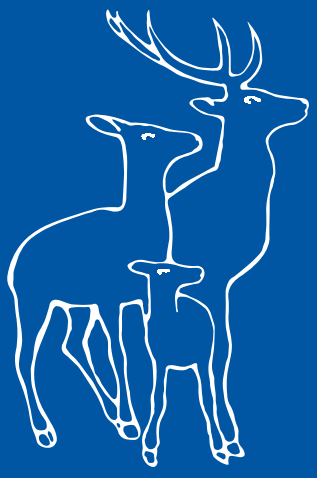

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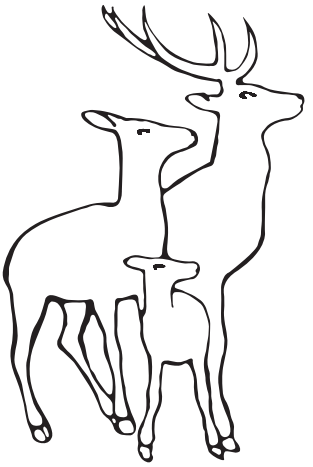

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CONTENTS

VOLUME: 64 NUMBER: 6 NOVEMBER-DECEMBER 2022

ORIGINAL ARTICLES

CONTENTS

VOLUME: 64 NUMBER: 6 NOVEMBER-DECEMBER 2022

CASE REPORTS

CONTENTS

VOLUME: 64 NUMBER: 6 NOVEMBER-DECEMBER 2022

LETTERS TO THE EDITOR

ERRATA

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Original Article

Clinical characteristics of firearm-related injuries in children in Turkey

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ABSTRACT

Background. A significant number of children are injured by or die from firearm-related incidents every year, although there is a lack of global data on the number of children admitted to pediatric emergency departments (PEDs) and pediatric intensive care units (PICU) with firearm injuries. This study is the most comprehensive analysis of firearm injuries sustained by children in Turkey to date.

Methods. This multicenter, retrospective, cohort study was conducted between 2010 and 2020 with the contributions of the PEDs, PICUs, intensive care units, and surgery departments of university hospitals and research hospitals.

Results. A total of 508 children were admitted to hospital with firearm-related injuries in the research period, although the medical records of only 489 could be obtained. Of the total admissions to hospitals, 55.0% were identified as unintentional, 8.2% as homicide, 4.5% as self-harm, and 32.3% as undetermined. The Glasgow Coma Scale (GCS) and ventilation support were found to be the most significant predictors of mortality, while head/neck injury, length of stay (LOS) in the hospital and surgical interventions were found to be the most significant predictors of disability. The overall mortality of firearm-related injuries was 6.3%, and the mortality for children admitted to the PICU was 19.8%. The probability of disability was calculated as 96.0% for children hospitalized with firearm injuries for longer than 75 days.

Conclusions. Head/neck injury, LOS in the hospital, and surgical interventions were found to be the most significant parameters for the prediction of disability. Hospitalization exceeding 6 days was found to be related to disability.

Key words: firearm injuries, disability, mortality, pediatric emergency medicine, pediatric intensive care.

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Firearm-related injuries are an important health problem, leading to both serious morbidity and death in children.[1,2] According to the American Academy of Pediatrics' 2012 policy statement on firearm injuries, firearm-related deaths are one of the three main causes of death among children in the United States aged 1–17 years, and the cause of 25.0% of the deaths in adolescents aged 15–19 years.[1] It is the second leading cause of injury-related death in this age group, surpassed only by motor vehicle injury deaths in the United States.[3] Many such injuries are present to pediatric emergency departments (PEDs) and pediatric intensive care units (PICUs). Pediatric emergency departments play an important role in the treatment of firearm-related injuries, with approximately 20,000 children presenting to PEDs with firearm-related injuries in the United States every year.[2]

Despite the significant number of child victims of firearm-related injuries and deaths, there is a distinct lack of data on children admitted to PEDs, hospitals and PICUs with firearm injuries. Previous studies of firearm injuries among children have focused on particular outcomes, such as death or hospitalization, or certain types of firearms injury, such as homicide or assault.

There is a lack of information about firearm-related deaths, sequelae and injuries in children and adolescents in Turkey. The aim of this study was to investigate the characteristics of children aged 0–18 years admitted to hospital with firearm-related injuries in Turkey to identify the factors determining the mortality and morbidity associated with firearm-related injuries.

Material and Method

This multicenter, retrospective, cohort study was conducted between January 2010 and August 2020 with the involvement of PEDs, emergency departments, PICUs, general intensive care units and surgery departments of university hospitals and research hospitals in Turkey. After the enrollment of 26 centers, patients under 18 years of age with admissions for firearm-related injuries were identified and included in the study. Diagnoses were established based on the International Classification of Disease 10th edition codes (W32-Accidental handgun discharge and malfunction, W33-Accidental discharge of hunting rifle, initial encounter, W34-Accidental discharge and malfunction from other and unspecified firearms and guns, X73-Intentional self-harm by rifle, shotgun and larger firearm discharge, X74-Intentional self-harm by other and unspecified firearm and gun discharge,Y23-Rifle, shotgun and larger firearm discharge, undetermined intent, Y24-Other and unspecified firearm discharge, undetermined intent, Y35.0- Legal intervention involving firearm discharge). Patients aged ≥18 years who presented to hospital with firearm-related injuries, and those whose file data could not be accessed, were excluded from the study. The study protocol was approved by the Ethical Committee of Ankara University (I7-448-20) and it was conducted in accordance with the principles of the Declaration of Helsinki.

For the purpose of the study, a firearm-related injury was defined as a gunshot wound or penetrating injury from a handgun, rifle, shotgun or other such weapon. The garnered demographic information included age at the time of admission to the hospital and gender. The patients were categorized into four groups based on their ages, similar to previous studies (0–4, 5–9, 10–14, and 15–17 years).[4,5]

There are standard definitions used for firearm-related injuries, including homicide, self-harm, unintentional and undetermined. The region of injury is defined based on eight categories, being head and neck, spine, chest, abdomen, extremity, skin, multiple and others. Multiple injuries are defined as three or greater in number in more than one body region. In the study, it is the injuries that are tabulated, not the patient, and so some patients may be included in more than one category. The setting in which the injury was sustained was defined in four categories: home, street, other and unknown.

The Glasgow Coma Scale (GCS) was used to evaluate patient state upon admission to the emergency department based on which they were categorized as mild (13–15), moderate (9–12) or severe (≤8). The Emergency Severity Index (ESI) triage instrument was used for patients who underwent an immediate life-saving intervention in the emergency department. Life-saving interventions were defined as those occurring within the first hour of arrival to the ED, and included airway and breathing support (intubation or emergent noninvasive positive pressure ventilation), electrical therapy (defibrillation, emergent cardioversion or external pacing), procedures (including chest, pericardiocentesis or open thoracotomy), hemodynamic support (significant intravenous fluid resuscitation in the presence of hypotension, blood administration or control of major bleeding) and emergent medications (naloxone, dextrose, atropine, adenosine, epinephrine or vasopressors).

All patients were evaluated in terms of hospitalization, LOS in the hospital and PICU, surgical procedures and discharge status according to the type of injury. The surgical procedures were tabulated rather than the patients, and so some patients may be included in more than one classification. Patients admitted to the PICU were evaluated in terms of LOS, respiratory support, circulatory support (fluid resuscitation, inotropes, blood product transfusion, and multiples thereof), and discharge according to the type of injury.

The World Health Organization International Classification of Functioning, Disability and Health (ICF) framework states that disabilities arise in the context of the dynamic interaction between a child's health conditions, functioning, activities, participation in life, and environmental/contextual and personal factors. In the present study, bodily function disability is defined based on the ICF classification (such as neuromusculoskeletal and movement-related functions, sensory functions and pain) and disability is defined based on the patient's status at discharge.

Statistical Analysis

Categorical variables were assessed with a $\chi 2$ test and expressed as number and percentage. Kolmogorov-Smirnov and Shapiro-Wilk tests were applied for the evaluation of the normality of continuous variables. Continuous variables (median [25^{th}–75^{th} percentile]; age, LOS in the hospital and PICU) were analyzed with a Kruskal-Wallis test. A Receiver Operator Characteristic (ROC) curve analysis was used to determine threshold values. The relationship between hospitalization days and disability was analyzed with a logistic regression analysis, and probability curves were plotted based on its outputs. The specificity, sensitivity, positive predictive value and negative predictive value of the relationship between hospitalization days and disability were determined.

The data analysis was conducted using IBM SPSS Statistics (Version 21.0. Armonk, NY: IBM Corp.) and MedCalc Version 12.5 software. A two-tailed p-value of <0.05 was considered significant.

Results

In the study period between January 2010 and August 2020, 508 children were admitted to medical centers in Turkey for firearm-related injuries, among which 489 met the inclusion criteria. The patients' median age was 13.66 [10–16] years. The firearm-related injuries of 55.0% were unintentional, while 8.2% were a result of homicide, 4.5% were self-harm and 32.3% were undetermined. Most admissions to hospital with firearm injuries were by those aged >10 years (p<0.0001). Of those admitted with firearm-related injuries, 77.9% were male, indicating a significant difference in gender (p=0.005). Unintentional injuries were more common in younger children (<10 years of age), but tended to decrease with age (0–4 years - 84.1%, 5–9 years - 76.6%, 10–14 - years 54.3%, 15–17 years - 40.6%.). In contrast, self-harming firearm-related injuries were predominant in children 10 years and older (especially >15 years) (Fig. 1). In terms of the setting in which

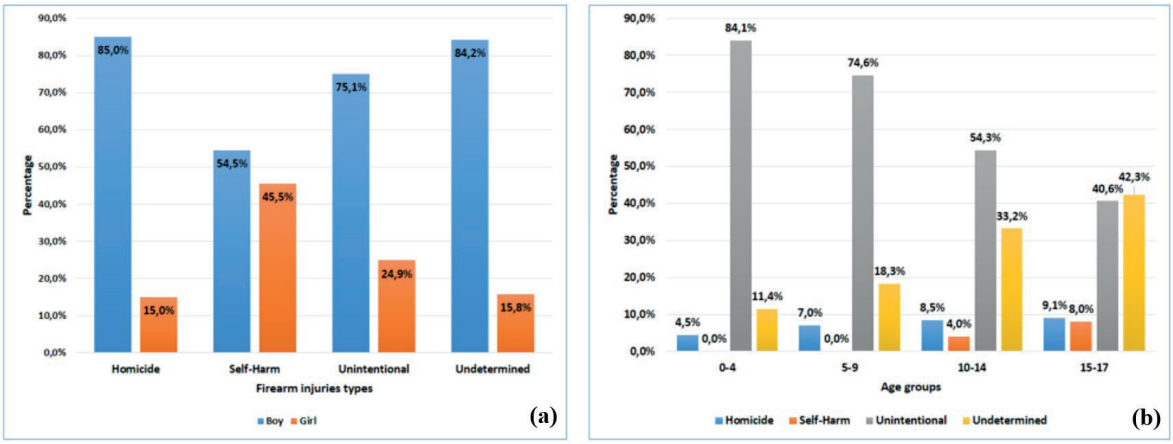

Fig. 1. Characteristics of firearm-related injuries in children, Firearm injury types by gender (a); Firearm injury types by age (b).

the injury was sustained, homicides were more common in the street, while self-harm were more common in the home (p<0.0001). In most of cases, the injuries were to extremities (49.5%). The demographic data of children with firearm injuries is shown in Table I.

Of the total, 41 (8.4%) of the patients admitted to hospital had a GCS of ≤8, and 29 of the 31 (93.5%) patients who died from their injuries had a GCS of ≤8. There were two deaths among the patients with an initial GCS above 12. Twenty-two of the 31 (71.0%) patients who died from their injuries had a head/neck injury, and their GCS was 8 or less. Furthermore, 30 of the 31 patients who died were intubated in the emergency room, and cardiopulmonary resuscitation was performed on 13 children, 12 (92.3%) of whom died. As a life-saving intervention, inotropic agents were administered to 18 patients who died. Of the 31 patients who died, 27 (87.1%) were in shock at the time of emergency room admission. The patients' characteristics data is presented in Table II.

Due to the lack of PICUs in some hospitals in our country, 2.1% of the patients were followed up in adult intensive care units. Despite the increase in the number of hospitalized patients over the years, there was no significant difference in the rate of hospitalized patients in need of PICU (Fig. 2). Hospitalizations with unintentional injuries were the most frequent, whereas hospitalizations resulting from self-harm were infrequent. Hospitalizations requiring admission to the PICU were most frequent among the self-harm patients. In children all of ages, among the hospitalizations necessitating admissions to PICU, unintentional injuries were the most common. The mean LOS in the hospital due to firearm-related injuries was 9.5±15.2 (1-123) days, with a median of 5 [2–10], and the mean stay in the PICU was 8.83±14.22 (1-107) days, with a median of 4 [2-10].

Ventilatory support was required in 68 of the 106 children admitted to the PICU. Of those who required ventilatory support, four (6.6%) needed noninvasive ventilator support and 57 (93.4%) needed mechanical ventilatory support, while the data for seven children were missing. The ventilatory support requirement rate was the highest among children admitted to the PICU due to self-harm (63.6%, p<0.0001). Circulatory support was required in 62 of the 106 patients admitted to the PICU, with the need for circulatory support being highest among those who sustained self-harm(45.5%, p<0.0001). Inotropes were the most commonly used circulatory support agents (66.1%). Of all the hospitalized children, 57.1% required surgical interventions for bullet/pellet removal (27.2%), wound repair (18.3%) and bone/tendon/nerve repair (17.6%) (Table III).

Table I. Demographic data of children with firearm injuries.

Characteristic	Homicide		Self-harm		Unintentional		Undetermined		Total		p
	n	%	n	%	n	%	n	%	n	%	
Gender											0.005
Male	34	(85.0)	12	(54.5)	202	(75.1)	133	(84.2)	381	(77.9)	
Female	6	(15.0)	10	(45.5)	67	(24.9)	25	(15.8)	108	(22.1)	
Age (years)											<0.0001
0-9	7	(17.5)	0	(0.0)	90	(33.5)	17	(10.8)	114	(23.3)	
10-17	33	(82.5)	22	(100.0)	179	(66.5)	141	(89.2)	375	(76.7)	
Season											0.010
Winter	7	(17.5)	6	(28.6)	48	(17.8)	39	(24.8)	100	(20.5)	
Spring	5	(12.5)	7	(33.3)	63	(23.4)	29	(18.5)	104	(21.4)	
Summer	9	(22.5)	4	(19.0)	100	(37.2)	46	(29.3)	159	(32.6)	
Autumn	19	(47.5)	4	(19.0)	58	(21.6)	43	(27.4)	124	(25.5)	
Setting of injury											<0.0001
Home	4	(10.0)	17	(77.3)	85	(31.6)	8	(5.1)	114	(23.3)	
Street	28	(70.0)	2	(9.1)	101	(37.5)	24	(15.2)	155	(31.7)	
Others	3	(7.5)	2	(9.1)	58	(21.6)	120	(75.9)	183	(37.4)	
Unknown	5	(12.5)	1	(4.5)	25	(9.3)	6	(3.8)	37	(7.6)	
Location of injury											
Head/neck	11	(27.5)	14	(63.6)	82	(30.5)	27	(17.1)	134	(27.4)	<0.0001
Spinal	4	(10.0)	1	(4.5)	11	(4.1)	2	(1.3)	18	(3.7)	0.064
Chest	10	(25.0)	5	(22.7)	53	(19.7)	29	(18.4)	97	(19.8)	0.799
Abdomen	12	(30.0)	3	(13.6)	52	(19.3)	21	(13.3)	88	(18.0)	0.076
Extremity	20	(50.0)	2	(9.1)	123	(45.7)	97	(61.4)	242	(49.5)	<0.0001
Skin	2	(5.0)	0	(0.0)	19	(7.1)	11	(7.0)	31	(6.5)	0.602
Multiple	4	(10.0)	0	(0.0)	10	(3.7)	5	(3.2)	19	(3.9)	0.162
Others	1	(2,5)	0	(0.0)	1	(0.4)	1	(0.6)	3	(0.6)	0.435

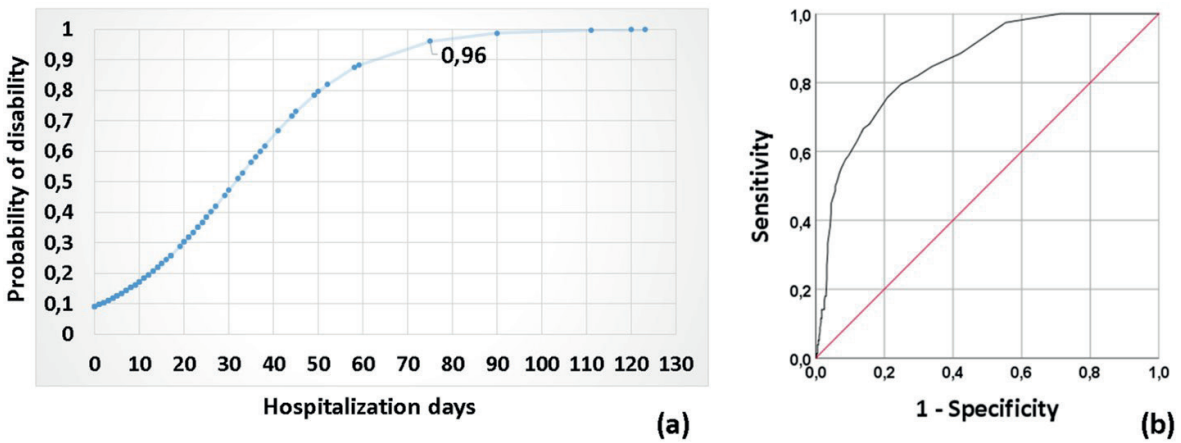

Fig. 2. Logistic relationship between the probability of disability and hospitalization days (a), and curve (b).

Table II. Patients characteristics of firearm-related injuries.

Characteristic	Homicide		Self-harm		Unintentional		Undetermined		Total		p
	n	%	n	%	n	%	n	%	n	%	
Total	40	(100)	22	(100)	269	(100)	158	(100)	489	(100)	
PED Admission	25	(62.5)	9	(40.9)	173	(64.3)	74	(46.8)	281	(57.5)	0.002
GCS											<0.0001
13-15	32	(80.0)	10	(45.5)	237	(88.1)	142	(89.9)	421	(86.1)	
9-12	3	(7.5)	1	(4.5)	16	(5.9)	7	(4.4)	27	(5.5)	
≤8	5	(12.5)	11	(50.0)	16	(5.9)	9	(5.7)	41	(8.4)	
Shock	8	(20.0)	11	(50.0)	31	(11.5)	19	(12.0)	69	(14.1)	<0.0001
Life-saving intervention	17	(42.5)	17	(77.3)	77	(28.6)	45	(28.5)	156	(31.9)	
BVM ventilation	4	(10.0)	2	(9.1)	22	(8.2)	12	(7.6)	40	(8.2)	0.546
Intubation	8	(20.0)	14	(63.6)	28	(10.4)	15	(9.5)	65	(13.3)	0.003
Surgical airway	1	(2.5)	2	(9.1)	4	(1.5)	2	(1.3)	9	(1.8)	0.505
IV fluid resuscitation	10	(25.0)	9	(40.9)	53	(19.7)	32	(20.3)	104	(21.3)	0.480
Blood administration	7	(17.5)	8	(36.4)	38	(14.1)	21	(13.3)	74	(15.1)	0.942
Control of major bleeding	3	(7.5)	2	(9.1)	19	(7.1)	14	(8.9)	38	(7.8)	0.392
Intraosseous access	1	(2.5)	0	(0.0)	0	(0.0)	0	(0.0)	1	(0.2)	0.042
Needle decompression	0	(0.0)	0	(0.0)	0	(0.0)	0	(0.0)	0	(0.0)	
Chest tube	6	(15.0)	6	(27.3)	13	(4.8)	11	(7.0)	36	(7.4)	0.204
Pericardiocentesis	0	(0.0)	0	(0.0)	0	(0.0)	0	(0.0)	0	(0.0)	
CPR	3	(7.5)	5	(22.7)	2	(0.7)	3	(1.9)	13	(2.7)	0.002
Inotropes	4	(10.0)	7	(31.8)	17	(6.3)	4	(2.5)	32	(6.5)	0.039

PEDs: pediatric emergency departments, GCS: Glasgow Coma Scale, BVM: Bag valve mask, IV: Intravenous, CPR: Cardiopulmonary resuscitation.

The overall mortality from firearm-related injuries was 6.3%, and 19.8% among those admitted to the PICU. The majority of firearm-related deaths were in males (58.1%) (p=0.06). The highest percentage of in-hospital deaths from firearm-related injuries was among those who attempted self-harm (40.9%, p<0.0001). Mortalities were more common in winter (40%) and summer (33.3%) than in spring (20.0%) and autumn (6.7%) (p=0.016). The most common causes of death were brain death (67.7%), sepsis (12.9%) and hemorrhagic shock (9.7%), while the other causes of death were cardiopulmonary arrest (6.4%) and pneumothorax (3.2%). The mortality rate of patients hospitalized in PICUs (19.8%) was higher than in the surgery departments (2.5%) (p<0.0001). Mortality was higher in the patients who received respiratory (77.4, p<0.0001) or circulatory support (71.0%, p<0.0001) or both (71.0%, p<0.0001). A logistic regression analysis assessing the relationship between the initial characteristics of the patient admissions and mortality revealed mortality to be positively associated with gender, season, setting of injury, injury category, head/neck injury, GCS, shock, life-saving interventions, CPR, PICU admission, LOS in the PICU, ventilation support and circulation support. GCS and ventilation support were found to be the most significant predictors of mortality (Table IV).

Of the total, 78 children were discharged from the hospital with some level of disability. The morbidity rate of patients hospitalized in the PICUs (29.7%) was higher than from the surgery departments (16.1%) (p<0.0001). The most frequent firearm-related disabilities were related to neuromusculoskeletal and movement-related function (60.3%), followed by sensory function and pain (30.8%), and functions of the digestive, metabolic and endocrine systems

Table III. Patients characteristics of firearm-related injuries related to hospitalizations and Pediatric Intensive Care Unit.

Characteristic	Homicide		Self-harm		Unintentional		Undetermined		Total		p
	n	%	n	%	n	%	n	%	n	%	
Total	40	(100)	22	(100)	269	(100)	158	(100)	489	(100)	
Hospitalization	33	(82.5)	19	(86.4)	202	(75.1)	119	(75.3)	373	(76.3)	0.497
PICU	8	(24.2)	10	(52.6)	72	(35.6)	16	(13.4)	106	(28.4)	<0.0001
Surgery Department	22	(66.7)	10	(52.6)	144	(71.3)	103	(86.6)	279	(74.8)	0.001
ICU	5	(15.2)	0	(0.0)	0	(0.0)	2	(1.7)	7	(1.9)	<0.0001
Length of stay - mean											
PICU											
Median [25th-75th percentile]	5 [2-21.75]		5 [2-15]		4 [2-10]		3 [2-5]		4 [2-10]		0.339
Hospital											
Median [25th-75th percentile]	6 [2.25-13.50]		3 [1-17]		5 [2-10]		4 [1-8]		5 [2-10]		0.304
Ventilation	7	(17.5)	14	(63.6)	33	(12.3)	14	(8.9)	68	(13.9)	<0.0001
Noninvasive	1	(14.3)	0	(0.0)	3	(9.7)	0	(0.0)	4	(6,6)	0.425
Mechanical	6	(85.7)	11	(100)	28	(90.3)	12	(100.0)	57	(93.4)	
Circulatory support	8	(20.0)	10	(45.5)	34	(12.6)	10	(6.3)	62	(12.7)	<0.0001
Inotropes	4	(50.0)	6	(60.0)	23	(67.6)	8	(80.0)	41	(66.1)	0.574
IV fluid resuscitation	3	(37.5)	1	(10.0)	7	(20.6)	3	(30.0)	14	(22.6)	0.509
Blood product transfusion	4	(50.0)	3	(30.0)	6	(17.6)	2	(20.0)	15	(24.2)	0.264
Multiple	3	(37.5)	1	(10.0)	7	(20.6)	4	(40.0)	15	(24.2)	0.324
Surgical intervention	25	(62.5)	11	(50.0)	156	(58.0)	87	(55.1)	279	(57.1)	0.733
Wound repair/ debridement/graft	4	(16.0)	1	(9,1)	23	(14.7)	23	(26.4)	51	(18.3)	0.117
Bullet/pellet removal	6	(24.0	0	(0.0)	58	(37.2)	12	(13.8)	76	(27.2)	<0.0001
Bone/tendon/nerve repair	3	(12.0	1	(9.1)	28	(17.9)	17	(19.5)	49	(17.6)	0.722
Maxilla/mandible reconstruction	1	(4.0)	2	(18.2	2	(1.3)	0	(0.0)	5	(1.8)	<0.0001
Head/neck surgery	1	(4.0)	1	(9.1)	5	(3.2)	3	(1.1)	10	(3.6)	0.790
Laparotomy	7	(28.0)	2	(18.2)	20	(12.8)	12	(13.8)	41	(14.7)	0.248
Eye surgery	1	(4.0)	0	(0.0)	8	(5.1)	0	(0.0)	9	(3.2)	0.163
Chest Tube	4	(16.0)	3	(27.3)	7	(4.5)	8	(9.2)	22	(7.9)	0.014
Artery/vein repair	0	(0.0)	1	(9.1)	10	(6.4)	10	(11.5)	21	(7.5)	0.229
Others	1	(4.0)	1	(9.1)	3	(1.9)	1	(1.1)	6	(2.2)	0.337
Discharge											
All patients											<0.0001
Home	29	(72.5)	11	(50.0)	209	(77.6)	128	(81.0)	376	(77.1)	
Died	5	(12.5)	9	(40.9)	8	(3.0)	9	(5.7)	31	(6.3)	
Disabilities	6	(15.0)	2	(9.1)	51	(19.0)	19	(12.0)	78	(16.0)	
Patient referral	0	(0.0)	0	(0.0)	1	(0.4)	2	(1.3)	3	(0.6)	
PICU patients											0.155
Home	4	(50.0)	4	(40.0)	35	(52.2)	8	(50.0)	51	(50.5)	
Died	3	(37.5)	4	(40.0)	8	(11.9)	5	(31.3)	20	(19.8)	
Disabilities	1	(12.5)	2	(20.0)	24	(35.8)	3	(18.8)	30	(29.7)	

PICU: Pediatric intensive care unit, ICU: Intensive care unit, IV: Intravenous.

Table IV. Logistic regression of initial characteristics for prediction of mortality in patients with firearm-related injuries.

Characteristic	Univariate regression model		Multivariate regression model	
	OR (95% CI lower-upper)	P	OR (95% CI lower-upper)	P
Gender	2.760 (1.306-5.832)	0.008		
Season	0.591 (0.414-0.846)	0.004		
Scene by injury	0.540 (0.351-0.831)	0.005		
Injury categories	0.569 (0.392-0.826)	0.003		
Head/neck injury	7.552 (3.379-16.878)	<0.0001		
GCS	29.227 (11.848-72.097)	<0.0001	11.683 (4.075-33.495)	<0.0001
Shock	66.857 (22.322-200.242)	<0.0001		
CPR	288.632 (35.665-2335.834)	<0.0001		
PICU administration	5.822 (2.749-12.328)	<0.0001		
Length of stay PICU	1.038 (1.008-1.069)	0.014		
Ventilation support	32.260 (13.149-79.144)	<0.0001	8.749 (1.162-65.869)	<0.0001
Circulation support	25.544 (11.021-59.208)	<0.0001		

GCS: Glasgow Coma Scale, CPR: Cardiopulmonary resuscitation, PICU: Pediatric intensive care unit.

(25.6%) (Table V). Unintentional injuries were the most common cause of disability (Table III).

A logistic regression analysis assessing the relationship between the initial characteristics of the patients at the time of admission and disability revealed disability to be positively associated with head/neck injury, GCS, shock, life-saving interventions, PICU administration, LOS in the PICU, LOS in the hospital, ventilation support, circulation support and surgical intervention. Head/neck injury, LOS in the hospital and surgical intervention were found to be the most significant predictors of disability (Table VI). The probability curve of disability from firearm-related injuries was calculated based on hospitalization days, and the probability of disability was calculated as 96.0% for children hospitalized for longer than 75 days (Fig. 2). The relationship between hospitalizations for more than 6 days and disability was calculated, and revealed a sensitivity, specificity, positive predictive value, negative predictive value and accuracy of 75.64% (64.60–84.65%), 79.16% (74.86–83.0%), 41.26% (35.86–46.88%), 94.38% (91.88–96.14%) and 78.6% (74.65–82.17%), respectively (Fig. 2).

Discussion

In this comprehensive, multicenter retrospective study of firearm-related injuries in children admitted to PEDs and PICUs in Turkey, firearm-

Table V. Firearm-related cause of disabilities characteristics.

Cause of disabilities	n	%
Neuromusculoskeletal and Movement-Related Functions	47	60.3
- Functions of the joints and bones	22	28.2
- Muscle functions	41	52.6
- Movement functions	47	60.3
Sensory Functions and Pains	24	30.8
- Sight and related functions	18	23.6
- Hearing and vestibular functions	1	1.3
Functions of the Digestive, Metabolic and Endocrine Systems	20	25.6
- Functions related to the digestive systems	20	25.6
Genitourinary and Reproductive Functions	8	10.3
- Urinary functions	7	9.0
- Genital and reproductive functions	1	1.3
Functions of the Skin and Related Structures	8	10.3
- Functions of the skin	6	7.7
- Functions of the hair and nails	3	3.8
Mental Functions	7	9.0
Voice and Speech Functions	5	6.4
Functions of Cardiovascular, Hematological, Immunological and Respiratory Systems	5	6.4
- Functions of respiratory systems	5	6.4

Table VI. Logistic regression of initial characteristics for prediction of disability in patients with firearm-related injuries.

Characteristic	Univariate regression model		Multivariate regression model	
	OR (95% CI lower-upper)	P	OR (95% CI lower-upper)	P
Head/neck injury	3.117 (1.892-5.136)	<0.0001	3.648 (2.013-6.610)	<0.0001
GCS	1.747 (1.240-2.459)	0.001		
Shock	3.615 (2.040-6.404)	<0.0001		
Life-saving interventions	2.677 (1.635-4.384)	<0.0001		
PICU administration	3.395 (2.028-5.686)	<0.0001		
Length of stay PICU	1.102 (1.056-1.150)	<0.0001		
Length of stay hospital	1.076 (1.052-1.101)	<0.0001	1.052 (1.029-1.074)	<0.0001
Ventilation support	3.707 (2.089-6.578)	<0.0001		
Circulation support	3.989 (2.216-7.181)	<0.0001		
Surgical intervention	8.457 (3.968-18.022)	<0.0001	5.549 (2.364-13.023)	<0.0001

GCS: Glasgow Coma Scale, PICU: Pediatric intensive care unit.

related injuries were found to vary by patient demographics, which is a pattern that has been well described in the pediatric population in the United States, but not in Turkey.[3] The findings in this article highlight the characteristics of firearm-related injuries among children in Turkey. To the best of our knowledge, this is the first multicenter study conducted to date analyzing the clinical characteristics of pediatric firearm-related injuries in Turkey.

The incidence of firearm injuries varies considerably between regions, age groups and genders.[6] In the present study, the majority (79.9%) of children affected by firearm-related injuries were male, concurring with previous studies in literature.[4,7] Risk also increases with age, with older adolescent males accounting for the majority of firearm-related injuries. These findings and those of previous studies suggest that efforts to prevent firearm-related injuries in children should begin at an early age, and should focus particularly on males.[6,7]

In 2019, there were 15,824 deaths among children aged 0–14 years in Turkey[8], among which more than 6.0% were due to "external causes of injury and poisoning".[8] In the study of Cunningham et al., firearm-related injuries were the second leading cause of death among children and adolescents in the United States, of which 59.0% were attributable to homicide and 35.0% to self-harm, while 4.0% were unintentional.[9] In contrast, among Turkish children, 29.0% were attributable to self-harm, 25.8% were unintentional, 16.1% were homicide and 29.0% were unknown in our study. The differences between the United States and Turkey are thought to arise from the differences in the laws governing the ownership of firearms.

Firearm injuries to the head are the most lethal, and many people do not survive after sustaining such an injury. Researchers have reported survival rates of 7.0–15.0%, and that more than 90.0% of people sustaining a firearm injury to the head eventually die, mostly within minutes due to respiratory and circulatory arrest.[10] In the present study, 22 of the 31 patients admitted to hospital who died due to firearm injuries had head/neck injuries, and a positive association with mortality.

Recent studies have identified a link between the GCS of a patient at the time of admission and the outcome of the patients, with an 85.0% likelihood of mortality in those with a GCS of less than 7.[10,11] In the present study, the GCS of 41 (8.4%) of the patients admitted to the hospital was ≤8, and 29 (70.7%) of these died. GCS was thus found to be one of the most accurate predictors of mortality, as in previous studies. In a study by Ewing-Cobbs et al.[12], the GCS score at baseline was able to predict moderate and severe disabilities in 69.0% and 23.0% of cases, respectively. Similar to this study, disability was found to have a positive association with GCS in the present study.

Firearm-related injuries lead to emergency department visits and hospitalizations, and around 40.0% of such injuries in children require hospitalization in the intensive care unit.[4,13,14] Pediatric emergency services are the entry point for many trauma patients, such as those who have sustained firearm injuries. In the United States, approximately 19 children receive treatment for, or die in the emergency department from firearm-related injuries every day.[15] As with all admissions to the PED, the ability to make effective interventions is of paramount importance. In the present study, early and effective life-saving interventions were shown to be important for the prevention of mortality and disability in cases of firearm-related injury. Intensive care unit interventions include the frequent use of invasive or noninvasive mechanical ventilatory support, circulatory support, such as vasopressor use, IV fluid replacement, and blood product transfusions. Our analysis revealed that firearm-related injuries resulting from self-harm required more frequent intensive care unit admissions (28.4%), and these patients needed more respiratory and circulatory support than those with other types of injury. This is because children are uniquely vulnerable, they have larger heads and torsos, densely calcified

bones are lacking, and skin and muscles are not sufficiently developed.[14] Furthermore, the high energy of a bullet leads to a wide field of tissue injury in children.[14] Children with firearm injuries are more likely to be admitted to the ICU than children with other penetrating injuries,[16] and PICU admission was found to be a significant predictor mortality and disability in the present study.

Firearm-related injuries lead to hospitalizations, and around 50.0–69.0% of children require surgical interventions.[13] Typically, these children must be cared for at tertiary hospitals and by a multidisciplinary team of pediatric surgery, neurosurgery, orthopedics and plastic surgery specialists.[14] In the present study, a large proportion (57.1%) of the patients required surgical interventions, although there were no significant differences in the surgical interventions made within the different firearm-related injury groups. The most common surgical interventions were bullet/pellet removal (27.2%), wound repair/debridement/graft (18.3%) and bone/tendon/nerve repair (17.6%).

Pediatric firearm injuries are associated with a higher likelihood of prolonged hospital or PICU stay.[14] Wolf et al.[16] reported a hospital LOS of children with firearm injuries of 5.0 days and an ICU LOS of 5.1 days. In the present study, the hospital LOS was 9.50±15.20 days and PICU LOS was 8.83±14.22days, which were longer than in Wolf et al.[16] ICU admission and longer LOS are associated with increased risk of morbidity and complications.[16] Our reported a probability of disability of 96.0% among children hospitalized with firearm injuries whose LOS was longer than 75 days, and a relationship between hospitalizations exceeding 6 days and disability in firearm injuries.

Pediatric suicides are common and are associated with the greatest odds of in-hospital mortality.[15,17,18] The CDC reported an increase in the suicide rate among children aged 15–19 from 2007 to 2015.[19] In the present study, the mortality rate was highest among those with self-directed firearm injuries than in the other groups, which is similar to the findings of earlier studies.[20-22] A continuous rise can be seen in cases of self-harm by firearms among children[20], and previous studies have reported that firearm-related injuries contribute to the rate of disabilities among children, making gun violence an important public health issue.[7] Around half of all children hospitalized with firearm injuries are discharged with disabilities.[14] Many hospitalized patients report a significant long-term decline in physical and/or mental health.[4,23] In a study by Discala et al.[4], more than one-third of the children developed short-term disabilities as a result of injuries to the extremities, while 6.5% developed long-term disabilities due to injuries to the central nervous system.[4] In the present study, we assessed the physical consequences of non-fatal firearm-related injuries at the time of discharge from hospitalization, classifying the disabilities according to the ICF. It was found that the most common disabilities resulting from firearm injuries were in neuromusculoskeletal and movement-related functions, accounting to 60.3%, sensory function and pain, with 30.8%, and functions of the digestive, metabolic and endocrine systems, with 25.6%. Unintentional firearm-related injuries were the most common cause of disability.

There are a few limitations to this study, the first of which is its retrospective design which prevented access to all the data on firearm-related injuries in children. One of the reasons for this was that the standard national database that is available today was not available for the period in question. This study is dependent on the accuracy and completeness of ICD-10. If these codes may be underreported in the data base the patients did not selected. As patient data were missing from the hospital database, the number of patients in the undetermined group of firearm-related injuries was high, and this high number of undetermined patients prevents the clarification of the exact distribution. This limitation may be eliminated in future prospective studies, or by informing

clinicians about the need to provide more detail for databases of firearm-related injuries.

Second, the centers in all cities of our country could not participate in our hospitals participating in this study are tertiary care facilities in major Turkey metropolitan cities. As such, while our findings cannot be generalized for all firearm-related injuries in Turkey, they do provide information about the distribution of firearm-related injuries in children in the country.

Third, firearm-related injuries that that resulted in mortality prior to hospital admission were disregarded in this study, leading to a low mortality rate for Turkey being recorded. Fourth, regarding the data on disabilities resulting from firearm injuries, we considered the condition of the patients at the time of discharge, while the long-term disabilities of patients are disregarded. A further study is thus needed to determine the long-term disabilities of firearm-related injuries in children in Turkey.

Fifth, our study made use of data only from centers with PEDs and PICUs in Turkey, while patients admitted to adult trauma centers were excluded from the study. Our results may thus not represent the true PICU requirement.

Our study found GCS and ventilation support to be the most significant predictors of mortality, while head/neck injuries, LOS in the hospital, and surgical intervention were found to be the most significant predictors of disability, along with hospitalization exceeding 6 days. Firearm injuries can be severe and can require frequent major therapeutic interventions, and are associated with high mortality and disability rates. Knowledgeable care teams that can provide the appropriate management and treatment for the long-term disabilities experienced by patients are thus needed. An assessment of the regional variations in pediatric firearm-related injuries may lead to the reduction of the rate of unintentional occurrences through the routine informing of families in high-risk areas.

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Ethical approval

The study protocol was approved by the Ethical Committee of Ankara University (I7-448-20) and it was conducted in accordance with the principles of the Declaration of Helsinki.

Author contribution

The authors confirm contribution to the paper as follows: study conception and design: GV,TK,DT,BU,HLY, DY; data collection: GV, TK, HLY, TN, MK, HA, AK, MÇ, ÖT, GB, MH, AEA, ODG, FB, PY, NY, DY, RD, DT, BU, Turkish Pediatric Firearm Study Group; analysis and interpretation of results: GV, TK, BU, HLY, TN, MK, HA, AK, MÇ, ÖT, GB, MH, AEA, ODG, FB, PY, NY, DY, RD, DT, Turkish Pediatric Firearm Study Group; draft manuscript preparation: GV,TK,DT,BU,HLY. All authors reviewed the results and approved the final version of the manuscript.

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Conflict of interest

The authors declare that there is no conflict of interest.

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Original Article

Evaluation of patients diagnosed with phenylketonuria and biotinidase deficiency by the newborn screening program: a ten-year retrospective study

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ABSTRACT

Background. Phenylketonuria (PKU) and biotinidase deficiency (BD) are autosomal recessive diseases. If they are not identified and treated early, severe intellectual disability and developmental delay occur. This study was conducted to calculate the ten-year incidence of PKU and BD in the Diyarbakır province of Turkey.

Methods. This cross-sectional study included patients born between 2011-2020 and diagnosed with PKU and BD. Patients with a clear diagnosis had their records evaluated retrospectively.

Results. Between 2011 and 2020, blood was taken from 417,525 newborns' heels in Diyarbakir province. As a result of further diagnostic testing, 53 PKU (Incidence: 1:7878) and 177 BD (Incidence: 1:2359) were detected. Of the patients with BD, 56% had profound BD and 44% had partial BD. The records of a total of 269 patients (PKU: 25; BD: 123; Hyperphenylalaninemia: 121) were examined. Parents of 65% (n=15) of the patients diagnosed with PKU and 46.6% (n=55) of the patients diagnosed with BD were consanguineous.

Conclusions. The incidence of both PKU and BD was found to be high in our region. The high number of consanguineous marriages was regarded as the most important explanation for the high frequency of these illnesses.

Key words: biotinidase deficiency, incidence, newborn screening, phenylketonuria.

Newborn screening programs are preventative health services that are extensively utilized across the world and play an essential role in public health programs. Robert Guthrie developed metabolic screening of neonates in the early 1960s by collecting blood samples on filter paper (Guthrie card) for phenylketonuria (PKU) screening using a bacterial inhibition technique.[1] Newborn screening programs include 1-50 disorders, depending on country or state.[2] The goal of newborn screening for PKU and biotinidase deficiency (BD) is to reduce the economic burden of diseases on society, raise public awareness about consanguineous marriages, detect disease symptoms in diagnosed infants before they appear, initiate the appropriate treatment to prevent diseases, and thus ensure that they attain normal intellectual capacity.[1,3]

Patients with PKU, which was first described in 1934, lack the phenylalanine hydroxylase enzyme. PKU is an autosomal recessive disease. The patient with classical PKU appears normal in the first few months, but as high levels of phenylalanine and its other toxic derivatives such as phenylpyruvate persist, vomiting, developmental delay, severe

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cognitive impairment, and dysmyelination ensue.[4] In children with PKU, developmental delay becomes visible after 5-6 months. They are unable to gain milestones such as sitting, walking, and speaking like their peers do. Microcephaly develops due to insufficient brain growth. The child may exhibit irrelevant, hyperkinetic or even autistic behaviors. Light hair, eye and skin color is seen in 60% of the cases. Moldy odor is noticeable in their body fluids and urine.[5] However, it is possible to prevent permanent damage to mental and motor development with early diagnosis and treatment.

The increase in the prevalence of genetic metabolic illnesses produces major societal health concerns in nations where consanguineous marriages are frequent. Due to consanguineous marriages, PKU is widespread in Turkey.[4,5] BD is another metabolic disorder that is commonly encountered in Turkey.[6] BD is also an inherited autosomal recessive neurocutaneous disorder. Seizures, skin rash, and alopecia, associated with acidosis and organic acidemia, emerge in individuals with untreated BD in the first few years of life owing to multiple carboxylase deficiency.[7,8] Clinical diagnosis may be challenging if BD is not detected by newborn screening since many children may mimic a variety of illnesses such as atopic dermatitis, neuromyelitis optica, optic atrophy, and myelopathy.[9] Early diagnosis and treatment can be life-saving, and the symptoms of the disease can be prevented. Early diagnosis and rapid initiation of oral biotin supplementation prevent neurological sequelae and clinical events.[8]

This study was conducted to calculate the incidence of phenylketonuria and biotinidase deficiency in the Diyarbakır province of Turkey.

Material and Methods

This study, conducted retrospectively using health records, comprises individuals born between 2011 and 2020 who were diagnosed with PKU and BD as a consequence of the newborn screening program. Patient information was acquired from their primary care providers. This study was conducted in compliance with the ethical principles according to the Declaration of Helsinki, and it was approved by the Ethics Committee of Health Sciences University, Gazi Yaşargil Training and Research Hospital (Number: 2021/855).

As per the algorithm of the Newborn Screening Program, capillary blood samples collected from the heels of newborns on the Guthrie card at health institutions are sent to screening laboratories designated by the Ministry of Health. If the phenylalanine level is 2.1 mg/dL or higher, the result is suspicious for PKU. If the biotinidase enzyme activity is low or absent (65 MRU or less), the result is suspicious for BD.[10] The screening laboratory's blood results for each disease are transmitted to the provinces via the Newborn Screening Program Web Application, and infants with suspected PKU and BD based on screening results are referred to the pediatric nutrition and metabolism clinics by their registered family physicians. In the patients included in this study, if the blood phenylalanine level was 2-10 mg/dL, the patient was diagnosed with hyperphenylalaninemia (HPA). If it is between 10-20 mg/dL, mild-moderate PKU, and if it is above 20 mg/dL, the patient was diagnosed with classical PKU.[11] In patients who were born in the years we included in the study and had high phenylalanine levels, the tetrahydrobiopterin (BH4) loading test was not performed. A low phenylalanine diet was initiated in all patients with blood phenylalanine levels above 10 mg/dL. In patients with blood phenylalanine levels between 6-10 mg/dL, protein intake is restricted to the safe lower limit according to their age. When the biotinidase enzyme activity was less than 30% of the standard value, BD was diagnosed. When the biotinidase enzyme activity was less than 10% of the normal level, profound BD was diagnosed; if the enzyme activity was between 10% and 30%, partial BD was diagnosed.[12]

Data for this study were gathered between August 1, 2021 and November 30, 2021. PKU and BD patients from Diyarbakir province were determined via the National Newborn Screening Program Web Application. Annual and ten-year incidence of disease were calculated. While calculating the incidence, Turkish Statistical Institute data was taken as the basis for the number of births.[13]

As a result of the screening, 443 patients were diagnosed with PKU, HPA or BD in 10 years. In the retrospective review of records, the data of 61% (n=269) of the patients were obtained. Because newborns do not have identification numbers when they are born, newborn screening records are created using the identification numbers of their mothers. The mother's identification number was used to locate the family medical unit where she was registered. The infant with PKU, HPA or BD was identified based on the date of birth from the family physician's records. The Family Medicine Unit's file records, in which patients with a clear diagnosis were registered, were evaluated retrospectively. It was questioned whether the patients had therapy, if their siblings/relatives had a similar disease and whether the parents were consanguineous or not.

The obtained data were loaded into the SPSS.21 statistical program, and the number, percentage, mean and standard deviation, median, minimum and maximum values were calculated. The normal distribution of quantitative data was evaluated with the Kolmogorov-Smirnov test. Kruskal-Wallis and Mann-Whitney U tests were used for comparison of quantitative data, and chi-square and Fisher Exact test were used to compare qualitative data. $p<0.05$ was considered statistically significant.

Results

Between 2011 and 2020, blood samples were obtained from the heels of 417,525 newborns in Diyarbakir. As a result of the screening, 1,122 infants were found to be suspicious for PKU, and 595 infants were found to be suspicious for BD. Among these, 122 infants (10.9%) with suspected PKU and 9 infants (1.5%) with suspected BD died before a diagnosis was made. The causes of death of the 9 patients with suspected BD were examined. It was determined that these nine patients died due to complications of congenital anomalies or prematurity. Two patients died from cardiac anomaly, two from hydrocephalus, one from meningomyelocele; and the other four patients died due to prematurity and sepsis within 7-28 days of follow-up in the intensive care unit. No findings suggestive of BD were found in these 9 patients who died. As a result of the tests performed in the pediatric metabolism clinic, the blood phenylalanine level of 266 newborns was found to be high. Of these, 53 were diagnosed with PKU (Incidence: 1:7878) and 213 with HPA (Incidence: 1:1960). 32.1% (n=17) of the patients with PKU had mild-moderate PKU, and 67.9% (n=36) had classical PKU. Furthermore, 177 BD (Incidence:1:2359) cases were discovered. 56% (n=100) of BD patients had profound enzyme deficiency (incidence:1:4175), whereas 44% (n=77) had partial enzyme deficiency (incidence:1:5422). In our study, 1 patient who was born in 2019 in our city and was residing in our country as a refugee and 3 patients who were born in 2020 were included in the calculation (Table I). In our study, no cases of PKU and BD co-existence were identified. When the distribution of illnesses by year is analyzed, it was seen that both the incidence of HPA and the incidence of BD increased in the previous three years. In particular, the incidence of BD has increased more than twice in the last three years compared to previous years (Fig. 1).

Data of a total of 269 patients (PKU: 25; BD: 123; HPA: 121) were analyzed. Parents of 65% (n=15) of the patients diagnosed with PKU and 46.6% (n=55) of the patients diagnosed with BD were consanguineous. It was determined that siblings or close relatives of 23.6% of the patients had a similar disease. On the other hand, it was determined that 52.0% of the patients diagnosed

Table I. Number of births by years, the incidence of phenylketonuria, hyperphenylalaninemia and biotinidase deficiency.

Year	Number of Births	PKU		HPA		BD			
						Profound	Partial	Total	
		Number	Incidence	Number	Incidence	Number	Number	Number	Incidence
2011	40,880	6	1/6813	17	1/2405	9	-	9	1/4542
2012	42,117	3	1/14039	23	1/1831	3	-	3	1/14039
2013	41,729	9	1/4637	26	1/1605	9	-	9	1/4637
2014	44,259	7	1/6323	14	1/3161	10	-	10	1/4426
2015	43,582	5	1/8716	16	1/2724	1	8	9	1/4842
2016	42,937	4	1/10734	14	1/3067	6	5	11	1/3903
2017	43,545	5	1/8709	14	1/3110	7	10	17	1/2561
2018	41,752	5	1/8350	26	1/1606	11	19	30	1/1392
2019	39,791	5	1/7958	33	1/1206	23*	18	41	1/971
2020	36,933	4	1/9233	30*	1/1231	21*	17*	38	1/972
Total	417,525	53	1/7878	213	1/1960	100	77	177	1/2359

BD: biotinidase deficiency, HPA: hyperphenylalaninemia without phenylketonuria, PKU: phenylketonuria
*One patient with profound BD, who was born in 2019 in our city and was residing in our country as a refugee, 2 patients with HPA, who were born in 2020; 2 patients with profound BD and 1 patient with partial BD were included in the table.

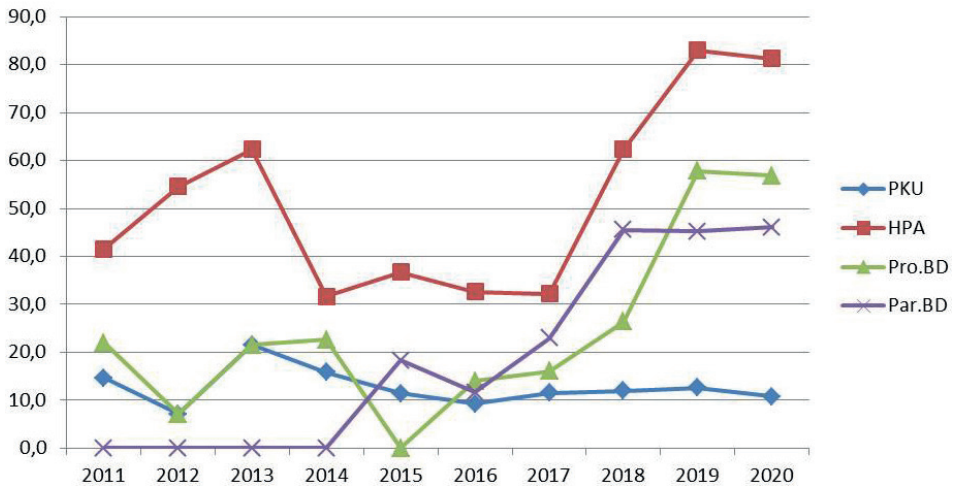

Fig. 1. Frequency distribution of PKU, HPA, profound biotinidase deficiency and partial biotinidase deficiency per 100,000 live births by years.

HPA: hyperphenylalaninemia without phenylketonuria, PKU: phenylketonuria, Par.BD: partial biotinidase deficiency, Pro.BD: profound biotinidase deficiency

with PKU and 17.9% of the patients diagnosed with BD had a similar disease in their relatives ($p<0.05$) (Table II).

49.4% (n=133) of the patients were male and 50.6% (n=136) were female. According to the latest follow-up, the mean age of the patients was 33.1±21.7 months (min-max: 2-117 months). At the time of the latest pediatric follow-up examination, the mean age of the patients with BD was 28.9±18.1 months, while the mean age of the patients with PKU was 44.1±24.1 months ($p<0.05$). While the majority of patients with PKU and BD were treated (86.6% and 91.8%, respectively), 40.6% of patients with HPA without phenylketonuria were treated ($p<0.05$).

Table II. Evaluation of the consanguinity status between the parents and the presence of a similar disease in siblings or relatives.

		BD n (%)	PKU n (%)	HPA n (%)	Total n (%)	P value
Are the parents consanguineous?	Yes	55 (46.6)	15 (65.2)	55 (48.7)	125 (49.2)	P=0.261
	No	63 (53.4)	8 (34.8)	58 (51.3)	129 (50.8)	
Have the patient's siblings/relatives been diagnosed with the same disease?	Yes	21 (17.9)	13 (52.0)	26 (23.2)	60 (23.6)	P=0.001
	No	96 (82.1)	12 (48.0)	86 (76.8)	194 (76.4)	

BD: biotinidase deficiency, HPA: hyperphenylalaninemia without phenylketonuria, PKU: phenylketonuria

24.1% of HPA patients do not receive therapy and are just observed (Table III). It was found that the patients with PKU were diagnosed after an average number of 44 days following the blood spot screening test; and the patients with BD were diagnosed after an average number of 76 days following the blood spot screening test and the treatment was initiated.

Disscussion

In this study, the screening results of 417,525 newborns born in Diyarbakir within ten years were evaluated for PKU and BD. The incidence of PKU was 1:7878, the incidence of HPA was 1:1960, and the incidence of BD was found to be 1:2359.

The incidence of PKU varies considerably between ethnicities and different geographical regions worldwide.[14] The incidence of PKU is high in Europe and some Middle Eastern countries: Italy (1:4,000), Ireland (1:4,545), Iran, Jordan (both 1:5,000) and Turkey (1:6,667). Northern European countries such as Denmark (1:13.434) and Finland (1:112,000) have the lowest PKU rates in Europe. In the American continent, PKU occurs in 1 of every 15,000 to 47,000 live births. The lowest PKU prevalence in the world has been reported in Asian countries such as Thailand (1:227,273), Japan (1:125,000), and the Philippines (1:116,006), with China being the exception (1:15,924).[15] According to a study from Saudi Arabia, the prevalence of PKU was one in every 28,316 live births.[2] It is estimated that one in every 23,930 infants suffer from it worldwide.[15] The increased incidence of consanguineous marriages in Iran, Jordan, Turkey, and Saudi Arabia explains the high prevalence of PKU.[2,13,14] Four out of every 100 people in Turkey are heterozygous carriers of PKU.[16]

We think that the prevalence of PKU is higher in our area. Because the records of some individuals who were initially monitored as

Table III. Evaluation of the age, gender and treatment status of the patients.

	BD n (%)	PKU n (%)	HPA n (%)	Total* n (%)	P value
Gender					P=0.495
Male	65 (52.8)	13 (52.0)	55 (45.5)	133 (49.4)	
Female	58 (47.2)	12 (48.0)	66 (54.5)	136 (50.6)	
Age (months); median (min-max)	24 (3-74)	49 (6-76)	27.5 (2-117)	28.5 (2-117)	P=0.005
Treatment status					P<0.001
Treatment ended	- (0.0)	3 (12.0)	19 (16.5)	44 (16.9)	
Treatment continues	108 (91.8)	19 (76.0)	28 (24.1)	128 (49.2)	
Not treated, being followed	- (0.0)	1 (4.0)	28 (24.1)	34 (13.1)	
Not followed	11 (9.2)	2 (8.0)	41 (35.3)	54 (20.8)	

BD: biotinidase deficiency, HPA: hyperphenylalaninemia without phenylketonuria, PKU: phenylketonuria
*: Because the patient files were examined retrospectively, some patients' data were missing or inadequate, hence instances with missing parameters were removed from the statistical analysis. For this reason, there were differences between the total figures in the table.

HPA and were later diagnosed with PKU were not updated, we believe the incidence of PKU is lower than expected. When PKU and HPA are taken as a whole (as phenylalanine hydroxylase deficiency), blood phenylalanine levels were found to be above the normal range in one out of every 1,570 newborns. In our investigation, the incidence of PKU was found to be higher than the global norm.

