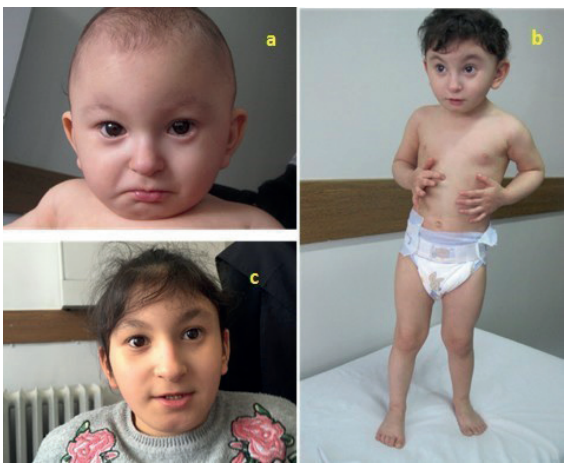

Fig. 1. The patient at 12 months (a), 42 months (b) and 8 years (c) of age. Note microcephaly, round facies, bitemporal narrowing, sparse hair, sparse and highly arched eyebrows, upslanted and short palpebral fissures, low set and large ears, thin upper lip and micrognathia with a chin dimple (a,b). She developed a long face with aging (c).

At the age of 4 years and 9 months, her height and weight were 91 cm (-3.25 SDS) and 12.5 kg (-2.57 SDS). Her bone age was compatible with the standards of 2.5 years. The GH response to GH stimulation test with clonidine was higher than 10 ng/ml, showing sufficient response. Due to her severe growth retardation, GH axis was reevaluated by IGF generation test to demonstrate if there was an IGF-1 response to exogenous GH. GH with a dose of 0.1 mg/kg/day was injected subcutaneously for 4 days. After the GH injections, baseline IGF-1 level (130.4 ng/ml, -0.6 SDS) reached the peak level (216.1 ng/ml, +0.9 SDS) with an increase ratio of 66% at the 5th day, showing a significant IGF-1 response to exogenous GH. Thus, GH treatment (0.2 mg/kg/week) was started. After the 1st year of treatment her height SDS was -3.16 and height velocity was 8.4 cm/year which was -3.65 SDS and 5.5 cm/year before treatment. After the 2nd year of the treatment her height SDS was -2.98 and height velocity was 7.1 cm/year. Growth velocity SDS in the first and second years of treament were +1.42 SDS and +0.79 SDS indicating a good response to the treatment. A positive affect on head circumference was not noted during GH treatment.

At 10 years of age, on the last examination, she was still receving GH treatment. Her height was 123.2 cm (-2.37 SDS). Her response to GH treatment was 7 cm/year (+0.61 SDS) with an IGF-1 response of 398.7 ng/ml (+2.35 SDS). Dysmorphic features including long facies, thin long fingers with wide fingernails, prominent bilateral sacral dimple, and skin eczema were noted (Fig 1c). She was receiving special education and was unable to read and write, yet.

Cytogenetic and molecular cytogenetic studies

Written informent consent was obtained from the parents. The patient had a normal

female karyotype in a peripheral blood sample (46,XX). Both SNP-array and array-CGH analyses of the genomic material extracted from peripheral blood cells revealed a 4.4 Mb-long microduplication of the 19p13.3 locus (Chr19: 259395_4615348) (Fig.2a,b). To define the location of the duplicated region, FISH analysis on metaphase spreads prepared from cultured lymphocytes was performed by using 19p subtelomeric and Smith Magenis / Miller Dieker (SM/MD) probes (dJ564C11, RAI1/ PAFAH1B1, Aquarius®, Cytocell Cambridge, UK). The analyses revealed an extra signal of 19p13.3 region on the short arm of chromosome 17 as a mosaic pattern (11/40 metaphases; 27.5%), and normal signal of SM/MD specific probe on the 17p region (Fig.3a, b, c). The FISH analysis of the buccal smear cells revealed the mosaicism ratio as 47%. The chromosomal analysis and FISH study of the parents excluded balanced translocations.