BD is seen approximately in one out of every 60,000 live births worldwide.[17] The estimated incidence of profound BD is one in 112,271 and the incidence of partial BD is one in 129,282.[18] According to two different studies conducted in Italy, the incidence of BD was 1:6,300 in Tuscany and Umbria, and 1:5,996 in the city of Verona. In both studies, approximately 90% of the patients had partial biotinidase deficiency.[8,19] In the study of Porta et al.[20] the incidence of BD in newborns was 1:61,000. Unlike the previous two studies conducted in Italy, 55% of the patients in this study had profound biotinidase deficiency. Due to high consanguinity rates, the prevalence of BD is high in some countries, such as Turkey and Saudi Arabia.[7] In a Saudi Arabian investigation, the frequency of BD was determined to be one in every 28,316 live births.[2] The incidence of BD was reported to be 1:11,614 in a study from Turkey, where seventy-eight percent of these individuals had profound BD.[21] When data from different cities in Turkey were evaluated, Aytaç et al.[22] discovered that the average incidence of BD in Adana was 1/11950. In their study in Şanlıurfa, Kazanasmaz et al.[23] found the incidence of BD as 1:1,177.

In our study, 56% of the patients with biotinidase enzyme deficiency had profound BD (incidence: 1:4,175), and 44% had partial BD (incidence: 1:5,422). The incidence of BD has increased more than twice in the last three years compared to previous years. In our study, BD was found to be higher compared to studies conducted in other countries and Turkey. Especially the increase in recent years is remarkable. In our study, one patient who was born in 2019 in our city and was residing in our country as a refugee (1/41) and 3 other such patients born in 2020 (3/38) were included in the calculation. However, even if the refugee patients were excluded from the table, the total incidence of BD in 2019 and 2020 (1/995 and 1/1055, respectively) would hardly change. The impact of the refugee patients on the increased BD rate in recent years was not significant. It was not possible to explain the reason for this increase with the refugee patients.

In Turkey, 23.2% of marriages are consanguineous. The region where consanguineous marriages are most common is the Southeastern Anatolia region, which includes Diyarbakir, with 42.6%.[3] In our study, parents of 65% of the patients diagnosed with PKU and 46.6% of the patients diagnosed with BD were relatives. Moreover, in our study, it was found that 52% of the patients diagnosed with PKU and 18% of the patients diagnosed with BD had a similar disease in their relatives. The high incidences of BD and phenylalanine hydroxylase deficiency in our study were thought to be due to the high rate of consanguineous marriages in our region. We believe that the reason relatives of PKU patients have identical disorders more commonly than relatives of BD patients is that PKU screening has been conducted for newborns in Turkey for roughly 30 years, whereas BD screening has only recently begun (in 2008). Considering the increase in BD in the last years, awareness studies and premarital counseling services for reducing consanguineous marriages are of great importance.

In patients who were born in the years we included in the study and had high phenylalanine levels, the BH4 loading test was not performed. Therefore, whether there were BH4-responsive patients was not determined. Our study's limitations include the fact that the records of some patients who were initially monitored as HPA and were later diagnosed with PKU were not updated, the lack of follow-up data in the family medicine records, and the low number of patients who came to the doctor's follow-up due to low health literacy in our region.

The incidence of both PKU and BD was found to be high in our region. The high prevalence of BD, the large proportion of patients with profound BD, and the cheap cost of screening tests all contribute to the relevance of screening infants. The parents of 65% of the patients diagnosed with PKU and 46.6% of the patients diagnosed with BD are consanguineous. It was found that the relatives of PKU patients were more likely to have a similar disorder than the relatives of BD patients. The main cause for the high incidence of these illnesses in our region was assumed to be the region's high rate of consanguineous marriages. For this reason, raising awareness and premarital counseling services to reduce consanguineous marriages are of great importance.

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Ethical approval

This study was conducted in compliance with the ethical principles according to the Declaration of Helsinki, and it was approved by the Ethics Committee of Health Sciences University, Gazi Yaşargil Training and Research Hospital (Number: 2021/855).

Author contribution

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

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Conflict of interest

The authors declare that there is no conflict of interest.

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Original Article

The effect of treatment with melatonin on primary school aged children with difficulty in initiation and maintenance of sleep

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ABSTRACT

Background. The present study was designed to evaluate the effect of melatonin on the sleep initiation, duration of sleep, quality of sleep and daily performance in healthy children suffering from insomnia.

Methods. This study was done as a double blind randomized clinical trial in the sleep clinic of Qods Hospital. Sixty healthy children between 7 and 12 years of age having sleep problems were chosen and randomly divided in interventional and placebo groups. Before the treatment, children's sleep habits questionnaire (CSHQ) was filled in both groups. Then, both groups were taught about sleep hygiene. Afterwards, the intervention group was treated with 3mg nocturnal dose of melatonin for one month and the other group with a placebo. Then, CSHQ was filled again for both groups. The intervention was Melatonin. The mean analyzed results of the variants in pre-test and post-test were compared and $p<0.05$ was regarded as significant.

Results. Results showed that Melatonin with no side effect is effective in improving: 1- The initiation and maintenance of sleep, 2- Sleep onset delay, 3- Sleep duration, 4- Sleep anxiety, 5- Nightly awakenings 6- Parasomnias and 7- daily performance; but is ineffective in bedtime resistance and sleep disordered breathing.

Conclusions. Our results indicate that melatonin is more effective than placebo in improving the initiation and maintenance of sleep and most of its subscales in primary school aged children.

Key words: difficulty in initiation and maintenance of sleep, primary school aged children, melatonin.

Difficulty in initiation and maintenance of sleep is observed in 15-25% of children and adolescents.[1] Insomnia is described as the decrease in daily performance due to sleep disturbances.[2] Children having insomnia suffer from fatigue, attention deficit, loss of concentration, irritability, loss of energy and anxiety. In chronic form, complications such as learning problems, decrease in school performance and depression do occur. Along with the problem, other family members' performance and sleep pattern are affected.[3]

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To evaluate the sleep problems, firstly, medical problems should be diagnosed and treated.[4] Then, sleep hygiene should be regarded in patients. Sleep hygiene practices comprises fixing the sleeping time and morning wake up time, fixing the amount of time expected for sleep appropriate to age, providing a restful and dark sleep environment, not being hungry at bed time, avoiding nicotine and caffeine compounds and including relaxation techniques before sleep, which is in turn composed of avoiding watching TV and playing computer and video games and encouraging to read before bed time.[5] Usually the steps above will lead to appropriate treatment, but in some resistant cases, drug therapy is necessary. To do this, benzodiazepines, antianxiety, antidepressants and antihistamines can be

used. But most of these drugs are not FDA approved for under 18 years of age.[4,6] For this reason, there is an increasing tendency to use natural hypnotics such as Melatonin for the treatment of insomnia in children. Naturally, the increase in the secretion of this hormone by pineal gland at night causes sleep within one to two hours. But contact electronic media and intense light, by changing the secretion amount of this hormone, could affect the initiation and maintenance of sleep and cause the corruption of natural sleep pattern and the beginning of sleep problems.[7] Exogenous melatonin causes the disruption of the body's internal clock to be resolved. In the past two decades, this drug has been used for the treatment of insomnia in children suffering from psychological disorders, ADHD, learning and developmental disorders and depression.[7,8] Nevertheless, it has been mostly used in children with concurrent somatic or psychological disorders, and its use for the treatment of sleep problems in healthy children otherwise, has been limited to only a small quantity and short term studies.[7-9] Besides, its exact dosage in children has not been yet specified and in different studies, a dosage range between 0.5 to 10 mg, 1 to 5 hours before sleep has been used.[9,10]

In 2001, Kayumov et al.[11] in a clinical trial, showed that melatonin without prolonging the duration of nightly sleep, significantly reduced the sleep onset delay and the amount of fatigue and sleepiness the following day.

In 2001, Takeuchi et al.[12] from Japan, compared the therapeutic effect of melatonin on REM sleep behavioral disorder with its choice drug clonazepam and showed that melatonin, quite similar to clonazepam, is strongly effective in improving the clinical symptoms of this parasomnia.

In 2001, Dodge and Wilson[13] in a double blind placebo controlled clinical trial, compared the therapeutic effect of melatonin in 20 children with developmental disorders with placebo. Significant decrease in sleep onset delay was seen, but the duration of sleep and the number of nightly awakenings did not change at all.

In 2001, Smits et al.[14] in a clinical trial, concluded that treatment of insomnia in children with 5 mg nocturnal dosage of melatonin significantly improves sleep onset delay and the duration of sleep. Only in one of the patients, mild generalized seizure occurred only once, but no other side effect was seen in the other patients.

In 2003, Smits et al.[15] in another similar study on 62 children with chronic idiopathic disorder of the initiation of sleep, showed that melatonin significantly improved the sleep onset and sleep wake-up time.

In 2011, Sánchez-Barceló et al.[16] in a study, reported numerous applications for melatonin in pediatrics even other than treatment of insomnia.

In 2012, Eckerberg et al.[17], in another interesting study finally concluded that daily administration of low dose of melatonin in the evening, even if the students in the last two-day of the weekend, continue abnormal sleep habits, can improve sleep duration and promote conscious state of the students during school hours.

In 2013, Ferracioli-Oda et al.[2] in a meta-analysis study concluded that melatonin reduces sleep onset delay, increases total sleep duration and improves the overall sleep quality.

The aim of this study was to evaluate the effect of Melatonin on the sleep

initiation, duration of sleep, quality of sleep and daily performance in healthy children suffering from insomnia.

Material and Methods

Study Design and Patients

This study was a randomized double blind clinical trial. Our samples were chosen from primary school students (7 to 12 years old, boys and girls) referred to the sleep clinic of Qods Hospital with the complaint of sleep problems. This hospital is the only center equipped with a sleep clinic in the Qazvin province of Iran.

The research was reviewed and approved by ethics committee of Qazvin University of Medical Sciences. The ethic number was 28/20/4329. All participants involved in the study signed an informed written consent. Also, this trial was registered with the Iranian Registry of Clinical Trials. The registration number was IRCT 2015111225008N1.

The number of samples in our study was 60. Sample size calculation was performed. According to study conducted by van Geijlswijk et al.[18], mean value and standard deviation considered as below: sleep initiation in treatment group with melatonin and control group were 24.1±20.2 and 48.3±36.1, respectively. Based on the following formula, 30 participants were computed in each group. We considered 0.05 for value of alpha, corresponding to a 95% level of confidence for avoiding a type I error, and 0.80 for value of beta, to detect a large effect size.

$$n = \frac{(Z_{1-\alpha/2} + Z_{1-\beta})^2 (S_1^2 + S_2^2)}{(\mu_1 - \mu_2)^2}$$

Participants (60 patients) were randomly allocated to intervention (n = 30) or to control group (n = 30). We used a specialized internet software application for randomization of participants.

In every 60 of these patients (i.e. the intervention and control group) and their parents sleep hygiene was asked. These children should have been physically and mentally healthy with the only problem of sleep disturbances, so they were examined by the physician, their underlying diseases, physical and psychological, ruled out and then randomly divided into intervention and control groups.

Inclusion criteria

Patients after interview and examination by the pediatric sleep medicine, were included in the study if they possessed the DSM-IV criteria for insomnia.

Exclusion criteria

Include the following: 1. Children with underlying neurological disorders, 2. children with psychological disorders including ADHD, 3. children with underlying organic problems including chronic cardiac, pulmonary, kidney and blood disorders, 4. history of drug use during the recent month including antibiotics, antihistamines and sedatives, 5. clear visible clinical symptoms suggestive of anemia. After assessing inclusion and exclusion criteria, all children were randomly divided into intervention and control groups.

Interventions

The 30 patients in the intervention group, were given Melatonin tablets (manufactured by Weber Nature Company) at a dosage of 3 mg at night and the 30 patients in the control group, were given placebo pills (taken from the pharmacy of Dr. Nozari in Qazvin). The administration of medication type was double-blind and the blinding of the medication to patients was carried by a pharmacist (Dr. Nozari) at the Qazvin university without the notice of the researchers. Both patients and control groups were blinded during the intervention. Medication intervention was of 4 weeks duration. All children were required to take medications at 7 pm. During the period of drug administration, none of the groups had the right to use another drug.

Measurement and statistical analysis tools

Our measuring tools in this study, was the Farsi translation of children's sleep habits questionnaire (CSHQ) Once before the treatment, and the second time at the end of the one month treatment, CSHQ was filled by the individuals in both groups by asking questions from their parents, so as to be used as pre-test and post-test scoring, by which the therapeutic response could be measured in both groups. Obtaining an overall score equal to or greater than 41 on this questionnaire indicates a sleep

disorder.[19,20] In our study although the basic sampling was done based on DSM IV criteria, the mean score of sleep disorders before the treatment was 56.57 for the intervention group and 53.96 for the control group, which suggests the existence of sleep disorders in both groups before intervention. Also, during the period of drug administration, parents were required to complete a sleep log every day for a month, to record bedtime, sleep onset, sleep duration, number of nightly awakenings after sleep, in other words to complete the 'evaluation of sleeping and waking hours' questionnaire.

In addition, during this period, researchers had weekly telephone contacts with all the patients in the two groups to be sure of drug consumption and to ask about possible adverse drug reactions and the effect of the drug on child's sleep based on the parents' observations.

The hypotheses of the study include the following:

1) The main hypothesis: Treatment with melatonin in primary school aged children with difficulty in initiation and maintenance of sleep, generally improves the sleep disorder.

2) First sub-hypothesis: Treatment with melatonin in primary school aged children with difficulty in initiation and maintenance of sleep, improves sleep onset. (It should be noted that the sleep onset is measured with two sub-scales of bedtime resistance and sleep onset delay.)

3) Second sub-hypothesis: Treatment with melatonin in primary school aged children with difficulty in initiation and maintenance of sleep, improves their sleep duration.

4) Third sub-hypothesis: Treatment with melatonin in primary school aged children with difficulty in initiation and maintenance of sleep, improves their sleep quality. (It should be noted that the quality of sleep is evaluated with four subscales of sleep anxiety, nightly awakenings, parasomnias, and breathing problems during sleep.)

5) Fourth sub-hypothesis: Treatment with melatonin in primary school aged children with difficulty in initiation and maintenance of sleep, improves their daily performance.

6) Fifth sub-hypothesis: Side effects of the prescribed Melatonin in primary school aged children with difficulty in initiation and maintenance of sleep, influences their treatment process.

In any case, in this way, the intervention and placebo groups were compared with each other from the viewpoint of sleep onset, sleep duration, sleep quality and daily performance improvement as well as possible adverse drug reactions, so that the differences among the melatonin and placebo-treated groups could be compared and declared. Then, the collected data were statistically analyzed with the SPSS version 18 software. The statistically significant number was considered at less than 0.05. The paired t-test was used to compare differences at before and after treatment in each groups, and between groups comparisons were made by independent student t-test.

Results

The overall mean age of the patients was 9.59 with a standard deviation of 2.03. The mean age of the patients in the intervention group was 9.79 with the standard deviation of 2.02, minimum age was 6 years and maximum was 12 years; in the placebo group 9.38 with a standard deviation of 2.05, minimum age was 7 years and maximum was 12 years. There was no statistically significant difference between two groups in terms of age ($P>0.05$).

All the children in both groups had parents with college education. The final results of our study has been shown in Table I, in which you can see all the variables' mean scores in pre-test and post-test in both the groups.

As it is observed, there is no significant difference before treatment for all the variables between the two groups.

On the other hand, except for bedtime resistance and sleep disordered breathing, based on the scoring system of CSHQ, the post- treatment mean scores show significant decrease between two groups after treatment with melatonin ($p<0.05$). But for the two variables of bedtime resistance and sleep disordered breathing, the mean score increased and insignificantly decreased, respectively, p value > 0.05.

Also, Table II shows results from paired t-test between before and after treatment in melatonin group. Results show that after treatment with melatonin, all variables have improved significantly except bedtime resistance.

Discussion

Thus, the findings of our study indicate that treatment with melatonin in primary school children, is in general effective in improving the difficulty in initiation and maintenance of sleep, reducing sleep onset delay, improving sleep duration, reducing sleep anxiety, reducing nightly awakenings, improving parasomnias, and improving daily performance.

Treatment with melatonin in primary school children, is not effective in improving bedtime resistance, and reducing sleep disordered breathing. In our study, melatonin did not have any side effects.

Table I. Comparison of mean scores of sleep disorder variables according to CSHQ , in placebo and melatonin groups.

	Before treatment		p-value	After treatment		p-value
	Placebo group	Melatonin Group		Placebo group	Melatonin Group	
Total	53.96±4.46	56.57±8.44	0.24	49.20±5.53	39.23±7.62	0.001
Bed time resistance	11.33±1.71	11.27±2.73	0.76	11.53±1.91	12.43±2.05	0.07
Sleep onset delay	1.63±0.67	2.2±0.85	0.63	2.33±0.77	1.37±0.67	0.001
Duration of sleep	4.90±1.16	4.73±1.20	0.61	4.13±1.38	4.11±0.99	0.001
Sleep anxiety	6.87±1.46	6.97±1.90	0.32	6.23±1.22	4.93±1.68	0.001
Nightly awakenings	3.90±0.88	4.20±1.35	0.76	3.50±0.9	2.60±1.16	0.002
Parasomnias	9.63±1.08	9.07±1.63	0.51	8.97±1.45	7.80±1.49	0.001
Sleep disordered breathing	4.97±1.10	5.43±1.36	0.93	4.90±1.49	5.20±1.52	0.87
Daily performance	10.87±2.58	10.80±3.39	0.46	9.97±2.30	9.25±3.56	0.002

CSHQ: Children's Sleep Habits Questionnaire

Table II. Comparison of mean scores of sleep disorder variables according to CSHQ before and after in the melatonin intervention group.

	Before treatment	After treatment	p-value
Total	56.57±8.44	39.23±7.62	<0.001
Bed time resistance	11.33±1.71	11.53±1.91	0.1
Sleep onset delay	1.63±0.67	2.33±0.77	<0.001
Duration of sleep	4.90±1.16	4.13±1.38	0.001
Sleep anxiety	6.87±1.46	6.23±1.22	0.003
Nightly awakenings	3.90±0.88	3.50±0.90	<0.001
Parasomnias	9.63±1.08	8.97±1.45	<0.001
Sleep disordered breathing	4.97±1.10	4.90±1.49	0.09
Daily performance	10.87±2.58	9.97±2.30	<0.001

CSHQ: Children's Sleep Habits Questionnaire

About the main hypothesis of our research, it should be noted that our findings are in line with the studies of Eckerberg et al.[17], Sánchez-Barceló et al.[16], Coppola et al.[21], Smits et al.[14], van Geijlswijk et al.[18], and Kayumov et al.[11]

All of them in their studies concluded that treatment with melatonin generally improves insomnia. In addition, Ferracioli-Oda et al.[2] too, in 2013 showed that melatonin reduces sleep onset delay, increases total sleep duration and improves overall sleep quality.

About the first sub-hypothesis of our research (effect of melatonin treatment on bedtime resistance and sleep duration), our findings are similar to the studies of Kayumov et al.[11], Smits et al.[14,15], Coppola et al.[21], Armour and Paton[22], Eckerberg et al.[17], Buscemi and Witmans.[23] All of them in their studies concluded that treatment with melatonin improves sleep onset.

About the second sub-hypothesis of our research (effect of melatonin treatment on improvement of sleep duration), our findings are in line with the studies of Smits et al.[14,15], Eckerberg et al.[17], Ferracioli-Oda et al.[2] They all concluded that treatment with melatonin increases sleep duration. Eckerberg et al.[17] in their study in 2012, finally concluded that daily administration of low dose melatonin in the evening, even if the students continue abnormal sleep habits in the last two days of the weekend, can improve sleep duration and promote students' conscious state during school hours. But our findings are not in line with the findings of Dodge and Wilson[13] and Armour and Paton.[22] But it should be noted that they studied the melatonin therapeutic effect on children with developmental disorders.

About the third sub-hypothesis of our research (effect of melatonin treatment on sleep quality), our findings are in line with the studies of van Geijlswijk et al.[18] and Takeuchi et al.[12] They also in their studies, concluded that Melatonin improves the sleep quality of the patients. Therefore, van Geijlswijk[18] suggested using the minimum dosage of melatonin i.e. 0.05mg/kg 1 to 2 hours before bed time for the treatment of the nocturnal insomnia in children due to initiation of sleep. Also, Ferracioli-Oda et al.[2] in their meta-analysis in 2013, emphasized the improvement of sleep quality after melatonin treatment. But our findings are not in line with Buscemi and Witmans' study.[23] Of course, it should be noted that they studied the Melatonin therapeutic effect on children with developmental disorders.

About the fourth sub-hypothesis of our research (effect of melatonin treatment on daily performance in school), our findings were similar to the studies of Kayumov et al.[11], and Eckerberg et al.[17] Their research results also showed that after melatonin administration for the treatment of sleep disorders, patients did not have sleepiness during the day and their daily performance increased. Eckerberg et al.[17] also emphasized that melatonin as well as increasing the duration of nightly sleep, promotes the students' conscious state during school hours.

And finally, about the fifth sub-hypothesis of our research (side effects of melatonin treatment), fortunately, in our study, melatonin caused no side effect on the patients; and this is in line with the studies of Smits et al.[14], Coppola et al.[21], and Buscemi et al.[23]

Of course, we should recall that in the study of Smits et al.[14] one of the patients had a period of mild generalized seizure which was not repeated for the second time.

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In the end, we the researchers, appreciate Dr. Nozari the pharmacologist for his unsparing efforts in supplying Melatonin and Placebo tablets and exact delivery of them to the patients of the two groups of our study.

Ethical approval

The research was reviewed and approved by ethics committee of Qazvin University of Medical Sciences. The ethic number was 28/20/4329.

Author contribution

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

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Conflict of interest

The authors declare that there is no conflict of interest.

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Original Article

Relationship between placental autophagy and inflammasome activities with morbidity of extremely preterm infants

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ABSTRACT

Background. The placenta is the major regulatory element of the in-utero environment, and alterations in placental cellular functions in infection, inflammation, and hypoxemia lead to adverse preterm birth outcomes. The importance of regulation of autophagy and inflammasome activities has been shown in the pathogenesis of morbidities in immature animal models. This study aimed to determine the relationship between placental autophagy and inflammasome activities with morbidity in extremely preterm infants.

Methods. Premature infants born between 24th to 29th gestational weeks were evaluated prospectively. Placental LC3B and NLRP3 immunostainings were performed to assess autophagy and inflammasome activities. Preterm morbidities including respiratory distress syndrome (RDS), patent ductus ateriosus: (PDA), intraventricular hemorrhage (IVH), necrotizing enterocolitis (NEC), periventricular leukomalacia (PVL), sepsis, bronchopulmonary dysplasia (BPD), retinopathy of prematurity (ROP) and mortality were evaluated.

Results. Fifty-nine infants with a mean gestational age of 26.9 ± 1.5 weeks were included. Anti-LC3B staining scores were moderate or intense positive in 75% of the placentas. Anti-LC3B activity was not associated with the existance of evaluated neonatal morbidities or mortality. Autophagy and inflammasome coexistence were demonstrated in 35 placentas (59.3%). Anti-NLRP3 staining score was moderate or intensely positive in 75% of the placentas. Infants with BPD had a lower rate of positive anti -NLRP3 staining than infants without BPD (42.9 vs 57.1%, p=0.048). Infants who had hemodynamic significant patent ductus arteriosus (hsPDA) and surgical-NEC showed significantly intense anti-NLRP3 staining compared to infants who did not (18.8% vs 0%, p= 0.027 and 33% vs 7.5%, p=0.048 respectively).

Conclusions. The results showed that autophagy and inflammatory activities were present in varying amounts in the placenta of preterm infants. Association of decreased or increased rates of inflammasome activities with certain diseases such as BPD, hsPDA and surgical-NEC indicates the role of the intrauterin inflammatory process and the importance of critical balance in inflammation. Because of the complex pathophysiology of preterm morbidities, placental autophagy and inflammasome activities seem worthy of further investigation.

Key words: premature birth, morbidity, placenta, autophagy, inflammasome.

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Very low birth weight (VLBW) infants' survival rate has been increasing over the last few decades, but morbidities associated with prematurity have been seen in almost half of the survivors. In-utero exposures to conditions like inflammation and maternal medical diseases (hypertension, diabetes, preeclampsia) predispose an adverse outcome

for preterm infants during neonatal intensive care unit (NICU) stay and later in childhood. The placenta provides all vital supplements for fetal development during pregnancy, and plays a critical role in maintaining a healthy in-utero environment.[1]

During pregnancy, the development of the placenta is interrelated with the oxygen concentration and autophagy acts as a crucial process in the placenta which is frequently affected by oxidative stress.[2] Autophagy is an intracellular lysosomal degradation process that contributes to basal cellular and tissue homeostasis, as well as developmental regulation in higher organisms. Autophagy primarily acts as a protective mechanism that may prevent cell death.[3] However, an imbalance between the protective and destructive mechanisms of autophagy appears to be linked with pregnancy-related disorders such as preeclampsia and fetal growth restriction, fetal hypoxia and inflammation.[2,4,5]

Oxidative stress and inflammation play roles as leading mechanisms that cause preterm delivery and play a crucial role in the pathogenesis of preterm morbidities. Recently, the critical effects of autophagy on inflammation induction have started to be understood. Inflammasomes are intracellular multi-protein complexes that act as sensor molecules that initiate the inflammatory pathway. Recently, a critical crosstalk between autophagy and inflammasome induction has been indicated.[6] Placental inflammasome activity present both in microbial and sterile inflammation, is associated with spontaneous preterm delivery.[7] Increased placental inflammasome activity is also reported in pregnancies complicated with preeclampsia and diabetes.[8]

Placental histological abnormalities were previously described as a risk factor in preterm infants with adverse outcomes. In this study, we hypothesized that intrauterine autophagy and inflammasome activities, and their balance, play a function in the healthy development of infants. Therefore, examination of placental autophagy and inflammasome activities could offer early signals for the development of preterm morbidities. The main objectives of this study were to i. investigate the placental autophagy and inflammasome activities in preterm infants by characterizing the expression levels of LC3B and NLRP3, and ii. explore the relationship between autophagy and inflammasome activities with preterm morbidities.

Material and Methods

Study Population and Clinical Definitions

This prospective cohort study was conducted at Dokuz Eylül University Hospital between June 2017 to August 2018. All infants born between the 24th to 29th gestational weeks were evaluated for eligibility. Exclusion criteria were major congenital anomaly, parental refusal and transfer to another hospital.

The study protocol was approved by Dokuz Eylul University Faculty of Medicine, Local Ethical Committee on Human Research (No:2017/25-02). Written informed parental consent for experimentation with human subjects was obtained.

Maternal and neonatal demographic and clinical data were collected from written and electronic folders. Data included i) neonatal demographics: gestational age determined as date of last menstrual period and/or first trimester ultrasonography, birthweight, gender, Apgar score at 5 minutes, mode of delivery, full course of antenatal corticosteroid administration[9], intrauterin growth status[10], multiple pregnancy, in-vitro fertilization (IVF) pregnancy; ii) maternal demographics: maternal age, preterm prelabour rupture of membrane (PPROM) and duration, chorioamnionitis, gestational hypertensive disorder, gestational diabetes, chronic conditions needed medical supervision; iii) neonatal morbidities: respiratory distress syndrome (RDS)[11], clinical and culture proven early onset neonatal sepsis

(EONS), hemodynamic significant patent ductus arteriosus (hsPDA) requiring treatment[12], intraventricular hemorrhage (IVH) > Grade II according to Papile staging[13], periventricular leukomalacia (PVL)[14], culture proven late-onset neonatal sepsis (LONS), retinopathy of prematurity (ROP) needed treatment[15], necrotizing enterocolitis (NEC) stage 2-3 according to Bell criteria[16], moderate and severe BPD at 36 weeks' postmenstrual age according to NICHD definition[17] and iv) neonatal mortality during hospital stay. Compound outcome was defined as any morbidity related to oxidative stress (IVH > grade 2, hsPDA, surgical NEC, cystic PVL, moderete-severe BPD and treatment required ROP), and/or mortality.

Placental Examination and Immunohistochemically Assessment

Placental histopathological evaluations were performed by two pathologists who were blinded to the neonatal clinical data. Placental tissue samples were fixed in 10% buffered formalin for 24 hours and embedded in paraffin. Tissue sections (4 μm) were cut from the maternal side, fetal side and umbilical cord, and then mounted on poly-L-lysine coated slides. Hematoxylin and eosin (HE) stained slides were examined under a light microscope (Olympus BX51; Olympus Corp. Tokyo Japan) at 100× magnification for histopathological evaluation. Placental histopathological findings were classified according to Amsterdam Placental Workshop Group Consensus Statement.[18] Villous maldevelopment such as infarcts, villous dysmaturity, accelerated villous maturation and vascular intramural fibrin deposition were classified as maternal/fetal malperfusion. Grading and staging of inflammation were defined as acute/chronic chorioamnionitis (or chorionitis) with/without a fetal inflammatory response.[18]

Immunohistochemical staining was performed for autophagy activity using LC3B antibody (rabbit monoclonal, Cell Cignaling, Lausen, Switzerland) at a dilution of 1:50 for 1 hour[19], and for inflammasome activity marker with NLRP3 antibody (rabbit polyclonal, Novus Biologicals, Littleton, CA, USA) at a dilution 1:200 for 1 hour.[20]

Placental tissues from four different sites for each patient were obtained for protein expression evaluation. Two pathologists scored LC3B and NLRP3 staining for all placental sites, and median values were used for the final score. Cytoplasmic NLRP3 staining in trophoblastic cells was considered positive and graded as negative, weak positive and strongly positive, due to the intensity of staining semi-quantitatively. Nuclear LC3B staining in trophoblastic cells was considered positive and scored due to the extensity of staining as 0: negative or positive in less than 10% of the cells, 1: positive in 10-50% of the cells and 2: positive in more than 50% of the cells.[21]

Statistics

SPSS version 22.0 (IBM SPSS Statistics, Chicago, IL, USA) was used in the study. Continuous data were expressed as mean ± standard deviation (SD) or median (interquartile range) according to the normal distribution pattern of data using the Shapiro Wilk test and analyzed with Student t or Mann Whitney U tests according to the distribution of data. Categorical unrelated data were analyzed using the chi-square or Fisher's exact tests as appropriate, and related data were analyzed using McNemar test. All reported p values were two-sided with a significance level of 0.05.

Results

Clinical characteristics

Among 62 eligible infants during the study period, 59 infants were included in the study. Two infants were transferred to other clinics and one infant was excluded due to major congenital abnormalities. The mean gestational age and birth weight of the infants were 26.9 ±1.5 weeks and 958±270 grams. Twenty-one of the infants (35.6%) were between 24-26 weeks of gestational age and the remaining were between

27-29 weeks of gestational age. Demographic and clinical characteristics of neonatal and maternal data are listed in Table I.

Placental Histopathological Findings

Fifty-nine placentas were examined, of which 32 (54%) had vascular malperfusion, 8 (13%) had inflammation (chorioamnionitis), 17 (29%) had vascular malperfusion plus inflammation, 1 (2%) had placenta previa and 1 (2%) was normal (Fig. 1. A, B).

Table I. Neonatal and maternal characteristics.

Characteristics	n=59
Gestational age (weeks) *	26.9 ± 1.5
Birth weight (grams) *	958 ± 270
Apgar score at 5min **	8 (7-8)
C/S delivery, n (%)	50 (87.7%)
Male gender, n (%)	25 (42.4%)
SGA, n (%)	6 (10.2%)
Multiple births, n (%)	16 (27.1%)
IVF pregnancy, n (%)	14 (23.7%)
Antenatal steroid, n (%)	32 (54.2%)
RDS, n (%)	50 (87.7%)
Surfactant dose **	1 (1-2)
hsPDA, n (%)	32 (54.2%)
IVH (> Grade 2), n (%)	2 (3.4%)
PVL, n (%)	6 (10.8%)
Early-onset sepsis, n (%)	15 (25.4%)
Late-onset sepsis, n (%)	19 (32.2%)
NEC (≥ Stage 2), n (%)	5 (8.4%)
BPD at 36w, n (%)	7 (11.8%)
Postnatal steroid treatment, n (%)	18 (30.5%)
ROP requiring treatment, n (%)	6 (10.2%)
The duration of NICU stay, days *	65 ± 24
Gestational age at discharge, weeks *	36 ± 2
Mortality, n (%)	3 (5.0 %)
Maternal age, (years) **	29 ± 6
PPROM, n (%)	22 (37.3%)
PPROM duration, (h) **	39 (26-120)
Histologic chorioamnionitis, n (%)	25 (42.3%)
Gestational hypertensive disorders, n (%)	19 (32.2%)
Gestational diabetes, n (%)	3 (5.0%)
Maternal obesity (BMI>30 kg/m^2), n(%)	7 (11.8%)
Maternal chronic disease, n (%)	4 (6.7%)

*Values are presented as mean±standard deviation
**Values are presented as median, an IQR (inter quartile range) 25-75 are given in parenthesis

Anti-LC3B and Anti-NLRP3 Immunohistochemical Staining

Overall, anti-LC3B and/or anti-NLRP3 immunohistochemical staining were positive in 53 patients (Fig. 2A). The intersection between the inflammasome and autophagy activities was shown through a Venn diagram in Figure 2B.

Anti-LC3B staining was scored as 0 in 15 placentas (25%), 1 in 25 placentas (43%) and 2 in 19 placentas (32%). Anti-NLRP3 staining was negative in 15 placentas (25%), weakly positive in 38 placentas (64%) and strongly positive in 6 placentas (11%) (Fig. 2. A, B, C).

Relationship Between Placental LC3B And NLRP3 Expressions With Preterm Morbidities And Mortality

Overall, anti-LC3B staining score was 1 or 2 positive in 75% of the placentas. Maternal illness, antenatal steroid administration, type of delivery, intrauterine growth status, and presence of histological chorioamnionitis did not significantly affect the staining pattern. When the relationship between morbidities and anti-LC3B activity was evaluated for each morbidity or mortality separately, anti-LC3B positivity rate was not associated with the existence of neonatal morbidities including RDS, hsPDA, IVH, PVL, sepsis, NEC, BPD, ROP or mortality. Anti- LC3B staining rate was 72.2% in the group of preterm infants who had compound outcomes and 75.6% for the infants who did not (p=0.783). Considering the intensity of autophagy, no significant relationship was found between the intensity of autophagy and evaluated morbidities.

Overall, anti-NLRP3 staining score was moderate or intensely positive in 75% of the placentas. Maternal illness, antenatal steroid administration, type of delivery, intrauterine growth status, and presence of histological chorioamnionitis did not significantly affect the results. Infants with BPD had a lower rate of positive anti-NLRP3 staining, than infants

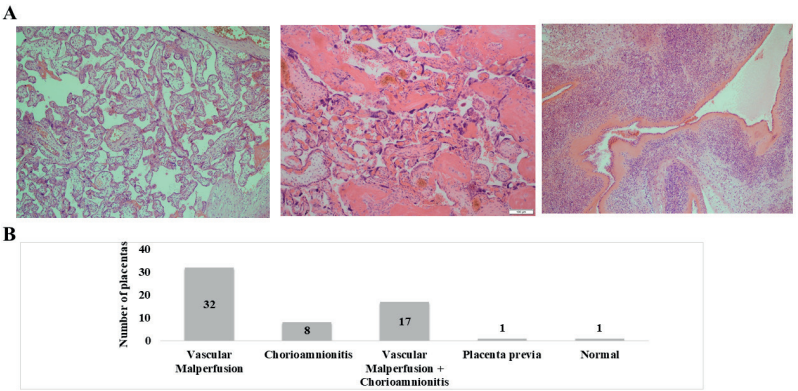

Fig. 1. A. Placental microscopic images (1x10 HE) representing a. normal placenta, b. vascular malperfusion and c. chorioamnionitis; B. Distribution of placental histological findings.

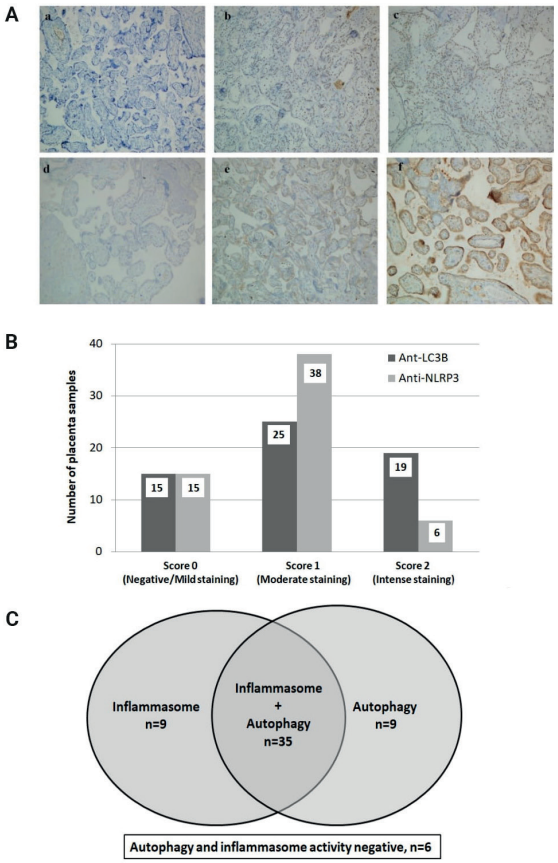

Fig. 2. Evaluation of Placental Anti-LC3B and Anti-NLRP3 activities: A. Evaluation of staining of placental anti-LC3B and anti-NLRP3 immunostainings (1x20): a. Anti-LC3B score 0 b. Anti-LC3B score 1 c. Anti-Anti-LC3B score 2 d. Anti-NLRP3 negative e. Anti-NLRP3 weak positive f. Anti- NLRP3 strong positive, B. Distribution of Anti-LC3B, Anti-NLRP3 scores across placentas C. Venn diagram demonstrating the overlapping between inflammasome and autophagy activities.

without BPD (42.9 vs 57.1%, p=0.048) (Table II). Infants who had compound outcome, positive anti-NLRP3 staining rate were 66.7% and the rate was 76.9 % for infants who did not (p=0.355). When an intense NLRP3 activity was evaluated, cases with early sepsis, hemodynamic significant PDA and surgical NEC had shown significantly increased NLRP3 activity. For infants who had hsPDA showed significantly intense anti-NLRP3 staining compared to infants who did not (18.8% vs 0%, p=0.027). Similarly, infants who developed NEC requiring surgery had a higher rate of intense inflammasome activity (33% vs 7.5%, p=0.048). All infants with mortality (n=3) had shown intense placental NLRP3 activity (p=0.279) (Table III).

Autophagy –inflammasome interplay

There was a significant overlap between autophagy and inflammasome activity (Mc Nemar test, p 0.05). Considering the relationship between autophagy and inflammasome, we formed four groups: i. Autophagy (without inflammasome): n=9 (15.3%) ii. Inflammasome (without autophagy): n=9 (15.3%), iii. Autophagy with inflammasome: n=35 (59.3%) iv. Negative or mild staining for autophagy inflammasome: n=6 (10.2%) (Fig. 2B). The above four groups did not show significant differences in terms of the diseases assessed.

Autophagy and/or inflammasome activities did not show a significant overlap with other placental histopathological findings (chorioamnionitis or vascular malperfusion) (Mc Nemar Test, p< 0.05).

Table II. The distribution of Anti-LC3B and Anti-NLRP3 staining, across neonatal outcome categories.

Neonatal Outcome	Anti-LC3B Score 1 or 2 Positive n (%)*	p	Anti-NLRP3 Weak or Strong Positive n (%)*	p
RDS				
No (n= 10)	6 (60.0)**	0.245	8 (80.0)	0.667
Yes (n=49)	38 (77.6)		36 (73.5)	
hsPDA requiring treatment				
No (n= 27)	20 (74.1)	0.935	20 (74.1)	0.935
Yes (n=32)	24 (75.0)		24 (75.0)	
IVH (> Grade 2)				
No (n= 57)	42 (73.7)	0.999	42 (73.7)	0.999
Yes (n=2)	2 (100.0)		2 (100.0)	
PVL				
No (n=51)	38 (74.5)	0.999	38 (74.5)	0.648
Yes (n=6)	5 (83.3)		4 (66.7	
Early-onset sepsis				
No (n=45)	34 (75.6)	0.757	31 (68.9)	0.072
Yes (n=14)	10 (71.4)		13 (92.9)	
Late-onset sepsis				
No (n=19)	12 (63.2)	0.285	15 (84.2)	0.478
Yes (n=40)	32 (80.0)		27 (67.5)	
NEC (≥ Stage 2)				
No (n=53)	40 (75.5)	0.638	40 (75.5)	0.478
Yes (n= 6)	4 (66.7)		4 (66.7)	
BPD at 36w				
No(n=50)	38 (76.0)	0.792	39 (78.0)	**0.048**
Yes (n=7)	5 (71.4)		3 (42.9)	
ROP requiring treatment				
No (n=51)	38 (74.5)	0.999	37 (72.5)	0.999
Yes (n=6)	5 (83.3)		5 (83.3)	
Mortality				
No (n=56)	42 (75.0)	0.999	41 (73.2)	0.564
Yes (n=3)	2 (66.7)		3(100.0)	
Compound outcome				
No (n=39)	29 (74.4)	0.957	30 (76.9)	0.563
Yes (n=20)	15(75.0)		14 (70.0)	

* % within outcomes (RDS, hs PDA, IVH, PVL, NEC, early sepsis, late sepsis, BPD, ROP, all morbidities, mortality, morbidies)

** existence of one of these outcomes or mortality: IVH > grade 2, hsPDA, surgical NEC, cystic PVL, moderete-severe BPD, ROP requiring treatment

RDS: respiratory distress syndrome, hsPDA: hemodynamically significant patent ductus arteriosus, IVH: intraventricular hemorrhage, PVL: periventricular leukomalacia, NEC: necrotizing enterocolitis, BPD: bronchopulmonary dysplasia, ROP: retinopathy of prematurity

Table III. The distribution of intense Anti LC3B and AntiNLRP3 staining acros selected neonatal outcome categories.

Neonatal Outcome	Anti-LC3B Score 2 Positive, n (%)*	p	Anti-NLRP3 Strong-Positive, n (%)*	p
hsPDA requiring treatment				
No (n= 27)	10 (37.0)	0.465	0 (0)	0.027
Yes (n=26)	9 (28.1)		6 (18.8)	
Early-onset sepsis				
No (n=45)	17 (37.8)	0.188	2 (4.4)	0.024
Yes (n=14)	2 (14.3)		4 (28.6)	
NEC (≥ Stage 2)				
No (n=53)	16 (30.2)	0.376	4 (7.5)	0.048
Yes (n= 6)	3 (50.0)		2 (33)	

* % within outcomes
# hsPDA: hemodynamically significant patent ductus arteriosus, NEC: necrotizing enterocolitis

Discussion

The placenta is the key regulatory element of the in-utero environment. Cellular homeostasis mechanisms such as the oxidative defense system, inflammatory pathway and autophagy are highly linked to neonatal morbidities.[22] This is the first study investigating the relationship between preterm morbidities and placental autophagy and/or inflammasome activities. The results demonstrated that placental autophagy and inflammasome activities were intersected significantly, and existed in the majority of the extremely premature infant population in varying degrees. Overall, no significant relationship was found between preterm morbidities and placental autophagy. Placental inflammasome activity seemed reduced in infants who developed BPD whereas intense inflammasome activity rate was significantly higher in infants who developed early sepsis, hsPDA and surgical NEC.

Evidence from previous studies has shown that the maternal inflammatory response, specifically chorioamnionitis, correlated with BPD, IVH, PVL and ROP.[23,24] Placental vascular malperfusion was also found to be a risk factor for BPD and IVH.[25,26] In this study, placental histological findings indicating chorioamnionitis and vascular malperfusion were not significantly associated with preterm morbidities. Most likely, the complicated intrauterine processes of the infants included in the study and the presence of at least one placental pathology in almost all of them may have suppressed the distinguishing feature of placental histology.

Autophagy plays a critical function in health and disease since it can be either a protector or detrimental.[27] Autophagy is one of the main mechanisms for maintaining cellular homeostasis, and is defined as the degradation of damaged intracellular proteins, organelles and microbial organisms. Autophagosome formation (double membrane vesicle) is essential for the process, and several proteins such as LC3B, Beclin-1, and p53 involve in molecular signaling. These proteins are widely used to assess autophagy activity. Microtubule-associated protein light chain 3B (LC3B) is the most commonly used marker for autophagosome formation in studies.[28] Autophagy is essential for normal placentation throughout pregnancy, and it contributes to operating appropriately in stressful conditions.[29] Placental autophagy is triggered by many pregnancy-related complications like preeclampsia, fetal growth restriction (FGR) and gestational diabetes.[30] Recent studies have demonstrated a close relationship between abnormal autophagy and prematurity, and autophagy has been found to be protective against preterm labor

by promoting the synthesis of progesterone.[31,32] In this study, we detected a high rate of placental autophagy activity in a premature infant population regardless of the existence of morbidity or mortality. Although varying in severity, placental autophagy activity was found in 75% of these babies. These data are inconsistent with previous ones indicating that the decrease in autophagy activity increases the risk of prematurity. Since our entire population was preterm, we could not demonstrate the effect of prematurity on autophagy.

In addition to the relationship between autophagy dysregulation and preterm birth, disorders in autophagy regulation are shown to be associated with certain preterm diseases. Previous preclinical studies showed impaired autophagy in oxygen-induced retinopathy model and BPD model, enhanced autophagy in the white matter injury model and NEC model.[33-36] Autophagy gene (ATG16L1) and NLRP3 gene were evaluated in preterm infants diagnosed with NEC, and a functional variant in ATG16L1 was associated with NEC.[37] In this cohort study, no relationship was found between autophagy activity and morbidities related to prematurity. The coexictance of autophagy and inflammasome did not make a significant difference. When we categorize the patients according to the severity of autophagy, the results did not change. Our evaluation in a partially homogeneous group consisting of entirely risky pregnancies and a small sample size may have prevented us from detecting meaningful results.

Gestational diabetes mellitus (GDM) and maternal obesity are risk factors for both mother and neonatal outcomes. Lipotoxicity associated oxidative stress results in altered autophagy in obese patients. Few studies have investigated autophagic activity from placental samples from obese pregnant women and women diagnosed with GDM.[38] In a previous study, decreased apoptosis and autophagy in the placentas from GDM women with LGA infants compared to those from normal pregnant women. However, it was reported that the biological significance of concomitant autophagy and apoptosis decreases in GDM placentas remains unclear. Several other studies showed inconsistent results in the placentas of women with gestational diabetes mellitus (GDM).[39] Anti-LC3B stainings in placental tissues from 10 women with GDM and obesity in our cohort resulted in 70% positivity. Overlapped conditions such as placental inflammation and vascular malperfusion rates were relatively high in our study, so it could affect our findings. Larger sample size studies are needed to pursue more detailed information about the association between preterm morbidities and placental apoptosis and autophagy in women with GDM and obesity.

Inflammasomes are cytosolic proteins that are part of the innate immune system regulating inflammation. NLRP3 (NOD-, LRR- and pyrin domain-containing protein 3) is a member of the inflammasome complex that activates proinflammatory pathways mainly via Caspase-1 activation. The NLRP3 is the most frequent marker to assess inflammasome formation.[40] The inflammasome activity is involved in implantation and as well as pregnancy.[41] Altered placental NLRP3 activation is also involved in placental disorders such as preeclampsia and preterm delivery with chorioamnionitis.[8,42] Rare data exist regarding preterm morbidities and inflammasome activation. The inflammasome activity was involved in preclinical studies including hyperoxic lung injury, endotoxin-induced lung injury and neonatal hypoxic-ischemic brain injury models.[43-45] In our study, we assessed the placental NLRP3 activity and its association with preterm morbidities. Our results showed a significantly increased high-intense inflammasome activity in patients who had surgical NEC and hsPDA. Surprisingly, a majority of the infants with BPD had lower inflammasome activity (Table II). Although decreased placental inflammasome activity in infants developing BPD may seem unexpected due to the role of antenatal inflammation in the pathogenesis of BPD development, it may be considered that exposure to fetal inflammation

may have both harmful and beneficial effects on the immature lung.[46] Repeated exposure to LPS or chronic chorioamnionitis in experimental animals reduces the development of BPD by leading to immune tolerance and a reduced inflammatory response. Exposure to antenatal inflammation can also induce a maturation effect through proinflammatory cytokines such as IL-1, IL-6, IL-8, and TNF-alpha, which enhance surfactant protein and lipid synthesis. Antenatal inflammation can correspondingly lead to structural modifications in the fetal lung and affect the expression of growth factors that are required for branching such as transforming growth factor-beta, connective tissue growth factor, fibroblast growth factor-10.[47]

Poor in-utero conditions cause increased placental oxidative stress with increased production of reactive oxygen species (ROS).[48] ROS is a major determinant of cellular damage by triggering autophagy and inflammation. Autophagy regulates inflammation by clearance of damaged mitochondria, ROS and inflammasome components. In this study, we demonstrated a significant overlap between autophagy and inflammasome activities. We also assessed the inflammasome activity with autophagy activity since the interaction between autophagy and inflammasome is complicated and two-sided. However, accompanying autophagy to inflammasome did not significantly change the results.

The strength of our study was its prospective design and relatively homogenous study population of preterm infants between 24-29 gestational age, who are especially at higher risk of adverse outcomes. However, several limitations exist. The first limitation was the small size of the study population. Autophagy and inflammasome activities are dynamic responses to certain triggers and they change activity over time. Studying the placenta at a single time point may have prevented us from demonstrating the dynamic processes taking place over time. Another limitation was the assessment of autophagy activation only via immunohistochemical LC3B staining. Autophagy is a dynamic process in human tissues, therefore using a single marker and method is challenging. Therefore, authophagy guideline recommends the use of multiple assays, whenever possible.[49]

In this study antenatal inflammatory process was assessed by only placental histopathological examination and inflammasome activity. Several studies in the literature have investigated umbilical cord inflammatory markers such as Il-6, procalcitonin and CRP, and the results were mostly controversial in terms of correlation between histological chorioamnionitis and neonatal outcomes.[50,51] Umbilical cord IL-6 is the most studied biomarker for predicting histological chorioamnionitis, and its sensitivity and specificity vary between 64-84%.[52] Therefore it is recommended that the histological examination of the placenta is more accurate and essential for predicting neonatal outcome. In regard to this limitation, the relationship between placental autophagy and the umblical cord inflammatory biomarkers are needed to be evaluated in future studies.

An important limitation was the lack of evaluation of postnatal factors in preterm morbidities in our cohort. Preterm morbidities are widely multifactorial affected by both antenatal and postnatal conditions. The aim of our study was evaluating placental autophagy and inflammasome as early biomarkers for predicting preterm morbidities. Hence various antenatal risk factors could affect placental findings, we evaluated antenatal risk factors in our cohort and found no relationship regarding placental findings. Further studies are needed to be carried out including postnatal risk factors for preterm morbidities based on our preliminary results on placental autophagy and inflammasomes.

In our study population, almost all placentas had an abnormality, demonstrating either inflammation or placental malperfusion findings. It is well known that these two conditions are involved in neonatal adverse outcomes. Autophagy and inflammasome

activities from pathological placentas could affect our results in terms of predicting preterm morbidities. Because of the natural causes of prematurity, a control group consisting of age-matched healthy placentas could not be set up. Because of the characteristics of our sample (high sectio rate, lower rate of antenatal steroid, higher complication rate) the data from this study population may be difficult to translate more widely.

In conclusion, the results signify the physiological role of these mechanisms in the intrauterine period. Association of decreased or increased rates of inflammasome activities with certain diseases such as BPD, hsPDa and surgical NEC indicates the role of the intrauterine inflamattory process and the importance of critical balance around the inflammation. Alongside conventional placental histopathological findings, the investigation of the placenta in terms of autophagy and inflammasome activity could bring new insights to clinical practice in neonatology. Understanding the interplay between autophagy and inflammasome in the pathogenesis of preterm morbidities may benefit a better understanding of molecular pathways, contribute to the development of new biomarkers, and provide new therapeutic options.

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Ethical approval

This study was approved by the Ethics Committee of Dokuz Eylul University Medical Faculty. (No:2017/25-02).

Author contribution

The authors confirm contribution to the paper as follows: study conception and design: FT, HO, ND, EO; data collection: BD, AA; analysis interpretation of results: BD, FT, HO, ND, draft manuscript preperation: BD, FT. All authors reviewed the results and approved the final version of the manuscript.

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Conflict of interest

The authors declare that there is no conflict of interest.

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Original Article

Comparison between oral melatonin and 24% sucrose for pain management during retinopathy of prematurity screening: a randomized controlled trial

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ABSTRACT

Background. Preterm neonates perceive multiple painful procedures during Neonatal Intensive Care Unit (NICU) stay, having long term neurobehavioral effects. This study aims to compare the analgesic efficacy of oral melatonin with 24% sucrose in neonates during retinopathy of prematurity (ROP) screening.

Methods. A prospective, non-blinded, randomized controlled trial was conducted in a tertiary care NICU. All preterm neonates with gestational age (GA) <34 weeks or birth weight (BW) < 2000 grams eligible for ROP screening were randomized into oral melatonin (4 mg/kg) and oral 24% sucrose (0.5 ml) groups. Both groups received standard non-pharmacological measures and topical proparacaine. The intensity of pain was measured by Premature Infant Pain Profile (PIPP) score during the procedure, at 1st and 5th minutes following the procedure and compared between the two groups by Mann-Whitney U test with p value <0.05 considered as significant.

Results. A total of 60 preterm neonates were randomized with 30 neonates in the melatonin (median [interquartile range] GA: 30.86 [3.78] weeks, BW: 1160 [430] grams) and 30 neonates in the 24% sucrose (median [IQR] GA: 29.29 [4.68] weeks, BW: 1070 [315] grams) group. The median PIPP score during the procedure in the melatonin and sucrose groups were 17 and 16, respectively (p=0.64). The median (Q1-Q3) PIPP score at the 1st minute was significantly lower among the melatonin group (7 [5.25-10]) vs 24% sucrose group (9.5 [7.25-11]) (p=0.02); and at the 5th minute, the median (Q1-Q3) PIPP scores in the melatonin group (5 [4-6]) was comparable to the 24% sucrose group (5.5 [3.25-7]) (p= 0.52).

Conclusions. Oral melatonin is not inferior to oral 24% sucrose for pain management during ROP screening.

Key words: retinopathy of prematurity, melatonin, analgesic, neonate.

Neonates are anatomically and physiologically capable of feeling pain; and inadequate pain management evokes long-term consequences.[1,2] A number of validated tools are used for pain assessment and various non-pharmacological (nesting, swaddling, non-nutritive sucking, facilitated tucking, kangaroo mother care etc.) as well as pharmacological (sucrose, dextrose, opioids, acetaminophen etc.) interventions are used for pain management in neonates.[3,4] Retinopathy of prematurity (ROP) screening is an essential procedure for the prevention of visual morbidity of preterm neonates. It inflicts severe pain during an eye examination with the persistence of residual pain up to 30 min after the procedure.[5] The recent recommendation includes various non-pharmacological measures, local anesthetics and oral 24% sucrose before ROP screening.[6]

Melatonin is a hormone synthesized and secreted by the pineal gland. In a meta-analysis,

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melatonin was effective in reducing chronic pain in the adult population.[7] There is only a single study evaluating the analgesic activity of intravenous melatonin in mechanically ventilated preterm neonates.[8] The present study aimed to explore the analgesic effect of oral melatonin and to compare its efficacy with 24% sucrose solution in neonates undergoing ROP screening.

Material and Methods

This prospective, non-blinded, parallel, randomized controlled trial was conducted in the neonatal intensive care unit of Kalinga Institute of Medical Sciences, Bhubaneswar from February 2021 to July 2021 after approval from the institutional ethics committee and drug trial (CTRI) registration (Institutional Ethics Committee, Kalinga Institute of Medical Sciences, CTRI/2021/02/031458).

Neonates with a gestational age <34 weeks or birth weight < 2000 grams undergoing ROP screening during Neonatal Intensive Care Unit (NICU) stay, and receiving partial paladai feeds (at least 7 ml/kg of breastmilk per feeding) were included in the study. Neonates on mechanical ventilation, ionotropic support, opioid analgesics, sedatives or anticonvulsants during ROP screening were excluded. As per unit protocol neonates with gestational age ≤ 28 weeks or birth weight ≤ 1250 grams were subjected to the first ROP screening in the second or third week of age whereas neonates of higher gestational age underwent the first screen by the fourth week of age. Some of them were subjected to repeated follow-up screens till full vascularization of the retina. The eligible neonates after randomization were included only once, out of several ROP examinations during their NICU stay, for study purposes. Parental consent was taken prior to case recruitment. Patients were assigned into the intervention and control groups, in a ratio of 1:1, by computer generated random list and allocation concealment was done in an opaque sealed envelope. Sample size calculation: In a previous study, 43% of neonates had no painful reaction during ROP examination with oral 24% sucrose vs 22% neonates with placebo.[9] Assuming 10% difference points in analgesic effect between 24% sucrose and oral melatonin, alpha error 5%, power 80% and 1:1 allocation ratio, the required sample size was 54 (27 in each arm). Considering a 10% attrition rate, calculated sample size was 60 (30 in each arm).

The baseline demographic neonatal characteristics, heart rate (HR) and oxygen saturation (SpO_2) denoted by Multipara monitors were recorded in a pre-structured proforma. Indirect ophthalmoscopy for ROP screening was done by a trained ophthalmologist. Prior to the procedure eye drop containing 0.8% tropicamide with 5.0% phenylephrine (Auromide Plus Drop by "Aurolab") was used four times at 10 minute intervals to dilate the pupils, and the infants were fed at least an hour before screening. Neonates in the intervention group were given melatonin (Syrup Trunap, 3mg/5ml, "Brio Bliss Life Science Pvt. Ltd") at a dose of 4mg/kg (~ 6.6ml/kg) orally 20 minutes prior to the procedure. The syrup Trunap contains melatonin as an active ingredient with the presence of minor ingredients as vehicle, preservative, flavoring agent similar to any other oral pediatric formulation. The control group received 0.5 ml oral 24% sucrose (Arbineo sachet by "Raptakos") 2 minutes prior to the procedure. Eye drop 0.5% proparacaine (Aurocaine drop by "Aurolab") was used for neonates in both arms just before the procedure. All the neonates were provided with non-pharmacological interventions such as nesting, swaddling and facilitated tucking by nursing staff in a dim light environment throughout the procedure. The chronological age (days), post menstrual age (gestational age plus chronological age in weeks) and weight of the baby at the time of the ROP examination, were duly noted in the proforma. Premature Infant Pain Profile (PIPP) score was used for the assessment of the severity of pain - during the procedure, at 1 minute and 5 minutes after the procedure. The parameters of the PIPP Scale are

gestational age, behavioral state, highest heart rate, lowest SpO_2, brow bulge, eye squeeze and nasolabial furrow wherein each parameter is scored from 0 to 3 with the maximum score being 21. The pain scoring was done by the primary investigator whereas timekeeping was done by the nurse educator with a stopwatch. The severity was categorized as mild/no pain (<6), moderate pain (6-12) and severe pain (>12). Complete pain relief was denoted by a PIPP score of less than 6 at any time during the study. Neonates recorded to have moderate to severe pain 5 minutes after the procedure (PIPP score ≥ 10) received oral paracetamol, 10 mg/kg.

Any adverse effects such as apnea, respiratory distress, arrhythmias, vomiting or feeding difficulties were monitored for the next 24 hours in both arms. Stoppage of the trial was planned in case of any major adverse effect such as any acute life threatening event noted in either of the groups.

Statistical analysis

All data was recorded in Microsoft Excel format. Statistical analysis was performed using SPSS version 20.0 (SPSS Inc., Chicago, IL, USA). Quantitative variables are expressed as mean with standard deviation (SD) or median with quartile range (Q1-Q3). The qualitative variables are described in terms of frequencies & proportion. The significance of the differences between the study groups was tested using the Mann-Whitney U test. Differences in categorical variables were tested using a chi-square test/ Fisher exact test. A p value < 0.05 was considered statistically significant in all statistical tests.

Results

A total of 108 preterm neonates were eligible for ROP screening and 48 neonates were excluded (25 discharged prior to ROP screening, 6 left against medical advice and 8 neonatal deaths before the screen, no parental consent for 9 neonates). The remaining 60 neonates were randomized into 30 in the melatonin and 30 in the 24% sucrose groups. Fig. 1 depicts the flow diagram of the study participants. The baseline characteristics of neonates were similar in both groups (Table I).

During the procedure, the median (Q1-Q3) PIPP score in the melatonin and 24% sucrose groups were 16 (14-17) and 15.50 (13.25-17) respectively and the neonates of both groups perceived severe pain. The median PIPP score was significantly lower in the melatonin vs 24% sucrose group at 1 minute after the procedure (p=0.02) but was not significantly different between the groups (p=0.52) at 5 minutes after the procedure (Table II). Only one neonate in the melatonin group had moderate pain at the 10th minute after the procedure (PIPP score-10) and needed an add on analgesic i.e. oral paracetamol. None of the neonates in the oral 24% sucrose group needed additional analgesics.

One neonate in the sucrose group had two episodes of apnea one minute after the procedure and was revived with tactile stimulation. One baby in the melatonin group had respiratory distress after a few minutes of the procedure requiring low flow oxygen at 1 L/min and it subsided within the next 30 minutes. Two neonates in the melatonin group developed one episode of non-bilious vomiting within one hour of the procedure. In neither of the arms, any of the neonates had difficulty in oral feeding after the procedure. There was no significant difference in adverse events between both groups.

Discussion

We have shown that the analgesic effect of oral melatonin is not inferior to oral 24% sucrose during the post-procedural period of ROP screening. The pain inflicted during ROP screening was not well relieved with either of the analgesic agents, in spite of the use of additional non-pharmacological measures and topical anesthetic agents. At 1 minute after the procedure, the median PIPP score in the melatonin group (7) was significantly lower (p=0.02) compared to the 24% sucrose

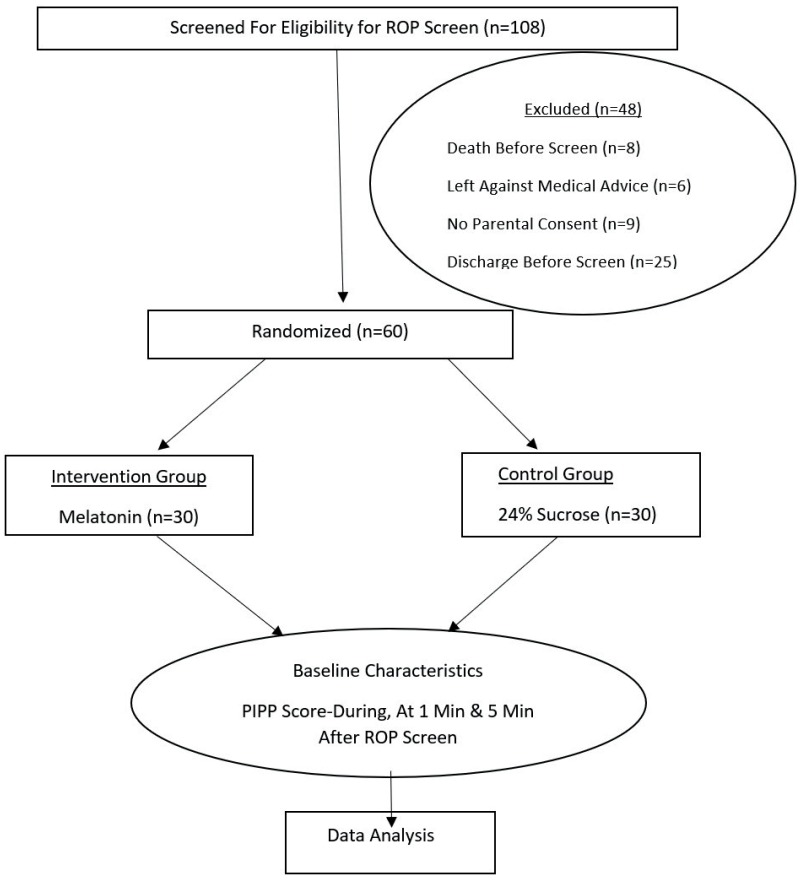

Fig. 1. Study participants flow diagram.
PIPP: Premature Infant Pain Profile, ROP: Retinopathy of prematurity

Table I. Comparison of baseline characteristics of neonates between Melatonin and Sucrose groups.

Variables	Median (Q_1-Q_3)		*p*-value
	Melatonin Group (N=30)	Sucrose Group (N=30)	
Gestational age (weeks)	30.86 (29.33-33.11)	29.29 (28.07-32.75)	0.15
Birth weight (grams)	1160.00 (1002.50-1432.50)	1070.00 (980.00- 1295.00)	0.23
Day of examination	26.50 (21.25- 41.50)	27.50 (20.25-40.00)	0.93
PMA at ROP screen (weeks)	35.64 (33.78-37.00)	35.86 (33.11-36.82)	0.59
Weight at ROP screen (grams)	1600.00 (1388.75-1787.50)	1485.00 (1240.00-1702.50)	0.13
Baseline heart rate (BPM)	149.00 (141.25-157.75)	146.50 (136.25-157.00)	0.49
Baseline SpO_2(%)	97.50 (96.00-99.00)	98.00 (95.00-99.00)	0.80

PMA: Post-menstrual age, BPM: Beats per minute, ROP: Retinopathy of prematurity

Table II. Comparison of pain scoring (PIPP score) between the two groups during and after examination.

Parameters	Median (Q_1-Q_3)			*p*-value
Pain Score	Melatonin Group (N=30)	Sucrose Group (N=30)	Total (N=60)	
PIPP score during examination	16.00 (14.00-17.00)	15.50 (13.25-17.00)	16.00 (14.00-17.00)	0.64
PIPP score at 1st minute	7.00 (5.25-10.00)	9.50 (7.25-11.00)	8.00 (7.00- 10.00)	0.02
PIPP score at 5th minute	5.00 (4.00-6.00)	5.50 (3.25-7.00)	5.00 (4.00- 6.00)	0.52

PIPP: Premature Infant Pain Profile

group (9.5) and around one third of neonates in the melatonin vs one tenth of neonates in the sucrose group had no pain (*p*=0.05). At the 5th minute after the procedure a majority of neonates in both the melatonin (median PIPP score-5, 86.66% with PIPP score <6) and sucrose (median PIPP score-5.5, 70% with PIPP score < 6) groups had no or minimal pain. To the best of our knowledge, this is the first study to explore the analgesic effect of oral melatonin and compares its efficacy with standard analgesic agent 24% sucrose in a randomized control trial.

The inconclusive effectiveness of either melatonin or sucrose during the ROP examination could be related to the severity of pain during the procedure. In a Cochrane systematic review, 24% sucrose was found to be a safe and effective analgesic agent in mild to moderate pain in neonates.[10] Grabska et al.[11] and Rush et al.[12] were unable to demonstrate a significant analgesic effect between a placebo and 24% sucrose during ROP screening in neonates. The analgesic effect of sucrose in combination with a pacifier was found to be greater than a placebo with a pacifier in two studies.[13,14]

The evidence of the analgesic effect of melatonin is very limited in neonates. The exact anti-nociceptive action of melatonin is not known and possible pathways are mostly explored from animal studies. Melatonin may regulate pain via various receptors i.e. MT1/MT2 –melatonin receptors, opioid l-receptors, GABA receptors present in both the central and peripheral nervous system, release of β-endorphins and the nitric oxide-arginine pathway.[15] In adult human studies, melatonin reduces acute pain during the post-operative period.[16] The anti-inflammatory cytokine pathway was found in a neonatal study for its late onset nociceptive effect.[8] However, the early analgesic effect of melatonin noticed in the present trial needs further studies to explore its mechanism.

We used oral melatonin suspension due to non-availability of intravenous formulations in this part of the country. To date, the safety and efficacy of melatonin have been established in the intravenous route at a dose of 3-10 mg/kg in neonates.[8,17-22] Based on an allometric evaluation, the oral melatonin dosage was estimated between 0.5-5 mg/kg for preterm neonates in a pharmacokinetic study.[23,24] The analgesic effect of melatonin was noticed with its oral administration 30 minutes prior to venipuncture in pediatric study participants aged between 1-14 years.[25] The paucity of pharmacokinetics data is the major limitation in establishing appropriate analgesic dosage of melatonin in neonates. The dose of melatonin used in the current study was extrapolated from available relevant literature.

The anti-inflammatory, antioxidant and neuroprotective behavior of melatonin have been studied in various neonatal diseases i.e. hypoxic ischemic encephalopathy, sepsis, respiratory distress syndrome, bronchopulmonary dysplasia and neonatal surgery.[18-22,26] The myelination in white matter of the preterm brain could be protected by melatonin and its metabolites.[27,28] Preterm neonates

usually require multiple painful procedures during their hospital stay. Considering its neuroprotective effect, melatonin could be explored as an analgesic drug that might be used multiple times. However, repeated use of sucrose analgesia has worse neurobehavioral development and physiologic outcomes in a preterm neonatal study by Johnston et al.[29] Again sucrose has no effect on pain related brain activity in an EEG based neonatal study by Slater et al.[30] In a systematic review, authors were concerned regarding the neurodevelopmental outcome with multiple times use of oral sucrose as analgesia.[31] Further research is needed for a head-to-head comparison of multiple doses of sucrose versus melatonin for the long-term neurodevelopmental outcomes.

In the 24% sucrose group, one neonate had apnea within one minute of the procedure. In a study by Dilli et al.[13], around one-third of total neonates had apnea and bradycardia following ROP screening both in the sucrose and placebo groups. Most of the neonates well tolerated the oral melatonin at 4 mg/kg dose apart from one episode of vomiting noted in two neonates and one neonate had transient respiratory distress. The vomiting episodes could be due to the adverse effect of melatonin or post-procedural pain and there was no persisting difficulty in paladai feeding. As 10% of neonates in the melatonin group faced some kind of adverse events, the safety of the drug needs to be evaluated in future studies.

This study has many limitations, one of them being a monocentric study with a relatively small sample size. The analgesic action of melatonin is evaluated only in a single procedure in hemodynamically stable neonates after completion of intensive care management. Hence its safety and effectiveness may not be generalized to critically ill neonates and also for different types of procedures. The sample size is relatively small to address the adverse effect of a novel drug like melatonin. Additionally, in this study, the analgesic effect of melatonin was measured along with standard non-pharmacological measures and topical anesthesia, thus the isolated effect of melatonin was not evaluated.

Oral melatonin may be an alternative medication to oral 24% sucrose for moderate to severe pain management in neonates. Hence further studies are needed to explore the analgesic effect of melatonin in neonatal practice with long term neurodevelopmental effects.

Ethical approval

Institutional Ethics Committee (KIMS/KIIT/IEC/535/2020) dt. 29/12/2020 CTRI Number – CTRI/2021/02/031458.

Author contribution

Study conception and design: SKP; data collection: SSB, AD; analysis and interpretation of results: BN, SKP; draft manuscript preparation: SKP, SSB, BN, AD. All authors reviewed the results and approved the final version of the manuscript.

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Conflict of interest

The authors declare that there is no conflict of interest.

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Original Article

Periostin and IFN-γ levels in serum and nasopharyngeal aspirate in infants with viral-induced wheezing – 2 year follow-up

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ABSTRACT

Background. The present study assesses the immune response in children with viral-induced wheezing by examining the two factors-interferon-gamma (IFN-γ) and periostin in serum and nasopharyngeal aspirate (NPA). The aim was to find a pattern with the severity and frequency of wheezing episodes.

Methods. Sixty-nine infants (40 boys and 29 girls), with a mean age of 11.4±6 (2 - 23) months, hospitalized with a first or recurrent episode of bronchial obstruction were enrolled in this study. The serum and NPA concentrations of IFN-γ and periostin were assessed by ELISA methodology. Fifty of the children (72%) were followed for 2 years.

Results. We detected lower NPA IFN-γ production in boys, infants with atopic status, family history of asthma, and respiratory syncytial virus infection. Recurrent wheezing in children was associated with a twice lower concentration of IFN-γ in NPA compared to those with the first episode (7.1 vs. 14.8 pg/ml, p=0.05). Higher serum periostin level was established in children over 12 mo in the group of recurrent wheezers with persistent manifestations compared to those without symptoms during the follow-up (410.5 vs. 269.7 ng/ml, p = 0.03). Multivariate logistical regression model assessed high level of serum periostin, male gender, atopy, family history of asthma, and severity of the attack as significant risk factors for persistent compared to intermittent wheezing ($r^2 = 0.87$, p = 0.04).

Conclusions. Our results demonstrated that recurrent viral-induced wheezing is associated with decreased IFN-γ production and increased periostin response and their correlation with severity and persistence of symptoms were the main outcome measures.

Key words: viral-induced wheezing, periostin, interferon-gamma (IFN-γ).

It is a challenge to determine the likelihood of recurrent wheezing in infants. It remains unclear what differentiates children with the first episode of wheezing from those who will switch to asthma in the future. Among the most common reasons for viral-induced wheezing is respiratory syncytial virus (RSV). Although our understanding of its immunopathogenesis is increasing, there are still no objective criteria to assess the risk for asthma, and results are limited and controversial.

The theory of imbalance in the T-helper 1 / T-helper 2 (Th1/Th2) immune response is the most widely accepted. In primary RSV infection, a type I response develops in which natural killer (NK) cells and Th1 lymphocytes are sources of interferon-gamma (IFN-γ), a major cytokine of cell-mediated immunity. IFN-γ inhibits allergic reactions and is an important molecule in antiviral protection. The lack of sufficiently

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strong polarization of T cells in the Th1 direction may allow the development of an unregulated Th2 response in RSV infection. In recent years, periostin has been used as a systemic biomarker for the Th2 immune response and eosinophilic inflammation.[1] Periostin is an extracellular protein, first described in 1993, after extraction from the periosteum of mice.[2] The role of periostin in Th2 inflammation and asthma in many aspects, includes eosinophil accumulation, increased mediator expression, and airway remodeling.[3]

The study aimed to assess the IFN-γ and periostin levels in serum and nasopharyngeal aspirate (NPA) in wheezing infants and to find a pattern with the severity and frequency of wheezing episodes.

Material and Methods

This prospective observational single-center study was carried out in the Pediatric Department of Alexandrovska University Hospital with the recruitment of patients for a one-year period from February 2018 to March 2019. Sixty-nine infants, aged 2 to 23 months, hospitalized with a first or recurrent episode (≥3) of bronchial obstruction were enrolled in this study. The inclusion criteria for the study were as follows: age 2–24 months, admission due to an acute bronchial obstruction diagnosed clinically based on tachypnea, expiratory dyspnea, prolonged expiration, and wheezing, born in gestational week 35 or later. Infants born less than 34 weeks gestation, those with bronchopulmonary dysplasia, congenital anomalies, cystic fibrosis, and other concomitant diseases (cardiac and neurological) were excluded.

We divided the patients into two groups, according to assessment:

- 30 children (44%) with one episode of bronchial obstruction up to 2 years of age, conditionally accepted as "internal control group"- first wheezing (FW) group.

- 39 children (56%) with three or more episodes of wheezing up to 2 years of age - "recurrent wheezing" (RW) group.

Medical history was collected at admission: family history of asthma (maternal, paternal, and first-line siblings); own atopic status - atopic dermatitis, allergy to cow's milk protein and other food allergens; duration, frequency, and severity of wheezing episodes. The disease severity was classified by Wainwright score[4] as mild in 5 (7%) cases, moderate in 53 (77%), and severe in 11 (16%) patients based on pulse oximetry, respiratory rate, and respiratory effort.

Paraclinical assessment at baseline included standard blood counts (white blood cells, differential count, C-reactive protein), nasopharyngeal aspirate (NPA), and blood sample collection done on the day of admission. Concentrations of periostin and IFN-γ in serum and NPA were determined by commercial enzyme-linked immunosorbent assay (ELISA, ABclonal, and R&D Systems) according to the manufacturer's directions. Detection limit for periostin was 0.004 ng/ml and 8.0 pg/ml for IFN-γ respectively. The viral etiology of the respiratory tract infections was determined using polymerase chain reaction (PCR) for a panel of 11 respiratory viruses (RSV A/B, human metapneumovirus (hMPV), influenza, and parainfluenza virus type (PIV) 1/2/3, rhinovirus (RV), adenovirus (AdV) and bocavirus (BoV)). Viral nucleic acids were extracted automatically from respiratory specimens using a commercial ExiPrep Dx Viral DNA/RNA kit (Bioneer, Korea). The detection and typing of influenza viruses were carried out by a real-time reverse transcriptase (RT) PCR method and the SuperScript III Platinum® One-Step qRT-PCR System (Invitrogen, Thermo Fisher Scientific, USA). The detection of RSV, hMPV, PIV 1/2/3, RV, AdV, and BoV was performed using singleplex real-time PCR assays and an AgPath-ID One-Step RT-PCR kit (Applied Biosystems, Thermo Fisher Scientific, USA).

The families were contacted by phone for information about new respiratory events and conducted treatment. Fifty of the parents (72%) responded to our phone survey and were prospectively followed-up during the next 2 years, the other 19 refused to be involved in the observation series.

The study protocol was approved by the Institutional Ethics Committee of the Sofia Medical University (No. 2781) and informed consent was obtained from the parents.

Statistical data processing was performed with the statistical package SPSS Version 23. The following were used: descriptive analysis, statistical dependence between qualitative variables (cross tabulation), parametric (t-test), and non-parametric methods (Mann-Whitney U-test, H-test of Kruskal-Wallis). The correlation of periostin and IFN–γ with the clinical variables was determined using Spearman's rank correlation coefficient. The odds ratio (OR) and 95% confidence interval (CI) were reported. Predictors of persistent wheezing were analyzed using a stepwise logistic regression model, with an inclusion $p < 0.05$ and an exclusion $p > 0.10$. The diagnostic values of IFN–γ and periostin levels to identify children with persistent wheezing were determined by receiver operating characteristic (ROC) curve analysis. Sensitivity and specificity were calculated for the selected cut-off point.

Results

Descriptive statistics

A total of 69 children with an average age of 11.4 months (range 2 to 23 months, SD ± 6 months) were enrolled. There was a slight predominance of boys in the gender distribution – 40 (58%) vs. 29 girls (42%). The full characteristics of the study subjects are outlined in Table I. We established a difference in the baseline demographic characteristics between the two groups – FW and RW, in terms of a higher age of enrolment, lower age of first wheezing, male gender prevalence, and older siblings in the RW group.

There were no differences between groups in terms of onset of symptoms, fever, indicators of inflammatory activity, and severity. Virological examination demonstrated RSV etiology of infection in 40 children (58%), RV in four, BoV virus in three, one child with hMPV, and 21 (30%) were negative.

Fifty of the children (72%) were followed for 2 years. Twenty-six (52%) of them were with persistent symptoms. The distribution by groups showed that in the RW group 23/33 (70%) were with persistent wheezing (RWP) compared to 3/17 (17%) in the FW group – OR 9.33 (7.3-39.4), p =0.001. As risk factors for persistence of manifestations were established: male gender – 20/26 (77%) vs. 12/24 (50%) OR=3.33 (0.99-11.21), p=0.04 and wheezing during the next 12 months, after the hospitalization – 69% (24/35)

Table I. Demographic characteristics.

	n (%)	Males	Average Age (months)	Family history of asthma	Atopy	Age of first wheezing (months)	Siblings
First wheezing (FW)	30 (49%)	12/30 (35%)	9 (2-23)	12/34 (35%)	5/34 (15%)	9.6 (3-23)	10/34 (29%)
Recurrent wheezing (RW)	39 (51%)	28/39 (80%)	11 (5-17)	13/35 (37%)	6/35 (17%)	6.7 (2-13)	23/35 (65%)
p =		0.001	0.001	0.5	0.5	0.003	0.003

of wheezers during the first year of follow-up were with persistent symptoms vs. 0/15 (0%) from patients without symptoms for that period, OR= 3.1 (1.95-5.19).

IFN-γ in serum and NPA

The mean IFN-γ level in serum was 14.28 (0-100.3) pg/ml and 10.93 (0-124.14) pg/ml in NPA. There was no relationship between the concentration of IFN-γ in serum and NPA. An increase in serum IFN-γ levels was observed with age – children over 18 months had 3 times higher IFN-γ 37,77 vs. 10.46 pg/ml, p=0.02. We detected lower IFN-γ in NPA in boys and infants with RSV infection. Recurrent wheezing in children was associated with a twice lower concentration of IFN-γ in NPA compared to those with the first episode (Table II). When distributed by gender, the male group is with lower IFN-γ NPA levels from the beginning (the first episode of wheezing), but not significant – boys 10.60 vs. girls 13.27 pg/ml, p=0.6. This discrepancy achieved statistical significance in the recurrent wheezing group – NPA IFN-γ levels: boys 5.48 vs. girls 15.11 pg/ml, p=0.008.

Periostin in serum and NPA

The mean serum periostin level was 418.73 (0-602.57) ng/ml. There was a significant inverse age correlation – periostin in serum decreased with age, Spearman r = - 0.51, p=0.005, Figure 1. The most significant decline was after 18 months, almost twice.

Table II. Comparison of IFN- γ and periostin levels in serum and NPA according to clinical variables in 69 participants.

	IFN-γ, serum (pg/mL)	P-value	Periostin, serum (ng/mL)	P-value	IFN-γ NPA (pg/mL)	P-value	Periostin NPA (ng/mL)	P-value
Age		**0.02**		0.005		0.2		0.1
2-12 mo	10.46		445.74		9.65		2.15	
12-18 mo	11.43		410.93		6.91		1.22	
>18 mo	37.77		239.44		7.07		0.01	
Gender		0.7		0.2		**0.007**		0.7
Male	10.00		419.85		7.03		1.81	
Female	17.34		404.40		16.07		1.74	
Atopy		0.8		0.1		0.2		0.9
Yes	10.94		440.81		7.01		1.79	
No	24.32		404.98		11.50		1.73	
Family asthma		0.4		0.1		0.1		0.3
Yes	20.29		443.05		8.77		1.62	
No	9.99		390.75		12.01		2.09	
Disease severity		0.6		**0.02**		0.4		0.4
Mild	17.20		401.80		1.60		1.60	
Moderate	15.15		422.41		1.12		1.12	
Severe	14.00		448.78		1.48		1.48	
Respiratory viruses		0.7		0.7		**0.01**		0,15
RSV	9.61		477.34		4.99		1.74	
Other than RSV	12.81		356.21		14.4		3.36	
Wheezing groups		0.5		0.3		**0.05**		**0.01**
FW	16.50		440.65		14.8		0.65	
RW	11.66		382.12		7.1		2.84	

FW: first wheezing, IFN-γ: interferon-gamma, NPA: nasopharyngeal aspirate, RW: recurrent wheezing

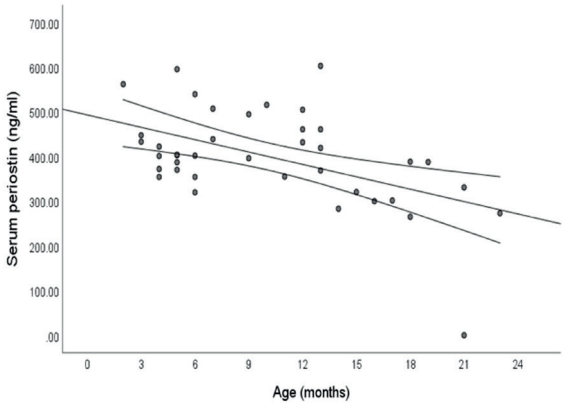

Fig. 1. Inverse correlation of the serum periostin by age.

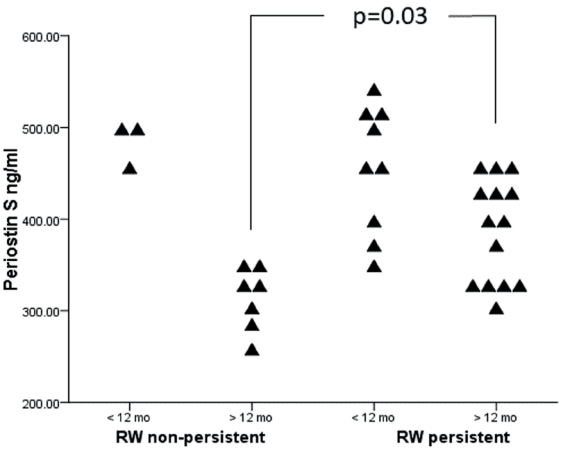

Fig. 2. Serum periostin level in recurrent persistent wheezers and non-persistent groups according to age distribution.

No differences in periostin levels were found according to gender. Slightly higher periostin concentration was reported in children with a family history of asthma, and atopy, but without statistical significance. A correlation between the higher serum periostin and severity of disease was observed, see Table II.

The mean periostin level in NPA was lower compared to serum - 1.75 (0-32.9) ng/ml. There was no correlation between periostin levels in serum and NPA. No significant differences in periostin NPA concentration were found according to age, gender, family history of asthma, atopic status, and severity of clinical manifestation. Children with RW were with a higher level of periostin in NPA- 2.84 vs. 0.65 ng/ml in the FW group, p = 0.01, see Table II.

We established higher serum periostin levels in children over 12 mo in the group of recurrent wheezers with persistent manifestations RWP (+) compared to those without symptoms during the follow-up RWP (-) - 410.5 vs. 269.7 ng/ml, r = 0.50, p = 0.03, as can be seen in Figure 2.

Risk factors for persistent wheezing

The analysis of risk factors for persistent wheezing during the 2 years follow-up by univariate logistic regression did not find a statistically significant difference in relation to the age, virus etiology, and NPA IFN-γ levels, Table III. The following factors were identified as potential risk factors: males with OR (95% CI) of 3.33 (0.99-11.21), p = 0.04, atopic status with OR (95% CI) of 3.11 (0.71-13.51), p=0.1, family history of asthma with OR (95% CI) of 2.33 (0.74-7.34), p=0.1, disease severity with OR (95% CI) of 4.05 (0.75-21.9), p=0.1, and serum periostin with OR (95% CI) of 5.62 (1.72-12.5), p=0.001, see Table III.

Multivariable regression and ROC analyses for wheezing persistence

The risk factors to predict persistent wheezing, with a p-value of 0.1 for inclusion, were further analyzed by logistic multivariable regression and by receiver operating characteristic (ROC) curve. High level of serum periostin, male gender, atopy, family history of asthma, and severity of the attack was significantly associated with persistent compared to intermittent wheezing, as can be seen in Table IV. Analysis, using ROC curves, identified the decreased NPA IFN-γ levels (AUC: 0.65, 95% CI: 0.51-0.78, p=0.03) with sensitivity 77% and specificity 67% respectively, and serum periostin with the best cut-off value of 310.92 ng/mL (AUC: 0.80, 95% CI: 0.45-1.00, P < .001) obtained with 81% sensitivity and 67% specificity respectively, see Figure 3.

Table III. Comparison of data between patients with and without persistent wheezing during the follow-up.

	PW (-) n=24 (%)	PW (+) n=26 (%)	OR (95% CI)	p
Age (mo)	10.42 (±6.35)	13 (±5.79)	1.07 (0.97-1.29)	0.2
Sex (males)	12 (50%)	20 (59%)	3.33 (0.99-11.21)	0.04
Atopy	3 (12%)	8 (31%)	3.11 (0.71-13.51)	0.1
Family asthma	8 (33%)	14 (54%)	2.33 (0.74-7.34)	0.1
Severe disease	2 (8%)	7 (27%)	4.05 (0.75-21.9)	0.1
RSV (+)	16 (67%)	14 (54%)	0.58 (0.17-1.83)	0.3
RW	10 (42%)	23 (88%)	0.73 (2.51-45.81)	0.001
IFN-γ serum (pg/mL)	14.26 (0-100.3)	11.38 (0-32.8)	1.11 (0.92-1.34)	0.3
IFN-γ NPA (pg/mL)	10.12 (0-61.2)	7.34 (0-49)	1.59 (0.95-3.02)	0.2
Periostin Serum (ng/mL)	292.5 (256.0-602.5)	411.3 (273-488)	5.62 (1.72-12.5)	0.001
Periostin NPA (ng/mL)	1.63 (0-11.44)	3.03 (0-32.9)	1.04 (0.93-1.38)	0.4

PW(+), patients with persisten wheezing episodes; PW(−), patients without persistent wheezing episodes, data are expressed as number and % of positive cases, mean ± SD or median (minimum-maximum).

Table IV. Multivariate logistical regression model on the involved in the follow-up patients for risk of persistence in children with recurrent wheezing.

	Univariate		Multivariate	
	OR (95% CI)	p	OR (95% CI)	p
Age (mo)	1.07 (0.97-1.29)	0.2		
Sex (males)	3.33 (0.99-11.21)	0.04*	2.98 (0.84-10.55)	0.009
Atopy	3.11 (0.71-13.51)	0.1*	2.44 (0.53-11.17)	0.2
Family asthma	2.33 (0.74-7.34)	0.1*	1.5 (0.48-4.65)	0.3
Severe disease	4.05 (0.75-21.9)	0.1*	0.46 (0.093-2.35)	0.35
RSV (+)	0.58 (0.17-1.83)	0.3		
RW	0.73 (2.51-45.81)	0.001**		
IFN-γ serum (pg/mL)	1.11 (0.92-1.34)	0.3		
IFN-γ NPA (pg/mL)	1.59 (0.95-3.02)	0.2		
Periostin Serum (ng/mL)	5.62 (1.72-12.5)	0.001*	4. 22 (1.55-11.82)	0.004
Periostin NPA (ng/mL)	1.04 (0.93-1.38)	0.4		

NPA: nasopharyngeal aspirate, RW: recurrent wheezing

Discussion

Pediatricians need biomarkers that allow early recognition of infants at risk of persistent wheezing. We examined two factors, IFN-γ-associated with viral immune response, and periostin- responsible for the Th2 immune reaction.

The age-dependent reduction in serum IFN-γ level that we observed - three times lower in the age group below 18 mo, suggests more frequent and severe respiratory infections in small infants. Reduced local interferon concentrations would be expected to permit increased viral replication, greater cytopathic effects, and increased shedding into the respiratory tract.[5,6]

Early studies have demonstrated a lower production of interferon in nasal washes during RSV infections when compared to other viral infections.[7] Our results confirm that RSV infection is associated with a significant decrease in IFN-γ responses in the NPA. This is in agreement with other authors and their suggestions for the suppression of Th1 cytokine response in the respiratory tract during RSV infections.[8] The protective role of IFN-γ in RSV

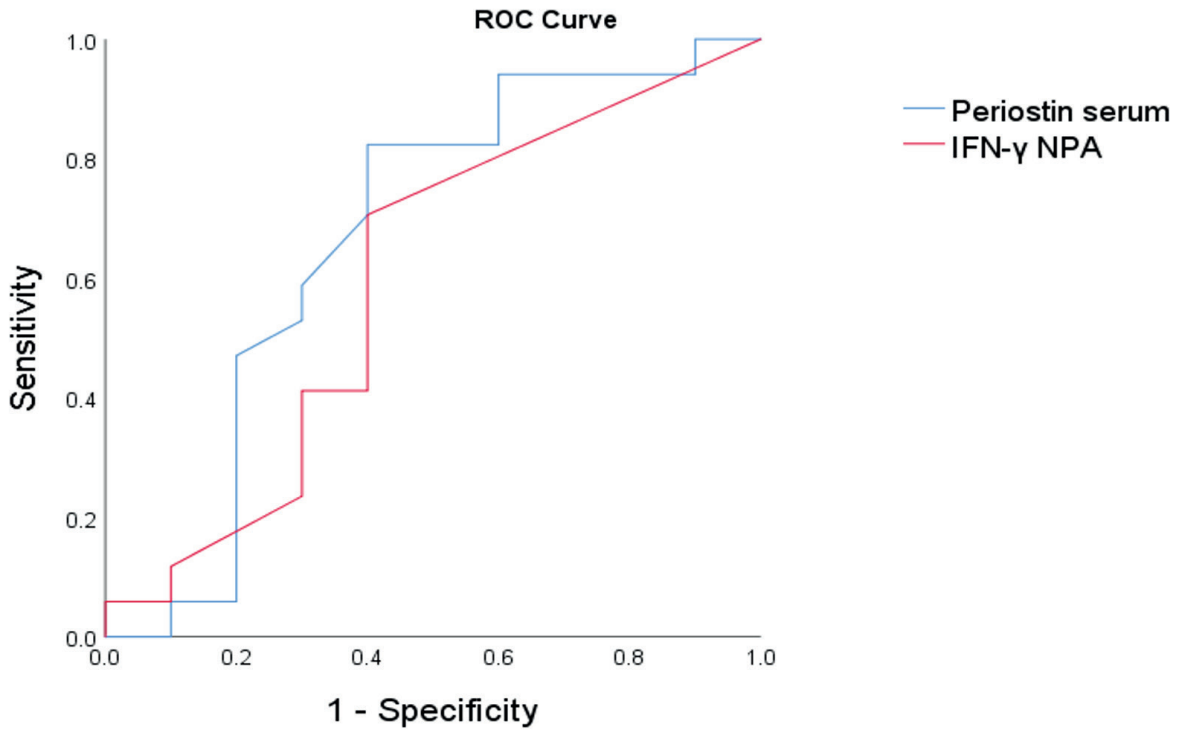

Fig. 3. ROC curve analyses of the sensitivity and specificity for the prediction of persistent wheezing by IFN-γ NPA (AUC: 0.65, 95% CI: 0.51-0.78, p=0.03) and serum periostin (AUC: 0.80, 95% CI: 0.45-1.00, P < .001).

infection can explain the association between reduced IFN-γ production and lower systemic proliferative responses to the severity of the disease and the need for mechanical ventilation.[9]

There is a lot of data that lower IFN-γ production is not a temporary condition, it was either present prior to bronchiolitis or induced by bronchiolitis and persists.[5,7,8] Renzi et al.[5] have shown that infants with the lowest IFN-γ production at the time of bronchiolitis are the most likely to develop asthma afterward and have abnormal pulmonary function later.

Twice the lower levels of IFN-γ in nasopharyngeal aspirate reported in our male group could be speculatively accepted as one of the reasons why males belong to the risk group for recurrent wheezing. In support of the proven risk factors for asthma, we and other authors have demonstrated reduced IFN-γ in NPA to viral infection in children with atopy and a family history of asthma, regardless of the viral agent.[10]

Recurrent wheezing according to our results was associated with a twice lower concentration of IFN-γ in NPA compared to those with the first episode and the lowest values were found in the male group. This confirms the concept that decreased levels of IFN-γ at early age determine susceptibility to common viral diseases and an altered viral immune response which is a risk for atopic asthma.[11] The imbalance between Th1 and Th2 responses in early life may create opportunities for the development of a Th2 response and the absence of a normal IFN-γ producing CD8+ T-cell response may skew towards a Th2 phenotype.[12]

During the last years, periostin was imposed as a systemic biomarker for the Th2 immune response and eosinophilic inflammation.[13] High osteoblast activity during linear growth in childhood causes extremely high levels of periostin- approximately 2- to 3-fold higher in children than in adult levels, with the highest values up to two years of age.[13] We observed

an inverse correlation between serum periostin and age with a double-declining after 18 mo of age.

García-García et al.[14] showed increased concentrations of periostin in NPA during viral bronchiolitis in RSV, HRV, HBoV, and hMPV-infected infants compared to healthy controls.

High levels of nasal periostin in infants with bronchiolitis suggest that respiratory viruses alter the immune response to Th2 and prove the presence of eosinophilic inflammation even at an early age.[15] These results indicate that periostin could be a biomarker for the prognosis of bronchiolitis. This was confirmed by data for high levels of periostin in children with severe pulmonary hypertension compared to those with mild form during RSV bronchiolitis.[16]

The observed positive relationship between severity of bronchoobstructive events and serum periostin levels in our study indicates that severe infection correlates with an altered profile of mediators to the Th2 immune response. Periostin plays a role in neonatal lung remodeling as well, because prolonged hyperoxic lung injury upregulates the expression of periostin, which in turn stimulates ectopic accumulation of myofibroblasts, followed by alveolar simplification.[17]

The role of periostin in asthma and type 2 inflammatory responses is an area of active research. Anderson and colleagues[13] demonstrated that high serum periostin level at the age of 2 years is a risk factor for asthma by school age. Results of several previous childhood studies have demonstrated an association between serum periostin levels and asthma. Multivariable logistic regression analysis demonstrated an association between serum periostin levels and asthma severity in children a value of 52 ng/mL emerged as the best cut-off level to differentiate children with severe asthma with high sensitivity and negative predictive values.[18]

The significantly higher values of periostin in the nasopharyngeal aspirate that we obtained in children with recurrent wheezing compared to those with the first attack, support the possible role of this protein in asthma development (2.84 vs. 0.65 ng/ml $p = 0.01$). We established higher serum periostin levels in children over 12 mo in the group of recurrent wheezers with persistent manifestations RWP (+) compared to those without symptoms during the follow-up RWP (-) (410.5 vs. 269.7 ng/ml, $r = 0.50$, $p = 0.03$). Our ROC curves analysis determining persistent wheezing children identified decreased IFN-γ NPA levels (AUC: 0.65, 95% CI: 0.51-0.78, p=0.03) with 77% sensitivity and 67% specificity, and serum periostin (AUC: 0.80, 95% CI: 0.45-1.00, $P < .001$) with the best cut-off value of 310.92 ng/mL obtained with 81% sensitivity and 67% specificity. Results of multivariable logistic regression analysis demonstrated an association between high serum periostin level, male gender, atopy, family history of asthma, and severity of the attack with a significant risk of persistent compared to intermittent wheezing, $r^2 = 0.87$, $p = 0.04$.

The mechanism of periostin's action is associated with its pleiotropic effects on airway epithelial cell function and on the development of airway fibroblasts, which promote airway remodeling in children with asthma.[19] Periostin appears to contribute to several pathogenic processes in asthma, including subepithelial fibrosis, eosinophil recruitment, and mucus production from goblet cells.[20] This is the reason why the gene coding for periostin is among one of the most highly up-regulated genes in asthma.[21] The increased secretion of periostin in the respiratory epithelium in children with asthma, compared to atopic and healthy controls, supports the hypothesis that periostin gene expression leads to subepithelial remodeling even in the growing pediatric lung.[19]

According to a lot of authors, periostin may be a useful biomarker for asthma diagnosis in children.[22,23] First, it allows patients with asthma to be distinguished from controls and is especially useful for a target small age group, with recurrent wheezing, who are unsuitable for lung function testing or FeNO

measurement.[22] On one hand, serum periostin level is accepted as a predictor of impaired forced expiratory volume 1 (FEV1) in asthmatic children and is comparable with FeNO in its usefulness as a biomarker for the diagnosis of pediatric asthma.[22,23] On the other hand, due to the insufficient data for the periostin levels in healthy children, without an accepted normal range, the study's interpretations are limited and contradictory, especially for preschool age.[3,24,25]

Contrary to the majority of reports in the study conducted by Castro-Rodriguez et al.[24], there wasn't a significant difference in periostin levels between preschool children with positive and negative asthma predictive index. Similar are the results in a more recent study that explores the role of periostin in young children with wheezing episodes for the prediction of asthma development. They did not confirm periostin as a predictive factor for future asthma in young children.[25]

There are several limitations of the current study that should be acknowledged. Selection of the examined children, with the severity of symptoms requiring treatment at a hospital, does not allow to draw generalized conclusions. As a limiting factor, we also consider the lack of a control group, which hinders a broader analysis of the established correlations. The short follow-up period of 2 years does not allow us to verify the observed correlations in children with a confirmed diagnosis of asthma. Long-term follow-up with repeated measurements over time is necessary in order to verify the diagnostic and prognostic value of these parameters and to confirm that the changes are not temporary events and could persist.

Our results demonstrated that recurrent viral-induced wheezing is associated with decreased IFN-γ production and increased periostin response and their correlation with severity and persistence of symptoms were the main outcome measures. Assessing the antiviral immune response would allow the identification of risk groups in children and the creation of new, more effective preventive strategies and therapeutic behaviors. The practical significance of the problem, as well as the limited number of studies in childhood, are a prerequisite for active scientific interest.

Ethical approval

The study was approved by the Ethics Committee of the Sofia Medical University (No.2781).

Author contribution

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

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Conflict of interest

The authors declare that there is no conflict of interest.

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Original Article

Clinical and radiologic manifestations of *Mycoplasma pneumoniae* infection in children

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ABSTRACT

Background. *Mycoplasma pneumoniae* (MP) is one of the most important etiological agents of community-acquired pneumonia in children.

Methods. The medical records of children with an MP infection between 2008 and 2015 were reviewed for their clinical, laboratory radiological features.

Results. This study included 244 patients (male 57.4%) with a median age of 80.5 months (IQR, 46.5-120 months). A total of 78 (32%) patients were < 5 years old, and 166 (68%) were ≥ 5 years old. The most common complaints before admission to the hospital were cough (84.8%), fever (57.4%), and weakness (18.9%). In the <5 years old age group, oxygen saturation was lower, and tachypnea was more common than in the ≥ 5 years old age group (p=0.02 and p=0.05, respectively). Similarly, the physical findings such as the prolonged expiration, presence of retractions, and rhonchi were more frequent in the < 5 years old age group (p=0.001, p=0.000, p=0.02, respectively). Extrapulmonary manifestations were present in 45 (18.4%) patients, and skin involvement was the most common one (7.7%). Two hundred-thirty-eight (97.5%) patients had chest radiographs, and 45 (18.4%) had normal radiography. The most common radiological involvement was peribronchial infiltration (n=70, 28.7%). Of the patients, 147 (60.2%) were hospitalized, and 97 (39.7%) were followed up as outpatients. It was determined that 156 (63.9%) patients had commenced macrolide empirically, and 61 (25%) patients were treated with positive serology results.

Conclusions. The prolonged fever, cough and expiration time, wheezing and rhonchi in younger children, and segmental-lober consolidation in chest radiography could be clues for MP infection. Further studies in different age groups can facilitate an understanding of MP infection's epidemic characteristics and clinical features that will provide early diagnosis and appropriate treatment.

Key words: children, infection, *Mycoplasma pneumoniae*, radiograph.

Mycoplasma pneumoniae (MP) is one of the most important etiological agents of community-acquired pneumonia in children. MP is responsible for 10-30% of community-acquired pneumonia cases in all age groups with insidious onset characteristics, mild pulmonary signs, and imaging specificity. MP infections are known to be also associated with extrapulmonary manifestations. In some cases, these manifestations can be independent of respiratory disease but are more often concomitant. These manifestations may originate from direct MP effects or autoimmune reactions.[1] Nervous system disease involvement such as encephalitis, acute disseminated encephalomyelitis, cerebellar ataxia, transverse myelitis, myocarditis, pericarditis, arthritis, Mycoplasma-induced rash and mucositis [MIRM] syndrome, hemolytic anemia, thrombocytopenic purpura are extrapulmonary and unusual manifestations.[2]

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Practical and sensitive laboratory diagnostic methods are the primary basis for diagnosing MP infection. There are many clinical methods for detecting MP, such as serology, and molecular-based methods. All have their advantages and disadvantages in clinical practice.[3] The serological assay is the most common method of diagnosing mycoplasma infection. The complement-fixation test, immunofluorescence, and enzyme immunoassays can be used as serologic tests. A fourfold rise in IgG antibody titer is a definite diagnosis. However, this test is not helpful in the acute phase of MP infection. IgM was regarded as an indicator of MP infection because it appears during the first week of the illness.[4]

This study aimed to describe the age-dependent clinical, laboratory, and radiologic features in children diagnosed with an MP infection.

Material and Methods

Selection of Patients and Data Collection

We conducted a retrospective study of children younger than 18 years of age tested for MP between 2008 and 2015 at Dr. Sami Ulus Maternity and Children's Research and Education Hospital. The patients who had positive *M. pneumoniae* IgM or 2 or 4-fold increase in *M. pneumoniae* IgG titers after 2 weeks of follow-up were enrolled. The patients with no increased IgG titers (2- or 4-fold) in 2 weeks of follow-up, and the patients who had a primary immune deficiency and hematological or oncological malignancies were excluded from the study.

The clinical, laboratory, and radiological features were evaluated from the medical records. Patients were grouped by age: < 5 years of age and ≥ 5 years for data analysis because *M. pneumoniae* epidemics are typically present in school-aged children and *M. pneumoniae* infection frequency is increasing in children aged 1-5 years so the clinical features may be different in these age groups. This study was conducted in compliance with the ethical principles according to the Declaration of Helsinki, and it was approved by the Dr. Sami Ulus Maternity and Children's Health and Diseases Training and Research Hospital Institutional Review Board (Number: 73799008-799).

Physical examination findings

Tympanic measurements of higher than 38°C were accepted as fever. The mean oxygen saturation percentages of the patients in room air were measured by transcutaneous pulse-oximetry. If the oxygen saturation was ≤92%, it was considered as low. Tachypnea and tachycardia were determined by evaluating the respiratory and heart rate per minute according to the age of the patient.[5]

Laboratory findings

Mycoplasma pneumoniae IgM and IgG antibodies were studied with the Enzyme-Linked Immuno-Sorbent Assay (ELISA) method on a Triturus grifols model device with the Vircell® commercial kit. The laboratory results included; blood count and differential, C-reactive protein (CRP), erythrocyte sedimentation rate (ESR), liver and kidney function tests, and microbiologic workup such as blood culture, throat culture, and respiratory multiplex polymerase chain reaction (PCR) results were recorded. Fast Track Diagnostics/ Respiratory Pathogens 21 (Luxemburg) (influenza virus A/ H1N1pdm, influenza virus B, rhinovirus, respiratory syncytial virus (RSV) A/B, human parainfluenzaviruses 1/2/3/4, coronaviruses OC43/229E/ NL63/ HKU1, parechovirus, enterovirus, adenovirus, human bocavirus, human metapneumovirus) commercial kit was used for the presence of respiratory tract viruses in the ABI 7500 Real-Time PCR (Applied Biosystems, USA) device.

Diagnosis

The patients' diagnoses were grouped into respiratory system diseases caused by MP and extrapulmonary system diseases. Pneumonia

was defined as fever, acute respiratory symptoms (cough, tachypnea, difficultly breathing), or both, plus the presence of a new infiltrate on chest radiography or consolidation not attributable to some other etiology.[6] Chronic cough, which is among the respiratory system symptoms, was defined as a cough that lasted for three weeks or more without improvement.[7] Prolonged fever was defined as fever lasting longer than expected for clinical diagnosis of a disease.[8] The diagnosis of encephalitis was made according to the specified criteria.[9]

Chest X-ray Findings

Chest radiographs were evaluated by the same radiologist. Findings were classified as hilar lymphadenopathy (LAP), peribronchial infiltration, peribronchial thickening, segmental-lobar consolidation, reticulonodular infiltration, atelectasis, and pleural effusion.

Statistical analysis

Clinical data of the separate groups were described by mean values and standard deviations or median and inter-quarter range, according to the type of variable. Students t-test and Mann-Whitney U-test were used to compare continuous variables between the group and the chi-square test and Fisher's exact test were used for categorical variables. A p-value of <0.05 was considered statistically significant.

Results

Two hundred forty-four patients were enrolled in this study, 140 (57.4%) were male, and the median age was 80.5 months [interquartile range (IQR), 46.5-120 months]. A total of 78 (32%) patients were < 5 years old, and 166 (68%) were ≥ 5 years old. The most common complaints before admission to the hospital were cough (84.8%), fever (57.4%), weakness (18.9%), and runny nose (16%). Patient characteristics and symptoms according to the age groups are presented in Table I. The seasons with the highest admissions were spring (33.2%) and summer (31.9%). Before admission, the fever duration (median (min-max), was 4.5 days (range, 1 day-30 days), and 8 (3.3%) patients had prolonged fever. The median cough duration was 10 days (range, 1day-300 days). A total of

Table I. Characteristics and symptoms of patients.

	Total	<5 years old	≥5 years old	p-Value
Number of children, n (%)	244	78 (32)	166 (68)	-
Male, n (%)	140	42 (53.8)	98 (59)	0.44
Respiratory symptoms, n (%)				
Cough	207 (84.8)	69 (88.4)	138 (83.1)	0.37
Rhinorrhea	39 (16)	17 (21.7)	22 (13.3)	0.13
Wheezing	26 (10.7)	16 (20.5)	10 (6)	0.01
Shortness of breath	21 (8.6)	7 (8.9)	14 (8.4)	1
Extrapulmonary symptoms, n (%)				
Fever	140 (57.4)	48 (61.5)	92 (55.4)	0.36
Vomiting	33 (13.5)	13 (16.7)	20 (12)	0.43
Rash	19 (7.8)	4 (5.1)	15 (9)	0.65
Headache	11 (4.5)	1 (1.3)	10 (6)	0.96
Abdominal pain	9 (3.7)	3 (3.8)	6 (3.6)	0.9
Diarrhea	7 (2.9)	3 (3.8)	4 (2.4)	0.45
Chest pain	6 (2.5)	-	6 (3.6)	-
Seizures	4 (1.6)	2 (2.6)	2 (1.2)	0.54
Speech impairment	2 (0.8)	-	2 (1.2)	-

155 (63.5%) patients had community-acquired pneumonia, 44 (18%) patients had a chronic cough, and 45 (18.4%) patients had only extrapulmonary involvement. A preexisting disease was present in 31 (12.7%) children, and 6/31 (19.3%) had been diagnosed with asthma.

On admission, physical examination of patients with pneumonia revealed that 103 (42.2%) had tachypnea, 59 (24.2%) had low oxygen saturation, 27 (11%) had a fever, and 14 (5.7%) had tachycardia. The body temperature (mean±SD) was 38.3±0.46°C, and the oxygen saturation (mean±SD) was 89.3±6.3%. In the <5 years old age group oxygen saturation was lower, and tachypnea presence was higher compared to the ≥ 5 years old age group (p=0.02 and p=0.05, respectively). Similarly, when we consider the patients' respiratory system findings, the frequency of the prolonged expiration, presence of retractions, and rhonchi was higher in the < 5 years old age group than in the group with ≥ 5 years old age. (p = 0.001, p<0.001, p=0.02 respectively).

Extrapulmonary manifestations were present in 45 (18.4%) patients. Skin involvement was the most common extrapulmonary finding (19/244, 7.7%). Central nervous system manifestations were seen in 16 (6.5%), hematologic involvement in 5 (2%), and musculoskeletal involvement in 5 (2%) patients. The summary of extrapulmonary manifestations are shown in Table II.

The comparison of patients with laboratory examinations by age group is shown in Table III. The leukocyte count and lymphocyte percentage in patients < 5 years old age group were statistically significantly higher than in the ≥ 5 years old age group. There was no statistically significant difference in ESR and CRP values between different age groups.

Table II. Extrapulmonary manifestations of *Mycoplasma pneumonia* infection.

	N (%)
Dermatological involvement	19 (7.8)
Maculopapular rash	7 (2.9)
Petechiae	4 (1.6)
Urticarial Plaque	2 (0.8)
Erythema Nodosum	2 (0.8)
Erythema Multiforme	2 (0.8)
Vesicle	1 (0.4)
Bulla	1 (0.4)
Neurological involvement	16 (6.6)
Suspected encephalitis	12 (4.9)
Guillain-Barré syndrome	4 (1.6)
Hematologic involvement	5 (2)
Immune thrombocytopenic purpura	4 (1.6)
Hemolytic anemia	1 (0.4)
Musculoskeletal involvement	5 (2)
Arthralgia/artritis	5 (2)

Lumbar puncture was performed in 14 (87.5%) of 16 patients with neurological findings at admission. The cerebrospinal fluid (CSF) cell count ranged from 0 to 150 cells/mm^3, with a median of 12 cells/mm^3. Analysis of CSF biochemical findings revealed the mean CSF protein was 51.2±34.1 mg/dl, and CSF glucose

Table III. The comparison of laboratory examinations by age group.

	< 5 years	≥ 5 years	P
Leukocyte count (mm^3)*	12.060±5450	10.400±4830	0.019
Neutrophil percentage (%)	55±18	59±15	0.1
Lymphocyte percentage (%)	44±17	39±15	0.02
Hemoglobin (g/dl)*	11.8±1.6	12.7±1.4	0.00
Platelet count (mm^3)*	373000±143400	357150±134200	0.4
ESH (mm/hr)*	45±34.7	48.8±33.9	0.5
CRP (mg/l), median (min-max)	14.5 (1-483)	16 (1-388)	0.6

*Mean±standart deviation; CRP: C-reactive protein; ESH: erythrocyte sedimentation rate.

was 77.2±21.9 mg/dl. All the CSF cultures were negative.

A total of 26 children were simultaneously tested with PCR for respiratory viruses. Viral coinfections were identified in 17 (65.4%) patients, mostly rhinovirus (5 cases, 19.2%) followed by influenza A and bocavirus (3 cases, 11.5% and 2 cases, 7.7% patients, respectively).

The chest radiographs of the patients were evaluated; 238 (97.5%) patients had radiographs. Chest radiographs of 45 (18.4%) patients were normal. The radiological characteristics of patients are summarized in Table IV. Peribronchial infiltration was more common in bilateral lower lobes (30%). Pleural effusion and segmental-lobar consolidation were found in the left lower lobe at 68% and 39.7%, respectively. The radiological findings according to age groups are shown in Figure 1.