Discussion

19p13.3 microduplication syndrome is a newly defined intrauterine onset growth retardation syndrome characterized by microcephaly, moderate intellectual disability, speech delay, and mild dysmorphic features.[1-6] The 19p13.3 region includes *PIAS4, ZBTB7A*, and *MAP2K2* genes, which were thought to be responsible for the characteristic findings of the syndrome. It was hypothesized that PIAS4 gene duplication has the essential role for microcephaly and intrauterine growth retardation.[5,6] PIAS4 protein is a member of the E3 SUMO-protein ligase family. It regulates histone modifications and plays a crucial role in transcriptional coregulation, genetic stability, and various cellular pathways like STAT, p53/TP53, Wnt, and steroid hormone signaling pathways.[8-13] Burn et al.[12] injected PIAS1, PIAS2, PIAS3, and PIAS4 RNAs into early frog embryos and observed failure in blastopore closure, shortened body

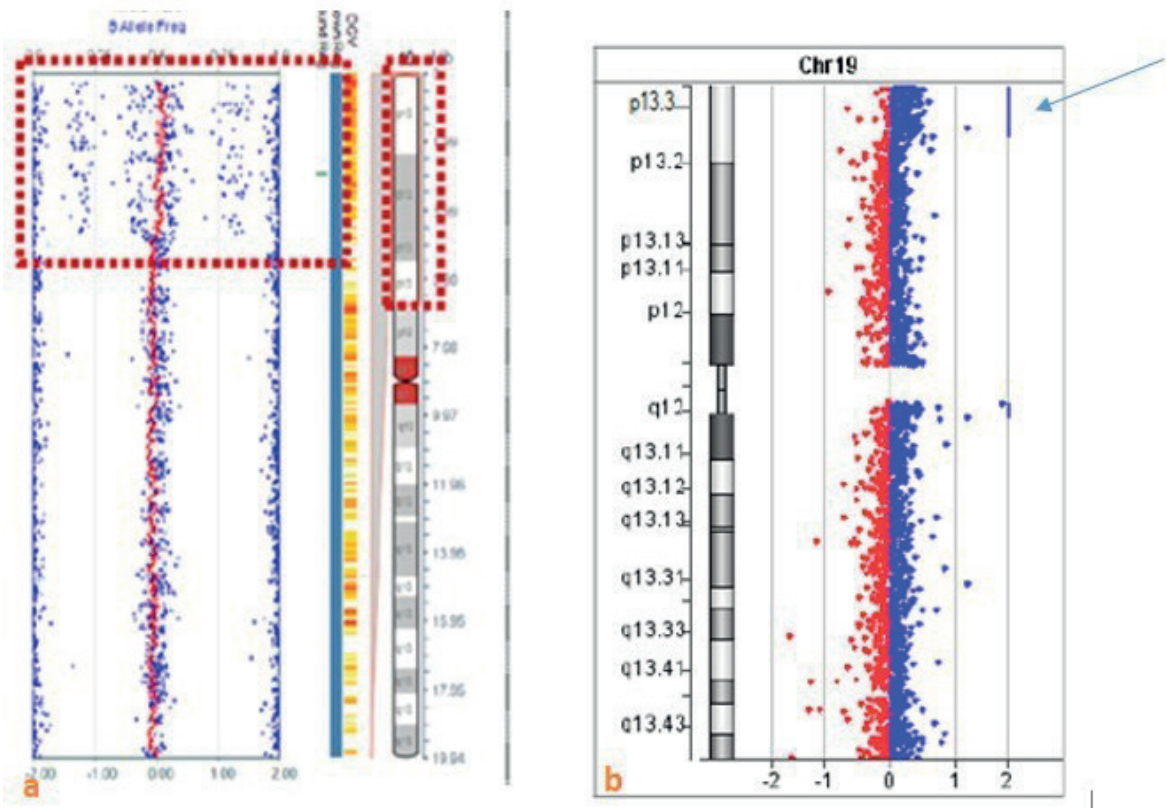

Fig. 2. SNP Array (a) and Array-CGH (b) analysis revealed a mosaic 4.4 Mb microduplication of 19p13.3 (Chr19: 259.395 – 4.615.348).

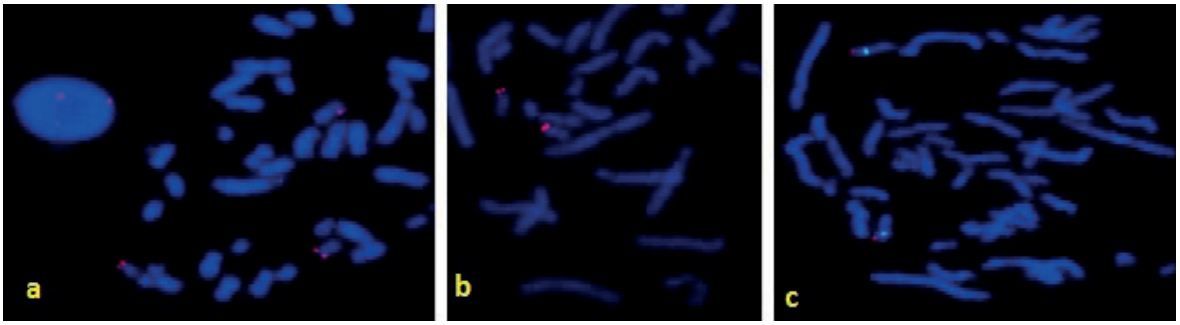

Fig. 3. FISH analyses revealed a third signal of 19p13.3 on the short arm of chromosome 17 in mosaic (in 11/40 metaphases) (a), among normal cells with two normal signals of 19p13.3 (b). FISH with the Miller Dieker syndrome-specific probe; green centromeric signals indicate chromosomes 17 (c).

axis, and defective head formation. *MAP2K2* is a regional gene potentially functioning in mitogenic activity, and mutations may cause growth retardation. Monoallelic mutations of this gene is associated more with cardiofasciocutaneous syndrome. *PIAS4* which encodes a member of the E3 SUMO-protein ligase family is thought to be the strongest candidate gene associated with growth retardation in 19p13.3 microduplication syndrome. Recently a case with single nucleotide variant in *PIAS4* that has common characteristics of 19p13.3 microdeletion syndrome was reported, supporting the hypothesis of *PIAS4* effect on head circumference and growth.[6] Nevado et al.[1] also evaluated the clinical findings of eleven patients with 19p13.3 microdeletion and two patients with 19p13.3 microduplication in a study in 2015. While one of the patients with microduplication without *PIAS4* duplication was normocephalic and short statured, the other patient with *PIAS4* duplication had microcephaly (-4 SDS) and short stature. They associated 19p13.3 region duplications with microcephaly as well as 19p13.3 region deletions with macrocephaly; and named both conditions as the 19p13.3 microdeletion/ microduplication syndrome.

We detected a *de novo* 4.4 Mb microduplication of 19p13.3 region by array-CGH analysis in a girl with microcephaly and IUGR. This duplication may originate by *de novo* unequal crossing over, or by abnormal segregation from parental balanced translocation or inversion. Since the parental karyotypes were normal, 19p13.3 duplication occurred as *de novo*. FISH analysis which was performed for defining the location of this duplicated region, revealed a mosaic 19p13.3 insertion on the chromosome 17p. This unbalanced insertion had caused a 19p13.3 microduplication, without 17p deletion. Mosaicism refers to a different genetic content in a group of cells from other cells in the body that arises during development. The percentage of cells with the mutation is related to the developmental stage at which the mutation arises.[14] The ratio between affected and unaffected cells or the corresponding cell types correlate with the severity of clinic outcome and probably with the response to growth hormone treatment.