Of the patients, 147 (60.2%) were hospitalized, and 97 (39.7%) were followed up as outpatients. A total of 60 (77%) patients < 5 years were hospitalized. It was determined that 156 (63.9%) patients had commenced macrolide empirically, and 61 (25%) patients were treated with positive serology results.

Table IV. Radiological characteristics of patients.

	N (%)
Peribronchial infiltration	70 (28.7)
Segmental-lobar consolidation	68 (27.9)
Reticulonodular infiltration	41 (16.8)
Pleural effusion	25 (10.3)
Atelectasis	14 (5.7)
Hilar lymphadenopathy	9 (3.7)

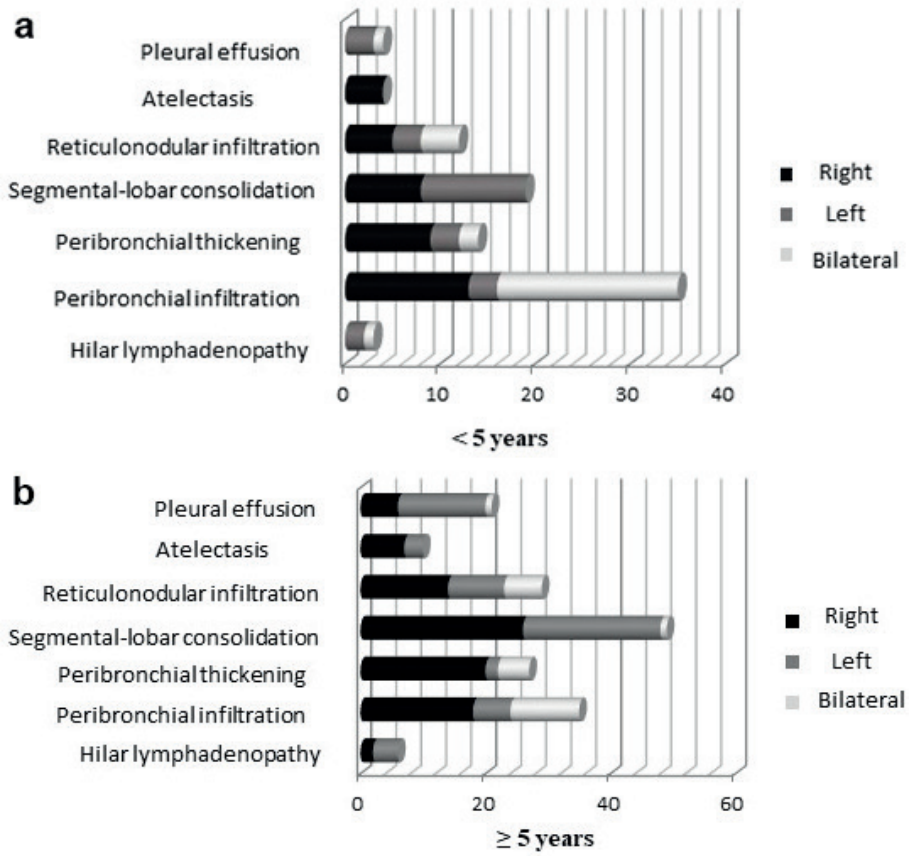

Fig. 1. The radiological findings according to age groups; a <5 years and b ≥5 years.

Discussion

Mycoplasma pneumoniae predominantly causes upper and lower respiratory tract infections in children and has various clinical manifestations.[10] Previous studies reported that MP community-acquired pneumonia is more prevalent in children 6-18 years old and less in preschool children. However, recent reports have shown that MP is also a significant respiratory pathogen in young and preschool children.[1] The epidemic outbreaks occur worldwide every 3-7 years and more frequently in winter and autumn.[11] In countries with temperate climates MP may be detected in summer and early autumn.[12] Our results showed that MP infection is more common in >5 years old children, and about two-thirds of the patients were admitted during the summer and spring seasons.

Despite some symptoms and signs supporting a possible diagnosis of MP infection, none of these are sufficient to confirm the diagnosis. A study comparing 302 pediatric patients diagnosed with MP positive and MP negative pneumonia found that fever and cough duration differed from patient to patient. The authors used age, cough, and fever duration as variables to predict the diagnosis and reported that the negative predictive value was 96%, and the sensitivity was 85%.[13] In our study, the mean fever duration was 4.5 days, and the mean cough duration was 10 days. A study evaluating 179 children with a persistent cough reported that the children with positive MP serology had a median of 39 days of cough.[14] The duration of the cough was thought to be a clue for possible MP infection if there is a suspicion of an infectious cause.

There may be age-related clinical differences at presentation in MP infection. A study from China that evaluated clinical features according to age groups reported fever for three days was more common in the 9 to <12 months age group.[15] A study describing the epidemiological and clinical features of infants and children during the MP epidemic in Denmark in 2010 and 2011 specified the clinical presentation as cough, asthma-like symptoms, and low-grade fever. They also suggest that small children with wheezing and rhinorrhoea should simultaneously be tested for MP and respiratory viral infections.[16] In both of these studies, the preschool age group had a higher hospitalization rate as well as more oxygen, and fluid requirement.[15,16] A study conducted during the MP epidemic in Norway reported a higher risk of severe pneumonia in preschool children.[17] Our results showed that MP infection was more common in children aged >5 years. However, preschoolers suffered a severe course of the disease, and approximately 70% of patients in this age group required hospitalization. The presence of wheezing, low oxygen saturation, tachypnea, retraction, rhonchi, and prolonged expiration under 5 years of age was found to be statistically significant compared to the older ones.

The respiratory system was primarily involved in MP infection; however, extrapulmonary manifestations may also cause various symptoms. Rash has been reported in 3% to 33% of children during infection.[18] In a study evaluating 353 pediatric patients positive for MP, extrapulmonary involvement was evident in 26% of all children. Skin involvement (18%) was the most common with nonspecific maculopapular rash or urticaria.[1] In this study, 18.4% of children had extrapulmonary manifestations, mostly dermatological (7.7%) and commonly observed as a maculopapular rash.

Approximately 1-10% of serologically confirmed MP infections may result in serious neurological complications requiring hospitalization.[19] The clinical manifestations of MP encephalitis are highly heterogeneous, and more than half of patients had seizures during the acute phase.[20] In a study evaluating 61 patients with neurological involvement with MP infection, encephalitis was diagnosed in 45 pediatric patients, and it was reported that patients presented with changes in consciousness (35%), seizures (45%), and meningeal irritation findings (78%).[21] In our study, 12 suspected

encephalitis associated with MP were observed. Patients were serologically MP positive with negative CSF results for other infectious pathogens. The most common complaints of these patients were headaches, vomiting, and seizures. GuillaineBarré syndrome (GBS) is an acute immune-mediated disorder characterized by acute, progressive weakness, hyporeflexia or areflexia, and elevated protein levels in the cerebrospinal fluid (CSF). Infection with MP has been reported in up to 5% of GBS patients.[22] We observed a 6.5% neurological involvement in our study population, and 1.6% were diagnosed with MP-related GBS.

The most common hematological complication of MP is hemolytic anemia secondary to cold agglutinin antibodies against I antigen on erythrocytes.[23] Thrombocytopenia associated with MP infection is rare. The pathogenesis of thrombocytopenia is autoimmune. In a study, 7 cases between 7 months and 44 years of age were discussed, and it was reported that five of these patients were under 8 years of age. The platelet counts were between 2000-66000/mm^3. It was stated that all patients were given effective antibiotics for mycoplasma in addition to IVIG, steroids, or both treatments.[24] In our study, four patients had thrombocytopenia, and these patients were given IVIG and clarithromycin treatment.

Mycoplasma pneumoniae associated arthritis in children has been reported between 0.9-3.0%. A study reported 13 cases of arthritis out of 1259 patients diagnosed with MP infection, five of them were children between the ages of 1-7 years, and monoarthritis was found in four of them.[25] In a study of 348 MP IgM positive patients aged 1-15 years, 4 (1.1%) patients were reported to have monoarthritis.[26] We found monoarthritis in 2.5% of the patients.

Regarding laboratory tests, a study from Italy that included 102 hospitalized children with MP pneumonia reported preschool children had a higher lymphocyte and monocyte count.[12] Gordon et al.[1] reported a higher number of white blood cells (WBC) and platelets in preschool children with MP infection. However, all these results were in normal ranges for age. In a study from Spain, 162 children were diagnosed with MP pneumonia, and higher WBC and lymphocytes were detected in infants.[27] Similarly, in our study, leukocyte counts and lymphocyte percentages were significantly higher in patients < 5 years of age.

In various studies, co-infection with viral pathogens has been reported ranging from 35% to 78%, depending on the age of the patients, the season, and the method used to detect the agent. In a study investigating the causative respiratory agent by PCR in 407 patients < 5 years of age, it was reported that two or more pathogens were detected in 19.3% of children with MP infection.[28]Another study identified 145 patients with MP, and viral co-infection was found in 16 (11%) patients, mostly RSV.[29] In the present study, concomitant respiratory tract virus infection was detected in 17 (7%) patients. The low co-infection rates in our study can be explained by the retrospective nature of the study and the low number of multiplex PCR requests sent from the study group. Co-infecting viruses were found as rhinovirus, RSV, and influenza virus A-B, in order of frequency.

Usually, chest radiography is the first imaging technique obtained to evaluate acute respiratory symptoms. However, the patterns of presentation of MP pneumonia on chest radiographs are nonspecific, consisting of patchy areas, consolidation, reticular interstitial infiltrates, or both.[30] In a study including 68 children with MP pneumonia, the most common chest X-ray findings were reported as perihilar linear opacity, reticulonodular infiltration, and segmental-lobar consolidation.[31] In another study evaluating 81 chest graphs of 102 pediatric patients with MP infection, 76 (93.8%) were interpreted as abnormal, and consolidation was reported as the most common and pleural effusion as the least detected finding. It was observed that interstitial changes were more common in children < 5 years of age, and consolidation was more common in children aged > 5 years.[12] Similarly, in our study, the

most common chest X-ray findings were peribronchial infiltration and segmental-lobar consolidation. Peribronchial infiltration was the most common chest X-ray finding at <5 years of age, while segmental-lobar consolidation was more common at ≥ 5 years and older. The least common finding in both age groups was hilar LAP. In addition, although there is no definite pattern for lobar involvement, it was determined that the lower lobes were more involved than the upper lobes. Pleural effusion was detected in 10% of patients. In various studies, MP-associated pleural effusion has been reported between 5-20%, mainly on the left side.[32,33]

The role of macrolide therapy in changing the clinical course of pediatric MP infection is controversial. Further studies are required to investigate this aspect, as suggested by a recent Cochrane Review.[34] In our study, macrolides were started empirically in the vast majority of patients, and one-fourth of them were started after they were proven serologically. About 10% of patients recovered without taking macrolides.

In conclusion, this study suggests that MP infection occurs mainly in the spring and summer seasons, mostly ≥ 5 years. Wheezing, tachypnea, retraction, prolonged expiration, rhonchi, and desaturation, as well as leukocytosis and lymphocytosis, were found to be more common in MP pneumonia in children < 5 years of age than the older ones. The clinical picture in young children is more similar to viral lower respiratory tract infections. Extrapulmonary MP findings support the current literature. There is no distinctive chest X-ray finding in MP infection, but segmental-lobar consolidation is more common in children aged ≥ 5 years, and the left lower lobe is more commonly involved. Clinicians should be aware of different MP clinical presentations to provide early diagnosis and appropriate treatment.

Ethical approval

This study was approved by the Dr. Sami Ulus Maternity and Children's Health and Diseases Training and Research Hospital Institutional Review Board (Number: 73799008- 799).

Author contribution

The authors confirm contribution to the paper as follows: study conception and design: MO, FNÖ, GT; data collection: MO; chest evaluated: HGÇ; analysis and interpretation of results: MO, FNÖ, GT; draft manuscript preparation: MO, FNÖ, GT. All authors reviewed the results and approved the final version of the manuscript.

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Conflict of interest

The authors declare that there is no conflict of interest.

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Original Article

Evaluation of the relationship between cardiopulmonary exercise test findings and clinical status in children and adolescents with congenital heart disease

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ABSTRACT

Background. The cardiopulmonary exercise test is accepted as a helpful diagnostic tool in risk stratification, evaluation of prognosis, and guiding treatment modality in adults with congenital heart disease. In this study, we present our experience with the use of cardiopulmonary exercise test in children with congenital heart disease in different physiological and anatomical classifications.

Methods. In this retrospective study, 25 children and adolescents who applied to the pediatric cardiology outpatient clinic between 2017 and 2020 with the diagnosis of different types of congenital heart disease were included. Demographic characteristics, electrocardiogram, echocardiogram, cardiopulmonary exercise test, spirometry, pro-BNP values, and in selected 20 patients; cardiac MRI data were examined. The modified Ross classification was used for heart failure grading.

Results. The mean age of the patients was 14.8 ±2.39 years. Fifteen (60%) of the patients were male and 10 (40%) were female. In the modified Ross classification, patients in group I-II had significantly higher maximum exercise time, heart rate reserve %, peak VO2, and VO2/kg values compared to those in group III (p=0.026, p=0.007, p=0.043, p= 0.018, respectively). Cardiopulmonary exercise test and spirometry values obtained from the patients were evaluated in the light of clinical and other laboratory findings, and surgical/interventional treatment was decided for 4 patients with the use of these test results.

Conclusions. Cardiopulmonary exercise test is a useful noninvasive diagnostic tool in guiding the treatment decision and predicting the prognosis of pediatric patients with congenital heart disease, who have borderline symptoms.

Key words: child, congenital heart disease, cardiopulmonary exercise test, Ross classification.

Today, thanks to advances in medicine and surgical techniques, children with congenital heart disease (CHD) can survive into adolescence and adulthood. However, residual lesions may persist after cardiac repair and these lesions may lead to progressive alteration of cardiac function.[1,2] The decision to treat the residual lesion depends on the extent of the lesion and the patient's symptoms of exercise intolerance. The regular bicycle or treadmill exercise tests and six-minute walking tests are used to determine the patient's exercise capacity with some information regarding the myocardial ischemia and occurrence of arrhythmias. The goal has been to detect problems before clinical symptoms are overt. Cardiopulmonary exercise tests (CPET) are performed by monitoring the individual's respiratory gas exchange, oxygen use (VO2: oxygen consumption), carbon dioxide production (VCO2), and minute ventilation (VE) during exercise, in addition to the electrocardiogram (ECG), blood pressure, and oxygen saturation monitoring, and can evaluate the patient's symptom-limited maximum increased exercise tolerance.[3] In studies, the superiority of CPET over other exercise tests

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has been stated for evaluation of the functional cardiovascular capacity of patients as well as the response of the respiratory system, muscular and metabolic systems to exercise.[4] CPET evaluates the cardiac performance using maximum oxygen uptake (VO2max) assessment in the clinical follow-up to evaluate morbidity and mortality.[5] In adults with chronic heart failure, VO2max correlates with both the quality of life and prognosis. Therefore, CPET has become the 'gold standard' for quantifying disease severity in adult cardiology. These results have also been found in adults with CHD, and CPET is now recommended in the follow-up of this particular population.[6-8]

Studies and experience regarding the use of CPET in the evaluation of functional capacity and heart failure in children with CHD, and in the direction of medical treatment of these patients are not sufficient as in adults. To contribute to the literature, we would like to present our experience with the use of CPET in children with CHD in different physiological and anatomical classifications.

Material and Methods

Study Population

In this retrospective study, 25 children and adolescents who were admitted to the pediatric cardiology outpatient clinic with different types of CHD between 2017 and 2020 were included. Patients' data were evaluated retrospectively. Demographic characteristics, electrocardiogram, echocardiogram, cardiopulmonary exercise test, spirometry, pro-BNP values, and in selected 20 patients cardiac MR data were examined. The existing pathologies of the patients were classified according to the anatomical classification in the 2018 American College of Cardiology (ACC)/ Adult congenital heart disease (ACHD) guidelinea.[5] The modified Ross classification was used for heart failure grading.[9] This research was reviewed and approved by the institutional Ethics Committee of our Koç University (approval number: 2020.265.IRB1.088).

CPET procedures

CPET was performed with a Carefusion (Vyntus® CPX, Germany) device using an automated sphygmomanometer with adapted pediatric cuffs, pediatric face mask, a calibrated gas analyzer, breath-to-breath measurements software, a 12-lead ECG monitor (GE CardioSoft® ECG, USA), a pulse oximeter, and a ramp protocol at 10 watts/minute incremental workload using with a bicycle ergometer. According to the incremental ramp protocol, the test consisted of 3 parts. The first part consisted of a 1-minute rest period, the second part consisted of a warm-up period of 2 minutes (0 Watt and a pedal speed of 60 rpm), and the third part consisted of an exercise period in which the workload was increased by 10 Watts per minute. Maximum heart rate was targeted by increasing the workload according to the patient's tolerance. The test was terminated due to fatigue, leg pain, chest pain, and shortness of breath. ECG, blood pressure and oxygen saturation values were monitored during the test for all the patients. From CPET data, oxygen uptake (VO2; ml/kg/min), carbon dioxide production (VCO2; ml/kg/min), respiratory exchange ratio (RER=VCO2/VO2), minute ventilation (VE; breaths/min), ventilatory equivalent for oxygen (VE/VO2), ventilatory equivalent for carbon dioxide (VE/VCO2), maximum workload (Watts), and oxygen pulse (VO2/HR; ml) were recorded.

Spirometry

Forced expiratory volume in 1 second (FEV1), forced vital capacity (FVC), FEV1/ FEVC ratio, predictive FEV1 values were obtained by performing a pulmonary function test with the spirometry mouthpiece of the Carefusion (Vyntus® CPX, Germany) device before and after the exercise test.

Statistical Analysis

SPSS Statistics 24.0 (IBM Corp., Armonk, NY, USA) statistical program was used for statistical analysis of the results. Categorical data were analyzed using the Chi-square test. For the

numerical variables, the 'Student-t test' was used for those with a normal distribution, and the Mann-Whitney-U Test for those with non-normal distribution. ANOVA test was used together with a post hoc test for comparisons between groups. Frequency and percentage in categorical data and mean±standard deviation values in numerical data were given as descriptive values. The limit of significance was accepted as $p<0.05$.

Results

Patients

A total of 25 children with CHD were included in this retrospective study. The mean age of the patients was 14.8 ±2.39 years (range 10-18 years,). Fifteen (60%) of the patients were male and 10 (40%) were female. Demographic characteristics, clinical, laboratory, and imaging data of the patients are given in Table I. Surgical/transcatheter procedure was performed in 15 of the patients due to the existing pathology, and 8 of them were under medical follow-up. Eight patients (32%) had marked dyspnea on exertion and palpitations at rest who are in Ross classification group III or IV. The remaining 17 (68%) patients had minimal symptoms.

12 lead ECG revealed right bundle branch block (RBBB) in 11 patients, left bundle branch block (LBBB) in one patient and complete atrioventricular block in one patient. Two patients had permanent pacemaker in dual chamber pacemaker (DDDR) mode. Two patients in group I and III had symptomatic tachycardias who needed antiarrhythmic therapy. One of them was diagnosed as atrioventricular nodal reentry tachycardia and the other was diagnosed as ventricular tachycardia and they were taking flecainide as antiarrhythmic treatment.

Diminished systemic ventricle ejection (EF<55%) was detected in two patients with L-TGA and single ventricular physiology who underwent bilateral Glenn anastomosis. Clinically significant valvular insufficiency was detected in 7 of the patients. Of these, 3 had aortic and 4 had tricuspid and pulmonary valve insufficiency together. Patients with bicuspid aortic valve have no severe aortic stenosis. Regarding the anatomical severity classification, there were 18 patients in group II and 6 patients in group III respectively. There were 17 patients in groups I and II and 8 patients in groups III and IV according to the physiologic severity classification (Ross Classification). In group 4, 1 patient had single ventricular anatomy and the other patient had moderate pulmonary valve stenosis who underwent arterial switch operation.

CPET Data

CPET and spirometry data of the patients are shown in Table II. Patients were compared according to the anatomical and modified Ross classification (Table III a,b). In anatomical severity classification comparison, no statistically significant findings were found between groups II and III in terms of the parameters.

When the CPET and spirometry values between the groups were compared according to the modified Ross classification; Maximum exercise time, heart rate reserve (HRR)%, VO2%, and VO2/kg values were significantly higher in patients in group I-II compared to group III-IV ($p=0.026$, $p=0.007$, $p=0.043$, $p=0.018$, respectively). No correlation was found between pro-BNP levels and exercise parameters.

CPET-spirometry values, clinical and other laboratory findings were used to decide the treatment modality as medical or surgical. Four patients underwent surgical-interventional treatment at a final decision with the substantial impact of the CPET findings (Table IV). 4 patients with tetralogy of Fallot had lower VO2% values (mean±SD:59.75±9.6) and higher VE/VCO2 (mean±SD: 32.6±1.56) and VE/VO2 (mean±SD: 32.95±2.15) values compared to the all the other patients (mean±SD: 72.85±19.9, mean±SD: 28.65±2.99, mean±SD: 27.74±3.63, respectively). The remaining 2 patients with tetralogy of Fallot

Table I. Baseline characteristics of the patients in the study.

Age (year)		14.8 ±2.39 (10-18)
Gender (M/F)		15 (60%)/10 (40%)
Body Mass Index		19.96±3.14 (14-26)
Conduction disorder	None	12
	LBBB	1
	RBBB	11
	CAVB	1
Resting Heart Rate (bpm)		80.48±11.13 (52-108)
Permanent Pacemaker		2
Distribution of the ACHD According to the 2018 ACC/ ACHD Anatomic Classification		
	Anatomic Severity Group 1 (4%)	1
	Congenital complete AV Block	1
	Anatomic Severity Group 2 (72%)	18
	Bicuspid Aortic Valve (balloon valvuloplasty)	2
	Bicuspid Aortic Valve (Medical Follow-up)	5
	VSD (surgical closure)	1
	AVSD (surgically corrected) with moderate/mild MR	2
	Tetralogy of Fallot (total correction)	6
	Mitral Valve Prolapse	1
	Hypertrophic cardiomyopathy	1
	Anatomic Severity Group 3 (24%)	6
	Double outlet RV+ VSD+ASD+ single ventricle (Fontan)	1
	cc-TGA	1
	Pulmonary atresia+ IVS (surgically corrected)+ PR (moderate)	1
	Ebstein	1
	Bilateral Glenn anastomosis+ RV hypoplasia+PS	1
	d-TGA (arterial switch)	1
Physiologic Severity Groups (Ross Classification)		
	I	6(24%)
	II	11(44%)
	III	6 (24%)
	IV	2 (8%)
	Systemic Ventricle EF (%)	70.76 ± 9.22 (52-89)
	Systolic Pulmonary Artery Pressure (mmHg)	35.08 ±12.79 (20-75)
	NT-ProBNP (ng/mL)	98.19±103.56 (8.5-429)
	Cardiac MRI Parameters	
	RV EF (%)	55.79±10.46 (25-73.5)
	LV EF (%)	57.21±6.41 (45-69)

ACHD: adult congenital heart disease, AV: atrioventricular, AVSD: atrioventricular septal defect, BNP: brain natriuretic peptide, CAVB: complete atrioventricular block, cc-TGA: congenitally corrected transposition of great arteries, d-TGA: dextropose transposition of great arteries, EF: ejection fraction, F: female, IVS: intact ventricular septum, LBBB: left bundle branch block, LV: left ventricle, M: Male, MR: mitral regurgitation, MRI: magnetic resonance imaging, PS: pulmonary stenosis, RBBB: right bundle branch block, RV: right ventricle, VSD: ventricular septal defect.

Table II. Cardiopulmonary exercise testing and spirometry parameters.

Parameters	mean±SD (range)
Exercise Duration (minutes)	12.3±5.14 (4.5-25)
Maximal Heart Rate (bpm)	159.68±25.95 (95-189)
Maximal Heart Rate %	83.88±13.98 (47-101)
HRR %	83.88±13.98 (47-101)
Peak respiratory exchange ratio	0.99±0.66 (0.83-1.13)
SaO2 at peak exercise %	98.44±1.47 (95-100)
VO2max (ml/min)	1558.04±490.50 (867-2673)
VO2max/kg (ml/min/kg)	30.05±7.63 (16-46.9)
VO2 %	71.72±18.98 (40-115)
VE/VCO2	28.57±3.31 (21.6-33.9)
VE/VO2	28.58±3.92 (21.6-36)
Peak O2 Pulse	10.15±2.76 (6.4-16.2)
FEV1/FVC (%)	91.18±10.76 (68-116)
FEV1 (% pred)	91.25±17.10 (56-137)
FVC (% pred)	88.04±15.84 (58.5-130.6)

FEV1/FVC (%): forced expiratory volume in 1 second/ forced vital capacity, HRR: heart rate reserve, pred: predicted, SaO2: oxygen saturation, VE/VO2: ventilatory equivalent for oxygen, VE/VCO2: ventilatory equivalent for carbon dioxide, VO2max: maximum oxygen uptake.

had similar values as the rest of the group and there were clinical and laboratory differences between the 4 patients with tetralogy of Fallot who had low VO2max values. Two patients with tetralogy of Fallot were in group II according to the Ross classification, and cardiac MRI RVEF values were higher and RVEDV indices were lower than the other 4 patients with tetralogy of Fallot.

In two patients who were operated on, CPET parameters improvement was observed in the follow-up (Table IV).

Discussion

Children with CHD account for 0.8 to 1% of all live births in developed countries.[10,11] Almost all pediatric patients reach adulthood with advances in surgery and percutaneous procedures. However, many of the patients are at risk of premature death from heart

Table III. a. Comparison of CPET variables between anatomic severity group II and group III.

	Group II (n=18)	Group III (n=6)	p
Maximal exercise duration	12.3±5.00	10.94±5.24	0.566
Maximal Heart Rate	163.88±21.97	150.66±36.57	0.673
Max. Heart Rate %	86.22±12.13	78.50±19.07	0.293
HRR %	86.22±12.13	78.50±19.07	0.255
Maximal RER	0.99±0.05	1.00±0.09	0.829
SaO2 at max. exercise	98.7±1.26	97.6±1.26	0.330
Peak VO2max%	73.55±17.55	60.50±16.84	0.237
VO2/kg	31.24±6.79	25.40±9.12	0.106
VE/VCO2	28.77±3.77	28.46±1.54	0.420
VE/VO2	27.88±3.91	28.73±3.21	0.310
Peak O2 Pulse	10.4±2.97	9.36±1.97	0.210
FEV1/FVC (%)	89.88±11.56	91.45±6.57	0.370
FEV1 (% pred)	91.98±19.79	88.46±7.63	0.330
FVC (% pred)	89.8±18.09	82.76±6.95	0.180

b. Comparison of CPET variables between Ross classifications group I-II and group III-IV.

	Group 1,2 (n=17)	Group 3,4 (n=8)	p
Max. Heart Rate %	87.23±8.4	85.66±14.44	0.108
HRR %	87.23±8.40	76.75±20.58	**0.007**
Maximal RER	1.00±0.05	0.99±0.07	0.202
SaO2 at max. exercise (%)	98.58±1.32	98.12±1.8	0.506
VO2 max %	79.88±15.66	54.37±13	**0.043**
VO2/kg	32.84±6.66	24.12±6.41	**0.018**
VE/VCO2	28.5±3.19	27.60±2.77	0.871
VE/VO2	28.02±3.1	26.97±3.94	0.103
Peak O2 Pulse	10.39±2.80	9.63±2.77	0.383
FEV1/FVC (%)	89.46±10.77	94.83±10.45	0.193
FEV1 (% pred)	91.77±19.75	90.16±10.48	0.107
FVC (% pred)	89.76±18.37	84.37±8.15	0.266

FEV1/FVC (%): forced expiratory volume in 1 second/ forced vital capacity, HRR %: heart rate reserve, Max: maximum, pred: predicted, RER: respiratory exchange ratio, SaO2: oxygen saturation, VE/VCO2: ventilatory equivalent for carbon dioxide, VE/VO2: ventilatory equivalent for oxygen, VO2max: maximum oxygen uptake.

Table IV. Characteristics of patients for whom surgical/interventional treatment decision was made based on CPET results.

	Patient 1	Patient 2	Patient 3	Patient 4
Age (year)	18	11	18	10
Diagnosis	Operated TOF	Operated TOF+PVR	Operated TOF+PVR	Operated TOF
Anatomic classification	2	2	2	2
Ross classification	3	3	2	2
Valvular pathology	TR+PR (significant)	TR+PR (significant)	TR+PR (significant)	TR+PR (significant)
Echocardiographic systemic ventricular EF value %	60	84	64	69
Cardiac MRI LV-RV EF %	60-58	60.5-67	45-25	55.4-51.4
Cardiac MRI RVEDV index (ml/m^2)	180	108.5	60	154
Pro-BNP (ng/dl)	123	43	198	102
VO2 max. % (pre/post-treatment)	48/61	56/80	66	69
VE/VCO2 (pre/post-treatment)	32.6/28.8	33.5/28.3	33.9	30.4
VE/VO2 (pre/post-treatment)	32.7/29.5	31/27.8	36	32.1
HRR % (pre/post-treatment)	47/67	81/85	86	81
FEV1/FVC (%)	116	82	96	102
FEV1 (% pred)	98	80.1	76	72.7
FVC (% pred)	87	80.1	64.6	70.3

EF: ejection fraction, FEV1/FVC (%): forced expiratory volume in 1 second/ forced vital capacity, LV-RV: left ventricle-right ventricle, MRI: magnetic resonance imaging, pred: predicted, Pro-BNP: pro-brain natriuretic peptide, PR: pulmonary regurgitation, RVEDV: right ventricular end-diastolic volume, TOF: tetralogy of Fallot, TR: tricuspid regurgitation, VE/VCO2: ventilatory equivalent for carbon dioxide, VE/VO2: ventilatory equivalent for oxygen, HRR %: heart rate reserve, VO2max%: maximum oxygen uptake.

failure or arrhythmias.[12-15] In these patients, exercise capacity is the most important factor that endangers their quality of life both preoperatively and postoperatively.[16,17] Impaired exercise capacity may result from prolonged volume or pressure overload, or heart failure resulting from increased myocardial tension or flow. However, all of the pulmonary, vascular, muscular, or metabolic systems may be affected in these patients.[4,11]

CPET is a diagnostic tool that can be used for objective and reproducible assessment of the cardiovascular, respiratory, and muscular systems.[5] In adult studies, CPET has been shown to have prognostic value in patients with a wide variety of CHD conditions.[6,7,18,19] In adult studies, it has been shown that the most valuable parameters in demonstrating the patient's exercise capacity in CPET are peak VO2, VE/VCO2 slope, and HRR.[17,20] However, studies and experience regarding the use of CPET In the evaluation of functional capacity and heart failure in children with CHD and the management of their medical treatment are insufficient. In this study, we aimed to show the contribution of CPET in the management of treatment by evaluating the exercise capacity of children with CHD in different anatomical and functional classes.

Peak VO2 is the most important indicator of cardiopulmonary function in CPET in patients with CHD, as it is associated with the cardiac output response.[21] Patients with CHD have impaired maximal VO2 compared to age-matched controls without heart disease.[4,20] In our study, the mean peak VO2 value of the patients was below the normal range, consistent with the literature. It may be useful

to compare peakVO2 with similar forms of CHD to determine whether a patient has an abnormality beyond what would be expected due to existing cardiac pathology.[7,22] Studies have shown that the decreased peak VO2 value has been linked to the severity of the underlying heart disease and patients with Eisenmenger syndrome have been shown to experience the greatest exercise intolerance.[6,7,18,23-25] In our study, the peak VO2 values of children with CHD in different anatomical and physiological classifications were compared. According to the modified Ross classification, the peak VO2 values of the children in the 3rd and 4th groups were statistically significantly lower than the other group (1-2nd), but there was no statistically significant difference between the peak VO2 values according to the anatomical severity.

VE/VCO2 slope, which is one of the other important indicators of CPET, is often called 'ventilation efficienc'' and an increase in this value indicates that a higher VE is required to remove CO2 sufficiently. High VE/VCO2 slope values are well defined in patients with respiratory muscle fatigue, inadequate muscle perfusion, and heart failure.[24,26] In our study, no significant difference was found in VE/VCO2 slope values between anatomical and physiological groups. However, VE/VCO2 slope values were higher than expected in 4 patients with operated tetralogy of Fallot and significant pulmonary valve insufficiency. Shafer et al.[27] found higher VE/VCO2 slope values in patients diagnosed with operative tetralogy of Fallot compared to other patient groups, and this was attributed to incorrect distribution of pulmonary blood flow secondary to pulmonary artery stenosis or insufficiency and as a result, ventilation /perfusion mismatch.

Low heart rate reserve (HRR) is common in adult congenital heart patients with an estimated incidence of 60%.[28] Chronotropic insufficiency may result from intrinsic or iatrogenic dysfunction of the conduction system. Therefore, the cardiac acceleration response to exercise is also low.[19,24] In previous studies, no matter how high the heart rate was, the maximum heart rate and HRR were found to be lower than normal.[29] In our study, although the mean maximum heart rate and HRR of the patients were within the normal range, the HRR values among the physiologically classified groups supported the literature. On the other hand, the fact that 2 patients in Ross classification 3 and 4 had permanent pacemaker may have affected the lower maximum heart rate compared to the other group.

When the spirometry values of the patients were examined, the mean FEV1/FVC (%), FEV1 (% pred), FVC (% pred) values were within the normal range. This result was associated with the younger age of the patients included in our study. Although there are several studies with the same conclusion as ours, they generally worsen with age, impairing exercise tolerance and contributing to respiratory comorbidities.[30-32]

In our study, when the CPET parameters of 4 patients were examined a surgical treatment was decided. The peak VO2 value of the patients is lower than normal and VE/VCO2 and VE/VO2 parameters are higher than normal. These findings were consistent with the results of studies in adult patients.[18,23-25]

There are several limitations to our study. These can be counted as the small number of patients and the absence of different anatomical and physiological groups with similar numbers of patients. We are planning studies with a larger number of patients. The other limitations are the absence of a control group and not having follow-up CPET measurements.

In conclusion, CPET may be a diagnostic method that can be used to evaluate the cardiac, pulmonary; and metabolic response to exercise, cardiopulmonary failure, exercise-related symptoms, and functional capacity of pediatric and adolescent patients with CHD in different anatomical and functional classes to guide their medical treatment. This test should be performed in patients at certain ages to reveal clues of clinical deterioration.

Ethical approval

Ethics/Institutional Review Board approval of institutional Ethics Committee of Koç University, İstanbul, Turkey (ethical approval no: 2020.265.IRB1.088).

Author contribution

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

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Conflict of interest

The authors declare that there is no conflict of interest.

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Original Article

Assessment of serum galectin-3 levels in acute rheumatic fever

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ABSTRACT

Background. Galectin-3 is a biomarker which takes a role in both acute and chronic inflammation as well as fibrosis and oxidative stress. Increased levels of it are associated with cardiovascular diseases. This study was performed to investigate the levels of galectin-3 in acute rheumatic fever (ARF).

Methods. 30 patients with ARF and 26 healthy children were included. Galectin-3 levels of the patients were compared with the controls, as well as within the patients before and after the treatment.

Results. The patients had significantly lower galectin-3 levels on admission than the control (p=0.02), but its levels were not significantly different between these groups at the end of treatment (p=0.714). The mean galectin-3 levels of the patients were increased after the treatment (p<0.001). Severity of carditis and galectin-3 levels were negatively correlated (r=-539, p=0.02).

Conclusions. Children with ARF have significantly reduced levels of galectin-3 and there is a negative correlation between the severity of the carditis and galectin-3 levels. Studies with larger sample sizes may give more accurate data about the role of galectin-3 in ARF.

Key words: galectin-3, acute rheumatic fever, children.

Acute rheumatic fever (ARF) is still an epidemic in developing countries.[1,2] Globally 471000 cases of ARF are diagnosed annually and deaths that occur due to ARF or rheumatic heart disease (RHD) are still not uncommon, especially in non-developed or developing countries.[3] Two separate pathways for diagnosis have been defined for low and moderate/high-risk populations in revised Jones Criteria, in 2015.[2] Joints and the heart are most commonly involved.[4]

Galectin-3 is a multifunctional β-galactosidase-binding lectin and plays a role in apoptosis and cell proliferation.[5,6] It plays a role in oxidative stress, inflammation and fibrosis.[7] It may mediate processes during inflammation including activation of mast cells, neutrophil activation and adhesion, chemotaxis and opsonization of apoptotic neutrophils.[8] Galectin-3 levels have been extensively studied in various viral infections and diseases affecting ophthalmological, renal, cardiovascular, and neurological systems.[9-13] Galectin-3 is reported to be associated with increased risk of heart failure, arrhythmias, atherosclerosis, and an indicator of the severity of heart diseases.[14] It was also proposed to be associated with poor prognosis in acute heart failure.[15]

We aimed to evaluate the role of galectin-3 in children with ARF.

Material and Methods

The study was carried out between October 2018 and October 2019. Informed constant was

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obtained from all. The study was approved by ethics committee of Van Training and Research Hospital (Approval date and number: 04.10.2018 - 2018/14).

The galectin-3 levels were compared between patients and the controls on admission and after the treatment. Also, the correlation between severity of carditis and galectin-3 levels was analyzed.

Study population

Thirty patients with ARF as study group and 26 children as control group were included. The study group was also subdivided based on the degree of carditis. Children with a history of any other diseases or any medication were not included.

History taking, physical examination, diagnosis, and management

All participants were asked to provide demographic information as well as a comprehensive history. A thorough physical assessment was conducted. Electrocardiography and echocardiography were performed for all individuals. The Jones Criteria, which were modified in 2015, were used to make the diagnosis of ARF.[2] All the patients were hospitalized at least for two weeks. The carditis was classified according to its severity in the light of report of Cannon et al.[16]

Benzathine penicillin G was used for the treatment of pharyngitis and for secondary prophylaxis.[4] Oral prednisolone was used in cases with severe or moderate carditis otherwise, naproxen sodium was used.[17] Appropriate treatment for heart failure and bed rest with activity restriction was decided.

Cardiac evaluation

A detailed echocardiographic evaluation of all participants was performed with a Vivid 7 Pro echocardiography device (GE Vingmed, Horten, Norway). Rheumatic valvulitis was diagnosed using the 2015 Revised Jones Criteria.[2]

Laboratory work-up

Galectin-3 levels and routine laboratory tests were assessed on admission and after treatment. The control group's galectin-3 levels were also tested. Each blood sample collected for galectin-3 analysis were coded by a nurse to have a blinded study. The blood samples were clotted for 2 hours on room air and centrifuged at 1000 × g at 2-8°C for 15 minutes. The serum was kept at −80°C until all the samples were tested. Samples were analyzed using commercial kits by micro-ELISA (Wuhan Elabscience Biotechnology Co. Ltd, China, LOT number WIKYLAGHTI).

Statistical analyses

The SPSS 20.0 statistics program was used to conduct the statistical analysis (IBM Corp., Armonk, NY, USA). For descriptive statistics, the mean, standard deviation, and frequency were applied. The groups were compared using the independent sample t-test. The subgroups were compared by using one-way ANOVA. The paired sample t-test was performed for comparison of the findings in the study group before and after the therapy. Correlation analysis was done by using Pearson's correlation analysis. The cut-off value, sensitivity, and specificity were determined using ROC analysis. The statistical significance was set at $p < 0.05$ and the confidence interval was set as 95%.

Results

The study and control group had mean ages of 11.30±3.11 and 12.42±2.75 years, respectively (p=0.161). There was a slight female dominance in both groups (53.3% of patients and 57.7% of the controls) (p=0.743).

The laboratory and clinical findings of the patients are shown in Table I. The distribution of carditis on admission was: 40% mild, 36.7% moderate, 23.3% severe. After treatment, there were no patients with severe carditis, and mild carditis was observed in 23 patients (76.7%).

Table I. Findings of the study group according to the diagnostic criteria.

	n (%)
Carditis	30 (100%)
Mild	12 (40%)
Moderate	11 (36.6%)
Severe	7 (23.4%)
Arthritis	16 (53.3%)
Fever	27 (90%)
Sydenham Chorea	2 (6.6%)
Elevated ESR and/or CRP	30 (100%)
Elevated ASO titres	25 (83.3%)
Positive throat culture for group A β-hemolytic streptococcus	13 (43.3%)
First degree AV block	16 (53.3%)

ESR: erythrocyte sedimentation rate CRP: C-reactive protein ASO: anti-streptolysin O

The mean CRP levels and ESR of the patients on admission were high (p<0.01).

The mean galectin-3 levels of patients on admission (7.18 ± 8.31 ng/ml) and after the treatment (22.29 ± 19 ng/ml, p<0.001) was significantly different. Figure 1 represents the distribution of galectin-3 levels of the patients on admission and the controls. The mean galectin-3 levels were significantly different by means of the severity of carditis (p=0.006). Mean galectin-3 levels were significantly higher in cases with mild carditis in comparison to ones with severe (p=0.012) and moderate (p=0.028) carditis. Severity of carditis and galectin-3 levels were negatively correlated (r=-539, p=0.02). Levels of galectin-3 in subgroups of the patients on admission are shown in Figure 2.

The mean galectin-3 levels of the cases with arthritis before and after the treatment were 8.41 ±8.73 ng/ml and 18.81 ±16.86 ng/ml, respectively. The mean galectin-3 levels of the cases without arthritis on admission and after the treatment were 5.76 ± 7.88 ng/ml and 26.26 ± 21.09 ng/ml, respectively. There was no significant difference between subgroups regarding the presence of arthritis before and after the treatment (p=0.523, p=0.873, respectively).

The whole study group's mean galectin-3 level was significantly lower than the control before the treatment (p =0.02) but, there was no significant difference after the treatment (p=0.714). Similarly, all except mild carditis had significantly lower levels of galectin-3 than the control group on admission, but mean galectin-3 levels were similar after treatment (Table II). Also, when the mean galectin-3 levels of subgroups defined by the presence of arthritis were compared with the control group, both subgroups had significantly lower mean galectin-3 levels (p=0.001 and p=0.002) than the controls on admission. The mean galectin-3 levels of the patients after treatment were not

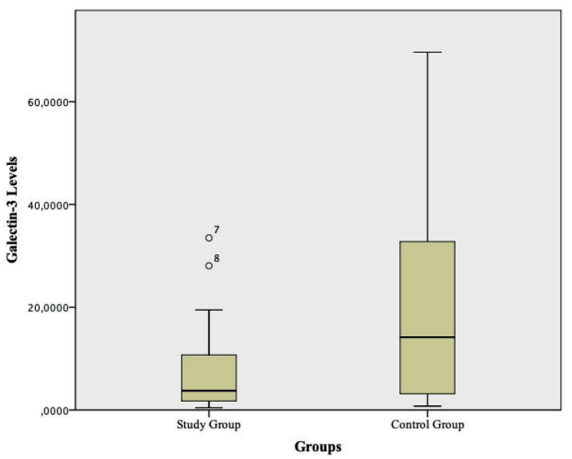

Fig. 1. The distribution of galectin-3 levels of the study group at the time of diagnosis and the control group.

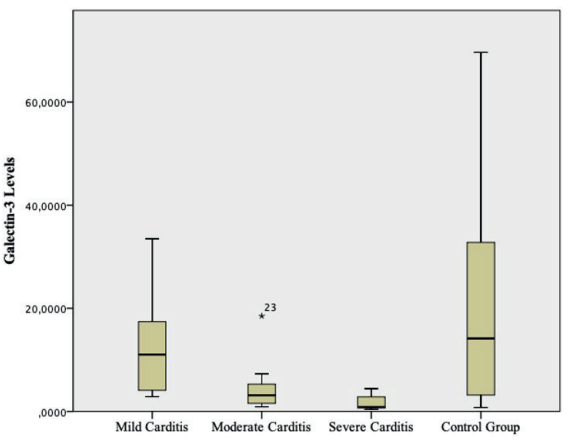

Fig. 2. The distribution of galectin-3 levels according to the severity of carditis at the time of diagnosis and the control group.

Table II. The comparison of mean galectin-3 levels of the study group and the control group.

Patient Group (n:30)		P value*	Control Group (n:26)	P value**
Before Treatment		***0.006***		
Mild carditis	12.6 ± 10.03			0.222
Moderate carditis	4.66 ± 5.05			***0.015***
Severe carditis	4.41 ± 1.81			***0.021***
All	7.18 ± 8.31		20.09 ± 19.59	***0.002***
After Treatment		0.995		
Mild carditis	22.73 ± 15.22			0.718
Moderate carditis	21.99 ± 13.83			0.808
Severe carditis	21.99 ± 31.62			0.862
All	22.29 ± 18.99		20.09 ± 19.59	0.714

Galectin-3 levels are expressed as mean±standart deviation and ng/ml. P values indicate the significance of the differences of galectin-3 levels *within the before and after treatment groups in relation to severity of carditis, and **compared to the control group. Bold italics indicate P < 0.05

significantly different when compared with the controls. One of our patients who had the most severe carditis and required treatment in the intensive care unit due to ARF with valve involvement and ventricular tachycardia had the lowest galectin-3 level (0.3 ng/ml) before treatment and the highest level (69 ng/ml) after treatment when compared with whole subjects.

ROC curve analysis determined a value of 5.46 ng/ml as a cut-off value for galectin-3 with a specificity of 65.4% and a sensitivity of 63.3% for the presence of carditis (Fig. 3).

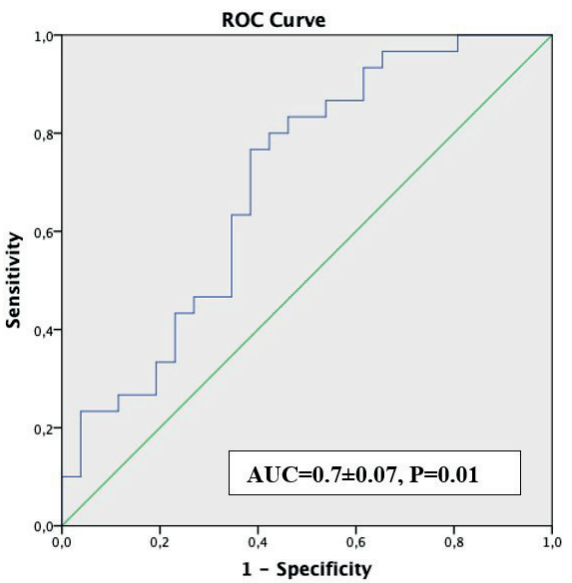

Fig. 3. ROC curve analysis of galectin-3 levels.

Discussion

Acute rheumatic fever is still common in low income countries and major cause of morbidity in our country. Galectin-3 is a biomarker used for various diseases including heart failure.

Acute rheumatic fever is most prevalent between the ages of 5-14 years and in the female gender (1.5-2 times higher risk).[17-19] The ages of the patients were between 6-17 years and 53.3% of them were female, in our study. Sydenham chorea, arthritis/polyarthralgia, fever and carditis was detected in 6.6%, 53.3%, 90% and 100% of patients, respectively. We did not observe any subcutaneous nodules or erythema marginatum. All cases had elevated levels of ESR and CRP on admission. The frequency of mild, moderate and severe carditis was 40%, 36.6% and 23.4%, respectively.

Fifteen galectins have been defined and galectin-3 is the only chimeric member found in humans.[20] It is found in various tissues in humans, but its expression varies according to the tissue type. Galectin-3 has intracellular and extracellular components which both regulate functions related to growth and development whereas extracellular galectin-3 also takes a role in homeostasis.[21] Galectin-3 plays role in inflammation with its both anti-inflammatory and pro-inflammatory properties which are determined according to the type of stimuli and the tissue.[22]

Galectin-3 has extensively been studied in diseases that affect various systems because of its unique properties.[9-13] It was shown to be an indicator of increased risk of heart failure, arrhythmias, atherosclerosis, and the severity of heart diseases.[14] Measurement of galectin-3 levels in patients with heart failure has been recommended in guidelines.[23] It's levels are reported to be low in a normal healthy heart and its expression increases in both chronic and acute decompensated heart failure. Although at the initial phases of heart failure it has a protective role, it leads to adverse remodeling and fibrosis over time.[20,24] In contrast to N-terminal pro-BNP, galectin-3 levels do not decrease rapidly with the resolution of the volume overload.[24] The natriuretic peptides are increased during hemodynamic stress; however, activated macrophages secrete galectin-3 which is associated with inflammation and ventricular remodeling. In contrast to BNP, galectin-3 do not decrease during heart failure in patients with total artificial hearts or ventricular assist devices.[24]

Considering previous data about the role of galectin-3 in cardiovascular diseases and heart failure, while planning this study we hypothesized that galectin-3 may be significantly higher in children diagnosed with ARF. But unexpectedly and surprisingly, the analysis and interpretation of the study data revealed that galectin-3 levels were significantly lower in children with ARF than the controls and this difference disappeared after the treatment. As supporting evidence of these findings, there was a significant difference in galectin-3 levels between subgroups that were determined according to severity of carditis. Patients with mild carditis had higher levels than the ones with moderate and severe carditis, and severity of carditis and galectin-3 levels were negatively correlated. ROC curve analysis determined a cut-off value of 5.46 ng/ml for galectin-3 with a specificity of 65.4% and a sensitivity of 63.3% for the presence of carditis in ARF.

Upon these findings, we searched the literature for decreased levels of galectin-3 in cardiovascular diseases, but we failed to find any report. Although there are some controversies, significantly decreased levels of galectin-3 have been reported in schizophrenia.[25] Galectin-1 and galectin-3 levels have been studied in patients with rheumatoid arthritis, and galectin-1 levels have been reported to be significantly increased.[22] In the same study, galectin-3 levels were significantly decreased, and were not correlated with the erythrocyte sedimentation rate as similar to our study. But in contrast to our study, they found a positive linear trend between galectin-3 levels and disease activity. Their findings were partly in contrast to previous studies, and they concluded that this difference should have resulted from the fact that patients in the study group were taking medication for rheumatoid arthritis. We performed further statistics to analyze any relation between galectin-3 and arthritis, but no correlation was detected. Galectin-3 has been found to be significantly decreased in diffuse cutaneous systemic sclerosis than in limited ones and healthy controls.[26,27]

Although most children will be exposed to group A Streptococcus, ARF develops in only a proportion and this is explained by the genetic susceptibility of the host.[28] Although the exact mechanism is not well established, molecular mimicry is thought to be the main reason. ARF may affect multiple tissues but cardiac involvement is the mainstay of the disease.[29] The similarity between exogenous proteins and human tissues is defined as molecular mimicry.[30] The innate immune system is activated after streptococcal pharyngeal infection, and then the bacterial antigens are presented to T cells. The activation of cellular and humoral immune responses leads to production of cross-reactive antibodies. Carditis is thought to be caused by these cross-reactive antibodies and T cells.[29] Although the pathogenesis of ARF may be related with numerous bacterial antigens, N-acetylglucosamine and M protein are the main epitopes accused of cardiac damage because of the similarity between the alpha-helical structure of the M protein and N-acetyl-beta-D-glucosamine to cardiac myosin.[31]

Galectin-3 mediates many processes during acute inflammation. It has both anti-inflammatory and pro-inflammatory roles. It can bind galactosides.[8] Galectins act as regulators of innate and adaptive immunity and they also have immunomodulatory properties.[32,33] Galectins have been shown to provide innate immunity against blood group molecular mimicry.[34,35] Also, they can recognize the antigenic determinants on the microbes and the host cells. Interestingly they can selectively damage the microbes with the same antigenic determinants but not the human cells. Galectin-4 and galectin-8, are shown to, recognize and destroy human blood group antigen-expressing *Escherichia coli*, and the C-terminal domains mediate this activity. This property of galectins is proposed to protect against molecular mimicry.[35] The regulatory role of galectins in immune response and the protection they provided against molecular mimicry can be the reason for lower levels of galectin-3 in patients with ARF in which the cardiac damage is thought to be mediated by molecular mimicry.

To us, this is the only study focusing on galectin-3 levels in ARF. Our study itself is not able to show the definite role of galectin-3 in ARF absolutely and to highlight the molecular mechanisms leading to decreased levels of galectin-3 in ARF. We think that the decreased levels of this biomarker in ARF are mainly due to its role in inflammation, its pro/anti-inflammatory role, and the protection it provided against molecular mimicry. Although our findings point to it being negatively correlated with the severity of carditis, this correlation may be through the severity of inflammation, rather than heart failure. If it was through heart failure, we might expect to observe increased levels of galectin-3 as stated in the literature. Unfortunately, there isn't any available data about galectin levels with valvular heart diseases although the main pathology in ARF is valvulitis. Further studies will help to highlight the role of this biomarker, in the pathophysiology, severity, and prognosis of the disease. The lowest galectin-3 level at the time of diagnosis and the highest level after treatment was seen in a 7-year-old male patient which was previously published as a case report.[36] Among all cases, he was the case with the most severe carditis (involving 4 valves and ventricular tachycardia). We think that this is supportive evidence that galectin-3 levels decrease in the acute period of rheumatic carditis and becomes to normal values after the treatment.

The study has certain limitations. The sample size is not large enough. Two measurements consisting of before and after treatment were performed rather than serial measurements which might give more reliable data.

In conclusion, children with ARF have significantly reduced levels of galectin-3 and its levels and the severity of the carditis are negatively correlated. In addition, after the treatment, galectin-3 levels return to normal values. More studies with serial measurements of galectin-3 would give more information and molecular studies may highlight the role of galectin-3 in ARF. We think that our study will lead to further studies that will make new contributions to the role of galectin-3 in cardiac diseases.

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Ethical approval

Ethical approval was obtained from Van Training and Research Hospital ethical committee. (Date: 04.10.2018 / No: 2018/14). All procedures performed were in accordance with the ethical standards and with the 1964 Helsinki Declaration and its later amendments.

Author contribution

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

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Conflict of interest

The authors declare that there is no conflict of interest.

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Original Article

Evaluation of thymic dimensions in patients with multisystem inflammatory syndrome

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ABSTRACT

Background. Multisystem inflammatory syndrome (MIS-C) is the most important complication of COVID-19 in the pediatric population. Unfortunately, this problem is an unpredictable situation in patients with COVID-19. We aimed to evaluate the effects of MIS-C on thymus dimensions in pediatric patients.

Methods. We retrospectively analyzed the files of 368 pediatric patients aged 2-18 years, who were diagnosed with COVID-19. Computer Tomography (CT) images of 22 patients diagnosed with COVID-19 and 10 patients diagnosed with MIS-C were evaluated in detail by two board-certified radiologists. Eighteen age and sex-matched patients who applied to the emergency department of our hospital for any reason and had a CT scan for any reason were selected as the control group. The data of both groups were statistically compared.

Results. Considering the differences between the groups in terms of laboratory data, monocytes, hemoglobin, and platelet were significantly lower in the MIS-C group than the other groups. Procalcitonin, C- reactive protein, neutrophil to lymphocyte ratio (NLR), platelet to lymphocyte ratio (PLR), and proBNP levels were statistically significantly higher in the MIS-C group compared to the other groups Regarding the differences in thymus dimensions, thymus AP diameter, transverse diameter, length, thickness, and volume were significantly higher in the MIS-C group than in the other groups There was a significant positive correlation between the transverse diameter of the thymus and CRP, procalcitonin, pro-brain natriuretic peptide (proBNP), and NLR levels.

Conclusions. Our study shows that thymus dimensions and acute phase reactants are higher in pediatric patients in the MIS-C group. Also, thymus transverse diameter, thymus thickness, and PLR values pose a risk for the development of MIS-C. More research is needed on the role of the thymus gland in the pathogenesis and diagnosis of MIS-C.

Key words: pediatric patient, COVID-19, multisystem inflammatory syndrome in children, thymus.