The patient presented here also had mild intellectual disability with aggressive behaviour patterns, speech delay, elongated face, sparse hair and eyebrows, bitemporal narrowing, short and upslanted palpebral fissures, thin upper lip, micrognathia, chin dimple, and long fingers. 19p13.3 microduplication syndrome has been reported in 9 patients to date. Table I summarizes the clinical findings in our patient and in previously reported patients with pure 19p13.3 duplications.[1-6] The majority of subjects including our patient had microcephaly (7/10). One of the normocephalic patients had a duplication without *PIAS4* gene, and the other patient with borderline microcephaly had a partial *PIAS4* gene duplication.[1-6] All patients had developmental delay and/or intellectual

Table I. The molecular and clinical findings of present and reported patients with 19p13.3 microduplication.

Features		Ishikawa (2)	Novikova (5)	Orellana (4)			Siggberg (3)	Nevado (1)		Tenorio (6)	Present Patient
				Patient 1	Patient 2	Patient 3		Patient 1	Patient 2		
Age (yrs)		3	11	10	2.5	39	9	6	3	5	1
Gender		F	M	M	M	F	M	F	F	F	F
Duplication size (Mb)		6.1	0.83	4.95	4.95	4.95	0.81	2.39	1.479	3.04	4.3
Including PIAS4 gene		+	Partial	+	+	+	+	+	-	+	+
Microcephaly		+	+	+	+	+	+	+	-	-	+
Birth	Length (SDS)	-3.3	NA	-0.9	-1.0	+1.1	-2.6	NA	NA	-1.35	-3.2
	Weight (SDS)	-2.9	NA	-2.1	-2.1	-1.0	-1.9	NA	NA	-1.39	-2.8
	HC (SDS)	-2.2	NA	NA	NA	NA	-2.2	NA	NA	-0,85	-3.2
Last examination	Height (SDS/age)	-5.0 / 3yrs	-1,6 / 11yrs	NA	-0.6 / 6yrs	-0.2 / 22 mo	–1.5 / 2yrs	Short	Short	NA	-2.8 / 8yrs
	Weight (SDS/age)	-3.4 / 3yrs	-2,0 / 11yrs	NA	+0.8 / 6yrs	-0.2 / 22 mo	NA	NA	NA	NA	-1.4 / 8yrs
	HC (SDS/age)	-4.0 /3	-1.7 /11	NA	-2.6 /6	-2.1 / 22 mo	NA	-4.4 / 6	N/3yrs	NA	-4.7 /8
Intellectualdisability		+	+	+	+	+	+	+	+	+	+
Speech delay		+	+	+	+	+	+	+	+	+	+
Hypotonia		NA	+	NA	NA	NA	NA	+	Mild	+	-
Behaviour phenotype		NA	ADHD	VSA	VSA	VSA	Adequate	ADHD	NA	NA	Aggre-sive
Elongated face		-	+	+	NA	+	NA	NA	NA	NA	+
Prominent forehead		NA	+	+	+	+	NA	+	+	NA	-
Temporal narrowing		-	-	-	NA	-	NA	-	-	NA	+
Sparse hair and eyebrow		+	+	-	NA	-	NA	NA	NA	NA	+
Short palpebral fissure		+	+	+	NA	+	NA	NA	NA	NA	+
Upslanted palpebral fissure		-	+	+	NA	+	NA	NA	+	NA	+
Hypertelorism		+	+	-	NA	+	NA	-	NA	NA	-
Thin upper lip		-	+	+	+	+	NA	+	-	NA	+
Micrognathia		+	+	+	NA	+	NA	NA	NA	NA	+
Chin dimple		-	-	-	NA	-	NA	NA	NA	NA	+
Small mouth		+	+	+	NA	+	NA	NA	NA	NA	-
Long finger		NA	-	+	+	+	NA	NA	NA	NA	+
GH treatment		-	-	-	-	-	-	-	-	-	+