The thymus, a lymphoid organ, is responsible for the formation and development of T cells. The process of change of the thymus begins when the progenitor cells enter the thymus with blood vessels in the cortico-medullary region. T cell development begins with the binding of the antigen to the T cell receptor (TCR). It is also the primary determinant of the fate of thymocytes. Thymocytes can be monitored for the presence or absence of surface markers such as CD4 and CD8. The maturing cells leave the thymus and migrate to peripheral lymphoid organs such as lymph nodes, spleen, and submucosal lymphoid tissue.[1,2] The thymus is very sensitive to endogenous or exogenous factors (infections, malnutrition). Depending on these changing factors, peripheral immune responses are likely to be impaired. Unfortunately, current studies are insufficient to evaluate the reflections of diseases on the thymus and its role in disease pathophysiology, focusing rather on the peripheral immune system.[1,3] The thymus, the producer of T lymphocytes, is particularly

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susceptible to infections because of its important function. There is evidence in the literature showing that viral pathogenic microorganisms can change thymic structure and physiology.[4-7] The pathophysiology of multisystem inflammatory syndrome (MIS-C) is currently not clarified. Initially asymptomatic or with mild symptoms in children, the infection may cause stimulation of helper T cells and activation of macrophages. In some children, this infection causes excessive release of cytokines such as tumor necrosis factor (TNF), interleukin (IL)-1β, 4, 6,12, 18, 23, interferon (IFN), and stimulation of macrophages. In addition, caspase 1, which causes stimulation of neutrophils and monocytes, is often co-produced with IL-1β in children with MIS-C. Increased levels of IL-18, which is converted from pro-IL-18 to IL-18 through its activation. IL-18 increases IFN levels and functional activities. Increased IL-18 and IFN levels and the cytokine storm can be seen in MIS-C.[8-10]

Currently, there is no sufficient number of articles in the literature showing the relationship between COVID-19 and MIS-C and thymus dimensions. Therefore, we aimed to evaluate the effects of COVID-19 and MIS-C on thymus dimensions in pediatric patients to develop new perspectives for the pathogenesis, treatment and prevention of MIS-C while the COVID-19 pandemic is still ongoing.

Material and Methods

Study group

This study is a retrospective single-center observational study. We retrospectively reviewed the files of 368 pediatric patients aged 2-18 years who were diagnosed with COVID-19, were positive for the COVID-19 PCR test and/or COVID-19 antibodies, and were examined at the time of admission. The files of patients admitted to the Pediatric Emergency Department between March 2020 and May 2021 were reviewed retrospectively. Pediatric patients with COVID-19, diagnosed with MIS-C according to the criteria of the World Health Organization (WHO) and Center for Disease Control and Prevention (CDC), with cardiac markers and chest computed tomography (CT) were included in the study.[11,12] Findings were obtained from hospital records. CT images of 22 patients diagnosed with COVID-19 and 10 patients diagnosed with MIS-C were evaluated in detail by two board-certified radiologists. Patients over 18 years of age, those without CT images, those with poor quality CT images, immunocompromised patients, those with liver failure, kidney failure, hematologic and/or oncologic disease, chronic obstructive pulmonary disease, and those whose COVID-19 PCR or COVID-19 antibodies were not investigated for the presence of COVID-19 were excluded.

Age and gender-matched patients who applied to the emergency department of our hospital due to trauma or non-opaque foreign body ingestion and who received a thoracic CT were selected as the control group. None of these patients had a lower respiratory tract infection COVID-19 and/or MIS-C clinic and diagnosis. Eighteen pediatric patients who met the criteria were selected as the control group.

The cases included in the study were grouped according to their ages as 2-6 years, 7-13 years, and 13-18 years.[13]

Computed tomography

Contrast-free CT images were obtained using a standard thorax CT protocol, 130 kVp regulating tube voltage, 100–350 mA tube current, a slice thickness is 3mm, 25 cm scanning field of view (FOV) and a 512×512 matrix with a high resolution thorax algorithm of a 64 detector array scanner (LightSpeed VCT, GE Medical Systems, Milwaukee, WI, USA).

Image evaluation and analysis

Thymus size and volume were evaluated in the axial plane on thorax CT images (using a 27-inch iMac computer, Apple Inc. Cupertino, 88 California, USA). Thymus volume was

calculated from the relevant region (ROI) mentioned in the literature in each case Fig. 1.[14] Quantitative measurements of the thymus (length and thickness of the right and left lobes of the thymus, width-length and anterior posterior diameters of the gland) were made by two radiologists from CT images. Diameter, length and thickness measurements of the thymus were made according to the definitions published in previous studies (Fig. 2).[15]

Laboratory data analysis white blood cell count (WBC), lymphocyte, monocyte, neutrophil, neutrophil-lymphocyte ratio (NLR), lymphocyte-monocyte ratio (LMO), hemoglobin (Hb), platelet (PLT), platelet-lymphocyte ratio (PLR), C-reactive protein (CRP), procalcitonin, and pro-brain natriuretic peptide (proBNP) values were analyzed retrospectively.

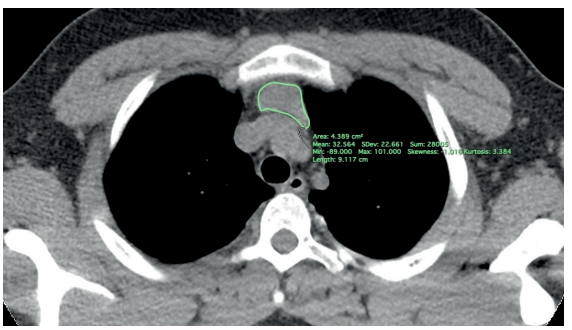

Fig. 1. The thymic volume was calculated in each case from the region of interest thorax axial CT images.

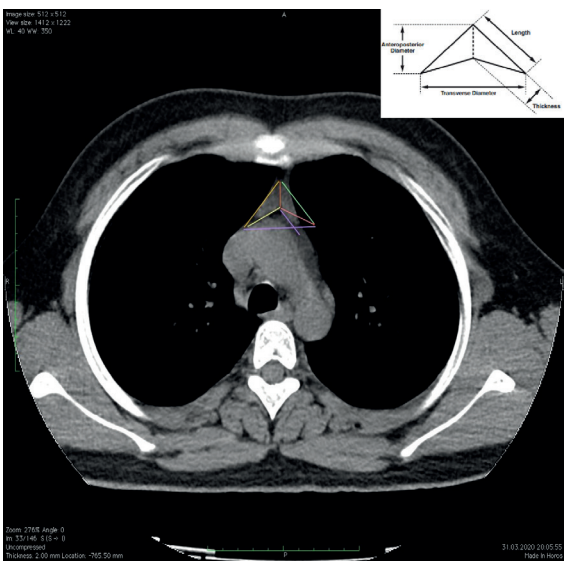

Fig. 2. Thymus thickness, anteroposterior diameter, transverse diameter and length measurement on thorax axial CT images.

The study was conducted in accordance with the principles stated in the Declaration of Helsinki. Ethical approval was obtained from the Ministry of Health and the local ethics committee of Kahramanmaras Sütcü İmam University before the study (Ethics committee date: 26.03.2021, Session: 2021/2022, Protocol no: 185, Decision no: 03)

Statistical analysis

Statistical data analysis was performed using SPSS 22 software. Descriptive statistics were presented as continuous variables (mean ± standard deviation) and categorical variables (%). Chi-square test and Fisher's exact test were used for the analysis of categorical variables. The Mann–Whitney U test was used to compare non-normally distributed continuous variables. Kolmogorov-Smirnov test was performed to check for normal distribution in continuous variables. A one-way ANOVA test was used to determine the difference of the mean value of a dependent variable between two independent groups. Correlation analysis was performed to determine the severity and direction of the relationship between two numerical variables. Pearson correlation coefficient was preferred for normally distributed data and Spearman rank correlation coefficient was preferred for non-normally distributed data. Receiver operating characteristic (ROC) curve analysis was performed to find the optimal cut-off, sensitivity, and specificity of the PLR and thymus dimensions in predicting MIS-C. For all tests, $p<0.05$ was considered statistically significant.

Results

Considering demographic and diagnostic data, the mean age of the patients was 10.85±4.52 years and there was no significant difference between the groups in terms of age or sex ($p=0.718$, $p=0.628$, respectively). COVID-19 PCR

(+) was detected in 19 (86.4%) of the patients in the COVID-19-infected non-MIS-C group and COVID-19 IgM was positive in 3 (13.6%). In the MIS-C group, PCR was positive in 3 patients (30%) and COVID-19 IgM was positive in 7 (70%). These diagnostic differences were statistically significantly different between the groups. Monocyte, Hb, and PLT were significantly lower in the MIS-C group than in the other groups. Procalcitonin, CRP, NLR, PLR, and proBNP levels were statistically significantly higher in the MIS-C group compared to the other groups (Table I). Considering the differences in thymus dimensions, thymus AP diameter, transverse diameter, length, thickness, and volume (Fig. 3) were significantly higher in the MIS-C group than in the other groups (Table II). When the relationship between laboratory findings and thymus dimensions was evaluated; There was a significant positive correlation between thymus transverse diameter and CRP, procalcitonin, proBNP and NLR. While there was a significant positive correlation between thymus length and CRP and NLR, there was a significant positive correlation between thymus thickness and CRP, proBNP and NLR. There was a significant positive correlation between thymus volume and CRP. However, there was no correlation between thymus AP diameter and laboratory findings (Table III). When the thymic dimensions were evaluated according to age groups, there was no statistically significant difference between the groups in terms of AP diameter, transverse diameter, length, thickness, or volume in the 2-6 age group. In the 7-13 age group, the AP diameter of the thymus was significantly higher in patients with COVID-19 and the transverse diameter and thickness of the

Table I. Comparison of laboratory data by groups.

	Control (n=18)	COVID-19 (n=22)	MIS-C (n=10)	P
WBC	8.97±4.74	8.85±3.83	10.27±5.81	0.702
Neutrophil	5.47±4.25	5.83±3.80	8.23±6.09	0.276
Lymphocyte	2.65±1.22	2.10±1.41	1.64±2.15	0.239
Monocyte	644.71±359.91	762.73±287.54	328.00±186.95	0.002
Hb	12.85±1.40	13.29±1.20	11.90±1.15	0.021
PLT	291.11±64.69	256.45±60.47	206.50±78.82	0.009
CRP	3.05±1.40	6.89±9.83	133.67±115.10	0.001
Procalcitonin	0.035±0.007	0.060±0.046	8.56±12.93	0.019
NLR	2.44±2.05	3.99±3.72	8.85±5.68	0.001
LMO	4.59±2.09	3.15±2.09	4.53±2.60	0.093
PLR	120.73±49.74	154.45±74.45	188.59±61.45	0.031
ProBNP	0.00±0.00	160.63±294.99	9976.80±8959.15	<0.001

Oneway ANOVA. Post Hoc Test (Scheffe)
CRP: C-reactive protein, Hb: hemoglobin, LMO: lymphocyte-monocyte ratio, MIS-C: multisystem inflammatory syndrome, NLR: neutrophil-lymphocyte ratio, PLR: platelet-lymphocyte ratio. ProBNP: pro-brain natriuretic peptide

Table II. Evaluation of thymus sizes by groups.

Thymus sizes	Control (n=18)	COVID-19 (n=22)	MIS-C (n=10)	P
AP diameter	1.20±0.29	1.39±0.32	1.47±0.18	0.039
Transverse diameter	3.67±0.35	2.96±0.91	4.66±0.84	<0.001
Length	2.29±0.36	2.09±0.64	2.84±0.59	0.003
Thickness	0.94±0.12	0.92±0.13	1.12±0.09	<0.001
Volume	12.46±3.11	9.88±4.45	14.71±4.06	0.007

Oneway ANOVA. Post Hoc Test (Scheffe)
AP: anterior-posterior, MIS-C: multisystem inflammatory syndrome

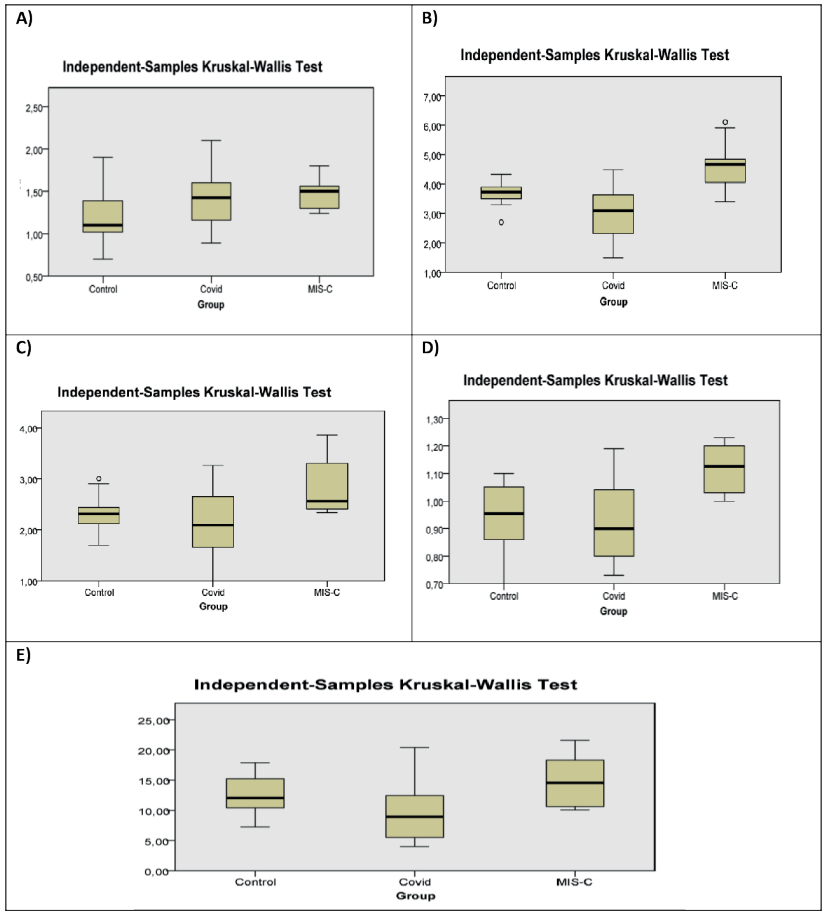

Fig. 3. A) Thymus AP diameter was significantly larger in the MIS-C group (p=0.033). B) Thymus transverse diameter was significantly higher in the MIS-C group (p<0.001). C) Thymus length was significantly higher in the MIS-C group (p=0.004). D) Thymus thickness was significantly higher in the MIS-C group (p=0.001). E) Thymus volume was significantly higher in the MIS-C group (p<0.001).

Table III. Evaluation of the relationship between the thymus dimensions of the patients and the laboratory data.

Thymus sizes		CRP	Procalcitonin	ProBNP	WBC	NLR	PLR	LMO
AP diameter	r	0.172	-0.027	0.221	0.098	0.221	0.246	-0.261
	p	0.308	0.884	0.182	0.564	0.127	0.141	0.070
Transverse diameter	r	0.596	0.379	0.416	0.166	0.367	0.094	0.174
	p	<0.001	0.036	0.009	0.326	0.009	0.582	0.232
Length	r	0.527	0.239	0.282	0.204	0.352	0.094	0.099
	p	0.001	0.195	0.086	0.225	0.013	0.579	0.499
Thickness	r	0.470	0.285	0.496	0.108	0.312	0.064	0.099
	p	0.003	0.120	0.002	0.525	0.029	0.706	0.499
Volume	r	0.362	0.203	0.226	0.040	0.123	-0.121	0.249
	p	0.030	0.282	0.178	0.818	0.404	0.481	0.087

Correlations. Pearson Correlation
CRP: C-reactive protein, Hb: hemoglobin, LMO: lymphocyte-monocyte ratio, MIS-C: multisystem inflammatory syndrome in children, NLR: neutrophil-lymphocyte ratio, PLR: platelet- lymphocyte ratio, ProBNP: pro-brain natriuretic peptide

thymus were statistically significantly higher in the MIS-C group compared to the other groups There was no significant difference between the groups in terms of thymus length or volume. In the 14-17 age group, there was no statistically significant difference in terms of AP diameter, thickness, or volume. The thymus transverse diameter and length were significantly higher in the MIS-C group (Table IV). A ROC curve analysis was performed to determine the best cut-off points for thymus dimensions and PLR levels to predict MIS-C. Accordingly, we found that if the AP diameter of the thymus tissue is ≥1.230 cm, it can predict MIS-C with 100% sensitivity and 47.5% specificity. We found that if the transverse diameter of the thymus tissue is ≥4,030 cm, it can predict MIS-C with 80% sensitivity and 92.5% specificity We found that if the length of the thymus tissue is ≥2.330 cm, it can predict MIS-C with 100% sensitivity and 62.5% specificity We found that if the thickness of the thymus tissue is ≥1.095 cm, it can predict MIS-C with 70% sensitivity and 92.5% specificity. We found that if the volume of the thymus tissue is ≥9.915 cm3, it can predict MIS-C with 100% sensitivity and 58.5% specificity Finally, if the PLR level is ≥168.61, it can predict MIS-C with 90% sensitivity and 96.4% specificity (Table V). According to the risk analysis for the development of MIS-C in pediatric patients with COVID-19 infection, the risk of developing MIS-C increases by 40 times if the thymus transverse diameter is ≥4.030 cm, by 14.778 times if the thymus thickness is ≥1.095 cm, and by 40.5 times if the PLR value is ≥168.61 (Table V).

Discussion

To the best of our knowledge, our study is the only one in the literature to show that thymus tissue enlarged significantly in the MIS-C group. A higher amount of proinflammatory cytokines (TNFα, IL-1, IL-6) and chemokines were recorded in patients with severe COVID-19 symptoms compared to patients with mild

Table IV. Comparison of thymus sizes by age groups.

Thymus sizes	2-6 age (n=11)			P
	Control (n=5)	Covid-19 (n=5)	MIS-C (n=1)	
AP diameter	1.22±0.34	1.48±0.29	1.50±0.00	0.635
Transverse diameter	3.69±0.08	3.21±0.62	4.06±0.00	0.164
Length	2.19±0.27	2.14±0.42	2.40±0.00	0.540
Thickness	0.95±0.15	0.90±0.12	1.21±0.00	0.198
Volume	14.44±3.52	6.69±3.17	14.90±0.00	0.188
		7-13 age (n=21)		
	Control (n=7)	Covid-19 (n=7)	MIS-C (n=7)	P
AP diameter	1.07±0.13	1.66±0.26	1.45±0.22	0.001
Transverse diameter	3.89±0.28	3.56±0.68	4.89±0.900	0.012
Length	2.39±0.31	2.63±0.49	2.82±0.54	0.217
Thickness	0.94±0.13	0.98±0.15	1.11±0.08	0.035
Volume	13.12±2.12	13.86±4.57	14.64±4.13	0.872
		14-17 age (n=18)		
	Control (n=6)	Covid-19 (n=10)	MIS-C (n=2)	P
AP diameter	1.34±0.37	1.27±0.27	1.53±0.04	0.410
Transverse diameter	3.39±0.40	2.42±0.91	4.18±0.54	0.014
Length	2.24±0.49	1.70±0.56	3.14±1.02	0.042
Thickness	0.94±0.09	0.89±0.14	1.13±0.14	0.183
Volume	10.06±2.47	6.89±2.20	14.88±6.78	0.060

Independent-Samples Kruskal-Wallis Test

Table V. Determination of the best cut-off points of thymus dimensions and laboratory findings to predict MIS-C and risk analysis for MIS-C.

Variable	Cut off value	AUC	Sensitivity	Specificity	Asymptotic 95% confidence interval	P*
AP diameter	≥1.230	0.692	1.000	0.475	0.547-0.838	0.062
Transverse diameter	≥4.030	0.911	0.800	0.925	0.798-1.000	<0.001
Length	≥2.330	0.820	1.000	0.625	0.700-0.940	0.002
Thickness	≥1.095	0.876	0.700	0.925	0.760-0.992	<0.001
Volume	≥9.915	0.719	1.000	0.585	0.553-0.885	0.034
PLR	≥168.61	0.777	0.900	0.821	0.591-0.963	0.007
Risk analysis with logistic regression						
	Cut off value		OD	95% confidence interval		P**
Transverse diameter	≥4.030 cm		40	4.779-334.765		0.001
Thickness	≥1.095 cm		14.778	2.395-91.195		0.004
PLR	≥168.61		40.5	3.929-417.434		0.002

*ROC Curve analysis, **Logistic regression analysis
AP: anterior-posterior, AUC: area under curve, MIS-C: multisystem inflammatory syndrome, OD: odds ratio, PLR: platelet-lymphocyte ratio

symptoms Research reports that the lung findings in the pathogenesis of COVID-19 are associated with increased serum cytokine and chemokine levels.[16] Also, decreased lymphocyte count and increased neutrophil-lymphocyte ratio have been reported in patients with severe symptoms.[17,18] We suppose that the significant growth of the thymus tissue in cases with severe COVID-19 infection and MIS-C is due to increased cytokines and chemokines secondary to an overstimulated immune response. Additionally, although the lymphocyte count was lower in the MIS-C group compared to the other groups, there was no statistically significant difference. This insignificance may be due to the small number of cases. In the literature, lymphocyte deficiency in MIS-C cases has been tried to be explained by the direct lymphocyte attack against the virus and migration to tissue and inflammation areas in some studies.[19,20] In our study, NLR was significantly higher in the MIS-C group, consistent with the literature, and this increase was positively correlated with the transverse diameter, thickness, and length of the thymus tissue. We think that the reason for this significant increase in NLR and thymus tissue may be due to the increased cytokines and chemokines mentioned in the literature.[17] Scientists have investigated why children have been protected during the coronavirus outbreaks; they reported that less outdoor activity, less international travel, and low angiotensin-converting enzyme 2 (ACE2) receptor expression were the primary factors responsible in children.[21,22] After viral infections, antigen-presenting natural killer cells and macrophages present the antigenic structure of the pathogen to T cells and activate adaptive immunity. Adaptive immunity is responsible for the production of T helper cells, activation of antibodies, cytokines, chemokines, and elimination of infected cells. The adaptive immune system, of which the thymus is primarily responsible for, is the most effective system for preventing damage to the body by invading microorganisms.[5] The thymus is quite active in the intrauterine and neonatal period; it begins to shrink after birth and continues its effectiveness until puberty. With increasing age, the function and activity of the thymus decreases.[23,24] This condition is called immune aging. After immune aging, the individual becomes prone to infections, cancer, and autoimmune diseases.[25,26] Our study included the pediatric population. In our evaluation according to age groups, there was no significant difference between the groups in terms of thymus dimensions in the

2-6 age group. In the school period (7-13 years), thymus AP diameter was significantly higher in patients with COVID-19 than in the control group. In the MIS-C group, however, we found thymus transverse diameter and thickness to be significantly higher than both the control group and COVID-19 group. In the adolescence period, thymus transverse diameter and length were significantly higher in the MIS-C group compared to the other groups.

We did not observe a significant difference in thymus dimensions between cases with COVID-19 and MIS-C during the 2-6 age period, which is the lowest age group of the pediatric population in our study. The number of MIS-C cases with CT evaluation was low in this age group. However, the fact that childhood vaccinations, seasonal corona virus, and other viral infection exposures are high in this age group may cause thymus dimensions to be high in healthy children. Therefore, we think exposure to COVID-19 did not cause a sufficiently significant increase in thymus dimensions in this age group.

In our study, thymus transverse diameter and thymus thickness measurements in other age groups over 7 years of age were found to be significantly higher in all COVID-19 cases compared to the control group. Also, a significant increase in thymus dimensions was observed in cases diagnosed with MIS-C compared to COVID positive cases. We believe that as age increases, T cells become more sensitive to antigenic structures similar to coronavirus and as a result of this sensitization, COVID-19 triggers an irregular immune response.

The clinical-pathological examinations of patients infected with COVID-19 by Qin et al.[17] support this idea. This review highlights that tissue damage in COVID-19 patients is the result of a cytokine storm associated with a dysregulated immune response. The positive correlation between thymus dimensions (transverse diameter, thymus thickness, thymus length, and thymus volume) and CRP and/or NLR, which we found in our study, as well as the higher CRP, NLR, and procalcitonin levels in the MIS-C group support this. Çakmak et al.[18] reported a significant correlation between the severity of lung imaging findings and the platelet-lymphocyte ratios of COVID-19 patients and that decreased platelet and lymphocyte counts led to noticeable increases in imaging findings. For COVID-19 patients, the severity of imaging findings has been reported to be significantly correlated with the level of fat involution in the patients' thymus tissue. In our study, the platelet count was significantly lower in the MIS-C group. We also showed that if the PLR is ≥168.61, it can predict MIS-C with 90% sensitivity and 82.1% specificity. However, we did not find a significant correlation between PLT and PLR values and thymus volume. This may be due to the absence of pulmonary involvement in any of the pediatric patients included in our study, unlike the literature. Also, unlike the literature, we determined cut-off points for thymus dimensions to predict MIS-C in patients with COVID-19 infection. We determined that it can predict MIS-C with 91.1% sensitivity and 80% specificity, especially when the transverse diameter of the thymus is ≥4.030 cm. According to our risk analysis with logistic regression over this cut-off value, the risk of developing MIS-C increases by 40 times in patients with COVID-19 infection with a thymus transverse diameter of ≥4.030 cm, by 14.778 times if the thymus thickness is ≥1.095 cm, and by 40.5 times if PLR is ≥168.61.

The size of the thymus is known to decrease with X-rays. In fact, X-rays were used in the past years to reduce the size of the thymus, and when it was found to be carcinogenic, this form of treatment was avoided.[27] However, the fact that this is the only study in the literature that compares thymus dimensions and laboratory findings in age and sex-matched pediatric patients with COVID-19 infection makes our study valuable.

Our study shows that thymus dimensions and acute phase reactants are higher in pediatric patients in the MIS-C group. It also shows that thymus transverse diameter, thymus thickness, and PLR values pose a risk for the development of MIS-C. This supports the evidence that MIS-C emerges with a dysregulated immune response, due to excessive thymus activity against the COVID-19. However, it is still a matter of debate why the clinical course of COVID-19 varies from patient to patient. For this reason, there is a need for prospective studies with large participation, including genetic analyses, thymus dimensions, and cytokine levels.

Ethical approval

Ethical approval was obtained from the Ministry of Health and the local ethics committee of Kahramanmaras Sütcü İmam University before the study (Ethics committee date: 26.03.2021 Session: 2021/2022 Protocol no: 185 Decision no: 03).

Author contribution

The authors confirm contribution to the paper as follows: study conception and design: SY, ŞG, AD; data collection: AD, NY, Sİ, UUG; analysis and interpretation of results: ŞG, AD, SY, UUG; draft manuscript preparation: AD, Sİ, NY, ŞG, All authors reviewed the results and approved the final version of the manuscript.

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Conflict of interest

The authors declare that there is no conflict of interest.

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Original Article

Significance of intestinal alkaline phosphatase in predicting histological activity of pediatric inflammatory bowel disease

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ABSTRACT

Background. Intestinal alkaline phosphatase (iAP) is an intestinal brush border enzyme that is one of the factors involved in the pathogenesis of inflammatory bowel disease (IBD). The aim of the study was to investigate the relationship between iAP enzyme and histological inflammatory activity in patients with IBD.

Methods. A total of 44 children were enrolled in this study including IBD patients (n=24; 12 Crohn's disease [CD] and 12 ulcerative colitis [UC]) and controls (n=20). Anti-human iAP antibody stained ileocolonoscopic biopsy specimens were graded for the terminal ileum and each section of the colon. Hematoxylin-eosin stained sections were used to determine inflammatory activity. Histopathological findings were compared in pre- and post-treatment biopsies of each group and with the control group (CG).

Results. A low grade of iAP staining was detected in IBD patients compared to the CG (p=0.02). iAP was remarkably concentrated in the terminal ileum (TI) and especially in region 1, which involved the apical surface, brush border, and epithelial cells. A significant negative correlation was found between the grade of iAP staining and inflammatory activity both in pre- and post-treatment biopsies (p=0.02, p=0.008, respectively) in the terminal ileum of CD patients. Likewise, pre-treatment biopsies of UC and CD patients and biopsies of the CG were compared with each other according to the grade of iAP staining. There were significant negative correlations for CD patients compared to UC and the CG in region1 of TI, and regions 1 and 2 (lamina propria and goblet cells) of the colon (p= 0.015, p= 0.006, p<0.001, respectively).

Conclusions. As a histological marker, iAP can be of value in monitoring the histological activity of IBD, particularly in remarkable inflammation in the small intestine.

Key words: inflammatory bowel disease, intestinal alkaline phosphatase, disease activity.

Alkaline phosphatases (APs), a group of enzymes, are classified into two subtypes; tissue non-specific APs and tissue-specific APs. Tissue-specific APs cover placental, germ cell, and intestinal AP (iAP).[1] IAP is an intestinal brush border enzyme that is expressed on the apical surface of the microvillus of enterocytes and exists in both membrane-bound and soluble forms.[2,3] The soluble form is situated in the area between the microbiota, the food, and the host. iAP has a pivotal role in maintaining intestinal mucosal defense mainly through the detoxification of bacterial endotoxins (e.g. lipopolysaccharide).[4] Other roles of iAP in controlling inflammation are to regulate intestinal bicarbonate secretion, to decrease bacterial translocation and toll-like receptor 4 expression.[4,5]

Inflammatory bowel disease (IBD) is a chronic and relapsing inflammatory disease of the

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gastrointestinal system whose prevalence has increased worldwide particularly in industrialized societies. Even though various interrelated genetic and environmental factors have roles in the development of IBD, the pathogenesis of the disease is not accurately known. iAP enzyme is thought to be one of the factors involved in the pathogenesis. As is already known both iAP expression and activity decrease in inflammatory processes including necrotizing enterocolitis, celiac disease, and IBD.[6-9]

There are limited data on the relationship between iAP and inflammation of IBD within the childhood age group. The aim of the study was to investigate the relationship between iAP enzyme and histological inflammatory activity in patients with IBD.

Material and Methods

The study was conducted at the Pediatric Gastroenterology, Hepatology and Nutrition Division of Hacettepe University. The study protocol was approved by Hacettepe University Non-Interventional Clinical Researches Ethics Committee of the University (study approval number 13/400-20). This was a retrospective, single-center, and non-randomized case-control trial. Twenty-four children (12 males, 12 females) with IBD and twenty children comprising the control group, were enrolled in the study. The diagnosis of IBD was established according to the criteria of Porto.[10] Twelve of the patients (6 male; mean age, 11.5 years) had Crohn's disease (CD) and the other 12 (6 male; mean age, 12.9 years) had ulcerative colitis (UC). Demographic characteristics of patients are summarized in Table I. Twenty children (11 male; mean age, 12.7 years) who underwent an ileocolonoscopy for various symptoms (ie. chronic diarrhea, chronic abdominal pain, and bloody stool) and had normal biopsy results in the histopathological examination were enrolled as the control group.

All patients had received standard bowel preparation before the ileocolonoscopy. A regular colonoscope (Olympus GIF-Q260 video colonoscope) had been used for the examination. One to two biopsies had been taken with forceps from TI and each colonic segment (cecum, ascending, transverse, descending colon, and rectosigmoid colon). In total two ileocolonoscopies were performed on the IBD patients group; one before and one after the treatment. The time interval between pre-treatment endoscopy and post-treatment endoscopy was 6-48 months (median: 12 months). Each patient in the control group underwent only one ileocolonoscopy.

Pediatric Crohn's Disease Activity Index (PCDAI) and Pediatric Ulcerative Colitis Activity Index (PUCAI) were used for all IBD patients to measure the disease activity before and after treatment. The relevance between disease activity indexes (PCDAI and PUCAI) and histological findings were also evaluated. We compared PCDAI and PUCAI with inflammation score and iAP staining for TI and each colonic segment.

Paris Classification was also used for predicting the IBDs course by using the age and growth failure of the patients as well as the location and behavior of the disease.

Biopsies were fixed in formaldehyde and embedded in paraffin. Hematoxylin-eosin (H&E) sections from all biopsies from the terminal ileum and five colon segments were used to evaluate the presence and extent of neutrophilic infiltration, cryptitis, crypt abscess, epithelial erosion, and ulceration, by modified Riley score.[11] Modified Riley score classifies histological activity as; 0; neutrophils in epithelium, none crypts involved; 1, neutrophils in epithelium, <%25 crypts involved; 2, neutrophils in epithelium, ≥ %25-≤ %75 crypts involved; 3, neutrophils in epithelium, >%75 crypts involved; 4, neutrophils in lamina propria, mild but unequivocal increase; 5, neutrophils in lamina propria, moderate increase; 6, neutrophils in lamina propria, marked increase; 7, erosion or ulceration.

For immunohistochemical staining, an anti-human intestinal alkaline phosphatase antibody (1:100 dilution, ab95462; Abcam, Cambridge, MA, USA) was used and the immunohistochemical staining was graded as Grade 0: no staining; Grade 1: 1-25%; Grade 2: 26-50%; Grade 3: 51-74%; Grade 4: ≥ 75% of the tissue section. Since positive iAP staining was observed in different parts of the biopsies, such as the apical surface, epithelia, or lamina propria, this grading system was applied for two localizations: Region 1, composed of apical surface, brush border, and epithelial cells, and Region 2, composed of lamina propria and goblet cells. An experienced, blinded pathologist performed all histological evaluations by using an Olympus microscope.

Biopsies of the IBD patients and the control group were first evaluated individually for each patient and then compared with each other. The grade of H&E-stained sections was compared with the grade of iAP antibody-stained sections for both region 1 and region 2 of TI and each colonic segment. An average grade was attained by dividing the sum of individual colonic segmental scores by the number of colonic segments (cecum, ascending, transverse, descending colon, and rectosigmoid colon) both for H&E and iAP antibody. The colon was included in the statistical analysis as a single data. Finally, pre- and post-treatment biopsies of the patient group were compared with each other according to the grade of staining with H&E and iAP antibodies.

Statistical analysis

The data were analyzed using IBM SPSS software, version 21 (SPSS Inc., Chicago, IL, USA). Statistical significance was assumed when the p-value was <0.05. All results were expressed as median (minimum-maximum). Spearman's rank test was used for the association between quantitative variables. Kruskal-Wallis test was followed by the Mann-Whitney U-test for comparison between groups. Wilcoxon rank-sum test was used to compare pre and post treatment results.

Results

A total of 44 children were analyzed (24 IBD patients, 20 control). Demographic and clinical characteristics of patients are shown in Table I. The patients with CD were admitted mostly with complaints of diarrhea, while the UC patients with bloody stool, and the control group with complaints of abdominal pain.

The patients with CD had neither stricturing and penetrating disease nor perianal disease. All patients, except one with inflammation only in his terminal ileum and cecum, had involvements in the terminal ileum and all colon segments. There was no distal colitis or proctocolitis among the UC patients. Most of the patients had pancolitis (E4) (83.4 %) or extensive disease (E3) (Table I).

In the individual comparison of pre-treatment biopsies of UC and CD patients, and biopsies of the control group we found a statistically significant negative correlation between the grade of H&E-stained sections and iAP antibody-stained sections only in region 1 of TI in CD patients (p-value 0.02, r- value -0.686). In UC patients and the control group, there was no statistically significant correlation between the TI and colon (Table II).

Post- treatment biopsies of each of the IBD patients were evaluated individually and we found a negative correlation (p-value 0.008, r-value -0.747) between the grade of H&E-stained sections and iAP antibody-stained sections in region 1 of TI in CD patients. There was a negative correlation between the grade of H&E-stained sections and iAP antibody-stained sections in the colon of CD patients but it was not statically significant. In UC patients we found no significant correlation between the grade of H&E-stained sections and iAP antibody-stained sections for TI and any colonic segment (Table II).

Pre-treatment biopsies of IBD patients and biopsies of the control group, taken from the same region, were compared with each other. The first comparison was done according to the

Table I. Demographic and baseline characteristics of patients at diagnosis.

	CD (n=12)	UC (n=12)	UC (n=12)
Male/Female	6/6	6/6	11/9
Age at diagnosis (years)			
mean±SD	11.5±4,58	12.9±3,47	12.7±3,37
Paris classification age. n (%)			
A1a	3 (25%)	3 (25%)	
A1b	9 (75%)	9 (75%)	
Paris behaviour, n (%)			
B1	12 (100%)		
Paris location, n (%)			
L1	1 (8.3%)		
L4	11 (91.7%)		
Paris growth delay, (G1), n (%)	3 (25%)		
Paris extent			
E3		2 (16.6%)	
E4		10 (83.4%)	
Paris severity (S1), n (%)		3 (25%)	
Main symptom (%)	Diarrhea (41%)	Bloody stool (91%)	Abdominal pain (85%)
Laboratory findings, mean±SD			
Hb (g/dl)	10.7±1.78	10.5±3.27	13.2±0.96
WBC (x10^3/μl)	13±6.1	11.8±5.4	7.9±2.9
Plt (x10^3/μl)	539±132.1	497±231.7	298±92.7
Alb (g/dl)	4.0±0.63	3.9±0.98	4.7±0.3
ESR (mm/h)	52.2±31.7	26.8±18.5	14.3±17.5
CRP (mg/dl)	7.9±8.44	1.9±2.06	0.9±2.9
Fecal calprotectine (μg/g)	423±264.6	824±814.8	46±122.9 (n=9)

Hb: Hemoglobin, WBC: White blood cell, Plt: Platelet, Alb: Albumin, ESR: Erythrocyte sedimentation rate, CRP: C-Reactive protein, CD: Crohn's disease, UC: Ulcerative colitis

Table II. Grades of pre-treatment and post-treatment ileocolonoscopic biopsy specimens stained for H&E and iAP according to the patient and control groups

		Hematoxylin-Eosin-stained sections		iAP antibody-stained sections			
		T. Ileum Mean (min-max)	Colon Mean (min-max)	T. Ileum Mean (min-max)		Colon Mean (min-max)	
				Region 1	Region 2	Region 1	Region 2
CD	Pre-treatment	4 (0-7)	2.94 (0-7)	1.4 (0-4)	2.02 (0-4)	2.13 (0-4)	2.25 (0-4)
	Post-treatment	2.5(0-7)	2.06(0-7)	1.81(0-4)	2.75(0-4)	2.19(0-4)	2.43(0-4)
	p-values	0.32	0.12	0.32	0.27	0.42	0.34
UC	Pre-treatment	1.3(0-7)	2.95 (0-7)	2.82 (0-4)	2.4 (0-4)	2.44 (0-4)	2.06(0-4)
	Post-treatment	1.27(0-7)	2.65(0-7)	2.87(0-4)	2.49(0-4)	2.48(0-4)	2.26(0-4)
	p-values	1.00	0.31	0.66	0.20	0.37	0.33
Control Group		0.68 (0-2)	1.02 (0-4)	2.84 (0-4)	2.05 (0-4)	2.45 (0-4)	2.8 (0-4)

Intestinal alkaline phosphatase. iAP; CD. Crohn's disease; UC. Ulcerative colitis; region 1. apical surface, brush border, and epithelial cells; region 2. lamina propria and goblet cells.

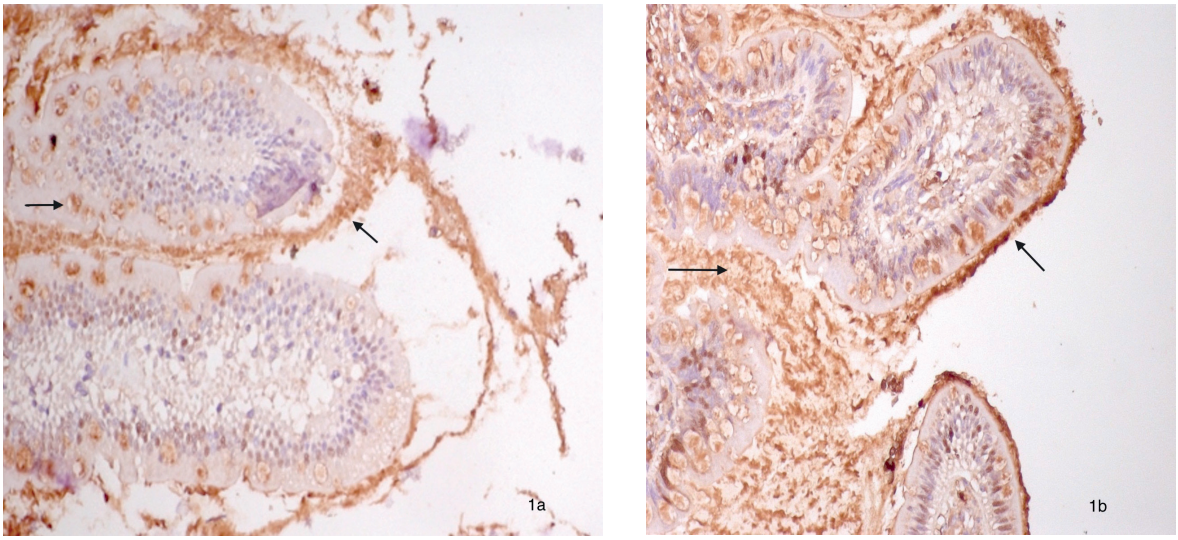

Fig. 1. Pre-treatment TI biopsy of a Crohn Disease patient stained for iAP (1a), low-intensity of iAP staining (arrow); TI biopsy of a control group patient stained for iAP (1b), high-intensity of iAP staining (arrow).

grade of H&E-stained sections. In the control group the grade of H&E-stained sections was lower both in TI and colon compared to IBD patients (p-value 0.005, < 0.001 respectively). Among the IBD patients, we determined that the grade of H&E staining was lower in TI of UC than in the CD patients but it was not statically significant. The second comparison was done according to the grade of iAP antibody-stained sections. In CD patients the grade of iAP staining of region 1 of TI was lower compared to patients with UC and the control group (p-value 0.015) (Fig. 1). We also found a significant difference between IBD patients and the control group, the grade of iAP staining of both region 1 and 2 of the colon were lower in IBD patients (p-value= 0.021, <0.001 respectively) (Fig. 2). This was the result of the low staining grade of CD patients with respect to the control group (p-value 0.006, <0.001 respectively).

Pre- and post-treatment biopsies of IBD patients were evaluated. We found a decrease in histologic grades of all CD (p-value for TI and colon 0.32, 0.12, respectively) and UC (p-value for TI and colon 1.00, 0.31, respectively) patients

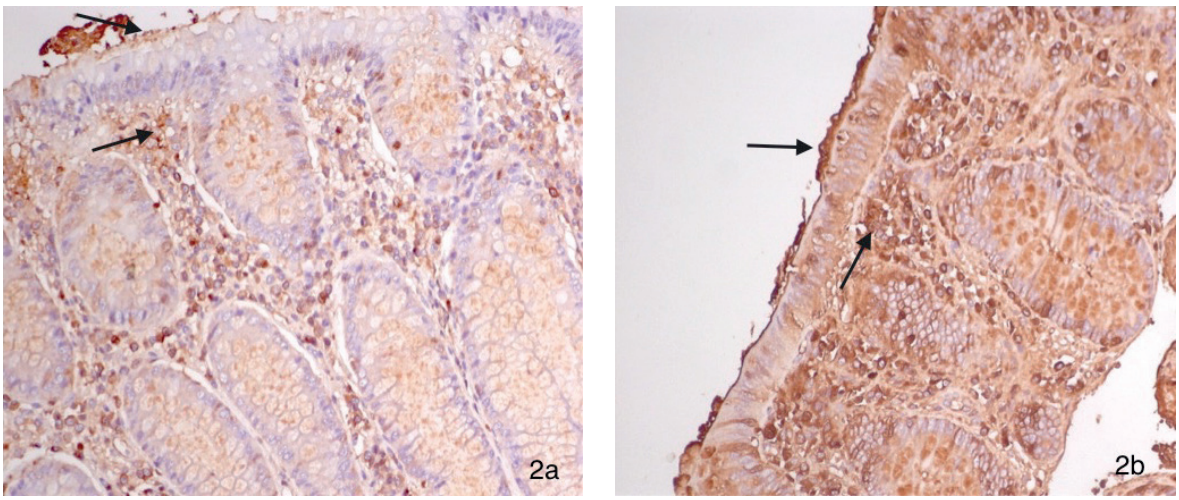

Fig. 2. Pre-treatment colon biopsy of a Crohn Disease patient stained for iAP (2a), low-intensity of iAP staining (arrow); colon biopsy of a control group patient stained for iAP (2b), high-intensity of iAP staining (arrow).

in post-treatment biopsies compared to pre-treatment biopsies (Table II). For TI and colon of CD and UC patients, we found no statistically significant difference neither for H&E nor for iAP staining grade.

We also evaluated the correlation between disease activity indices and histologic activity of IBD patients. IBD activity indices were calculated before and after treatment. Before treatment PCDAI scores were between 15-50 (median; 30), and PUCAI scores 15-75 (median; 42.5); after treatment 0-17.5 (median; 5), 0-55 (median 7.5), respectively. The time interval between pre-treatment endoscopy and post-treatment endoscopy was 6-48 months (median: 12 months). There was a positive correlation between PUCAI / PCDAI and the grade of staining with H&E and a negative correlation between PUCAI / PCDAI and the grade of staining with iAP in TI. However, this was not statistically significant.

Discussion

The interaction between gut microbiota and the innate immune system has a significant effect on intestinal homeostasis. It is thought that dysregulation of the balance between these two systems plays a considerable role in the pathogenesis of the inflammation in IBD and that luminal bacterial products may not trigger the disease but have a role in its progression by stimulating the local process.[8,12] The activation of Toll-like receptor 4 (TLR4) by bacterial lipopolysaccharide contributes to disease progression.

IAP is secreted into the intestinal lumen from the apical and basolateral domain of intestinal epithelial cells in 90-nm-diameter luminal vesicles, which also contain other functional proteins.[9] The physiological role of iAP in the intestine has remained a mystery for decades. As far as is known iAP is one of the major factors of mucosal defense. It attenuates the LPS-mediated inflammation by dephosphorylating and detoxifying lipopolysaccharide (LPS), the toxic cell component of the outer membrane of Gram-negative bacteria, which triggers the innate immune system by activating TLR4.[13,14] It has been shown that iAP reduces the activation of NF-κB by preventing the activation of TLR4, thereby inhibiting the MyD88-dependent inflammatory pathway. Although its pathomechanism is not fully understood, iAP deficiency and its decreased activity are thought to impair intestinal protective mechanisms in IBD patients.[8] In this context, we hypothesize that the more severe the grade of inflammation in IBD patients, the lower the iAP intensity and so the grade of staining with the iAP enzyme.

Previous studies have indicated that iAP is expressed substantially on the apical surface of the enterocytes and iAP enzyme expression is highest in the duodenum and least in the stomach and colon.[2,3,15] The existence and intensity of iAP were evaluated histologically in our study. We demonstrated the existence and the intensity of the enzyme itself with iAP specific antibody. We stained TI and colon specimens with anti-human iAP specific IgG type antibody and found that iAP was present, even in CD and UC patients and in the healthy control group, along the small intestine and colon with varied intensity. We also determined that it was concentrated predominantly in region 1 of TI, which involved the apical surface, brush border, and epithelial cells. The secretion of iAP from enterocytes could explain the more intense concentration of staining in region 1 than in region 2.

Histological assessment of biopsy specimens stained with H&E combined into clinical findings has already been used to predict diagnosis and to evaluate the response to treatment and disease activity. Modified Riley score was used to indicate the severity of inflammation for H&E stained specimens and in the study we used it in the grading of H&E stained specimens, as it is one of the most commonly preferred histological scoring systems of IBD to date.[16] We demonstrated that the grade of H&E-stained sections was positively correlated with the intensity of inflammation, as it was lowest in the healthy mucosa of TI and colon of CG.

Besides this existing histological assessment score, some studies have been reported about the utility of iAP as an additional negative inflammatory marker of the gastrointestinal tract. Some of these studies have reported that iAP is negatively correlated with the degree of inflammation.[3,8,17] Comparing the iAP activity and mRNA levels of iAP in inflamed and non-inflamed mucosa of IBD patients, Tuin et al.[8] found that both iAP activity and mRNA levels of iAP were reduced in inflamed mucosa and that iAP mRNA levels in the ileum were found to be 30 times higher than those in the human colon. Likewise, Molnár et al.[3] demonstrated that iAP protein level in the inflamed mucosa of children with CD and UC was significantly decreased compared to the control group. In the present study, as mentioned above, we evaluate the existence and intensity of iAP in TI and the colon. In the apical surface of TI of the children with CD, we found that as the degree of inflammation increases, the grade of staining with iAP decreases which were compatible with the findings of Molnár and Tuin.[3,8] We also found no significant correlation between the grade of H&E and iAP staining for TI and any colonic segment in UC patients and the control group. Tuin et al.[8] determined that when the epithelial layer within the colon is intact, iAP activity is absent in rats and they hypothesized that iAP only plays a role in the colon after the damage to the intestinal wall. Thus the negative correlation between histologic activity and iAP in TI of CD patients can be caused by the fact that there is intense inflammation in TI in CD and that there is mild or no inflammation in TI of UC patients. However, in the comparison of IBD patients and the control group we did not determine a decrease in the grade of staining with iAP despite obvious inflammation in the colon of UC patients. Following this finding iAP can only provide beneficial results when it is used in the case of both the presence of intense inflammation and the involvement of the small intestine.

In the comparison of pre-and post-treatment biopsies of CD and UC patients, we detected that histological score decreased and iAP staining score increased both in TI and colon in the post-treatment biopsies but this was not statistically significant. This finding can be explained by the fact that mucosal healing after therapies is associated with several variables including sex, presenting symptoms, disease extension, etc.[18] Additionally, mucosal healing lags behind symptomatic improvement depending on the chronicity of inflammation.[19,20]

PCDAI and PUCAI are frequently used in clinical practice because they are noninvasive and easy ways of predicting and monitoring disease activity in contrast with endoscopic procedures. But there are controversies about their accuracy in reflecting disease activity in children. Some reports claim that PCDAI is a utilizable measure of disease activity in children.[21] On the other hand Zubin et al.[22] determine that PCDAI is unreliable for endoscopic disease severity assessment.[23] Our results are consistent with the study of Zubin et al.[22] in that the correlations between both PUCAI / PCDAI and H&E and iAP staining were not statistically significant.

The first limitation of the study is the small sample size; larger-scale studies are required to evaluate the significance of iAP in IBD. There is no data about the alteration of iAP intensity by age in the gastrointestinal tract. Therefore age heterogeneity might reduce the comparability between the patients and the control group. The last limitation is that we were not able to use iAP activity and/or mRNA levels besides the histological intensity of iAP. This would have helped to strengthen the results and add some further information on this topic.

In conclusion, iAP as a histological marker can be of value in monitoring IBD activity, particularly in remarkable inflammation in the small intestine. The utility of iAP in predicting disease activity of the colon is a matter of debate.

Ethical approval

This study was conducted in adherence to the Declaration of Helsinki and approved

by Hacettepe University Non-interventional Clinical Researches Ethics Board with study approval number 13/400-20 and the participation involved informed concent.

Author contribution

The authors confirm contribution to the paper as follows: study conception and design: BBA, INST; data collection: BBA, HHG, BT; analysis and interpretation of results: BBA, EK; draft manuscript preparation: BBA, HD, HO, INST. All authors reviewed the results and approved the final version of the manuscript.

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Conflict of interest

The authors declare that there is no conflict of interest.

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Original Article

Management of thrombocytopenia-associated multiple organ failure: plasma infusion vs plasma exchange

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ABSTRACT

Background. Thrombocytopenia-associated multiple organ failure (TAMOF) causes a high ratio of mortality in pediatric patients. Only anticoagulants and profibrinolytic molecules can be replaced with plasma infusion (PI), while therapeutic plasma exchange (TPE) eliminates antifibrinolytic and thrombogenic molecules and charges inadequate anticoagulants and profibrinolytic molecules. This study aims to compare the efficacy of plasma exchange to plasma infusion in pediatric TAMOF patients.

Methods. Twenty-seven patients with TAMOF were included and the efficacy of PI and TPE was compared. The demographic data, admission laboratory values, Pediatric Logistic Organ Dysfunction (PELOD) scores before the beginning of treatment and PELOD at the end of treatment, and outcomes of groups were compared.

Results. Sixteen children were in the plasma infusion group, eleven children were in the plasma exchange group. The total mortality rate of all patients was 37%. The PELOD scores were significantly reduced on the 5th day of treatment in both groups and also PELOD scores were significantly higher on the 5th day of study in the non-survivor group (p: <0.001). The fifth day of PELOD scores and ferritin had a significant effect on mortality (OR: 1.85, 95% CI: 1.02-2.69; p: 0.04, OR: 1.43, 95% CI: 0.97-2.03; p: 0.05). The overall mortality ratio was not different between TPE and PI groups (p: 0.12).

Conclusions. Although there was no difference in mortality rates in children who received plasma exchange compared to children who received plasma infusion, mechanical ventilation and length of pediatric intensive care unit (PICU) day were shorter in the TPE group. The small patient population may be the major cause for the lack of significant statistical difference.

Key words: thrombocytopenia-associated multiple organ failure, plasma exchange, plasma infusion, pediatric logistic organ dysfunction, pediatric.

Thrombocytopenia-associated multiple organ failure (TAMOF) is a spectrum of microangiopathic disorders related to disseminated microvascular thrombosis such as disseminated intravascular coagulopathy, secondary thrombotic microangiopathy, and thrombotic thrombocytopenic purpura/hemolytic uremic syndrome (TTP/HUS).[1] Exposure to infection, autoimmune diseases, cardiopulmonary bypass, chemotherapy, radiation, and transplantation are the reason for hyper-inflammation that can cause microangiopathic endothelial damage.[2-4] Antifibrinolytic and prothrombotic reactions can occur in the setting of systemic endothelial damage and may cause thrombocytopenia, systemic thrombosis, and multiple organ failure.[5] After the endothelial injury, ultra-large von Willebrand factor (ULVWF) protein clusters are released, under normal conditions, ULVWF is cleaved into smaller and less thrombogenic forms by ADAMTS-13.[6-9] Sepsis-induced many inflammatory mediators inhibit or inactivate a disintegrin and metalloproteinase with thrombospondin motifs (ADAMTS-13), and a deficiency of ADAMTS-13 may cause disseminated platelet-/VWF-rich microthrombi

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that are associated with TAMOF.[10] Therapeutic plasma exchange (TPE) restores ADAMTS-13 and eliminates ULVWF and ADAMTS-13 inhibitors for the reason that it becomes a classic treatment option for patients with TAMOF.[5]

Straat et al.[11] found that plasma infusion increased levels of ADAMTS-13 and was associated with decreased levels of von Willebrand factor in critically ill patients. As a result, increased ADAMTS-13 preserved endothelial condition by increasing the ability to cleave ULVWF.[11] Some patients with atypical HUS or TTP may benefit from plasma infusion therapy.[12] The purpose of plasma infusion is to replace ADAMTS-13 that are inadequate in the affected patient. We preferred plasma infusion (PI) therapy for patients with TAMOF when could not be able to reach central venous access or plasma exchange could not be performed due to some technical limits of exchange equipment. This research aimed to compare the efficacy of plasma exchange to plasma infusion in pediatric patients with TAMOF. We know that a comparison of plasma exchange and plasma infusion efficacy has not been studied previously in patients with TAMOF.

Material and Methods

Patient population

This retrospective study was conducted in the tertiary level institution to assess the clinical process, laboratory values, and patients' outcomes meeting the criteria of TAMOF. The period of study was from February 2020 to March 2022.

This study was approved by the Afyonkarahisar Health Science University Faculty Ethics Committee (date: 03.12.2021, no: 2021/13).

Patients 1 month to 18 years old were included for record if patients met the following criteria within 30 hours of screening: 1) New onset thrombocytopenia as defined by a thrombocyte number less than or equal to 100,000/μL or a minimum 50% reduction in initial thrombocyte number if the initial platelet count was less than or equal to 100,000/μL; 2) new-onset organ failure in at least 2 failing organs of five organ systems defined as an Organ Failure Index score greater than or equal to 3 for less than or equal to 30 hours, histologic and biochemical evidence of a thrombotic microangiopathic pathology; 3) organ failure etiology was due to systemic infection, shock, and chemotherapy.

Exclusion criteria

Children were excluded if they had treatment with any form of plasma exchange procedure or plasma infusion within 30 days before research entry or if a terminal disease was being considered. Informed consent was obtained from the parents of patients before TPE or plasma infusion initiation.

Collection of blood samples

Children were divided into two groups; the plasma infusion group and the plasma exchange group. The selection of therapy between PI and TPE was mainly based on the preference of the physician and the possibility of vascular access. The following data were collected; demographic and clinical data, admitting diagnosis resulting in TAMOF, PICU and hospital length of stay, pediatric risk of mortality score III (PRISM III score), need for mechanical ventilation duration, and renal replacement therapy. Pediatric logistic organ dysfunction (PELOD) scores, vasoactive-inotropic score (VIS), and count of failing organ systems were calculated at the beginning of the research entry and consecutively for 5 days. Laboratory values of hemoglobin, thrombocyte count, creatinine, alanine aminotransferase (ALT), fibrinogen, ferritin, D-dimer, international normalized ratio (INR), lactate dehydrogenase (LDH), and outcome of patients were extracted from files of the patient.

Plasma exchange was performed with a centrifugal cell separator (As-Tec 204, COM TEC, Fresenius, Bad Oeynhausen, Germany). The inlet blood flow rate set ranged between 10–

80 mL/min (minimum rate of 10 mL/min). The total blood/anticoagulant ratio was adjusted according to the patient's weight and total plasma volume. The predicted whole plasma volume was calculated as 80 × kg × (1 − Htc)/100 and 1.5 volume plasma was exchanged for the first day of therapy, continued with one volume each day until × 5 days or thrombocyte count over 100,000/μL whichever was shorter in duration. Before the beginning of TPE, the target thrombocyte count kept to greater than 50,000/mm^3. Patients' vital signs and ionized calcium levels were closely monitored during the procedure. Citrate was used for anticoagulation so ionized calcium levels were monitored closely. Calcium was routinely applied to prevent hypocalcemia symptoms. Each plasma exchange cycle was performed for approximately 2-3 hours.

The volume of infused plasma was 15 mL per kilogram for the first 24 hours of the procedure, followed by 10 mL per kilogram each day after the first day. Plasma infusion was continued until the platelet count was above 100,000/μL. If the plasma infusion caused symptoms of fluid overload diuretics were used.

Statistical Analysis

All variables were analyzed by SPSS Statistics 22 software (IBM, Armonk, NY, USA). The comparison of baseline properties of the two groups was evaluated by the Mann-Whitney U test for quantitative features and with Fisher's exact test for qualitative variables. A p-value of less than 0.05 was considered statistically significant. Two-way analysis of variance (ANOVA) was applied to understand the effects between and within groups in course of time. Parameters were included in multiple logistic regression analyses to identify the independent risk factors of mortality.

Results

Twenty-seven pediatric patients meeting the criteria of TAMOF were enrolled in this retrospective study. Sixteen children were in the plasma infusion group, eleven children were in the plasma exchange group. Sepsis was the predominant admitting diagnosis, followed by respiratory failure secondary to infections of the respiratory system. The research included other reasons for TAMOF, including malignancy, multi-trauma, gastroenteritis, and an inborn error of metabolism presenting with acute crises. All patients needed mechanical ventilator support. Renal failure and fluid overload were managed by renal replacement therapies. Demographics, clinical characteristics, and laboratory data were shown in Table I. The median age was 108 months (IQR, 35) in the TPE group and 96 months (IQR, 47) in the PI group, the TPE group was nonsignificantly older than the PI group (p: 0.42). The male/female ratio of the two groups was similar (p: 0.57). Although the median platelet number was lower, and the mean PELOD score and VIS were higher in the TPE group at admission, there were no differences between groups (p: 0.65; p: 0.21; p: 0.47). While the mean PRISM III score and the number of failing organ systems were lesser in the PI group, these differentiations were not statistically significant (p: 0.09; p: 0.12). Length of PICU and hospital stay were higher in patients who received plasma infusion (p: 0.01; p: 0.01). Having the chronic disease was nonsignificantly higher in the PI group (p: 0.54). PI group had significantly longer mechanical ventilation days than the TPE group (p: 0.04). Patients receiving TPE had nonsignificantly increased creatinine levels, but requiring renal replacement therapy was greater in PI receiving cases (p: 0.46; p: 0.08). The PELOD scores were significantly decreased on the 5th day of treatment when compared to PELOD scores before the beginning of treatment in each group.

The median levels of hemoglobin, platelet, ALT, INR, ferritin, fibrinogen, LDH, and D-dimer levels were similar in the two groups. No patients in TPE and PI groups had any treatment complications. During plasma exchange procedures, ionized calcium levels were closely monitored, and no symptomatic hypocalcemia occurred.

Table I. Demographics, clinical characteristics, and laboratory data of the TPE group and PI group.

Variables	TPE group (n:11)	PI group (n:16)	p value
Age (months)*	108 (35)	96 (47)	0.42
Sex			0.57
Male	6	9	
Female	5	7	
Admission diagnosis			0.89
Sepsis	5	6	
Respiratory Failure	2	4	
Malignancy	2	2	
Multi-trauma	1	2	
Gastroenteritis	-	1	
Inborn error of metabolism	1	1	
Hgb (gr/dL)*	9.7 (3.1)	10.2 (1.28)	0.28
PLT $\times 10^3$ /μL*	76000 (53000)	82000 (77000)	0.65
ALT (U/L)*	205 (114)	168 (73)	0.35
D-dimer (ng/dL)	25.33 ± 7.69	26.3 ± 9.25	0.78
INR	2.26 ± 0.45	2.44 ± 0.43	0.18
Fibrinogen (mg/dL)	141.25 ± 38.17	146.18 ± 37.21	0.34
Ferritin (ng/mL)	963.21 ± 318.94	924.47 ± 234.21	0.23
LDH (U/L)	1753.64 ± 273.22	1725.70 ± 241.16	0.12
Creatinine (mg/dL)*	0.86 (0.15)	0.83 (0.14)	0.46
PELOD score day 1	30.38 ± 2.65	28.54 ± 4.27	0.21
PELOD score day 5	21.62 ± 4.14	22.46 ± 6.35	0.87
PRISM III score	17.41 ± 2.28	16.2 ± 2.61	0.09
Vasoactive-Inotropic score day 1 *	35 (5)	30 (7)	0.47
Number of failing organ systems*	5 (2)	5 (1)	0.12
Requiring renal replacement therapy	5	6	0.08
Mechanical ventilation day*	9 (4.2)	11 (5)	**0.04**
Length of PICU stay	13.6 ± 3.42	18.52 ± 6.15	**0.01**
Length of hospital stay	19.54 ± 4.38	23.64 ± 5.12	**0.01**
Chronic disease	2	4	0.54
Hematologic-oncologic disease	1	-	
Metabolic disease	-	1	
Neurologic disease	-	1	
Respiratory disease	1	2	
Mortality	4	6	0.12

*Median (IQR)
Hgb: hemoglobin, PLT: platelet, ALT: alanine aminotransferase, INR: international normalized ratio, LDH: lactate dehydrogenase, PICU: pediatric intensive care unit, TPE: therapeutic plasma exchange, PELOD: Pediatric Logistic Organ Dysfunction, PRISM III: Pediatric, Risk of Mortality Score III, SD: standard deviation, IQR: interquartile range

The total mortality rate of all patients was 37%, mortality for patients receiving TPE was 36.3% (4/11), and 37.5% (6/16) for patients with PI (p: 0.12). The survival rate of the two groups was similar. Demographics, laboratory data, and clinical characteristics of each group are shown in Table II. There was no difference between the survivor and non-survivor groups in terms of age and gender. The median PELOD scores and PRISM III scores were similar between groups

Table II. Demographics, clinical and laboratory variables of survivors and non-survivors.

Variables	Survivors (n:17)	Non-survivors (n:10)	p value
Age (months)*	38 (62)	43 (51)	0.85
Sex			0.61
Male	10	6	
Female	7	4	
Hgb (gr/dL)*	9.7 (2.6)	10.1 (0.73)	0.75
PLT $\times 10^3$ /μL*	85000 (72000)	82600 (61500)	0.53
ALT (U/L)*	128 (96)	135 (74)	0.32
D-dimer (ng/dL)	24.53 ± 6.74	23.78 ± 7.24	0.87
INR	2.16 ± 0.6	2.33 ± 0.21	0.34
Fibrinogen (mg/dL)	148.62 ± 37.18	160.63 ± 41.50	0.28
Ferritin (ng/mL)	850.40 ± 154.20	1052.14 ± 262.51	**0.01**
Lactate dehydrogenase (U/L)	1740.62 ± 325.41	1742.24 ± 215.62	0.85
Creatinine (mg/dL)*	0.84 (0.22)	0.86 (0.43)	0.54
PELOD score day 1	27.51 ± 65	29.48 ± 7.34	0.16
PELOD score day 5	18.14 ± 3.42	30.64 ± 5.74	**<0.001**
PRISM III score	15.32 ± 2.71	19.38 ± 2.41	0.09
Vasoactive-Inotropic score day 1*	21.7 (16)	35 (8)	**0.03**
Number of failing organ systems*	7 (2)	5 (1)	0.21
Requiring renal replacement therapy	4	7	0.81
Mechanical ventilation day*	10 (2.8)	7 (6.2)	**<0.001**
Length of PICU stay	15.74 ± 4.38	12.71 ± 6.34	0.19
Length of hospital stay	20.41 ± 2.24	17.6 ± 6.1	0.07
Chronic disease	4	2	0.21
Hematologic-oncologic disease	-	1	
Metabolic disease	1	-	
Neurologic disease	1	-	
Respiratory disease	2	1	

*Median (IQR)
Hgb: hemoglobin, PLT: platelet, ALT: alanine aminotransferase, INR: international normalized ratio, LDH: lactate dehydrogenase, PICU: pediatric intensive care unit, PE: therapeutic plasma exchange, PELOD: Pediatric Logistic Organ Dysfunction, PRISM III score: Pediatric Risk of Mortality Score III, SD: standard deviation, IQR: interquartile range

on admission. Both in the survivor's group and non-survivors group, the PELOD scores were significantly decreased on the fifth day of treatments when compared to admission days of PELOD scores however, PELOD scores were significantly higher on the 5th day of TPE in the non-survivor group (p: <0.001). Day of mechanical ventilation was longer in the survivor group (p: <0.001). The length of PICU and number of hospitalization days were not different between the groups (p: 0.19; p: 0.07). Requiring renal replacement therapy and several failing organ systems were not different between survivor and non-survivor groups (p: 0.81; p: 0.21). Although ferritin level was significantly higher in non-survivors, the mean levels of fibrinogen, LDH, INR, D-dimer and median levels of hemoglobin, platelet, alanine aminotransferase and creatinine were not different between groups (p: 0.01; p: 0.28; p: 0.85; p: 0.34; p: 0.87; p: 0.75; p: 0.53; p: 0.32; p: 0.54, respectively). Non performed plasma exchange, ferritin, VIS, mechanical ventilation day, and fifth day of PELOD score were added

to the multivariate logistic regression model via forwarding stepwise technique in Table III. The fifth day of PELOD score and ferritin had a significant effect on mortality (OR: 1.85, 95% CI: 1.02-2.69; p: 0.04, OR: 1.43, 95% CI: 0.97-2.03; p: 0.05, respectively).

Consecutive measurements of the PELOD score in the first 5 days were shown in Fig. 1. Two-factor ANOVA was used to measure the PELOD score differences over time between the two groups. The test for the primary effect of plasma exchange or plasma infusion on PELOD by the time presented a nonsignificant effect (F: 0.623, p: 0.43).

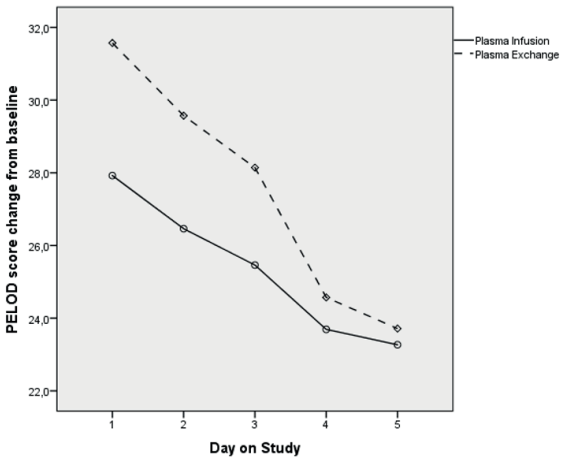

Fig. 1. Pediatric logistic organ dysfunction (PELOD) score comparison of groups who received plasma exchange and receive plasma infusion.

Discussion

TAMOF is a clinical phenotype described by new-onset thrombocytopenia with progression to multiple organ failure. The reason for the decrease in the platelet count is disseminated microvascular thrombosis, which also leads to the deterioration of organ functions.[5] There is currently no standard therapeutic strategy for TAMOF and researchers continue to find efficient medical treatment approaches for clinical phenotype TAMOF. Although plasma exchange is believed to be essential to improve sepsis-induced multi-organ failure syndrome as it restores ADAMTS-13 activity and removes inflammatory mediators, ADAMTS-13 inhibitors, and ULVWF multimers, the American Society of Apheresis guidelines suggests a level C evidence recommendation for the utilization of TPE in sepsis-induced multiple organ failure.[10,13] In addition to TPE, different extracorporeal treatments are used in patients with sepsis or severe sepsis to modulate hyper-inflammation.[14] Some researchers showed that plasma infusion was effective for critically ill patients to increase the ADAMTS-13 activity and decrease the proinflammatory parameters.[11]

The present research is a retrospective clinical study that investigates and compares the effect of TPE and PI on the survival of pediatric patients with TAMOF.

Adult research reported that plasma exchange was superior to plasma infusion for hematological remission and reducing mortality of patients with TTP.[15] The use of TPE was associated with a decreased mortality rate in pediatric patients with TAMOF and secondary hemophagocytic lymphohistiocytosis/macrophage activation syndrome.[16] Sevketoglu et al.[3] previously reported that plasma exchange was more effective than standard medical treatment of sepsis in Turkish children with thrombocytopenia associated with multiple

Table III. Multivariate Logistic Regression Analysis of Survivors vs Non-survivors.

Variables	Odds Ratio	95% Confidence Interval	p value
PELOD score day 5	1.85	1.02-2.69	**0.04**
Ferritin (ng/mL)	1.43	0.97-2.03	**0.05**
Inotrope score day 1	1.12	0.84-1.41	0.42
Mechanical ventilation day	0.76	0.37-1.52	0.38
Receiving plasma infusion	0.62	0.4-1.24	0.62

PELOD: Pediatric Logistic Organ Dysfunction

organ failure. Fortenberry et al.[17] demonstrated that plasma exchange increases survival and improves organ dysfunction. Church et al.[18] hypothesized that plasma infusion is related to increased nosocomial infections, prolonged length of hospital stay, the occurrence of new-onset organ dysfunction, and mortality in critically ill pediatric patients. The use of fresh frozen plasma may unfavorably influence the survival in severe meningococcemia, which was revealed by Busund et al.[19] In contrast to previous data, we found that PI did not have an unfavorable effect on mortality. There was no difference found in the mortality of patients who received PI compared with children receiving TPE.

Some studies found that the PELOD score was not statistically different between plasma exchange (+) and plasma exchange (-) groups in patients with TAMOF at admission, however plasma exchange significantly decreased the PELOD score and facilitated the resolution of organ failures.[3,17] In our study, the baseline PELOD score was similar between TPE (+) and PI (+) groups and the changes from baseline in PELOD scores in the two therapy groups were not different at the end of the study.

Garcia and colleagues showed that high levels of ferritin were associated with increased mortality in children with severe sepsis.[20] Bennett and colleagues showed that an increase in ferritin levels increases mortality.[21] According to Demirkol and colleagues, serum ferritin levels were significantly higher in non-survivors.[16] Similarly, in this present study, higher ferritin levels were associated with mortality.

The use of TPE did not affect PRISM III scores and PELOD scores in patients with TAMOF and secondary hemophagocytic lymphohistiocytosis / macrophage activation syndrome.[14] In contrast, Sevketoglu et al.[3] showed that the baseline PRISM score, PELOD score, and OFI (Organ Failure Index) score of non-survivors were higher in children with TAMOF. In our present study, non-survivors had a higher PELOD score on the fifth day and this factor was independently associated with mortality, however, there were no difference found in the PRISM III score, baseline PELOD score, and several failing organ systems between the TPE and PI groups.

We also found that the mean length of PICU stays, hospital stays and mechanical ventilation days were longer in the PI (+) group than in TPE (+) group. These conditions can be clarified by the fact that ADAMTS-13 activity was restored more rapidly and antifibrinolytic and thrombogenic molecules eliminated the use of plasma exchange treatment and less time was needed for recovery of organ dysfunction.

Our study has some limitations to consider when evaluating. First, this was a retrospective study done with a small patient population which was the most important limitation, results need to be confirmed in an extensive sample group. Second, some coagulation complexes such as ADAMTS-13 activity and ultra-large Von Willebrand factor multimers could not be measured before and after treatments to evaluate the effect on a net complex level.

In a conclusion, although this retrospective study supports the beneficial effect of PI in patients with TAMOF, TPE is still the more effective treatment option. Even though there was no statistical difference in the effect of both treatments on mortality, the duration of mechanical ventilator, intensive care and hospital stay were longer in the plasma infusion group. The main reason for the lack of statistically significant differences may be the small patient population. Various technical reasons limit us in applying some kind of invasive procedures such as plasma exchange, dialysis, etc. Physicians particularly experience such technical problems in patients with low weight so this research will encourage the use of plasma infusion in children with multiorgan failure.

Ethical approval

Approval was obtained from the Afyonkarahisar Health Science University Faculty Ethics Committee (date: 03.12.2021, no: 2021/13).

Author contribution

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

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The authors declare the study received no funding.

Conflict of interest

The authors declare that there is no conflict of interest.

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Original Article

Evaluation of hair structural abnormalities in children with different neurological diseases

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ABSTRACT

Background. Hair microscopy is a fast and effortless diagnostic method for many diseases affecting hair in daily practice. Many diseases can present with hair shaft disorders in pediatric neurology practice.

Methods. Children with pathological hair findings were included in our study. Microscopic evaluation of the hair was performed under light microscopy. The clinical findings, pathological hair shaft findings, laboratory tests, and final diagnosis of the patients were evaluated.

Results. In our study, 16 patients with rare pathological hair findings were identified. Of these 16 patients, nine were diagnosed with giant axonal neuropathy, three with Griscelli syndrome, two with Menkes disease, and two with autosomal recessive woolly hair disease. In hair inspection, curly and tangled hair in patients with giant axonal neuropathy; silvery blond hair in patients with Griscelli syndrome; sparse, coarse, and light-colored hair in patients with Menkes disease; and hypotrichosis in patients with autosomal recessive woolly hair were remarkable findings. Dystrophic hair was detected in most of the patients on light microscopy. In addition, signs of trichorrhexis nodosa, tricoptylosis, and pili torti were found. In particular, pigment deposition in the hair shaft of two patients diagnosed with Griscelli syndrome and pili torti findings in two patients with Menkes disease were the most important findings suggestingthe diagnosis.

Conclusions. Detection of hair findings in the physical examination and performing light microscopic evaluation facilitates the diagnosis of rare diseases accompanied by hair findings. A hair examination should be performed as a part of physical and neurological examinationson eachpatient regardless of thecomplaint.

Key words: hair microscopy, giant axonal neuropathy, Griscelli syndrome, Menkes disease, child.

Hair is a skin appendage that affects the appearance of the person and can be easily examined by a glance in the routine examination. The hair findings can give clues in terms of a child's healthy growth, development, and nutrition, and sometimes they can be the first sign of many congenital or acquired diseases. Examination of the hair structure with a microscope is a simple yet important method in diagnosing many hair-related diseases. There are different types of hair findings in neurogenetic diseases such as giant axonal neuropathy (GAN), Menkes disease, Cockayne syndrome, and Gricelli syndrome (GS).[1]

Anomalies of the hair shaft are divided into two; congenital and acquired. Hair shaft anomalies are also divided into two regarding the presence of hair loss. The first group is hair shaft anomalies that cause hair loss by breaching which are: monilethrix, trichorrhexis nodosa, pseudomonilethrix, trichorrhexis invaginata, pili torti, trichodystrophia, and pili bifurcate. The second group is hair shaft anomalies that do not cause hair breakage or shedding including woolly hair and pili annulati.[1-3]

The normal hair shaft consists of the outer cuticula, the cortex below it, and the innermost medulla. Normally, the hair shaft should be

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thick at the root and slightly thin at the ends, but the hair should be of the same thickness and color throughout its length. Trichorrhexis nodosa is the most common hair shaft anomaly. It is the longitudinal separation of hair in one or more areas along the hair shaft. As the fibers close to the exterior of the hair are separated outward, the hair diameter appears to be enlarged in these areas. These areas appear to be a few nodules along the hair shaft. From this point, the hair breaks easily and the hair is short in clinical evaluations. The ends of the broken hair look like a paintbrush. White dots on the hair that can be seen even with the naked eye indicate these nodules. It is frequently detected in Netherton syndrome, Pollitt syndrome, Tay syndrome, Basex-Dupre-Christol syndrome, Tricho-hepato-enteric syndrome, argininosuccinic aciduria, Menkes disease, Kabuki syndrome, ectodermal dysplasia, and biotin deficiency diseases.[1-3] Trichopitylosis is the fringing of hair fibers at the ends of the hair shaft by separating from each other. It can be seen in healthy people at the ends of long hair. If this is observed at the ends of short hair, many hair shaft anomalies should be considered. The woolly hair has irregular serpentine folds along the hair shaft and often broken hair. This is called Woolly hair nevus, which may be autosomal dominant or autosomal recessive. Woolly hair can also be seen in many genetic diseases such as giant axonal neuropathy. Pili torti is the 180-degree rotation of the hair shaft around its axis; which may cause hair breakage and shedding. Such findings occur in Menkes disease.[1-4]

In this study, the hair findings of 16 pediatric patients who were admitted to our outpatient clinic and were found to have hair structural abnormalities on examinations were evaluated with along their clinical findings. Thus, the important contribution of hair microscopy to the diagnosis of neurological diseases was revealed.

Material and Methods

Patients who applied to Gaziantep University Faculty of Medicine, Pediatric Neurology Outpatient Clinic between August 2020 and September 2021 and who had pathological hair findings in their physical examination were included in this study. During the study period, approximately 9000 patients applied to our outpatient clinic. All of these patients underwent a detailed physical examination. In the physical examination, hair findings were detected by inspection in 46 patients. In 10 of these patients, cosmetic treatment in the form of hair dye was applied to the hair and these patients were not evaluated further. No features were found in the detailed hair examination and hair microscopy of 20 patients. The remaining 16 patients were included in the study. Detailed clinical and laboratory findings of these patients were evaluated and recorded.

Before starting the study, ethical approval was obtained from the hospital's non-interventional clinical research ethics committee (Gaziantep University Non-Interventional Clinical Studies Ethics Committee dated 30.06.2021 and decision no: 2020/430). This study was supported by Gaziantep University Scientific Research Projects Management Unit with the project numbered TF.UT.21.27.

Before the genetic analysis of all patients included in the study, an informed consent form approved by the families of the patients was obtained. Written and signed consent forms were obtained from the patients and their families for the use of photographs.

Hair samples were obtained from the top and lateral regions of patients with common hair differences. In cases with localized hair differences, hair samples were taken from those dissimilar areas. Hair samples were obtained by cutting with scissors. Microscopic evaluation of the hair was made using a light microscope.

The preparations were prepared dry between slides and coverslips. At least 40-50 hair strands were examined from each patient's hair sample. Hair strands were evaluated under the light microscope at X4, X40, and X100 magnifications. Pathological hair structural findings detected in each patient were recorded. The relations between the clinical findings, hair findings, laboratory tests, and final diagnosis of the patients were evaluated.

Results

Totally sixteen patients were included in the study; nine with giant axonal neuropathy, three with Griscelli syndrome, two with Menkes disease, and two with autosomal recessive woolly hair disease.

Patients with Giant Axonal Neuropathy

Of nine patients with giant axonal neuropathy, six (62.5%) were female and three (37.5%) were male, the mean age was 10 years (5-16). All patients had first-degree parental consanguinity.

There was no affected family member except siblings with a similar disease. Patients 1 and 2; 3 and 4; 5 and 6 were siblings. None of the patients had a history of prenatal, natal, or postnatal problems. All of these patients have lived in the South eastern Anatolia Region. In patients 2, 4, and 6, developmental delay was described by the families since infancy. The complaints of all patients started between the ages of 2-4 years. The disease started with gait disturbance in the form of unsteady gait and frequent falls in all patients and the complaints progressed rapidly within a few years. Varying degrees of cerebellar findings were present in all patients. Again, deep tendon reflexes could not be obtained in any of the patients.

Complete blood count, routine biochemical tests, serum vitamin B_{12}, and vitamin E levels, thyroid function tests, serum ammonia and lactate levels, blood-urine amino acids, urinary organic acids, and very long-chain fatty acids were found to be normal in all patients. A lumbar puncture was not performed on any patient. Ophthalmological examination of all patients was normal.

All patients had coarse, dense, curly, and woolly hair on physical examination (Fig. 1 and 2). In the hair microscopy of the patients, the hair strands were circular and compatible with the curly hair (Fig. 3 and 4). Except for patient 2, hair shaft disorders in the form of trichorrhexis nodosa and tricoptylosis were detected (Fig. 4).

The diseases of patients 2, 4, and 6 were severe with no ambulation, strabismus, scoliosis, cavus deformity, and contractures. All three patients had moderate intellectual disability.

Brain magnetic resonance imaging (MRI) was performed in all patients except patient 6. In brain MRI, hyperintense white matter lesions were present in all patients except patients 3 and 7 (Fig. 5 and 6). There was cerebral and cerebellar atrophy in three patients (Patients 2, 8 and 9) and only cerebellar atrophy in two patients (Patients 1 and 5). An asymptomatic pineal gland cyst was detected in patient 7 (Fig. 5). There was a variation of the cavum septum pellucidum in four of the patients (Fig. 5, 6, and 7). The lateral ventricles of patient 2 were found to be asymmetrically dilated (Fig. 5).

Electroencephalogram (EEG) was performed on all patients. Patient 8 was also under follow-

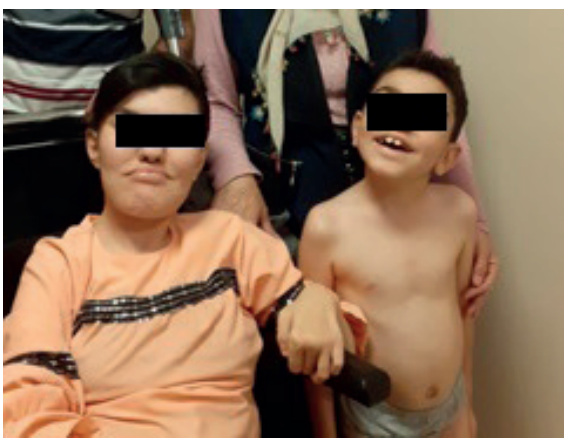

Fig. 1. The appearance of the tangled and curly hair of patients 1 (right) and 2 (left).

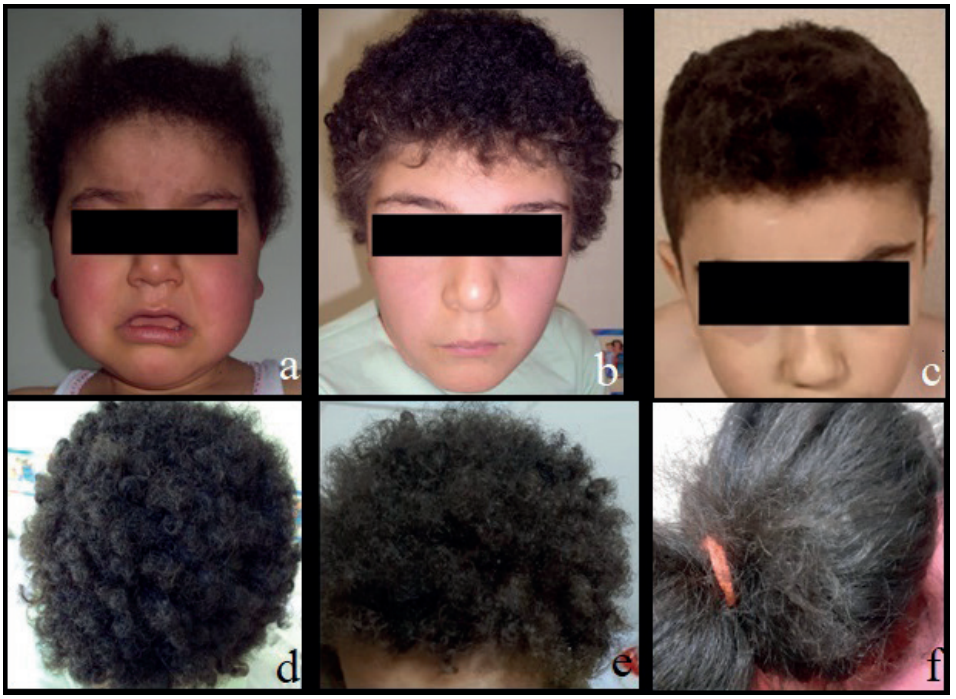

Fig. 2. The appearance of the tangled and curly hair of Patient 3 (a), Patient 4 (b), Patient 5 (c), Patient 6 (d), Patient 7 (e), and Patient 8 (f).

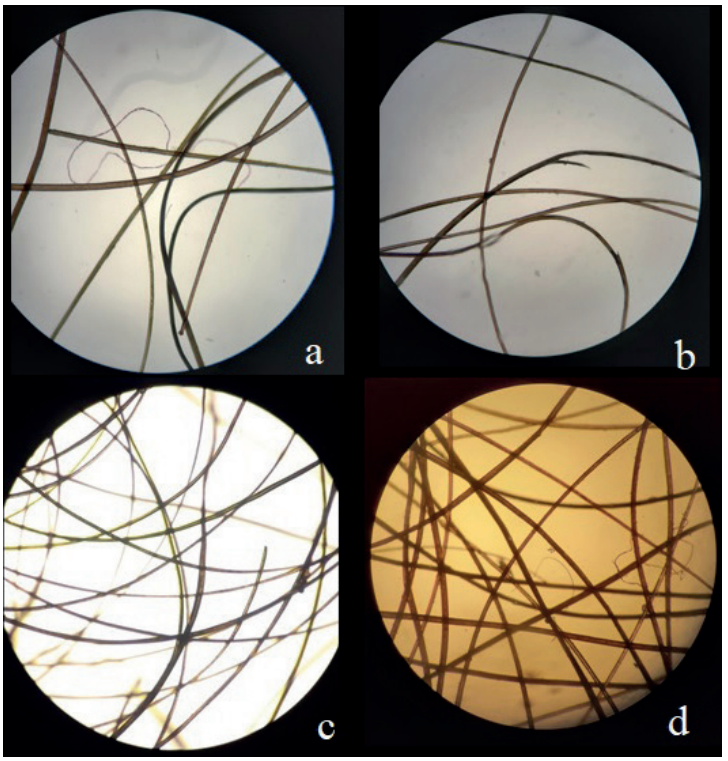

Fig. 3. Light microscopic appearance of hair strands of patient 3 at 4X magnification. The appearance of curly and dystrophic hair strands with different thicknesses (a-d). Sign of trichoptylosis (b).

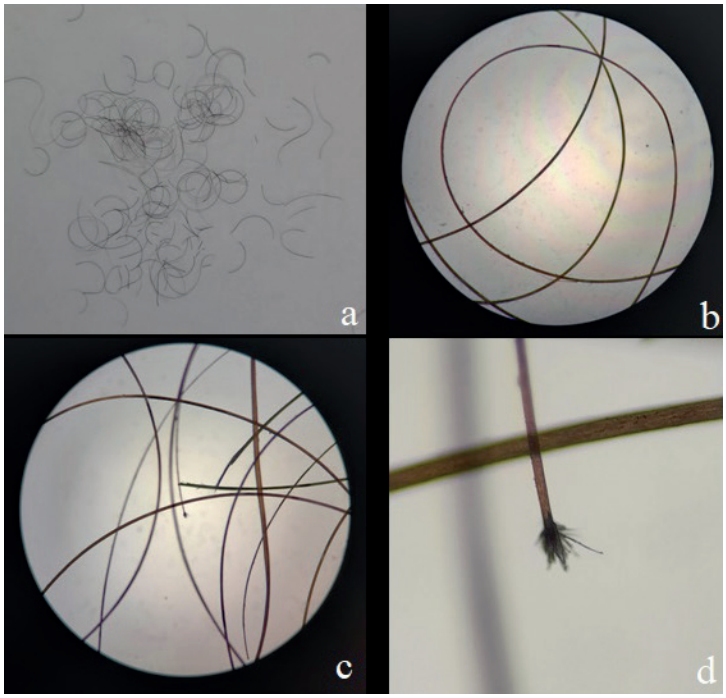

Fig. 4. Curly hair of patient 9 (a), circular hair strands at 4X magnification in microscopic examination (b, c), the appearance of trichorrhexis nodosa sign at 4X (c) and 10X (d) magnifications.

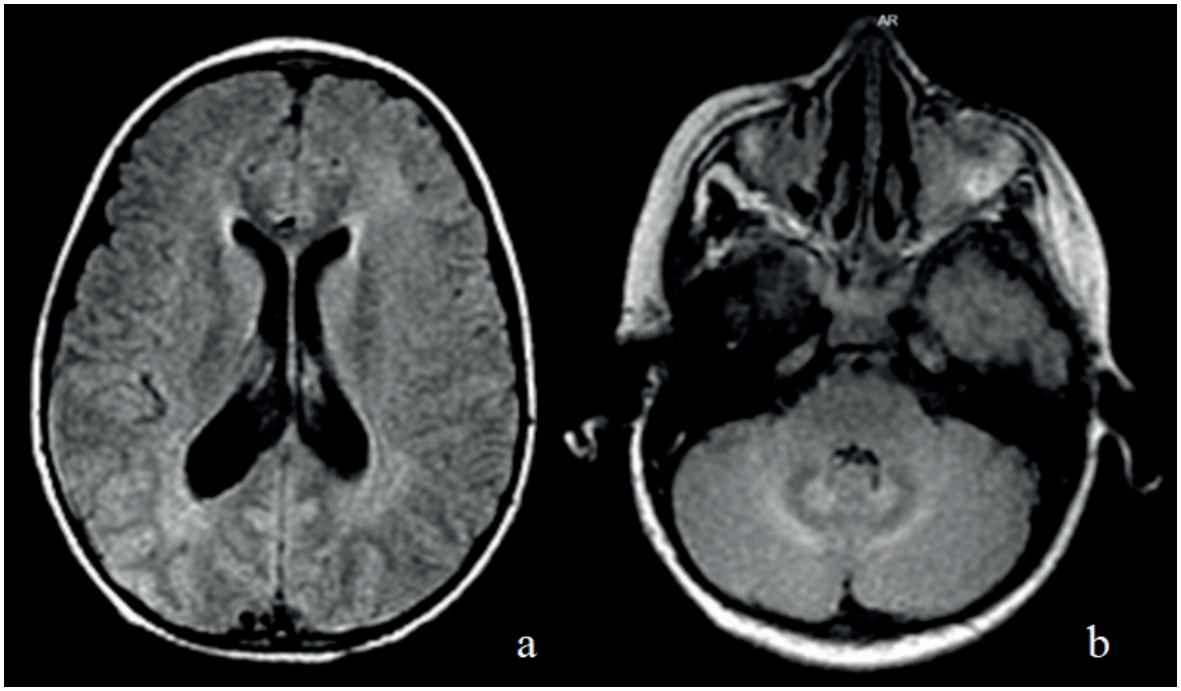

Fig. 5. Asymmetric ventricular dilatation (a) in axial T2-FLAIR sections of patient 2 (a, b), especially in posterior localization, hyperintense periventricular (a), subcortical, cerebellar deep white matter areas (b), and hypointense dentate nuclei (a, b).

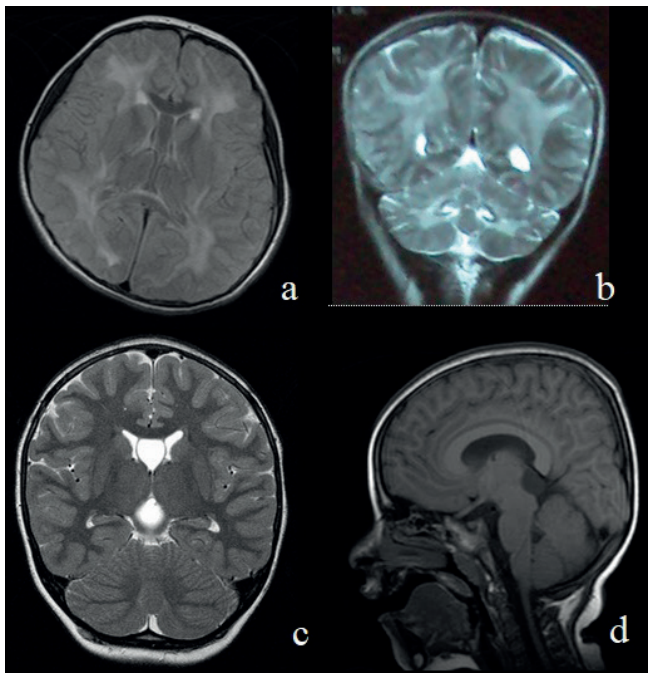

Fig. 6. Patient 3, Cranial MRI appearance of hyperintense areas in the periventricular white matter and variation of the cavum septum pellucidum on T2-FLAIR-weighted axial sections (a). The appearance of hyperintensity in the subcortical white matter areas and dentate nuclei of the cerebrum-cerebellum in the T2-weighted coronal sections of patient 4 (b). The appearance of a cavum septum pellucidum variation (c) in the T2-weighted-coronal section and pineal gland cyst in the pineal gland localization (d) in T1-weighted sagittal sections (d).

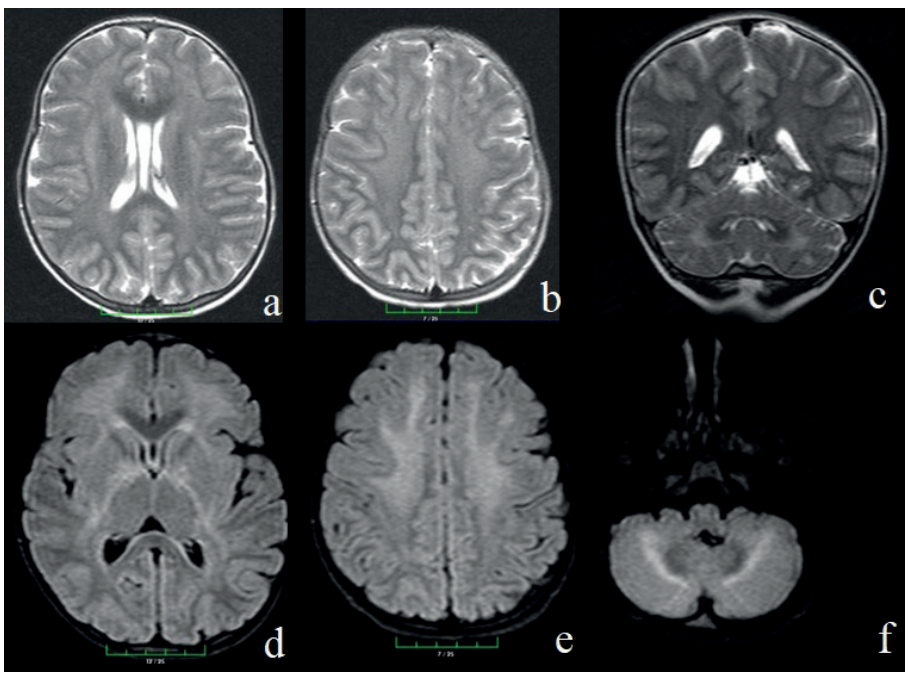

Fig. 7. T2-weighted (a-c) and T2-FLAIR (d-f) axial sections of patient 8 with hyperintense areas in periventricular-subcortical (a, b, d, e) and cerebellar white matter (c, f), internal capsule posterior leg areas and hypointense areas in and dentate nuclei. The appearance of cerebral (a-e) and cerebellar atrophic (c) areas and cavum septum pellucidum variation (a, d) with prominence in occipital and perirolandic areas.

up (for the last 3 years) with the diagnosis of epilepsy. In his EEG, there were bilateral asynchronous parietotemporooccipital sharp waves. Seizure control was achieved with levetiracetam, valproic acid, and clonazepam treatments. Electroencephalogram was unremarkable in other patients.

Electromyography (EMG) examination was performed on all patients. As a result of nerve conduction studies, all patients had sensory-motor neuropathy findings, especially in the axonal weight. Needle EMG was performed in cases 1 and 7, and chronic denervation findings showing partial reinnervation were obtained.

Gene analysis was performed in patients 1, 3, 5, 7, 8, and 9. While homozygous [IVS9 (+1G>T)] was detected in patient 9, c.1502+1 G>T homozygous mutation was detected in the mutation analysis of the other five patients. Heterozygous mutations were found in the parents of these patients.

A nerve biopsy was not performed on any of our patients. The clinical findings of the patients are summarized in Table I. Genetic counseling was given to the families of all patients.

Patients with Griscelli syndrome:

Case 1

A 2.5-year-old boy, was admitted with the complaint of intermittent high fever for about two months. Due to the high fever, he was hospitalized in another center, and treated with antibiotics, but no etiological cause was revealed. He was admitted to our clinic for further evaluation. The patient was consulted with the pediatric neurology department due to developmental delay. On physical examination, his hair, eyebrows, and eyelashes were silvery gray. The patient's skin was rough and dry. There were punctuated hypopigmented areas on the face, arm, and leg skin (Fig. 8a). Height and weight were below 3rd percentile. He had abdominal distention and hepatosplenomegaly. Routine blood tests were normal except for the findings of anemia at the time of admission. Peripheral smear showed findings consistent with hypochromic microcytic anemia. The erythrocyte sedimentation rate was 20. C-reactive protein, coagulation functions, electrolytes, ferritin, uric acid, and kidney and liver functions were within normal limits. Hepatitis A, B, and C, HIV-1, cytomegalovirus (CMV), Ebstein Barr virus (EBV), tuberculosis, salmonella, and mycoplasma serologies were normal. Antinuclear antibody (ANA) and anti-double stranded DNA were negative. The immunoglobulin panel was normal.

Hepatosplenomegaly was detected in the abdominal ultrasonography (USG). Thorax tomography was normal. Bone marrow aspiration was considered normal. Lumbar puncture showed normal cerebrospinal fluid (CSF) opening pressure. No cells were seen, CSF protein was 20 mg/dL, and glucose was 45 mg/dL. Brain MRI could not be performed owing to poor condition of the patient. The patient died on the 21st day of hospitalization due to a severe pulmonary infection. Natural killer cells (NK) activity and CD25 levels of the patient could not be evaluated.

Case 2

Case 2 was the sister of case 1. His 11-year-old sister was hospitalized simultaneously in the same ward due to weakness, fatigue, inability to walk, and poor general condition. In her physical examination, she also had silvery gray hair, eyebrows, and eyelashes (Fig. 8b). The patient's skin was rough and dry. There were punctuated hypopigmented areas on the face, arm, and leg skin. Height and weight percentiles were below the 3 percentiles. Deep tendon reflexes were absent in the lower and upper extremities. She had no organomegaly. There were thenar and hypothenar atrophy of the hands. Neurogenic findings were observed in EMG, there was severe mixed neuropathy, predominantly as sensory-motor axonal

Table I. Clinical data of the patients with giant axonal neuropathy.

Date	Patient 1	Patient 2	Patient 3	Patient 4	Patient 5	Patient 6	Patient 7	Patient 8	Patient 9
Age/Gender	10/M	16/F	10/F	16/F	6/F	15/F	5/M	6/F	6/M
Onset age	2	3	4	4	3	4	3	2	3
The symptoms at onset	Gait disturbance, falling	Gait disturbance	Gait disturbance	Gait disturbance	Gait disturbance, falling	Gait disturbance, falling	Gait disturbance, falling	Gait disturbance	Gait disturbance
Curly and tangled hair	+	+	+	+	+	+	+	+	+
Microscopic findings	TN	-	TN, TP	TN, TP	TN	TN	TN	TN	TN, TP
Intellectual Disability	-	+	-	+	-	+	-	-	-
Scoliosis	+	+	-	+	-	+	-	-	+
Areflexia	+	+	+	+	+	+	+	+	+
Cerebellar findings	+	+	+	+	+	+	+	+	+
Babinski sign	+	-	-	-	+	-	+	-	-
Peripheral neuropathy	+	+	+	+	+	+	+	+	+
Epilepsy	-	-	-	-	-	-	-	+	-
Extremity deformity	-	+	-	+	-	+	-	-	-
Motor function	walks	unable to walk	walks	unable to walk	walks	unable to walk	walks	walks	walks
GAN mutation	+	Not analyzed	+	Not analyzed	+	Not analyzed	+	+	-

GAN: Giant axonal neuropathy, TN: Trichorrhexis nodosa, TP: Trichoptilosis

neuropathy. The patient was followed up in another center for three years with the diagnosis of acute demyelinating encephalomyelitis (ADEM), and steroid and intravenous immune globulin treatments were administered. In the first brain MRI, diffuse involvement was present in the cerebellar and cerebral white matter areas (Fig. 9). Follow up brain MRI performed during her hospitalization which was obtained 3 years after the inital MRI, both cerebral and cerebellar involvement and diffuse atrophy were observed in the white matter areas (Fig. 10). Hair microscopy of both siblings revealed hypopigmentation and pigment deposition (Fig. 8). The case was transferred to the pediatric oncology department for with presumed diagnosis of hemophagocytic lymphohistiocytosis (HLH) with central nervous system involvement. However, the patient died without a definitive diagnosis due to pulmonary infection during follow-up. Both siblings were diagnosed as Griscelli Syndrome based on clinical and laboratory examinations. Genetic testing was not available, yet genetic counseling was provided for the family.

Case 3

A 14-year-old female patient was admitted because of poor school performance. On physical examination, she had silvery gray hair, eyebrows, and eyelashes (Fig. 8c). The patient's skin was rough and dry. There were punctuated hypopigmented areas on the face, arm, and leg skin. In the hair microscopy, clustered melanin granules were seen in the hair shaft (Fig. 8f).

Complete blood count, blood chemistry were normal. Psychometric evaluation revealed mild intellectual disability. The patient was diagnosed with Griscelli syndrome type 1 based on clinical and microscopic findings. In the gene analysis, a homozygous pathogenic variant c.5152C>T (p.Gln1718*) mutation was detected in the *MYO5A* gene, and the clinical diagnosis was confirmed.

Fig. 8. The appearance of silvery blond hair (a-c), eyebrows (a, c), and eyelashes (a) of patients 1 (a), 2 (b), and 3 (c) and punctate and circular hypopigmented lesions on the face of Patient 1. Light microscopic view of abnormal melanin pigment clusters along the hair shaft at X10 and X40 magnifications (d-f).

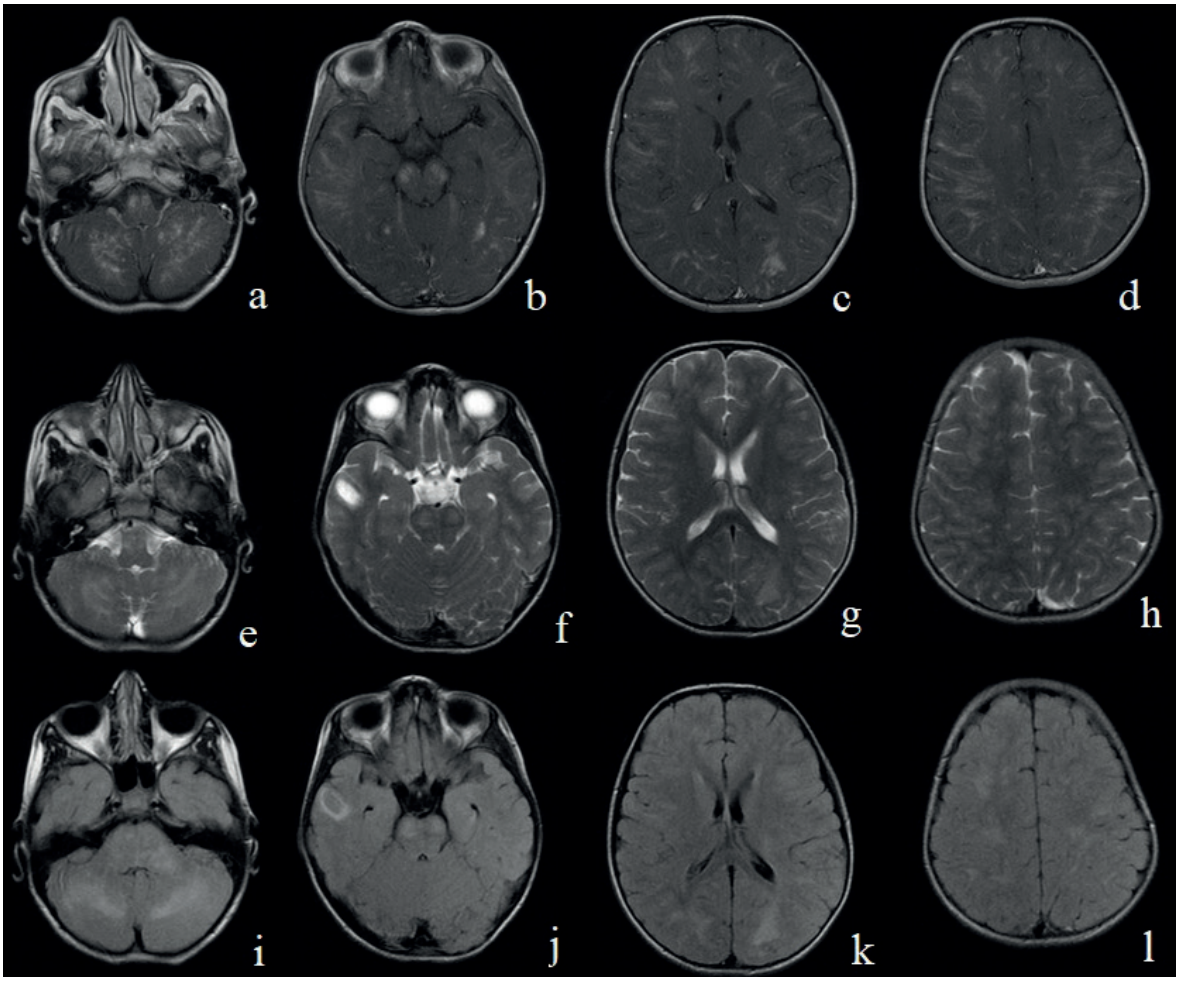

Fig. 9. The appearance of hyperintense areas on subcortical deep white matter areas in the cerebral (b-d, f-h, j-l), cerebellar (a, e, i), brain-stem (a, e), pontocerebellar (i), and mesencephalon tegmentum (b, f, j) on T1-weighted (a-d), T2-weighted (e-h), and T2FLAIR (i-l) sections of axial images of brain magnetic resonance imaging of the patient.

Patients with Menkes Disease:

Two patients were diagnosed with Menkes disease. The first patient was a 15-month-old male with complaints of laxity and developmental delay compared to his peers. His prenatal history was unremarkable. He was born in the hospital with a normal spontaneous delivery with a birth weight of 3250 grams. Family history revealed parental consanguinity. In physical examination; he was restless, and hypoactive. His body weight was 10.1 kg (50th percentile), height was 76 cm (50th percentile), and head circumference was 43 cm (10-25th percentile). He was hypotonic on examination. There was no eye tracking. In laboratory examinations, complete blood count, routine biochemical tests including renal and liver functions, serum ammonia and lactate levels, coagulation parameters, congenital metabolic screening tests, quantitative amino acid levels in the blood, serum Vitamin B12 and biotinidase levels, and urinary organic acid levels were found within normal limits. Toxoplasmosis, cytomegalovirus, rubella, and herpes simplex virus types 1 and 2 serum IgM and IgG tests were unremarkable. Serum copper was <10 μg/dL (85-190 μg/dL) and ceruloplasmin level was 0.07 g/L (0.15-0.48 g/L). Cardiac examination, echocardiography,

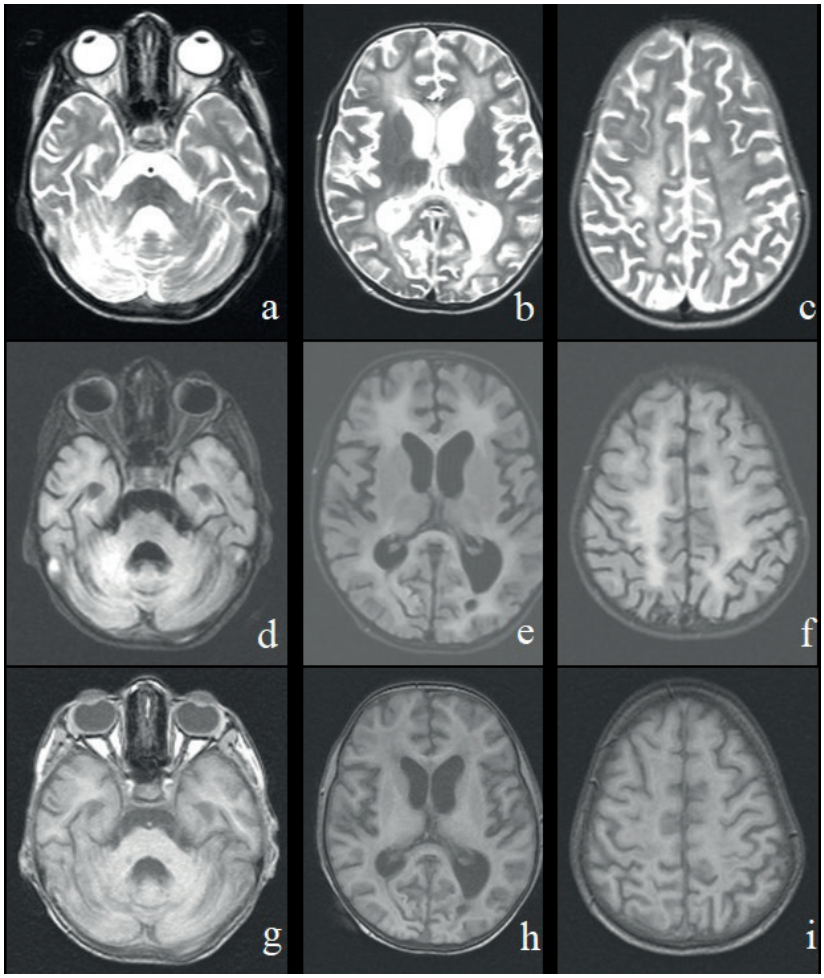

Fig. 10. The appearance of diffuse cortical atrophy in the brain (a-i) and cerebellum (a, d, g) on T2-weighted (a-c), T2-FLAIR (d-f), and T1-weighted (g-i) sections on brain magnetic resonance imaging. The appearance of asymmetric wide lateral ventricles (b, e, h) and enlarge 4th ventricle (a, d, g) secondary to diffuse atrophy. Also, the appearance of diffuse white matter atrophy and hyperintensity in all sections, especially in T2-weighted ones.

and abdominal ultrasonography were found to be normal. Brain MRI revealed atrophy in the brain parenchyma (Fig. 11). In hair examination, his hair was sparse, weak, and light-colored. In the microscopic examination of the hair, trichorrhexis nodosa and pili torti findings were detected (Fig. 11). Gene analysis revealed a homozygous mutation in the ATP7A gene.

The second patient, a 9-month old male patient, was admitted with complaints of weakness in head control and inability to sit with support. He was born after a normal spontaneous delivery at term with a birth weight of 3080 grams. His parents were relatives. His body weight was 7.5 kg (25-50th percentile), and his height was 68 cm (25-50th percentile). On neurological examination; he was restless, hypoactive and hypotonic. Fundus examination was normal. Deep tendon reflexes were hypoactive. In laboratory examinations, complete blood count, routine biochemistry tests including liver and renal function tests, coagulation tests, metabolic screening tests such as serum ammonia and lactate levels, amino acid levels in the blood, serum vitamin B12 level, and biotinidase

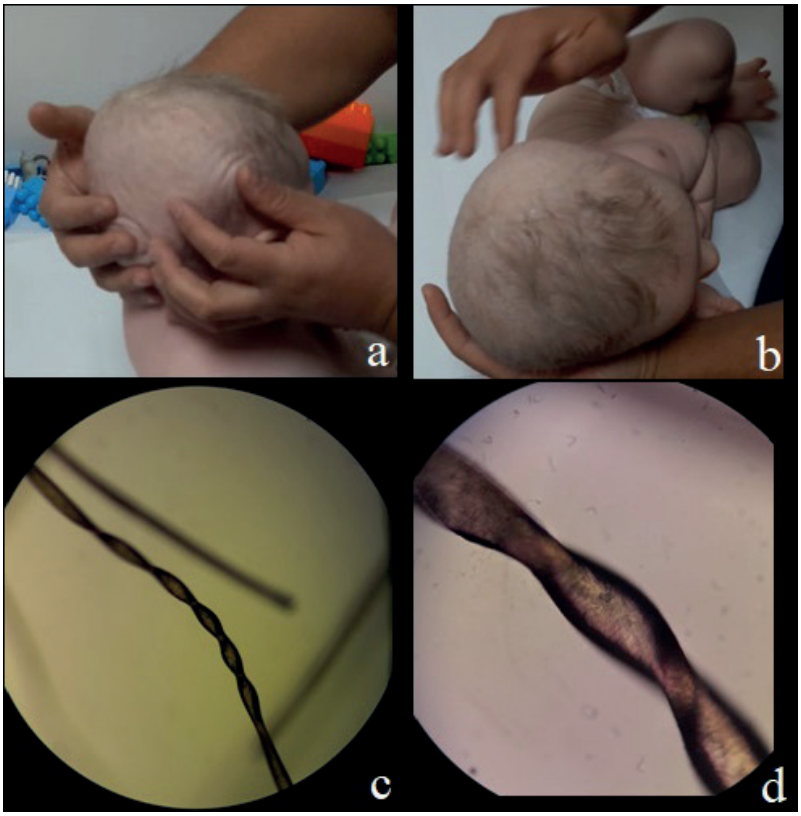

Fig. 11. The appearance of the sparse, weak, and light-colored hair of the patient with the diagnosis of Menkes disease. The appearance of the Pili torti signs at 4X (c) and 10X (d) magnifications on light microscopy (c, d).

activity, and urinary organic acid levels were found within normal limits. Toxoplasmosis, cytomegalovirus, rubella, Epstein-Barr virus, and herpes simplex virus types 1 and 2 serum Ig M and Ig G tests were negative. Serum copper was 36 μg/dL (85-190 μg/dL) and ceruloplasmin level was 0.12 g/L (0.15-0.48 g/L). Brain MRI showed atrophy in the brain parenchyma.