ADHD: attention-deficit/hyperactivity disorder, GH: growth hormone, NA: not-available, VSA: very sociable and affectionate; mo: months, yrs: years, N: normal, HC: head circumference, SDS: standard deviation score.

disability. Common craniofacial findings were prominent forehead (6/7), sparse hair and eyebrows (3/5), upslanted palpebral fissures (5/6), short palpebral fissures (5/5), thin upper lip (6/8), small mouth (4/5), micrognathia (5/5), and long fingers (4/5). Other uncommon features were early-onset puberty, joint abnormalities (hip dislocations, hypermobility), and cardiac defects.[2-4]

Growth hormone stimulation test of the patient presented here revealed adequate GH responses. Despite normal baseline IGF-1 levels we performed IGF-1 stimulation test to see the increment of IGF-1 in response to GH, which showed a significant increase and led to treatment with GH. GH treatment improved growth in our patient in the short term and she achieved a good response. To our knowledge, the response to GH treatment in patients with 19p13.3 microduplication syndrome has not been examined to date. However long term effects and final height might show a real benefit of GH treatment. It is difficult to explain the exact mechanism of growth failure in this disorder in which several genes are located adjacently. It is also difficult to explain why our patient responded to GH because there was no GH nor IGF-1 deficiency. Especially *PIAS4*, a transcriptional coregulator in various cellular pathways including STAT, p53/TP53 and growth hormone (GH) signalling, may be involved in growth failure by affecting post receptor signalling of GH. Although IGF-1 levels increased after GH stimulation showing a functional receptor, the patient might have had a partial insufficiency in post receptor signalling, thus achieving higher GH levels following administration of GH might have improved her growth.

In conclusion, we have described typical findings of 19p13.3 microduplication syndrome in a patient with mosaic 19p13.3 microduplication, and demonstrated that there may be a prominent response to GH treatment in this syndrome.

Acknowledgement

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Headache, cataract, and unilateral visual loss: unusual features of *DARS2* variants in LBSL

Josef Finsterer

Klinik Landstrasse, Messerli Institute, Vienna, Austria.

Dear Editor,

With interest we read the article by Cavusoglu et al.[1] about a 12 year old male with bilateral congenital cataract, right-sided visual loss, recurrent frontal headache since age 9, paraspasticity, T2-hyperintensities of the corporis and genu callosum, posterior limb of internal capsule, inferior cerebellar peduncles, and mesencephalic portion of nucleus-V.[1] Spinal magnetic resonance imaging (MRI) revealed T2-hyperintensities of the corticospinal tract, the spinocerebellar tracts, and the dorsal columns.[1] Magnetic resonance spectroscopy (MRS) revealed a lactate peak.[1] Based on these findings leukoencephalopathy with brainstem and spinal cord involvement and lactate elevation (LBSL) was suspected and confirmed by documentation of the variant c.455G>T (p.C152F) (p.Cys152Phe)/c.228-21_228-20delTTinsC in *DARS2*.[1]

We have the following comments and concerns.

Headache is an unusual phenotypic feature of LBSL. The authors should inform us about how the headache was classified (tension-type, migraine, migraine-like, cluster). Since migraine-like headache is a frequent feature of mitochondrial disorders (MID)[2], we should know if the headache was regarded as a feature of LBSL or a second problem independent of LBSL. The authors should also mention how the headache was treated and if the treatment was effective.

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Congenital cataract is also unusual. Though congenital cataract has not been reported in association with LBSL, it is quite likely that it was a feature of the MID, since MIDs frequently go along with cataract in early ages.[3,4] Cataract has been also reported in patients carrying *IARS2* mutations.[5] As *DARS2, IARS2* encodes a tRNA-synthetase required for charging of tRNAs with their cognate amino acid for translation.

Missing in this report is an extensive family history and genetic workup of first-degree relatives. Thus, it remains unclear if the *DARS2* variant in the index case occurred spontaneously or was inherited. We should be informed if any of the first-degree relatives were clinically affected or not.

Bladder dysfunction in the form of a spastic bladder is an occasional feature of LBSL.[6] It would have been interesting to find out if the index patient presented with bladder dysfunction and if steroids were beneficial as has been previously reported.[5]

Some of the LBSL patients present with double vision, spontaneous nystagmus, cognitive impairment[6], or developmental delay.[7] Information concerning if any of these features were also found in the index patient or not should have been provided.

Extensive involvement of the spinal cord may not only occur in LBSL but has been also reported in other MIDs due to impaired aminotransferase[8], such as *ISCA2*.

Unexplained remains the visual loss of the right eye. The authors did not inform us if the visual loss was due to complications from cataract surgery, damage of the optic nerve, or of the

post-chiasmatic visual pathways. We should be informed about ophthalmologic investigations and the results of the visually-evoked potentials.

Overall, this interesting case could profit from the resolution of some shortcomings, such as a more detailed description of headache and congenital cataract, provision of an extensive family history, assessment of bladder function, comparison with rare features previously reported, and explanation of the unilateral visual loss.

Key words: myasthenia, seropositivity, acetylcholin-receptor antibodies, depression, quality of life, immunosuppression.

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Reply
Response to "Headache, cataract, and unilateral visual loss: unusual features of *DARS2* variants in LBSL"

Dilek Çavuşoğlu[1], Nihal Olgaç Dündar[2]

[1]Department of Pediatric Neurology, Faculty of Medicine, Afyonkarahisar Health Sciences University, Afyon; [2]Department of Pediatric Neurology, Faculty of Medicine, İzmir Katip Çelebi University, İzmir, Turkey.

Dear Editor,

This letter is a reply to the paper written by Josef Finsterer entitled "Headache, cataract, and unilateral visual loss: unusual features of DARS2 variants in LBSL" In his letter, Finsterer comments on a previous case presentation written by us entitled "The first pediatric case of leukoencephalopathy with brainstem and spinal cord involvement and lactate elevation (LBSL) from Turkey".[1] We would like to thank Finsterer for their comments and giving us the opportunity to further explain this interesting case.

The first question brought up concerned the type of headache experienced. We classified the headache as tension-type. There was no feature associated with migraine. We did not consider the headache to be a feature of LBSL. We firstly

proposed life style modification and as the headaches were mild he was followed with non pharmacologic treatment.

The second comment raised concerned the patients congenital cataract and the fact that this is an unusual finding. We agree with this comment and the believe the cause of cataract in our patient to be associated with mitochondrial dysfunction. It is known that LBSL is a mitochondrial disease caused by homozygous or compound heterozygous mutation in the DARS2 gene encoding mitochondrial aspartyl-tRNA synthetase (mtAspRS).[2] The visual loss of the right eye was informed to us by the family and was reported to be a consequence of the cataract operation performed in early ages. We are afraid there was no available results concerning recent ophthalmologic investigations or any result of the visually-evoked potentials. Moreover, our patient did not present with double vision, spontaneous nystagmus, cognitive impairment or developmental delay.

In their letter Finsterer discussed bladder dysfunction as an occasional feature of LBSL. Our case had no bladder dysfunction and therefore was not treated for this condition.

His family history did not include a similar case with features of LBSL. Genetic analysis was performed for variants in the parents. Our patient had a compound heterozygous mutation. As a result of these genetic analyses, it was shown that one of these mutations was taken from the mother and the other from the father.

We would like to thank you for the opportunity to respond to the issues addressed in Finsterers letter and to clarify aspects of our audit in relation to these concerns and believe all comments have been answered.

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