His hair was evaluated because it was light-colored. Pili torti and trichorrhexis nodosa were detected in the microscopic examination of the hair (Fig. 12). A homozygous mutation in the ATP7A gene was detected in the genetic examination.

Patients with autosomal recessive woolly hair:

Two patients were diagnosed with autosomal recessive woolly hair disease. The first patient was a 15-month-old male patient. He has been followed up for febrile seizures. Hair findings had been noticed by his family since he was born. There was no sweating complaint. Family history was negative for similar hair findings. On physical examination, his hair was light colored, brittle, woolly, and sparse. He had long eyelashes and light blond eyebrows (Fig. 13). His nails and teeth were normal. Physical examination was otherwise normal. Brain MRI and EEG were normal. Hair microscopy showed irregular and dysmorphic findings. Trichoptilosis and Trichorrhexis nodosa findings were present (Fig. 13). A whole exome analysis was performed on the patient, and a homozygous *LPAR6* gene mutation (c.373_374delAA) was detected. He was diagnosed with ARWH type 1.

The second patient was a 16-month-old male patient. He has been followed up for

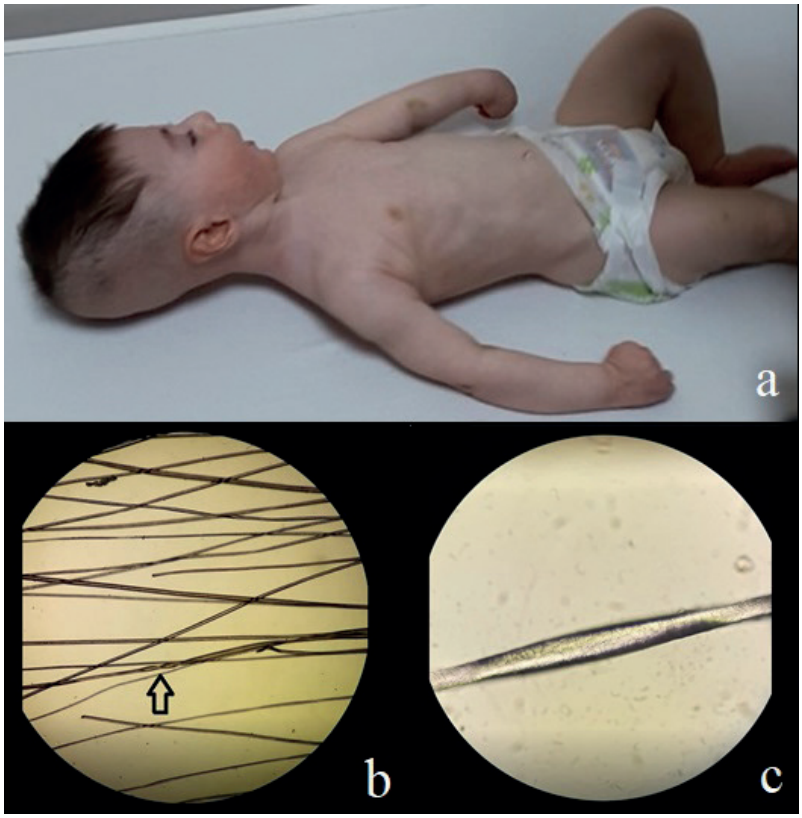

Fig. 12. The appearance of sparse hair on the occipital and parietal regions, but more frequent hair on the upper and front regions, of the patient with the diagnosis of Menkes disease (a). The appearance of hypopigmented, light-colored hair strands, and the Pili torti sign at 4X (c) and 10X (d) magnifications on light microscopy (b, c).

complicated febrile seizures. Hair findings had been noticed by his family since birth. Family history was negative for similar hair findings. On physical examination, his hair was light-colored, fragile, woolly, and sparse (Fig. 14). He had long eyelashes and light blond eyebrows. Nails and teeth were normal. Physical and neurological examinations were otherwise normal. Brain MRI and EEG were normal. Hair microscopy showed irregular and dysmorphic findings. Trichoptilosis and trichorrhexis nodosa findings were present (Fig. 14). In the *LIPH* gene analysis, homozygous missense mutation [c.501C>A, p.(Tyr167*)] was found in the 6th exon of the *LIPH* gene, which was heterozygous in both parents. The patient was diagnosed with ARWH type 2.

Discussion

Giant axonal neuropathy (GAN) is a severe, progressive, and rare autosomal recessive disease affecting the nervous system. Clinical manifestations usually begin around three years of age. Patients have a similar appearance, generally with tangled and curly hair, and neuropathy.[5,6] Life expectancy is usually between 10 and 30 years.[7] The hallmark of GAN in nerve biopsy is axonal swelling due to intermittent neurofilament deposition. Histopathologically, the presence of nodal or internodal axons with segmental enlargement in diameter is diagnostic finding.[8] It is suggested that patients with severe early-onset peripheral motor and sensory neuropathy, distinctly

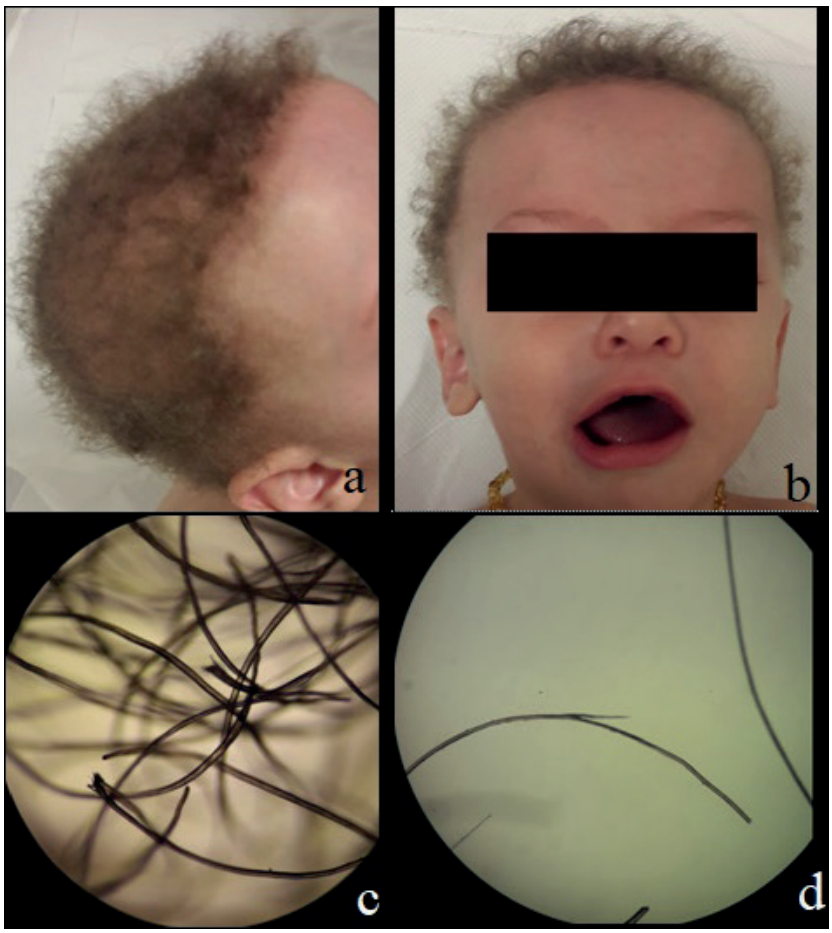

Fig. 13. The appearance of the patient's sparse, weak, fine, and woolly hair that is consistent with hypotrichosis (a, b). The appearance of dystrophic and circular hair strands (c, d), Trichorrhexis nodosa (c), and Trichoptilosis (d) findings at X4 magnification on light microscopy.

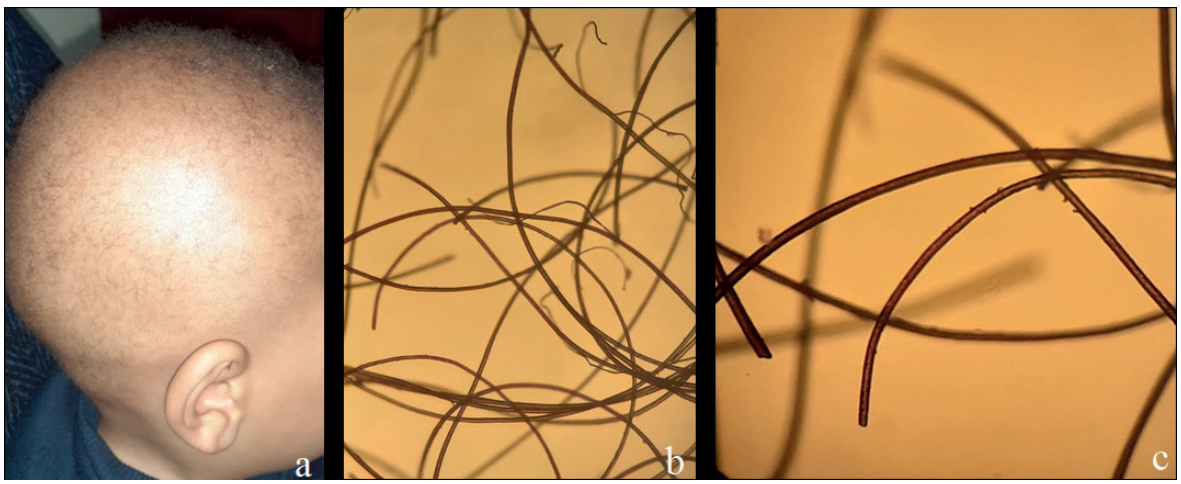

Fig. 14. The appearance of the patient's sparse and woolly hair that is consistent with hypotrichosis (a). The appearance of dystrophic, thin, and curled hair strands of different thicknesses and thickness at X4 magnification on light microscopy (b, c).

dense and curly hair different from parents, intellectual disability, cerebellar, and pyramidal findings should be considered as GAN.[9]

Typical clinical findings of GAN present with both motor and sensory involvement, including sensory dysfunction, clumsiness, weakness, absence of deep tendon reflexes, and marked gait disturbance.[5,9] Affected patients may show symptoms that progress from clumsy gait to marked difficulty with ambulation. In our study, phenotypic findings were consistent with GAN in all of our patients. Symptoms started in the age range of 2-4 years. Besides, a nerve biopsy was not performed because the definitive diagnosis was made with clinical and genetic findings.

Lesions in the CNS are the cause of intellectual disability, epileptic seizures, spasticity, dysmetria, nystagmus, and dysarthria. Scoliosis, ophthalmoplegia, and optic atrophy have been reported less frequently. Cranial nerves, especially the 3rd and 4th nerves, may also be involved.[5,10-13]

In most of the reported cases of GAN, CNS involvement was identified early in the disease.[11] In addition; diffuse demyelination with preservation of U-fibers, atrophy of the brain, brain stem, cerebellum, and corpus callosum can be seen in brain MRI. Most cases are accompanied by the cavum septum pellucidum variations.[14,15] Most of our patients had hyperintense white matter lesions, and varying degrees of brain and cerebellum atrophy, consistent with the literature. Cavum septum variation was detected in four of our patients. A cyst was detected in the pineal gland localization in one of our patients. To our knowledge, this association was not previously reported in the literature. However, Almeida et al.[16] reported the presence of a pituitary cyst in a patient with GAN. In one of our patients, asymmetrical dilatation of lateral ventricles was detecte attributed to cerebral atrophy.

Tangled or curly hair is a characteristic finding in patients with GAN. Also, patients have a characteristic facial appearance with pale skin, a wide forehead, and long eyelashes. Hair findings are usually seen at an early age.[17,18] Wavy, tangled, curly, and coarse hair has been associated with abnormal keratinization. It is thought to develop as a result of the segmental axonal expansion of peripheral nerves due to abnormal neurofilament aggregation.[19] In our study, all patients had similar hair findings. In our patients, the first and the most important finding that attracted our attention was the hair findings, leading to the diagnosis during the initial admission of the patients. Lycklama et al.[20] reported that patients with GAN may have trichorrhexis nodosa on their hair ends. In another study, it is reported that the pili torti sign, frequently seen in Menkes disease, can also be seen in patients with GAN.[21] However, hair strands in Menkes patients may be distinguished from patients with GAN by their lighter color and easy-breaking characteristics.[21] In our study, we found trichorrhexis nodosa in most of our patients, and trichopitylosis in fewer patients, in microscopic evaluation. To our knowledge, the finding of trichoptilosis has not been reported in previous studies. Almeida et al.[16] detected the finding of pili canaliculi as a longitudinal depression on the hair shaft in the electron microscopic evaluation of a pediatric patient.

Central nervous system involvement may cause epileptic seizures and intellectual disability. Intellectual disability usually begins before the age of 10.[7,14] All but three of our patients had normal intelligence.

In the literature, disorganised background activity involving spikes, and paroxysmal activities have been reported in the EEG.[14,22] In our study, only Patient 8 was diagnosed with epilepsy. In his EEG, there were parietotemporooccipital sharp-waves. Seizure control was achieved with levetiracetam, valproic acid, and clonazepam treatments. The GAN gene is localized on chromosome 16q24. This gene encodes the protein gigaxonin and mutations cause the loss of function of this protein.[13,23] In addition, it has been

reported that the disease is not associated with 16q24 in some families. These patients showed slower progression without signs of CNS involvement.[24] C.1502+1 G>T and R293X mutations have been described in previously reported Turkish patients. The geography and some clinical findings of patients with these mutations were found to be similar. The patients with c.1502+1 G>T mutation were from Southeastern Anatolia Region of our country. In addition to the classical findings in patients with this mutation, the association of cavum septum pellucidum variation in brain MRI has been reported.[13,17]

Significant periventricular hyperintense areas were not observed on T2-weighted MRI in patients 3 and 7. Patient 7 had a pineal gland cyst. This finding has never been reported before in the literature in patients with GAN. In the literature, various systemic diseases such as puberty precocs, gastrointestinal diseases such as constipation, reflux, regurgitation, and lactose intolerance, dermatological problems such as ichthyosis and keratosis pilaris, diabetes, and renal tubular acidosis have been reported in patients with GAN.[18] In our study, no other accompanying disease was detected in any of our patients.

Severe early-onset neuropathies [(Charcot-Marie-Tooth disease type (CMT) 2e, CMT4A, CMT4B, CMT4C, CMT4E), Friedreich's ataxia, distal hereditary motor neuropathy], leukodystrophies (metachromatic leukodystrophy and globoid cell leukodystrophy), spinal muscular atrophy, and some rare diseases such as infantile neuroaxonal dystrophy should be considered in the differential diagnosis. Typical giant axons can be seen in some cases of CMT. Other causes of neuropathy such as toxic neuropathy due to smelling glue, n-hexane toxicity, Acrylamide toxicity, and vitamin B_{12} deficiency should also be excluded.[9,18,25] When the CNS is affected, Alexander disease, Fazio-Londe disease, and Menkes disease should be considered in the differential diagnosis.[7] All examinations of our patients were normal and there were no findings suggestive of other genetic neuropathies. Another disease that should be considered in the differential diagnosis is chronic inflammatory polyneuropathy disease (CIPD) with similar clinical findings of neuropathy, progressive progression, and EMG findings.[26]

Griscelli syndrome is a rare autosomal recessive disease with three different subtypes.

Type 1 (*MYO5A*), Type 2 (*RAB27A*), and Type 3 (*MLPH*) develop as a result of related gene mutations. All 3 subtypes have partial albinism affecting the hair and skin. Neurological problems develop in Griscelli syndrome type 1, which is associated with type 2 immune system dysfunction and the development of HLH. Neurological problems such as seizures, and ataxia may develop secondary to CNS involvement. GS3 is presented with dermatological findings.[11,27-29]

Hemophagocytic lymphohistiocytosis is a hyper-inflammation syndrome characterized by fever, hepatosplenomegaly, cytopenia, elevated biomarkers, and sometimes CNS involvement. Primary HLH is associated with genetic defects in the familial HLH genes *UNC13D*, *PRF1*, *STXBP2*, and *STX11*, and the X-linked lymphoproliferative disease genes *SH2D1A* and *XIAP*. The genes involved in pigment transportation such as *NLRC4*, *CDC42* and *LYST*, *RAB27A*, and *AP3B1* genes are also included. All of the proteins encoded by these genes have been involved in lymphocyte cytolytic activity.[11,30,31] RAB27A, a member of the GTPase family, is required for the distribution of pigment-containing melanosomes in melanocytes and the release of cytolytic granules from T cells and natural killer cells. Thus, RAB27A controls these functions through two different cells by interacting with different effective proteins. Primary HLH is usually fatal with a rapid course and the only curative treatment is hematopoietic stem cell transplantation.[11,28-31]

The clinical and neurological findings observed in two siblings with GS were consistent with

the GS phenotype. However, the diagnosis of classical HLH could not be established at the first stage because the routine examinations and bone marrow aspiration findings were normal and the family history did not meet the criteria. However, HLH symptoms and laboratory findings (cytopenia, high CD25, low NK cell activity) appear as the disease progresses. During HLH, CNS involvement may develop at the beginning or at any time of the disease. CNS involvement can be seen as seizure, epilepsy, ataxia, seven nerve paralysis, spasticity, or coma.[32-34] In both patients, the clinical presentation probably developed as CNS HLH and not as typical systemic HLH.

Similarly, in a study, it was reported that HLH may merely affect the CNS.[34] In that study, it was reported that pathogenic mutations of *PRF1*, *RAB27A, UNC13D, LYST*, and *STXBP2* were detected in these patients; and the definitive diagnosis of HLH patients presenting with CNS involvement was established in an average of 28 months.[34] Our case 2 could be evaluated in this regard, approximately three years after the onset of complaints. Since the diagnosis could be established late, there was no chance of treatment.

Treatment regimens for patients with GS are determined depending on the subtypes. In type 1 cases, the treatment is adapted according to the patient's symptoms and clinical presentation. Because type 2 is fatal, it requires bone marrow transplantation. Type 3 does not require any treatment. In a retrospective study, the presence of neurological symptoms before bone marrow transplantation (BMT) in children diagnosed with Griscelli syndrome type 2 indicates a poor prognosis, and the 5- year survival rate was reported to be 50±12.5%.[35] Bone marrow transplantation was not performed in our two patients, who were siblings, because the diagnosis could not be made on time. Both patients died during follow-up. In a similar case with GS, cure was provided with early diagnosis and treatment.[36] Since there were isolated dermatological findings in Case 3, no specific treatment was given.

Menkes disease is a rare X-linked recessive disease that is predominantly affected by the CNS and shows GAN-like hair findings. Copper is a trace element required as a cofactor for many enzymes. The protein that ensures the release of copper from the intracellular environment to the outside of the cell and its transport in the intracellular environment is ATP7A. In the absence of this protein, copper accumulates in the cell and causes the dysfunction of copper- dependent enzymes. Clinical findings in Menkes disease result from dysfunction of copper- dependent enzymes (tyrosinase, cytochrome c oxidase, dopamine beta-hydroxylase, lysyl- oxidase). Patients with Menkes disease show normal development until the first two to three months of their life. Symptoms and signs include hypotonia, feeding difficulties, seizures, dysmorphic facial appearance, and mental and motor retardation, which usually begin in the first months of life. Since these symptoms or signs are not specific, the differential diagnosis includes many chronic neurological diseases.[37] In our study, in both patients, the present findings suggested neurometabolic diseases. Clinical and hair findings of both patients suggested Menkes disease. The diagnosis of Menkes disease is usually made with suspicion in infants with typical neurological changes and hair findings. Hair color is usually white, silvery, or gray. The hair is short, sparse, coarse, shiny, and twisted. Pili torti and monilethrix are usually seen as hair shaft abnormalities. Although rare, patients may have normal hair at birth, but this hair later evolves into light-colored, short, brittle, woolly hair.[37,38] Both patients had light-colored, brittle, woolly hair. Hair microscopy revealed pili torti and trichorrhexis nodosa findings. Light-colored hair and pili torti are not specific to Menkes disease and can also be seen in some other metabolic and hereditary diseases (such as phenylketonuria, trichorrhexis nodosa, and biotin deficiency).[37,38] These diseases were excluded with the family history, clinical, imaging, and laboratory findings of our patients. With cranial imaging, infarcts, cortical atrophy, torsion in intracranial vessels, and hypoplasia

in the cerebellum can be observed.[37] Cerebral atrophy findings were detected in both of our patients. Menkes disease is diagnosed by low serum copper and ceruloplasmin levels. Serum copper and ceruloplasmin levels were found to be low in both of our patients. The definitive diagnosis is made by genetic analysis. Both of our patients had homozygous mutations. There is no effective treatment method for Menkes disease. Symptomatic treatments and genetic counseling for the family are recommended.[37-39]

Isolated autosomal recessive woolly hair/hypotrichosis (ARWH) is a rare genetic hair disorder characterized by tightly curled sparse hair. These patients consist of a genetically heterogeneous group. ARWH type 1, type 2, and type 3 develop as a result of *LPAR6*, *LIPH*, and *KRT25* mutations. The frequency of these mutations varies according to ethnicity and country/geographic location. In the majority of ARWH cases, the *LIPH* mutation has been identified in Pakistan, Japan, and Russia. Loss of LIPH and LPAR6 function resulting from the mutation causes a decrease in the LIPH-LPA-LPAR6 signaling pathway and results in a decrease in the transactivation of EGFR signal. This causes regression of the phenotypic development of the hair. There is no definitive treatment for the disease. In prospective studies, it has been suggested that topical minoxidil application may be a promising treatment in patients due to *LIPH* mutation.[40] Interestingly, both of our patients presented with febrile seizures. To our knowledge, no cases were reported in literature presenting with a neurological complaint. Hair findings were noted in the physical examination which indicated the diagnosis. The first patient was diagnosed with ARWH type 1 with LPAR6 gene mutation, and the other patient with ARWH type 2 due to *LIPH* gene mutation. No problems were encountered in their follow-ups.

In this study, we aimed to establish the importance of light microscopic evaluation of hair, which is a simple and inexpensive examination, in the diagnosis of some rare neurogenetic diseases. Hair examination should be a part of physical examination in pediatric neurology practice. Underlying diseases should be considered in every patient with a local or general abnormality in their hair. The main limitation of this study is the low number of patients with hair findings.

In conclusion, hair shaft disorders have many etiological causes, especially genetic diseases. Since consanguineous marriages are common in the Southeastern Anatolia Region of Turkey, where we practice, autosomal recessive genetic diseases are not uncommon in our routine practice. The diagnosis of genetic diseases require detailed clinical evaluation and phenotyping and relavant genetic tests. A careful clinical evaluation including hair examination may provide hints regarding rare neurogenetic diseases and guide genetic tests.

Ethical approval

The study was carried out following the Declaration of Helsinki of the World Medical Association and approved by the Research Ethics Committee of Gaziantep University (Gaziantep University Non-Interventional Clinical Studies Ethics Committee dated 30.06.2021 and decision no: 2020/430).

Author contribution

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

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Conflict of interest

The authors declare that there is no conflict of interest.

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Original Article

Cerebral developmental venous anomalies in children with mismatch repair deficiency

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ABSTRACT

Background. Constitutional mismatch repair deficiency (CMMRD) is one of the rare cancer predisposition syndromes. The aim of this study was to evaluate the cerebral developmental venous anomalies in children with central nervous system tumors associated with CMMRD, an area in which there is extremely little experience.

Methods. Data from children diagnosed with medulloblastoma and high grade central nervous sytem tumor were retrospectively collected. According to the European CMMRD criteria, nine patients were diagnosed as CMMRD syndrome and the others consisted of the group without CMMRD. All radiological examinations of these children were retrospectively reviewed. Whole exome sequencing was performed to index cases' germline DNA.

Results. Nine children from four families, six females and three males, were studied. The median age at the first tumor diagnosis was 4.5 years (range, 9 months to 14 years). All CMMRD patients had café au lait spots, but none fulfilled the diagnostic criteria for neurofibromatosis. The patients developed high-grade glial tumor (n: 7) and medulloblastoma (n: 2). The affected genes in the three families were *MSH6* [c.478C>T (p.Gln160Ter)], *MSH6* [c.2871dupC (p.Phe958LeufsTer5)] and *MLH1* [c.236G>A(p.Arg79Lys)], respectively. Seven patients had multiple developmental venous anomalies; six patients had leptomeningeal enhancement; and five patients had cavernomas. None of these findings were present in the group without CMMRD.

Conclusions. Constitutional mismatch repair deficiency should be considered when multiple developmental venous anomalies, cavernomas, and leptomeningeal enhancement are detected, especially in patients with café au lait spots.

Key words: constitutional mismatch repair deficiency, central nervous system tumor, developmental venous anomalies, leptomeningeal enhancement, cavernomas.

In developed countries, central nervous system (CNS) tumors are the second most common malignancy in childhood. Recently, genetic syndromes associated with these tumors have attracted interest. The most frequent syndromes are familial adenomatous polyposis, hereditary nonpolyposis colorectal cancer (HNPCC), constitutional mismatch repair deficiency (CMMRD), Li–Fraumeni syndrome, Gorlin syndrome, neurofibromatosis types 1 and 2, tuberous sclerosis complex, and von Hippel–Lindau disease.[1-3]

Constitutional mismatch repair deficiency which has been known for two decadesis a rare autosomal recessive cancer predisposition syndrome.[4] The studies and experiences on

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this subject are increasing daily. The related mismatch repair (MMR) genes are *MLH1*, *MSH6*, *MSH2*, and *PMS2*. Monoallelic mutations are associated with Lynch syndrome (or HNPCC), whereas biallelic mutations are associated with CMMRD. The characteristic features in in this syndrome includes early childhood onset of some malignant diseases, especially hematological and CNS malignacies, and findings similar to cutaneous features of neurofibromatosis type 1 (café au lait spots).[5-7] Due to the rarity of CMMRD, there is little experience in radiological findings of pediatric CNS tumors associated with CMMRD. A scoring system including malignancy or premalignant lesions, and additional features (such as skin alterations, diagnosis of Lynch syndrome and carcinoma from Lynch syndrome spectrum in relatives, pilomatricoma(s) in the patient, parental consanguinity and deficiency/ decreased levels of IgG2/4 and/or IgA) is used for the diagnosis of CMMRD in the European Union (EU)-Consortium 'Care for CMMRD' (C4CMMD) recommendations. The consortium suggests that if the score is three and above, CMMRD should be suspected.[6]

In neuroimaging of children with CMMRD, it has been reported that developmental venous anomaly (DVA), spontaneous cavernoma (CA) and corpus callosum agenesis can be observed in addition to central nervous system tumors (especially high grade glial tumors).[6-11] The relationship between CNS tumors and DVAs was investigated, and the prevalence of DVAs in patients with CNS tumors was found to be higher than in the control groups.[8] Recently, Shiran et al.[9] reported multiple DVAs in patients with CMMRD; all the patients had at least two DVAs in the brain, and none of them had any clinical signs of bleeding. The authors did not detect a relationship between brain tumor location and DVA locations. In addition, no new DVAs developed during patients' follow-up. Subsequently, two similar articles were reported with venous anomalies and malformations in CMMRD.[10,11]

The aim of this study was to evaluate the cerebral vascular malformations including DVA(s) and CA(s), and additional neuroimaging findings, if any, in children with CMMRD, an area in which there is little experience.

Material and Methods

Local institutional review board approval was obtained (Selcuk University, Dec 30, 2020, No: 2020/26). The written consent forms were obtained from the guardians of all participants. The principles outlined in the Declaration of Helsinki were followed.

Between 2006 and 2018, the oncological charts and neuroimaging of all patients with high-grade glial tumors (n=17) and medulloblastoma (n=50) were retrospectively analyzed, fulfilling a score above three according to the EU-Consortium C4CMMRD. Inclusion criteria were as follows: 1) Patients under 18 years of age, 2) Histopathologically proven diagnosis of high grade glial tumor or medulloblastoma according to WHO 2016 classification[12] 3) A score above or equal to three according to the EU-Consortium C4CMMRD criteria; and 4) Having magnetic resonance imaging (MRI) study before surgery. Exclusion criteria was presence of cancer predisposition syndromes other than CMMRD. According to the EU-Consortium C4CMMRD criteria, nine patients had a score above or equal to three and these patients were classified as e "probable CMMRD group". Other patients with a score less than three were classified as the group without CMMRD. In the probable CMMRD group, genetic studies of six patients were completed and the diagnosis of CMMRD was confirmed. Unfortunately, the genetic studies of the remaining three patients are yet to be completed.

Whole-exome sequencing (WES) was performed for genetic diagnosis using NimbleGen liquid-phase arrays and the Illumina Hi-Seq 2000 instrument on DNA obtained from the index cases' blood from the first, second and third families as described previously.[13,14]

Although all MRI studies were not performed

atour hospital, all patients had MRI with different pulse sequences. All patients, except one, underwent axial susceptibility-weighted imaging (SWI) or gradient echo imaging. In our hospital for such patients, we prepared a scanning protocol composed of axial T1-weighted imaging, T2-weighted imaging, SWI, fat saturation (FATSAT)-FLAIR imaging, sagittal T1-weighted imaging, coronal FATSAT-FLAIR imaging, and postcontrast axial T1-weighted and 3D T1-weighted imaging.

In all of these patients MRI studies were retrospectively reviewed by the same radiologist. In addition to CNS tumors, the presence or absence of other findings, such as CAs, cerebral DVAs, and leptomeningeal enhancement (LME), were evaluated. If these patients' parents underwent cranial MRI, the images were also re-examined accordingly.

In MRI, CA was defined as a popcorn or berry-like appearance and loss of environmental signal due to the surrounding hemosiderin. In addition, it had been kept in mind that in T1 and T2 images, there might be a signal change depending on the age of the blood components within the CA. The numbers and diameters of the CAs were recorded.

Cavernomas, cavernous hemangiomas, and cerebral cavernous malformations are a cluster of abnormal blood vessels, and they were more prominent on SWI sequence than gradient echo images. They have a typical popcorn-shaped appearance. Although it is difficult to differentiate small CAs from small bleeding and postradiation angiopathy-related bleeding, there was no predisposition to hemorrhage in our cases, and CAs were detected before radiotherapy. Another hallmark is the outer border, CAs tend to be more irregular with some interior heterogeneous bubbly signals. A hemorrhage is shown as a more homogenous dark signal and rather regular borders than CAs.

Having a diffuse or focal gyriform or serpentine enhancement has been defined as LME. The images were carefully examined, especially since they should be distinguished from veins.

Statistical analysis

Statistical Package for the Social Sciences version 15 software (SPSS Inc., Illinois, USA) was used for all statistical analyses. For nominal data, percentage values were given. Since the data were not normally distributed, the median values (minimum-maximum values) were used for numerical variables. The Mann-Whitney *U* test was used to compare the variables of the independent groups without normally distributed data. In order to compare the qualitative variables, Fisher's exact test was used, and the alpha (*p) values* < .05 was considered to be statistically significant.

Results

According to the EU-Consortium C4CMMRD, nine patients had a score above or equal to threethree and these patients were included in the probable CMMRD group. The demographic data of the patients with and without CMMRD are seen in Table I.

Patient characteristics

In patients with CMMRD, there were six females and three males with a median age of 4.5 years at the time of diagnosis of the first tumor (range, nine months to 14 years). All CMMRD patients had *café au lait* spots, but none fulfilled the neurofibromatosis diagnostic criteria. In patients with CMMRD, it was observed that the CNS tumor occurred statistically at an earlier age (Table I).

The first family previously reported by our group[14], and had pathogenic homozygous c.478C>T p.(Gln160*) mutations in *MSH6* (NM_000179.3) gene. The family had three children and all three were affected and detected mutation was segregated within the family (Fig. 1A). High grade glial tumors developed in all children of this family. The second child with spinal cord anaplastic astrocytoma developed

Table I. Comparison of the central nervous system tumors patients with and without constitutional mismatch repair deficiency.

	The patients without CMMRD (n: 48)	The patients with CMMRD (n: 9)	p
Age years (minimum-maximum)	9.5 (0.3-18)	4.5 (0.75-14)	.041
Gender			ns
Male, (%)	29 (60.4 %)	3 (33.3 %)	
Female, (%)	19 (39.6 %)	6 (66.7 %)	
Radiological findings			
Developmental venous anomalies, (%)	0 (0%)	7 (77.8 %)	< .0001
Cavernoma, (%)	0 (0%)	5 (62.5%)	< .0001
Leptomeningeal enhancement (%)	0 (0%)	6 (66.7 %)	< .0001

CMMRD: constitutional mismatch repair deficiency, ns: not significant

a CNS tumor approximately four years later. However, the family refused any surgical intervention. Radiotherapy and chemotherapy were -administered to the patient for the treatment of the CNS tumor. In the sixth month of this treatment, the patient developed acute myeloid leukemia.

Pathogenic c.2871dupC p.(Phe958Leufs*5) mutation in *MSH6* (NM_000179) gene is a well-known pathogenic variant (National Center for Biotechnology Information. ClinVar; [VCV000089364.24], https://www.ncbi.nlm.nih.gov/clinvar/variation/VCV000089364.24 accessed on Sept. 18, 2021) and was found to be homozygous state in both affected siblings in the second family. The first child of this family was diagnosed with mixed hepatoblastoma at the age of nine months and glioblastoma at the age of four years. The second child of this family developed pre-B cell acute lymphoblastic leukemia (cranial radiotherapy was used for CNS prophylaxis) at the age of seven years, and glioblastoma at the age of 10 years.

In the third family, c.236G>A p.(Arg79Lys) mutation, a rare, novel, heterozygous mutation, was identified in *MLH1* (NM_000249.3) gene and glioblastoma developed in a seven years old child.

In the fourth family (Fig. 2A), mothers of patient 7 and patient 8 are siblings and their fathers are siblings as well. In addition, the father of patient 9 is cousins with the mothers of patient 7 and patient 8. Patient 7 was diagnosed as medulloblastoma at the age of three years and acute lymphoblastic leukemia at the age of 4.5 years. Patient 8 was diagnosed as medulloblastoma at the age of 4.5 years. Unfortunately, relapse developed at the age of seven years. The patient was operated on again, and was diagnosed with glioblastoma by pathological examination. The patient, then progressed rapidly, died in a short period of time. In patient 9, gross total resection of the lesion was performed and after pathologic examination, anaplastic oligodendroglioma was diagnosed.

The genetic analysis of the fourth family has not been performed.

Radiological findings

The comparison of radiological findings of CNS tumor patients with and without CMMRD is summarized in Table I. Developmental venous anomalies, CAs, and LMEs, which were seen in cranial MRI in addition to CNS tumors of CMMRD patients, were not seen in in the group without CMMRD.

The cranial MRI of some patients with CMMRD are shown in Fig. 1B and Fig. 2B. In addition to CNS tumors, multiple DVAs were detected in different parts of the brain in seven of nine patients; six patients had diffuse LME, and five patients had CA (Table II).

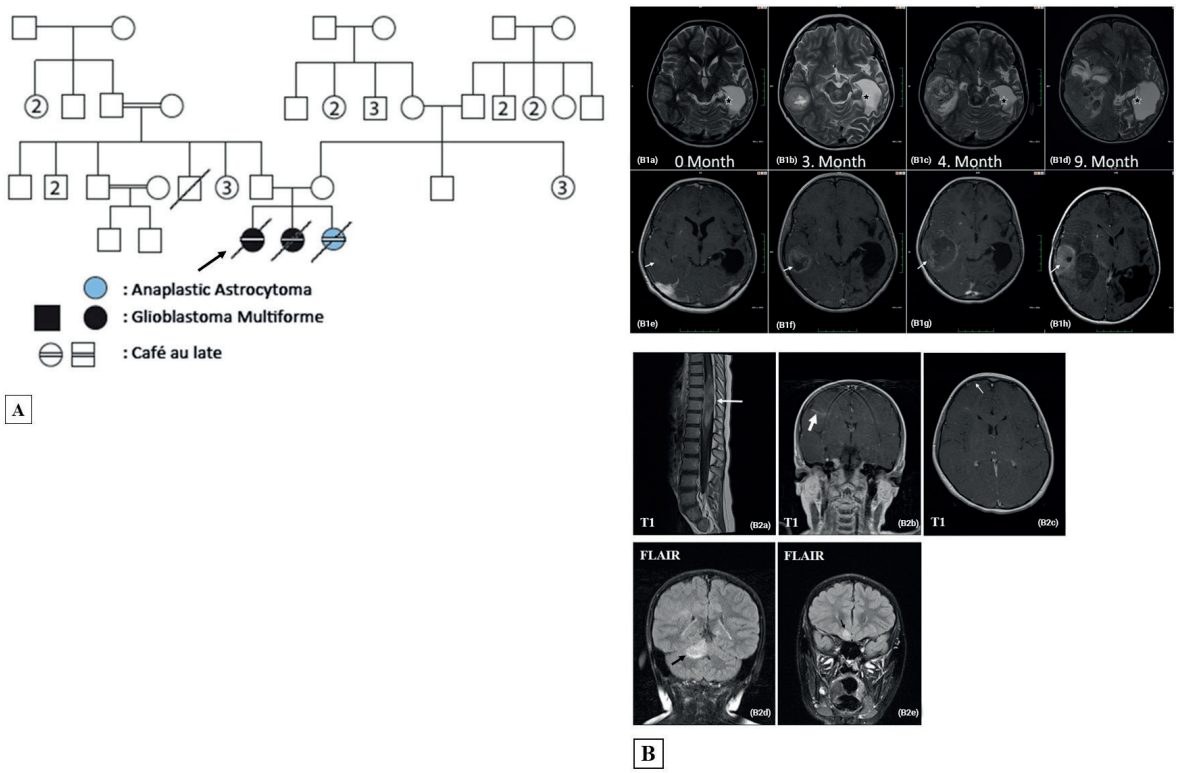

Fig. 1. A: The pedigree of the first family. B1) An 8-year-old female patient with a history of an operation for a left-side glioblastoma (star). During follow-up, she developed a symmetric mass on the right side. During the 0, 3, 4, and 9-month follow-ups (arrow) (B1a-d: T2 weighted imagines, and B1e-h: post-contrast T1 weighted imagines) (The first patient). B2) When the patient was three years old, she was diagnosed with spinal cord anaplastic astrocytoma (long white arrow). Developmental venous anomaly (thick white arrow), leptomeningeal enhancement (thin white arrow). The patient developed masses in the frontal lobe and cerebellum when she was seven years old (black arrows) (B2a: sagittal T1 weighted imaging, B2b: post-contrast coronal T1 weighted imaging, B2c: post-contrast T1 weighted imaging, and B2d-e: coronal FLAIR weighted imagines). (The second patient).

In patients with a cerebral DVA, the number of developmental vascular malformations ranged from 3 to 20 (median, 6). The diameter of the largest DVAs ranged from 1.5 to 3 mm (median, 2 mm) (Table II). We did not find any relationship between the location of CNS tumors and DVAs. Deep venous anomalies were detected in other areas besides the tumor.

In patients with CAs, the number of CAs ranged between 2 and 20 (median, 3), and the largest diameter of CAs ranged from 4 to 20 mm (median, 9 mm) (Table II). No bleeding was observed in these CAs at the time of diagnosis. In one patient, CAs could not be evaluated as SWI sequences were not obtained.

Two patients did not have any other additional imaging abnormality.

There was no evidence of seeding on cerebrospinal fluid sampling of the patients with medulloblastoma and anaplastic oligodendroglioma, also, the LME was always stable during followup neuroimaging studies.

Neither DVAs, CA, nor LME were observed on neuroimaging studies of parents who had cranial MRI (Table II).

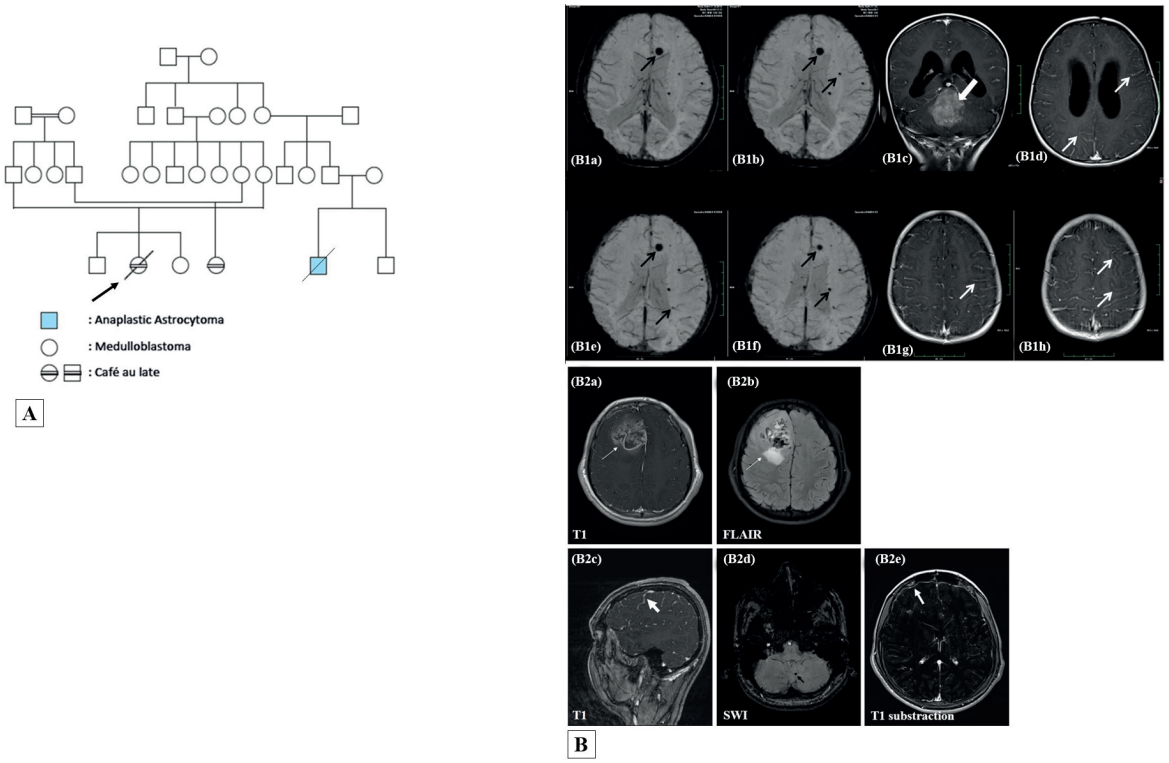

Fig. 2. A: The pedigree of the 7th, 8th, and 9th patients. B1) A 3-year-old female patient had medulloblastoma in the posterior fossa (thick white arrow), diffuse –leptomenengeal enhancement (thin white arrows), and multiple cavernaomas (thin black arrows) (3a-b: axial SWI imagines, 3c: post-contrast T1 weighted imaging, 3d: post-contrast axial T1 weighted imaging, 3e-f: axial SWI imagines, and 3g-h: post-contrast T1 weighted imagines) (The seventh patient). B2) A 14-year-old male patient had anaplastic oligodendroglioma on the right frontal lobe (thin white arrow), multiple DVAs (thick white arrow), CA (thin white arrow), and diffuse LME (thick white arrow). (B2a: post-contrast T1 weighted imaging, B2b: axial FLAIR weighted imaging, B2c: post-contrast T1 weighted imaging, B2d: axial SWI weighted imaging, and B2e: axial post-contrast subtraction T1 weighted imaging) (The ninth patient).

Discussion

In CMMRD, known as a rare cancer susceptibility syndrome, CNS tumors including high-grade glial tumors, medulloblastoma, and supratentorial primitive neuroectodermal tumor, and hematological malignant diseases are seen in early childhood.[6,15,16] Recently, it has been reported that patients with CMMRD may have multiple DVAs and spontaneous CAs in the CNS.[9-11] In our study, seven patients with CMMRD had multiple DVAs; six patients had LME; and five patients had CAs. None of these lesions were observed in the group consisting of patients with brain tumors but without CMMRD.

The related mismatch repair genes are *MLH1*, *MSH6*, *MSH2*, and *PMS2*. The pathogenic homozygous c.478C>T p.(Gln160*) mutations in *MSH6* gene detected in the first family was reported by our group.[14] The pathogenic c.2871dupC p.(Phe958Leufs*5) mutations in *MSH6* gene, reported as pathogenic previously in the literature[17,18], was found to be in homozygous state in both affected siblings in the second family. The c.236G>A variant was reported in heterozygous state ones with a $3.98x\ 10^{-6}$ allele frequency in GnomAD exomes and never reported in GnomAD genomes.[19] Of note, the variant has also never been reported in the NHLBI GO ESP Exome Variant Server, Exome Aggregation Consortium (ExAC), 1000

Table II. Patients' characteristics and radiological findings.

Family	Mutation	Zygosity	No	Gender	Age**	Café au lait Spots	Tumor	Score§	Radiological findings at diagnosis				
									DVA		CA		LME
									Number	Diameter‡ (mm)	Number	Diameter‡ (mm)	
1	*MSH6*	Homozygous	1	F	8	+	1º Glioblastoma	9	8	2	3	20	-
	c.478C>T				11		2º Glioblastoma						
	(p.Gln160Ter)	Homozygous	2	F	3	+	1º Cord anaplastic astrocytoma	8	9	2	-	-	+
					7		2º Brain masses (refuse surgery)						
					7.5		3º Acute myeloid leukemia						
		Homozygous	3	F	2	+	1º Glioblastoma	8	3	2	Unknown	Unknown	+
		Heterozygous	Father	M		-	-	-	-	-	-	-	-
		Heterozygous	Mother	F		-	-	-	-	-	-	-	-
2	*MSH6*	Homozygous	4	M	0.75	+	1º Hepatoblastoma	7	20	2	3	10	+
	c.2871dupC				4		2º Glioblastoma						
	(p.Phe958LeufsTer5)	Homozygous	5	F	7	+	1º Acute lymphoblastic leukemia	7	6	3	3	9	+
					10		2º Glioblastoma						
		Mandatory Heterozygous	Father	M		-	-				No imaging		
		Mandatory Heterozygous	Mother	F		-	-	-	-	-	-	-	-
3	*MLH1*	Heterozygous	6	M	7	+	1º Glioblastoma	5	-	-	-	-	-
	c.236G>A	Unknown	Father	M		-	-				No imaging		
	(p.Arg79Lys)	Unknown	Mother	F		-	-				No imaging		
4	Unknown*	Unknown*	7	F	3	+	1º Medulloblastoma	5	3	1.5	2	8	+
					4.5		2º Acute lymphoblastic leukemia						
		Unknown*	Father	M		-	-	-			No imaging		
		Unknown*	Mother	F		-	-	-			No imaging		
	Unknown*	Unknown*	8	F	4.5	+	1º Medulloblastoma	5	-	-	-	-	-
					7		2° Glioblastoma						
		Unknown*	Father	M		-	-	-	-	-	-	-	-
		Unknown*	Mother	F		-	-	-	-	-	-	-	-
	Unknown*	Unknown*	9	M	14	+	1º Anaplastic oligodendroglioma	+	4	2	20	4	+
		Unknown*	Father	M		-	-	-			No imaging		
		Unknown*	Mother	F		-	-	-			No imaging		

*Not determined yet, whole exome sequencing analysis is ongoing. **: age at which the tumor develops, (+): positive, (-) negative, §: According to the EU-Consortium Criteria for CMMRD [6], ‡: the largest diameter

F: Female, M: Male, DVA: Developmental venous anomalies, CA: Cavernoma, LME: Leptomeningeal enhancement, [a] CNS prophylaxis was performed.

Note: Mothers of patient 7 and patient 8 are siblings and their fathers are siblings as well. The father of patient 9 is cousins with the mothers of patient 7 and patient 8.

Genomes or Greater Middle East Variome Project databases. Based on these data detected mutation is rare and predicted to be pathogenic by most of the in slico prediction tools (MutationTaster,[20] PolyPhen2,[21] SIFT,[22] REVEL,[23] and MetaLR.[24]). Applying American College of Medical Genetics and Genomics and the Association for Molecular Pathology criteria for *MLH1*:c.236G>A as PM1, PM2, PP3 and PP4, leading to a likely pathogenic classification.[25] Sanger confirmation and segregation studies are ongoing. Since the patient's phenotype is in line with the CMMRD syndrome, for the other allele, del/dup analysis of *MLH1* is planned.

In our study nine children with CNS tumors were included. In two patients, a CNS tumor developed as the second primary tumor who had primary diagnosis of hepatoblastoma and acute lymphoblastic leukemia. In patients with CMMRD, the most common CNS tumors are high grade glial tumors, whereas medulloblastoma and supratentorial primitive neuroectodermal tumors have been also reported. In our patients, interestingly two of nine patients had medulloblastoma. Another finding in our study was that the patient with hepatoblastoma was the first case with CMMRD to our knowledge.

Publications on vascular malformations in patients with CNS tumors have been widely reported.[8,26-32] In a retrospective study, the relationship between CNS tumors and DVAs was investigated. The prevalence of DVAs in patients with CNS tumors and the control group were 10.17% and 5.34%, retrospectively, and this difference was statistically significant. No statistically significant associations were found between DVA prevalence with tumor type and the localization of DVA within the brain.[8] In particular, venous angiomas in different CNS tumors were reported by Uchino et al.[27] In another study investigating vascular malformations associated with CNS tumors in children, three patients with astrocytoma had arteriovenous malformations, and two patients with astrocytoma had CAs.[28] In another article, a case of metachronous occurrence of cavernous hemangioma and medulloblastoma in a pediatric patient with neurofibromatosis type 1 phenotype was reported, in whom the criteria other than *café au lait* spots were not met. The diagnosis of acute lymphoblastic leukemia with similar findings in this patient's brother also suggested CMMRD in these siblings; genetic research was performed, but the result was not reported in the article.[31]

Cavernomas, cavernous hemangiomas, and cerebral cavernous malformations are a cluster of abnormal blood vessels. Although the cause of CAs is unclear, genetic causes are emphasized. Exposure to radiotherapy in children is a cause of CA. In 2014, Wimmer et al.[6] published an article entitled "Diagnostic Criteria for Constitutional Mismatch Repair Deficiency Syndrome." In this publication, spontaneous CA was one of the diagnostic criteria. In our study, five patients had CAs, and no bleeding was observed in these CAs at diagnosis.

In a study by Shiran et al.[9], 10 patients with CMMRD were retrospectively evaluated. They detected two or more DVAs in the cranial MRI of all patients. However, they did not find a relationship between the locations of DVAs and tumor location. In addition, they did not detect any new vascular anomalies. In our study, we found DVAs in seven of nine patients. We detected DVAs at different frequencies and with different diameters. Similar to Shiran et al.[9], we did not find a relationship between DVAs and tumor location and any emerging DVAs. Considering the study by Shiran et al.[9] and our study, DVA detection may be a valuable finding in the diagnosis of CMMRD. Following the article by Shiran et al.[9], MRI of 14 children with CMMRD revealed DVAs, CA and nonspecific subcortical white matter T2 hyperintensities. In a more recent article, malformations of cortical development were reported in addition to DVAs.[10,11]

Leptomenengeal enhancement refers to a diffuse or focal gyriform or serpentine enhancement, it should be differentiated from vessels. In LME,

all pia mater shows uninterrupted enhancement whereas in contrast, vessels show interruptions. Therefore, images should be carefully examined. In our study, we detected LME in six patients. Leptomenengeal enhancementis a combination of two processes: (i) intravascular enhancement may reflect neovascularity, vasodilation, hyperemia, and shortened transit time, and (ii) interstitial enhancement is associated with changes in the permeability of the blood-brain barrier. The most common causes of LME are infections, encephalitis, tumors, hemorrhage, uncomplicated lumbar puncture, granulomatous conditions, and late findings of postoperative or posttraumatic conditions.[29,33] In our patients with diffuse LME, an important problem to resolve for LME was whether it was metastatic or merelyan artefact. Our patient with medulloblastoma had negative cytology in a cerebrospinal fluid sample obtained preoperatively. In the patient with spinal cord anaplastic astrocytoma, cerebral LME was present despite no tumor being detected on neuroimaging at the beginning and no subsequent local treatment was administered, including surgery andradiotherapy. These findings suggest that LME was not tumoral. However, preoperative cerebrospinal fluid cytological examination and, where appropriate, biopsy may be performed for definitive diagnosis in patients with LME.

The most important limitations of our study are that genetic analysis of the fourth family was not performed and the segregation analysis of the third family has also not been performed.

In conclusion, CMMRD is a distinct and rare cancer predisposition syndrome with autosomal recessive inheritence, including hematological malignancies, CNS tumors, and intestinal tract tumors and its awareness is increasing. Cavernomas and multiple DVAs are the most common radiological findings of CMMRD alongwith CNS tumor. Constitutional mismatch repair deficiency should be considered when DVAs, CAs, and LME are detected, especially in patients with *café au lait* spots.

Ethical approval

Local institutional review board approval was provided (Selcuk University, Dec 30, 2020, No: 2020/26).

Author contribution

The authors confirm contribution to the paper as follows: study conception and design: BK, YP, YK; data collection BK, YP, AOÇ, NS, HA, YK; analysis and interpretation of results: BK, YP, AOÇ, NS, HA, YK; draft manuscript preparation: BK, YP, AOÇ, NS, HA, YK. All authors reviewed the results and approved the final version of the manuscript.

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Conflict of interest

The authors declare that there is no conflict of interest.

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Case Report

Spontaneous remission of nephrotic syndrome associated with COVID-19 infection in an 8-year-old boy

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ABSTRACT

Background. It is already known that viral infections, exclusively upper respiratory tract infections may trigger relapses of nephrotic syndrome. Recently, COVID-19 disease has also been reported to be related with relapse of nephrotic syndrome in a few pediatric cases.

Case. Here we present an 8-year-old boy who had relapse of nephrotic syndrome due to COVID-19 infection. He was asymptomatic except for mild edema. He was managed supportively, no medication was started and went into spontaneous remission in 7 days.

Conclusions. Viral infections particularly upper respiratory tract infections may trigger relapse of nephrotic syndrome. COVID-19 has also been reported to be related with relapses of nephrotic syndrome in a few pediatric cases. Spontaneous remission in our patient indicates the importance of close monitoring of patients before starting long term treatment with steroids.

Key words: COVID-19, SARS-CoV-2, nephrotic syndrome, child.

Although kidneys are among the most affected organs in COVID-19 disease and there is an increasing number of publication on this area, the number of studies regarding the clinical profile of COVID-19 in children with known kidney disease is still scarce.[1] A global survey on children receiving immunosuppressive medications for kidney diseases showed that most of the patients had a mild clinical course of COVID-19.[2] In an Italian national study including 159 children with nephrotic syndrome on chronic immunosuppression with B cell depleting agents, none of the patients reported clinical symptoms of COVID-19.[3] In a recent study from Turkey, Canpolat et al.[4] demonstrated that although the clinical course was mild the frequency of COVID-19 was increased in children undergoing chronic dialysis with a kidney transplant.

There are limited number of case reports describing simultaneous occurrence of new onset nephrotic syndrome and COVID-19.[5,6] There are also few case reports presenting relapse of nephrotic syndrome with COVID-19.[7,8] In these reports both the cases with new onset nephrotic syndrome and the relapsing cases had favorable response to oral steroids.[5-8]

Here we present an 8-year-old boy who had a relapse of nephrotic syndrome with asymptomatic COVID-19 infection and went into spontaneous remission in seven days without any treatment. To our knowledge this is the first case of asymptomatic COVID-19 infection triggering relapse in nephrotic syndrome.

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Case Report

An 8-year-old boy was admitted to our emergency department because of swelling in the eyelids and legs. He was diagnosed with nephrotic syndrome four years prior, he had two relapses following the first two years after diagnosis and was in remission for two years without any medication. Recently his father was diagnosed with the COVID-19 infection. At admission, physical examination revealed: height 136 cm, weight 27 kg, blood pressure 106/60 mmHg, heart rate 84/min, respiratory rate 20 /min, O_2 saturation 98%; and he was afebrile. Except periorbital and pretibial edema physical examination was normal. On laboratory examination dipstick urine analysis revealed 4+ proteinuria. Spot urine protein-to-creatinine ratio was 2.2 (mg/mg). Biochemistry parameters were as follows: hemoglobin 14.1 g/dl, leukocytes 5,190/mm^3, absolute neutrophil count 2,060/mm^3, lymphocyte count 2,640/mm^3, CRP negative, serum albumin 1.8 g/dl, creatinine 0.4 mg/dl, cholesterol 298 mg/dl and triglycerides 200 mg/dl. Serum electrolytes and liver function tests were in normal limits. Chest X-ray was normal. With these clinical and laboratory features the patient was diagnosed as relapsing nephrotic syndrome. To identify the triggering factor, he was tested with nasopharyngeal multiplex PCR assay for upper respiratory tract infections with influenza, parainfluenza, adenovirus and rhinovirus and all were negative. Because he had a close household contact, he was also tested for SARS-CoV-2 and real-time PCR (RT-PCR) assay was positive, so he was also diagnosed to have asymptomatic COVID-19 infection. He was hospitalized in order to observe the possible undesired course; but because he was asymptomatic no medication was started for COVID disease; he was also managed conservatively for nephrotic syndrome. On the third day of his admission urine output increased and spot urine protein-to-creatinine ratio decreased to 1.8 mg/mg. Because his vital signs were in normal limits and he did not have weight gain and had improving edema, we decided to manage him without steroids. His edema improved day by day and proteinuria decreased. On the seventh day of his admission spot urine protein-to-creatinine ratio was 0.2 mg/mg and serum albumin was to 2.9 g/dl. SARS-CoV-2 RT-PCR also turned to negative. No fever or any new complaints developed during hospitalization; so he was discharged with complete spontaneous remission. After two weeks of discharge he was still in remission.

Written informed consent was obtained from the parents of the patient for publication.

Discussion

Renal involvement is reported to be relatively common particularly among hospitalized adult patients with COVID-19. The spectrum ranges from isolated hematuria and/or proteinuria to acute kidney injury requiring renal replacement therapy.[9-11]

Although there is mounting evidence about kidney involvement of COVID-19 in adults, the data regarding renal complications in children and how the disease effects this age group with underlying nephropathies are scarce.[2,3] Angeletti et al.[3] evaluated 159 children with nephrotic syndrome who had received B cell depleting therapy and were on chronic immunosuppression. Among these only 6 children who had close household contacts were tested positive for SARS-CoV-2 by nasopharyngeal swab, but none of these patients developed symptoms.[3] A multicentric study hosted by European Rare Kidney Diseases Reference Network revealed that most children with kidney diseases on immunosuppressive medication had a mild disease of COVID-19.[2] The authors also demonstrated that the number of the immunosuppressive drugs did not affect the severity of COVID-19 in children.[2]

A handful of cases with renal manifestations of COVID-19 has been reported in the pediatric age group (Table I). Macroscopic hematuria was described in a 10 year old boy during mild upper respiratory tract infection due to

Table I. Published reports of pediatric patients with renal manifestations due to COVID-19 infction.

Reference	Age	Gender	Symptom	Renal manifestations/ disease	Renal dysfunction	Renal biopsy	Treatment	Outcome
Almeida FJ et al.[12]	10	F	Fever, cough, sore throat	Gross hematuria	No	No	Supportive	Hematuria resolved in five days
Alvarado A et al.[5]	15	M	Dyspnea	Edema anasarca, new onset nephrotic syndrome	No	No	PMP (5 days), chloroquine, azithromycin	Respiratory symptoms and edema improved (no data for duration)
Enya T et al.[7]	3	M	Fever	Eyelid edema Relapse of nephrotic syndrome	No	No	Oral prednisolone (2mg/kg/day)	Remission of nephrotic syndrome in one week
Basiratnia et al.[9]	17	M	Feeling unwell for last two weeks, nausea, vomiting	Decreased urine output, hypertension, glomerulonephritis	Yes (serum creatinine: 17 mg/dl)	Acute necrotizing glomerulonephritis	PMP (3 days) followed by oral prednisolone, hemodialysis, enoxoparine	Follow-up on hemodialysis
Basiratnia et al.[9]	16	M	Fever	Oliguria, tea colored urine, glomerulonephritis	Yes (serum creatinine:15 mg/dl)	Acute necrotizing glomerulonephritis; negative for COVID-19	PMP (3 days) followed by oral prednisolone, hemodialysis, enoxoparine	Remission achieved in 2 weeks (last serum creatinine 0.8 mg/dl)
Shah AS, Carter H[6]	8	M	Vomiting, diarrhea	Facial swelling, new onset nephrotic syndrome	No	No	Oral prednisolone 30 mg	Remission in seven days

PMP: pulse methylprednisolone

COVID-19.[12] New onset nephrotic syndrome associated with COVID-19 in two boys and acute necrotizing glomerulonephritis in two adolescents were described.[5-6,9] Also relapses of nephrotic syndrome due to COVID-19 have been observed.[7] Except for the two adolescents with necrotizing glomerulonephritis, the other patients reported with relapses and new onset nephrotic syndrome did not undergo renal biopsy because their clinical picture was highly suggestive of minimal change nephrotic syndrome and they responded well to steroid treatment.[5-7,9] In adult studies it is demonstrated with renal biopsies that COVID-19 may cause a spectrum of podocytopathy ranging from minimal change disease to collapsing glomerulopathy. The underlying mechanism of podocyte injury in COVID 19 yet remains unknown.[10]

It is already known that viral infections, particularly upper respiratory tract infections are associated with the onset or exacerbations and relapses of nephrotic syndrome. Among these influenza, parainfluenza and adenoviruses are reported to be the most common pathogens triggering relapses.[13] Additionally, new onset nephrotic syndrome or relapses has been previously reported during other viral outbreaks such as H1N1.[14] The underlying mechanism by which infections cause relapses is not exactly identified but might be related to upregulation of T cells, altered balance of T helper 1/T helper 2 and as a result of these cytokine mediated podocyte injury.[14] Various studies demonstrated T lymphocyte abnormalities in COVID-19 infection and as in other viral infections these alterations may be responsible for the relapses of nephrotic syndrome.[15]

Except for multisystem inflammatory syndrome, the most remarkable feature of COVID-19 is the lower risk of severe forms of disease in children when compared with adult counterparts. But even asymptomatic disease course may be responsible for relapses in nephrotic syndrome patients as demonstrated here. Although we did not study the antibody profile of the patient; because all possible causal factors were excluded, and because he had close household contact with COVID-19 along with a positive RT-PCR test result, we attributed the relapse of nephrotic syndrome to COVID-19. We think this report shows that as in other viral infections even an asymptomatic COVID-19 infection may trigger relapse of nephrotic syndrome. As far as we know this is the first asymptomatic case of COVID 19 resulting in relapse in nephrotic syndrome. It is well known that relapses of nephrotic syndrome, which was triggered by an upper respiratory tract infection can go into remission spontaneously in up to 20% of the patients.[16] Considering the relatively mild course in children we think that before administrating steroids that will last for months, patients should be clinically observed for a couple of days for spontaneous remission.

Ethical approval

Written informed consent was obtained from the parents of the patient for publication.

Author contribution

The authors confirm contribution to the paper as follows: study conception and design: ÖM, AY; data collection: GAD, ÖM; draft manuscript preparation: AKA, GAD. All authors critically reviewed the manuscript and approved the final version of the manuscript.

Source of funding

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Conflict of interest

The authors declare that there is no conflict of interest.

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Case Report

Priapism associated with COVID-19: a pediatric case

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ABSTRACT

Background. Urological involvement is rare in patients with coronavirus disease 2019 (COVID-19). Priapism, one of the urological involvements, was reported as one of the COVID-19 comorbidities in the elderly male patient group but has rarely been reported in the pediatric age group.

Case. Herein, a previously healthy 8-year-old patient with COVID-19-associated priapism, which is rare in children, is presented.

Conclusions. During the pandemic, in pediatric cases with priapism of unknown etiology, COVID-19 should be one of the diagnoses to be considered.

Key words: priapism, COVID-19, child.

Despite the fact that respiratory system involvement is prominent in patients with coronavirus disease 2019 (COVID-19) since the pandemic various other clinical findings have been presented such as urological involvement.[1] Priapism was reported rarely in elderly cases with COVID-19.[2] Here, a pediatric case that was admitted to the hospital with priapism and was also found to be positive for COVID-19 is described.

Case Report

A previously healthy 8-year-old boy, with no history of a surgical procedure, was admitted to the University of Health Sciences, Dr. Behçet Uz Pediatric Diseases and Surgery Training and Research Hospital, pediatric surgery clinic, because of an ongoing painless erection that had lasted for 48 hours. Medical history revealed that the patient had a fever and malaise 5 days before admission and had no additional respiratory or any other symptoms. Fever and a painless erection occurred on the same day. The patient had no history of penile trauma or drug use. On physical examination, there was no fever (37.2 °C), blood pressure (100/60 mmHg) and oxygen saturation (99%) were normal, and penile erection was present, but there were no signs of penile skin change, discoloration, or tenderness. Other systemic examinations were normal. A nasopharyngeal swab sample taken at hospital admission was positive for severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) real-time polymerase chain reaction. There was no other individual with COVID-19 in the family. Blood cell count, peripheral blood smear, biochemical parameters and coagulation parameters including prothrombin time / international normalized ratio, activated partial thromboplastin time, fibrinogen level, and D-dimer levels, were all within normal range. The C-reactive protein was 1.53 mg/dl and slightly elevated (normal level <0.2 mg/dL). The patient was consulted to the pediatric hematology department, atypia and blast were not detected in the peripheral smear. In addition to the patient's medical history and laboratory

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evaluation, sickle cell anemia and thalassemia were excluded by hemoglobin electrophoresis and sickling test. His penile Doppler ultrasonography (USG) imaging revealed a "biphasic flow in the dorsal penile artery and cavernous arteries in the proximal part of the penis with an erect peak systolic flow velocity of 10-15 cm/sec (resistant arterial flow)". In the treatment, ice was applied to the penile area and enoxaparin sodium was initiated. Repeat Doppler USG on the sixth day of hospitalization revealed "both corpus cavernous parenchyma echogenicity was homogeneous and no space-occupying formation and both cavernous arteries were patents and high resistive flow was observed in erection". The patient recovered without any requirement for cavernous aspiration. Enoxaparin was discontinued after 5 days and the patient was discharged to continue outpatient follow-up and after discharge, no anticoagulant or other medication was started. Physical examination was normal in the outpatient clinic controls, and no additional problems were encountered.

Informed consent was obtained for publication from the family.

Discussion

Priapism is defined as an abnormal persistent penile erection lasting longer than 4 hours, beyond or unrelated to sexual arousal.[3,4] The incidence of priapism in men is estimated at 0.3-1.5 per 100,000 per year and is most common in men in the fifth decade. Although there are no clear data about priapism in children, it seems rare.[4]

There are three widely accepted types of priapism: ischemic (low-flow, veno-occlusive), stuttering (intermittent, recurrent ischemic), and non-ischemic (high-flow, arterial).[4] Ischemic priapism is the commonest type seen in children and is typically painful. Stuttering priapism describes recurrent "unwanted and painful erections" which are often self-limiting but may precede an "unrelenting" ischemic priapism. Non-ischemic priapism is a partial erection due to unregulated cavernous arterial flow which is usually painless. SARS-CoV-2 has been linked to thromboembolic complications.[2] The reports presented on COVID-19 and priapism have shown that the type of ischemic priapism can be seen in older men.[5-7] Non-ischemic priapism is derived from unregulated arterial inflow within the penis and is less well characterized than ischemic priapism.[8] Non-ischemic priapism is mostly a result of trauma. Although the pathogenesis has not been fully defined: it is suggested that it may also be due to impaired autonomic regulation of penile blood flow, vasculopathy or iatrogenic injuries caused by blood aspirations or shunt.[9] In the case reports published during the pandemic period, post-COVID-19 ischemic priapism cases were seen in the elderly patient group with underlying comorbidities, and in a 12-year-old patient without any underlying disease in the pediatric age group, ischemic priapism was detected after COVID-19 infection.[6,10,11] Unlike our case, the 12-year-old patient had COVID-19 7 weeks before the diagnosis of priapism, but in the adult age group cases, as in our case, priapism and COVID-19 were detected simultaneously. While priapism was ischemic in detected cases, it was nonischemic in our case.

This case was diagnosed as non-ischemic priapism, which is less common than the other types of priapism. This present case is one of the first reports of a child with COVID-19 presenting with priapism. However, it is not clear whether this finding is a complication of COVID-19 or it is a coincidental situation. On the other hand, we thought that the priapism might be associated with COVID-19, since non-ischemic priapism can originate from irregular arterial inflow in the penis, and COVID-19 may cause a slowdown in flow with hyperviscosity, and the patient had no history of trauma and no signs of hematological disease. Due to the wide spectrum of clinical presentation of COVID-19 infection during the ongoing pandemic, COVID-19 should be kept in mind in pediatric cases with priapism with unknown etiology.

Ethical approval

Consent was obtained from the parents for the publication of this case report.

Author contribution

The authors confirm contribution to the paper as follows: study conception and design: AAK, AO; data collection: MYÇ, EB, MD, ABU; analysis and interpretation of results: AAK, AŞ; draft manuscript preparation: MYÇ. All authors reviewed the results and approved the final version of the manuscript.

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Case Report

Complete atrioventricular block due to multisystem inflammatory syndrome in children: a case report

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ABSTRACT

Background. Cardiac manifestations in multisystem inflammatory syndrome in children (MIS-C) may involve the conduction system. The incidence and publication is still very limited.

Case. We report the case of a 2-year-old girl who presented with complete atrioventricular (AV) block with a current infection of SARS-CoV-2 and fulfilled the criteria of MIS-C. After observation for 2 weeks of the SARS-CoV-2 convalescence phase and temporary pacemaker insertion, the complete AV block was not resolved. The intrinsic junctional escape beat was only 40 beats/minute. We decided to implant a dual-chamber epicardial permanent pacemaker to maintain synchrony between atrium and ventricle and furthermore provide hemodynamic stability. We observed persistent complete AV block 9 months after SARS-CoV-2 infection in long-term follow up of this patient.

Conclusions. Complete AV block in MIS-C could persist months after its onset. Our case could give additional knowledge regarding the natural history of cardiac involvement after SARS-CoV-2 infection

Key word: MIS-C, complete AV Block, permanent pacemaker.

Two years since the start of COVID-19 pandemic, scientist have gained more knowledge about its ability to affect many organ. Although children with COVID-19 mostly show mild symptoms there is growing risk to develop multisystem inflammatory syndrome in children (MIS-C) during or after the infection. Cardiac manifestation in MIS-C has varied signs and symptoms and poses worse prognosis.[1] Conduction disturbance in particular could be irreversible far beyond MIS-C onset.

Case Report

A 2-year-old girl presented with headache and syncope since one day prior. Her electrogram (ECG) revealed complete atrioventricular (AV) block (Fig. 1). The SARS-CoV-2 PCR swab test showed positive. She had a history of 5 days of fever, vomiting, constipation, and gastrointestinal infection 2 weeks before the first hospitalization at another hospital. She was admitted to the previous hospital for 4 days and her vital signs were unremarkable. Three days after her first hospital discharge, on current admission, we sent her to the pediatric intensive care unit (PICU) for urgent temporary pacemaker. Echocardiography prior to temporary pacemaker placement showed moderate left ventricular dysfunction (ejection fraction [EF] 40%; fractional shortening [FS] 18%) and no structural heart defect.

Bedside temporary pacemaker was inserted urgently under hemodynamic monitor guidance. The lead was inserted via the right jugular vein and placed at right ventricular high mid-septal, initial setting was set at sensitivity 3 mV, output 5 mA, and rate 80 bpm. Following

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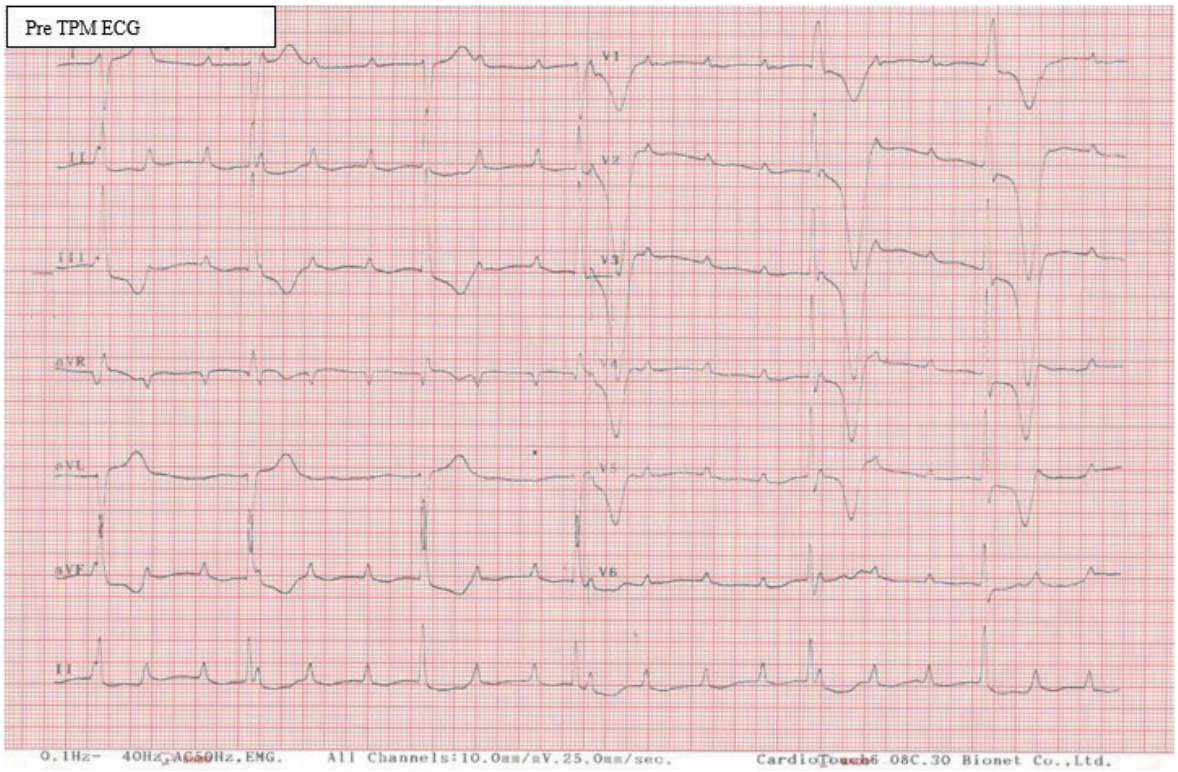

Fig. 1. Complete AV block ECG (Before temporary pacemaker placement).

the implantation, the surface ECG showed all the pacing spike captured the ventricular myocardium with a heart rate of 80 bpm (Fig. 2). The blood pressure was 104/70 mmHg, and oxygen saturation on room air was 100%. Left ventricular dysfunction resolved (EF 56%; FS 28%) after temporary pacemaker placement.

On the following day, the blood serum investigation revealed: leucocytes 13.15 $x10^3/\mu L$ (N: 5000-15,000 /μL) and lymphocyte percentage 13.1% (N: 20-40%). C-reactive protein 27.2 mg/L (N: <5 mg/L), procalcitonin 0.14 ng/mL (N: <0.05 ng/mL), D-dimer 720 μg/L (N: <440 μg/L), fibrinogen 712 mg/dL (N: 200-400 mg/dL), and Troponin I 140.9 pg/mL (N: <15.6 pg/mL). On day 2 of admission, SARS-CoV-2 PCR showed negative results and immunoglobulin G antibody of SARS-CoV-2 was reactive. On day 6 of admission, D-dimer level increased to 3300 μg/L. She was given heparin 10 unit/kg/hour and methylprednisolone 15mg every 12 hours for two weeks.

Complete AV block with an intrinsic junctional escape rhythm at 40 bpm persisted after 2 weeks of convalescence phase of SARS-CoV-2. Therefore, dual chamber epicardial permanent pacemaker was implanted in right atrial appendage and mid right ventricle septum. The permanent pacemaker (PPM) mode was dual chamber pacing (DDD) with lower rate set at 70 bpm (Fig. 3). Parameter of atrial lead showed the sensitivity 2.5 mV, and impedance 250 Ohm, whereas, parameter of ventricular lead was sensitivity dependent (<1.5 mV), and impedance 349 Ohm. Five months after PPM implantation, during PPM check, the intrinsic ventricular rate was 20 bpm, with atrial sensing 86.9 %, and ventricular pacing 86.9 %. On the last outpatient visit, 9 months after PPM implantation, we still observed persistent complete AV block but patient condition was stable. Parents gave the authors permission to publish this case.

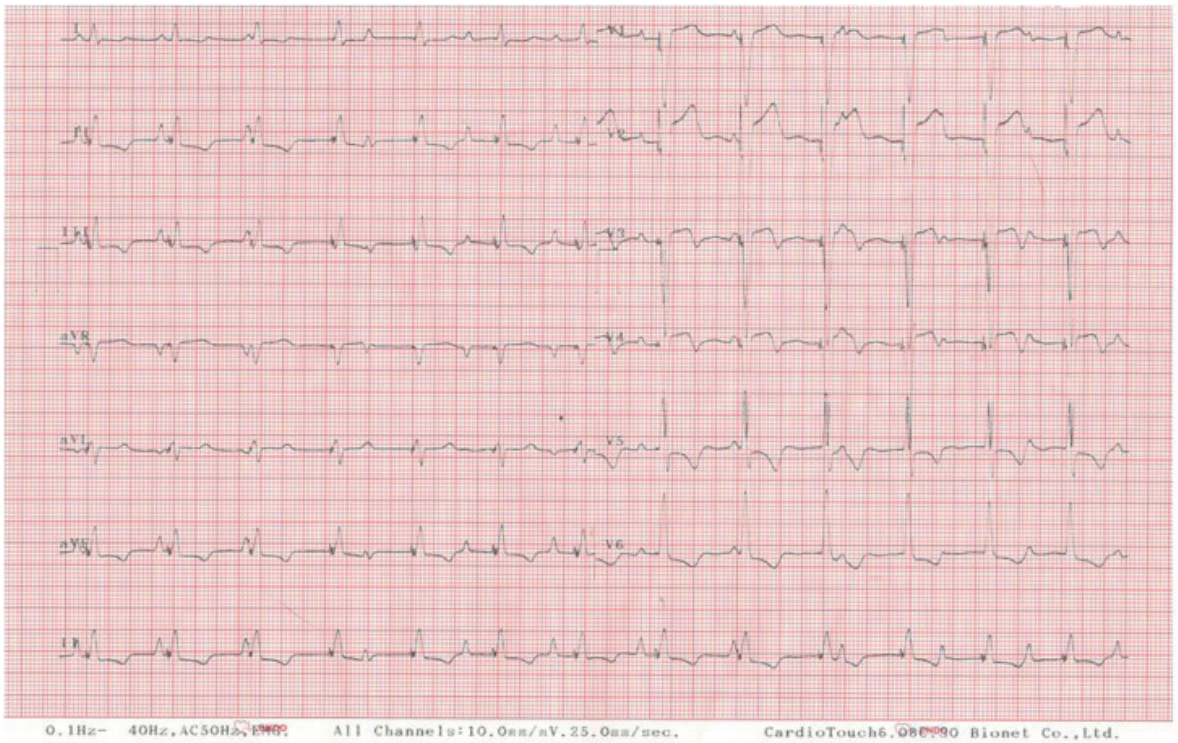

Fig. 2. Post Temporary Pacemaker ECG (VVI rate 80 bpm, sensitivity 3 mV, output 5 mA).

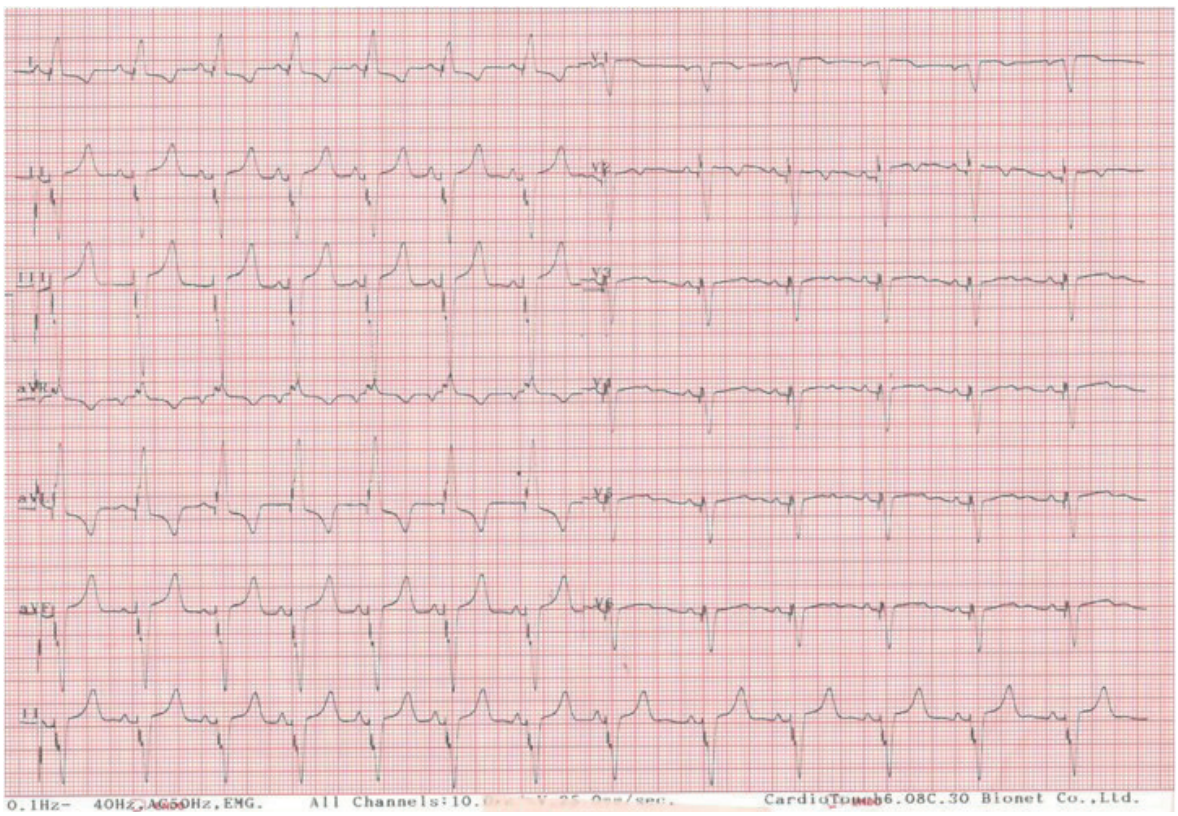

Fig. 3. Post permanent pacemaker ECG (ECG showing atrial-sensed and ventricular-paced rhythm after permanent pacemaker (PPM) implantation at a lower rate of 70 beats per minute).

Discussion

The Centers for Disease Control and Prevention (CDC) definition of MIS-C is an individual aged <21 years with fever greater than 38°C, evidence of inflammation on laboratory examination, multisystem organ involvement, no alternative plausible diagnoses and positive for current or recent SARS-CoV-2 Infection.[1] In our case, the patient presented with history of 5 day-fever, headache, history of syncope before admission, complete atrioventricular block, positive result of SARS-CoV-2 infection, history of gastrointestinal problems, elevated inflammatory marker and elevated coagulation profile. The child in our case met the criteria of MIS-C.

Among children with COVID-19, 0.14% of them developed MIS-C.[2] Cardiac involvement was estimated in 80% of children with MIS-C.[3] The cardiac manifestations include ventricular dysfunction, coronary artery aneurysms, conduction abnormalities, and arrhythmias. Bradyarrhythmia in children with MIS-C varied from sinus bradycardia, first degree AV block, second degree AV block and complete AV block.[3] Data from small retrospective cohort study reported that 4% of children with MIS-C developed complete AV block and manifested within the first week after onset of fever in children with MIS-C.[4] Children under 5 years of age with MIS-C tended more often to have cardiac involvement.[5] Our patient was 2 years of age and complete AV block was identified 3 weeks after onset of fever.

The etiology of AV block is still unknown, but it might be a secondary response due to the inflammation and the result of direct virus invasion. It has been reported that with immunomodulatory and antiviral therapy, the inflammatory markers trended downward, and episodes of heart block resolved, thus indicating that inflammation is likely the main cause of conduction.[6] Recent literature showed most of bradyarrhythmia in children with MIS-C was transient.[6-8] Nevertheless, other literatures involving adult subjects with COVID-19 reported persistent complete AV block requiring permanent pacemaker placement.[9,10] Our case showed irreversible complete AV block until 9 months following permanent pacemaker implantation.

Permanent pacemaker placement is indicated in children with complete AV block due to irreversible cause.[11] After 2 weeks of temporary pacemaker implantation, our patient still had persistent complete AV block that warranted the need for a permanent pacemaker. We planned to do a permanent pacemaker evaluation in the hospital. Current guidelines suggest doing pacemaker evaluations regularly, every 6-12 months.[11] We hope our case will give additional knowledge regarding the natural history of cardiac involvement after SARS-CoV-2 infection.

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Ethical approval

The authors have received parental permission to publish this case.

Author contribution

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

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Case Report

Familial clustering of nasopharyngeal carcinoma in the family of an adolescent with nasopharyngeal carcinoma

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ABSTRACT

Background. Nasopharyngeal carcinoma (NPC) is one of the rare malignant diseases of childhood, of which only 1% occurs in children. In recent years, genetic factors have attracted attention in NPC. A very limited data have been reported about clustering within families.

Case. Herein, the familial clustering of nasopharyngeal carcinoma in the family of an adolescent with nasopharyngeal carcinoma is presented.

Conclusions. There is familial clustering in nasopharyngeal carcinoma (NPC), but our knowledge on this subject is limited, especially in children or adolescent populations. Therefore, we should be more careful in NPC in childhood, especially in first-degree relatives.

Key words: adolescent, nasopharyngeal carcinoma, familial nasopharyngeal carcinoma.

Nasopharyngeal carcinoma (NPC) is one of the rare malignant diseases of childhood, of which only 1% occurs in children.[1] Some subjects in NPC are well known, including geographical and ethnic distribution, the bimodal age distribution in some countries, a 2-3 times higher diagnosis in males than females, and the association with Epstein-Barr virus (EBV). NPC is common in Southern China, Southeast Asia, the Mediterranean basin countries, and Alaska but rarer in Japan, Europe, and North America. It is bimodal in some populations in North America and the Mediterranean region. Moreover, the similarity between age distribution patterns has been hypothesized to be related to the similarity of latency patterns as in NPC and Hodgkin lymphoma.[2]

In recent years, genetic factors have attracted attention in NPC.[3] A very limited data have been reported about clustering within families.[4-9] Studies suggest that having a first-degree relative with NPC increases the risk of NPC sevenfold, and therefore recommend screening for NPCs among first-degree relatives.[5,10] The rate of familial NPC was reported as 15.5%. Additionally, the influence was more common among siblings, with a relatively short interval between affected siblings.[5] Studies associated NPC with human leukocyte antigens (HLA) and emphasized a possible relationship between NPC and especially human leukocyte antigen (HLA-A).[11-13] In a genome-wide search conducted in families at high risk for NPC, the evidence of a major susceptibility locus for NPC on chromosome 4 in a subset of families was determined.[14] However, our knowledge of NPC and genetics is quite limited. Additionally, familial clustering has not been emphasized much in childhood NPC series.[15-18]

Herein, familial clustering of NPC in the family of an adolescent with NPC is presented with a related literature review.

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Case Report

A 17-year-old male applied to the dentist because of new-onset toothache and neck swelling. The patient was referred to our hospital after the dentist detected a mass. The patient's medical history was unremarkable, but his family history revealed that one of his aunts and two uncles had NPC and one of his uncles died due to NPC (Fig. 1). Initial physical examination revealed a firmly fixed mass of 70 × 80 mm in the left angle of the mandible and 50 × 30 mm conglomerated lymphadenopathy in the left cervical region. Additionally, the oropharyngeal examination determined an anteriorly deviated left tonsil due to the mass. The patient did not have any other findings including café au lait spot on physical examination. The complete blood count and biochemical analyses were within normal limits.

The imaging studies revealed a normal chest computed tomography. Computed tomography and magnetic resonance imaging (MRI) revealed a 65 × 55 × 45 mm mass on the left side of the nasopharynx, which obstructs the torus tubarius and eustachian tube (Fig. 2). Additionally, 35 × 25 mm conglomerated lymphadenopathy was detected in the left submandibular and upper cervical region. 18-F FDG PET/CT images revealed a mass on the left of the nasopharynx (SUV_{max}: 15) and lymphadenopathies (SUV_{max}: 16.2) in the area that extend to the left parapharyngeal in the left upper and lower cervical region.

Undifferentiated NPC was diagnosed from the pathological examination of the nasopharyngeal mass biopsy. The patient was staged as IVA (T4N1M0) according to the American Joint Committee on Cancer NPC staging classification after a metastatic work-up completion. Polymerase chain reaction analysis of EBV DNA in the sera was 33,000 ug/dL (N: 0–1000 ug/dL). The patient was instituted on an NPC chemotherapy regimen containing cisplatin, docetaxel, and 5-fluorouracil.[18] Complete response was achieved after the fourth cycle of chemotherapy. Intensity-modulated radiotherapy (IMRT) with megavoltage (6 MV photon) radiotherapy was delivered once daily to the primary tumor and regional lymph nodes. The gross tumor volume included the disease in the primary lesion and cervical lymph nodes as observed on MRI studies with gadolinium contrast (T1 and T2 sequences). The clinical target volume included the gross tumor volume and all sites of potential subclinical disease with 1-cm margins. An additional margin of 3 mm (depending on local policy) was added to define the planning target volume. The primary tumor received a total dose of 70 Gy and 66 Gy for those with involved left level IB and II cervical lymph nodes. All nasopharyngeal regions, including

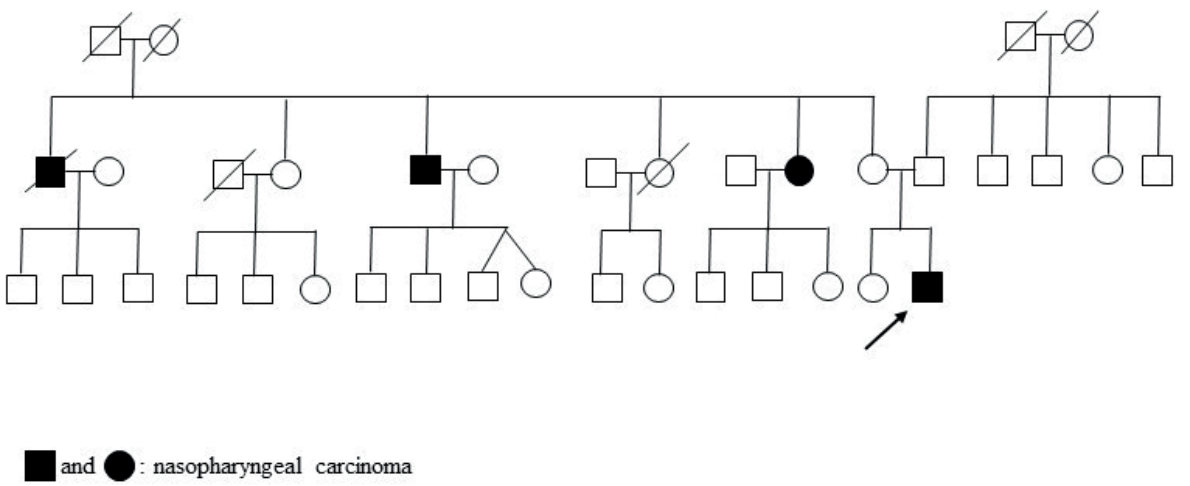

Fig. 1. The familial clustering of nasopharyngeal carcinoma on the patient's pedigree.

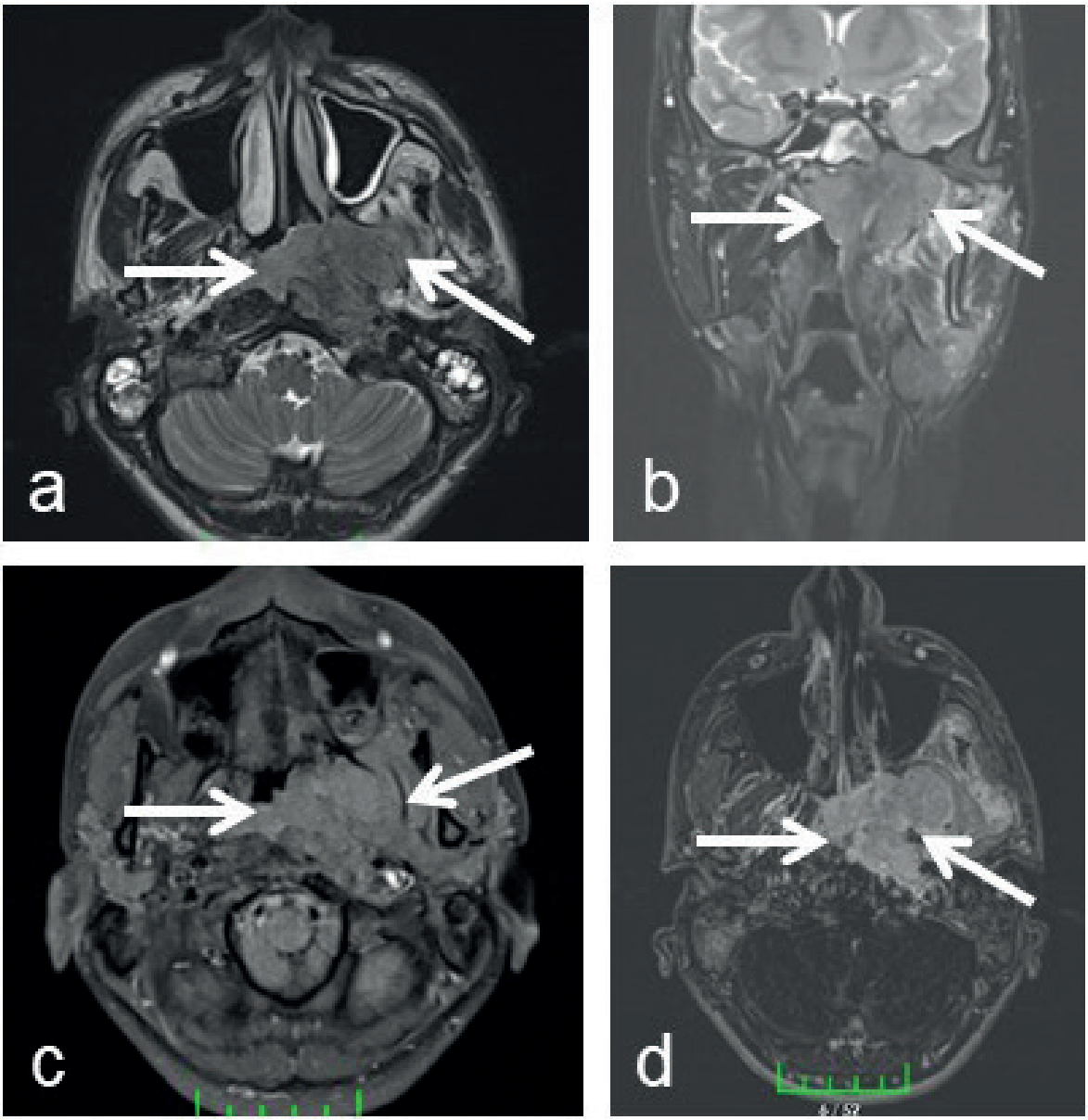

Fig. 2. Axial T2 (a), coronal T2 (b), fat-suppressed precontrast axial T1 (c), fat-suppressed postcontrast axial T1-weighted images (d) show a mass lesion filling the nasopharynx with marked contrast enhancement.

clivus and skull base, received a total dose of 60 Gy, whereas uninvolved regional lymphoid areas were irradiated with a total dose of 54 Gy with a simultaneous integrated boost technique for 33 fractions. The patient has been in follow-up for 6 months with the tumor-free disease.

Our patient, his uncle, and his aunt were taken into genetic analysis. However, mutations in known cancer susceptibility genes including *APC, ATM, BARD, BLM, BMPR1A, BRCA1, BRCA2, BRIP1, CDH1, CDK4, CDKN2A, CHEK2, EPCAM, MLH1, MRE11A, MSH2, MSH6, MUTYH, NBN, PALB2, PRSS1, PTEN, RAD50, RAD51C, RAD51D, SLX4, SMAD4, STK11, TP53*, and *VHL* were not detected.

Written consent for publication of this case report and accompanying images was obtained from the patient and the parents of the patient.

Discussion

NPC is a rare malignant childhood disease, with interesting epidemiological features, such as geographic and ethnic distribution, gender and age distribution, and secular trend, and etiological features such as EBV, Human Papillomavirus (HPV), diet, and heredity.[1,3]

NPC is endemic in Southern China, including Hong Kong, whereas rare in the United States and Western Europe. Southeast Asia, North Africa, the Middle East, and the Arctic are intermediate-risk regions. The age distribution has peaked in the sixth decade in high-risk regions; bimodal age distribution can be seen in some regions, such as Southeast Asia, the Middle East/North Africa, and the United States (minor first peak in adolescents and young adults); and its incidence increases with age in low-risk regions. The median age of diagnosis is 13 years and the first peak of incidence in childhood is 10-20 years. The age distribution is different in these endemic regions. Children under the age of 16 years account for 1–2% of all NPC in China, while in the Mediterranean basin and Africa they account for 10-18%. Sex ratio ranges from 1.7 to 4.8 in the pediatric series. The geographic variation of NPC supports the fact that it develops with a multifactorial etiology.[19,20]

Viral infections, EBV, lifestyles, and especially dietary habits are among the main causes of high-risk incidence in endemic or intermediate-risk regions.[1,3] In endemic populations, the risk appears to be due to an interaction of several factors: EBV infection, environmental factors (such as high consumption of preserved foods and smoking), and genetic predisposition. In addition, the increased incidence in younger adults in high- and intermediate-risk areas suggests that exposure to a common pathogen at a young age is a determining factor.[3,21] In the last few decades, a decreased incidence of NPC has been observed in some endemic areas such as Hong Kong, Singapore, and Taiwan. The reasons for this decline are not exactly known; however, the role of lifestyle changes associated with rapid economic development is considered.[22]

There is ample evidence supporting the role of EBV as a primary etiologic agent in the pathogenesis of nasopharyngeal carcinoma. This includes detection of gene expression of both EBV DNA and EBV in precursor lesions and tumor cells, and it is also important that the nonkeratinizing subtype accounts for most cases in endemic areas (> 95%) and is predominantly associated with EBV infection.[23] Another viral pathogen less associated with nasopharyngeal carcinoma is HPV, and this pathogen is usually observed in nonendemic areas.[24] Dietary habits which contain salt-cured food, high consumption of preserved or fermented foods can lead to NPC. Because they contain high levels of nitrosamines that are direct genotoxins and EBV reactivating substances.[3]

In NPC, heredity is also emphasized, although detailed information is limited about its etiopathogenesis, unlike EBV and diet.[1,3] However, in recent years, rare publications on genetic clusters increased interest in genetics in NPC. Some NPC-associated chromosomes have also been reported. These are 3p21.31-21.2, 4p15.1-q12, and 4.[6,14] The rate of familial NPC was reported as 15.5%. Additionally, the influence was more common among siblings, with a relatively short interval between affected siblings.[5] For example, a case-control study from Taiwan suggests that having a first-degree relative with NPC increases the risk sevenfold.[10] A study from Sweden, where NPC is not endemic, investigated the cancer risk in the relatives of patients with NPC and revealed that relatives of NPC propands are at risk for both NPC and other cancers. Additionally, they concluded that the environmental risk factors, such as EBV infection and smoking, could explain this association, but that the shared genetic predisposition should not be ignored.[9] Another study emphasized the risk factors of NPC, such as environmental factors and dietary habits, as well as the possible association of *CYP2E1* polymorphism with familial NPC.[8,25] There were familial clusters; however, no

difference was found between familial and sporadic cases in demographic and clinical features.[10]

Generally, screening for first-degree relatives, especially siblings, of individuals with NPC is recommended; however, more information is necessary on NPC and genetics.

No familial NPC was reported in the large childhood NPC series from Turkey.[15-17,26] Herein, we present the familial clustering of NPC in the family of an adolescent with NPC. To our knowledge, it is the first reported familial NPC cluster in Turkey. However, so far, mutations in known cancer susceptibility genes were not detected.

In conclusion, there is familial clustering in NPC, but our knowledge on this subject is limited, especially in children or adolescent populations. Therefore, we should be more careful in NPC in childhood, especially in first-degree relatives.

Acknowledgement

We are grateful and thank all the medical doctors and healthcare team who contributed to the diagnosis, treatment, and follow-up of the children with Hodgkin lymphoma over the years in the Department of Pediatric Hematology and Oncology, Selcuk University.

Ethical approval

Written consent for publication of this case report and accompanying images was obtained from the patient and the parents of the patient.

Author contribution

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

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Conflict of interest

The authors declare that there is no conflict of interest.

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Case Report

Prenatal diagnosis of congenital megalourethra: case report and literature review

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ABSTRACT

Background. Congenital megalourethra is an uncommon cause of lower urinary tract obstruction that is rarely prenatally diagnosed in second trimester sonographic examination as a cystic genital mass.

Case. In the presented case, the megalourethra was accompanied with bilateral mild pelviectasis. The newborn had no morbidity during follow-up period. To review the literature, electronic databases including PubMed, Web of Science and Google Scholar were searched up to February 15, 2021. In 51 prenatally diagnosed cases in the literature, most of the cases had accompanying congenital anomalies, especially structural abnormalities in the genitourinary.

Conclusions. In the absence of associated abnormalities, the condition of the upper urinary tract is the main determinant of postnatal outcome. The outcome of congenital megalourethra may be good as in our case, but there may also be serious disorders such as renal failure, pulmonary hypoplasia, erectile dysfunction and fertility issues.

Key words: congenital megalourethra, lower urinary tract obstruction, obstructive uropathy, prenatal diagnosis, prenatal sonography.

Lower urinary tract obstruction, a pediatric end-stage kidney disease cause, is seen in 3.34 of 10,000 live births.[1] Congenital megalourethra is a rare form of lower urinary tract obstruction, resulting from the dysgenesis of penile erectile tissue.[2] Congenital maldevelopment of mesodermal penile erectile structures, which normally supports the urinary tract during micturition, can cause urine stasis and functional dilatation.[3]

Megalourethra is classified into two types according to urethrographic findings. In the scaphoid variant which is the most common type, a bulging occurs in the ventral urethra due to the hypoplasia or aplasia of corpus spongiosum. Whereas in the fusiform variant, associated with worse prognosis, the defect of spongy and cavernous tissues leads to a circumferential enlargement of the urethra. However, there are intermediate phenotypes between these two types and it is not always easy to precisely classify patients.[4]

While it is usually diagnosed in the neonatal period, the number of prenatally reported cases is gradually increasing with advances in sonographic imaging. However, since being described for the first time by Benacerraf, in 1989, the number of prenatally reported cases is not very large.[5,6]

In this report, we aimed to analyze the prenatal sonographic findings and postnatal outcomes of a congenital megalourethra case, and to review the previous prenatally diagnosed cases in the relevant literature.

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For the literature review, the following search strategy was carried out: the keywords of 'congenital megalourethra' and 'prenatal diagnosis' were searched in PubMed, Web of Science and Google Scholar databases up to February 15, 2021 and the references of previous studies were reviewed. Inclusion criteria comprised case reports written in English and published in peer-reviewed journals, prenatally diagnosed cases and congenital megalourethra. Studies that did not provide sufficient clinical data on gestational age and ultrasound findings were excluded.

In this way, we retained 30 studies, which reported a total of 51 prenatally diagnosed congenital megalourethra cases; 6 of these studies reported at least two cases. The largest series included 10 cases.[2] We reviewed these 51 cases according to the following criteria: gestational week at diagnosis, pregnancy outcome, prenatal ultrasound findings, postnatal findings and fetal interventions. The results are summarized in Table I.

Case Report

A 28-year-old woman, gravida 1, was referred to our clinic with suspicion of ambiguous genitalia due to a cystic structure in the fetal penis. Her past medical history was unremarkable, with no teratogenic exposure and no family history of hereditary disease, congenital abnormalities and consanguinity. The first trimester examination and combined test were performed in the referring clinic and there were no abnormalities.

At first ultrasound examination in our clinic, fetal growth was appropriate for gestational age, 21-week, and amniotic fluid was normal. A cystic mass that was ballooning up from the penile shaft between two legs was detected (Fig. 1). There was no vascularization in color Doppler. The urinary bladder was of normal size (Fig. 2). Both renal pelvises were 4.5 mm in diameter (Fig. 3). The case was diagnosed as megalourethra with bilateral mild pelviectasis.

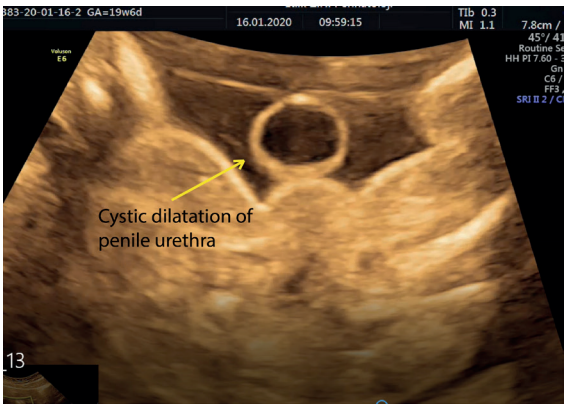

Fig. 1. Ultrasound showing penile shaft as a cystic mass between two legs.

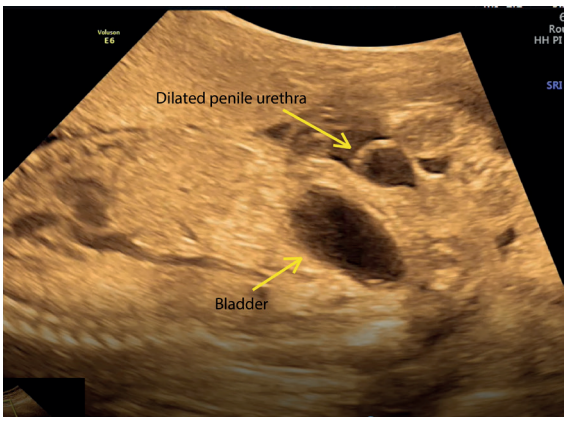

Fig. 2. Ultrasound showing normal sized urinary bladder and cystic dilatation of penile urethra.

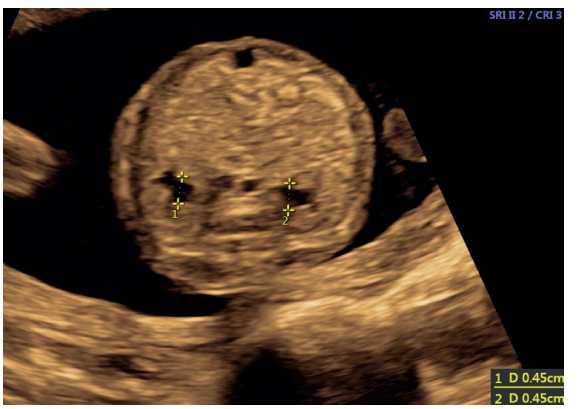

Fig. 3. Ultrasound revealing mild bilateral pyelectasis (both renal pelvises were 4.5 mm in a diameter) and normal amniotic fluid.

No other fetal structural defect was detected and fetal echocardiography was normal. Patient underwent amniocentesis for genetic anomaly evaluation. Fetal karyotype and chromosomal

Table I. Review of 51 prenatally diagnosed megalourethra cases reported by previous studies.

Publication	GW at diagnosis/ delivery (pregnancy outcome)	Obstructive uropathy/ amniotic fluid index	Prenatal additional findings	Postnatal additional diagnosis/ renal functions	Fetal interventions
Benacerraf et al., 1989 [6]	21/36 (live birth)	Bilateral/Polyhydramnios	-	Prune-belly variant	-
Fisk et al., 1990 [7]	18/ - (TOP)	Bilateral/Oligohydramnios	PBS	-	In utero cystourethrogram, bladder aspiration
Simma et al., 1992 [13]	22/39 (live birth, neonatal death)	Unilateral/Oligohydramnios	Right renal dysplasia	Esophageal atresia, imperforate anus, malformed sacrum, VSD rectovesical fistula, splenic duplication,	Bladder aspiration
Sepulveda et al., 1993 [14]	24/37 (live birth)	Bilateral/Normal	-	PBS, normal kidney function at 7 day	Bladder aspiration
Stephens and Fortune, 1993 [15]	14/ Abortion	Megacystis only/Normal	Gastrochisis, scoliosis, rectal atresia	Abortion	-
Dillon et al., 1994 [16]	16/term (live birth)	Unilateral/-	Imperforate anus, renal dysplasia	-	-
Wu et al., 1995 [17]	18/ - (TOP)	Bilateral/Oligohydramnios	Renal dysplasia	PBS	Bladder aspiration
Smith et al., 1996 [18]	24/37 (live birth)	Bilateral/Normal	-	PBS	-
Lam and Tang, 2000 [19]	13/ - (TOP)	Bilateral/Normal	-	-	Transabdominal fetoscopy
Perrotin et al., 2001 [20]	23/38 (live birth)	Bilateral/Normal	Umbilical cord cyst, cryptorchidism	Renal impairment 6-year-old	Bladder aspiration
	21/37 (live birth)	Bilateral/Oligohydramnios	-	Renal impairment 3-year-old	Bladder aspiration
Krapp et al., 2002 [21]	13/ - (TOP)	Megacystis only/ Oligohydramnios	Tracheoesophageal fistula, anal atresia, spina bifida, SUA, unilateral renal agenesis	-	Distal urethral aspiration
Ardiet et al., 2003 [22]	20/36 (live birth, twin)	Bilateral/Normal	VACTERL association	Normal renal function at 2 year	-

GIS: gastrointestinal system, GW: gestational week, PBS: prune-belly syndrome, SUA: single umbilical artery, TOP: termination of pregnancy, VSD: ventricular septal defect.

Table I. Continued.

Publication	GW at diagnosis/ delivery (pregnancy outcome)	Obstructive uropathy/ amniotic fluid index	Prenatal additional findings	Postnatal additional diagnosis/ renal functions	Fetal interventions
Nijagal et al., 2004 [8]	14/36 (live birth)	Bilateral/Normal	-	Cryptorchidism, normal function at 10 year	Bladder aspiration
	20/38 (live birth)	Bilateral/Normal	-	Cryptorchidism, normal function at 2 year	Bladder aspiration
	18/term (live birth)	Bilateral/Normal	-	Normal function at 2 year	-
Misseri et al., 2004 [23]	15/38 (live birth)	Megacystis only/ Oligohydramnios	-	Normal kidney function, anterior urethrotomy	Bladder aspiration
Sepulveda et al., 2005 [24]	20/ - (TOP)	Bilateral/Oligohydramnios	-	-	-
	24/28 (live birth, neonatal death)	Bilateral/Oligohydramnios	Multicystic dysplastic kidney	Pulmonary hypoplasia	-
	28/term (live birth, neonatal death)	Bilateral/Oligohydramnios	-	PBS, pulmonary hypoplasia	-
	28/31 (live birth, neonatal death)	Bilateral/Oligohydramnios	-	Pulmonary hypoplasia	-
Torcia et al., 2007 [25]	21/term (live birth)	Not present/Normal	-	Intrauterine resolution after 3 weeks	-
Gandhi et al., 2008 [26]	22/ - (TOP)	Not present/ Oligohydramnios	Hypoplastic left heart, imperforate anus	Gastrointestinal abnormalities	-
Wax et al., 2009 [9]	21/38 (live birth)	Bilateral/Normal	-	Resolution of dilatation at 32 weeks, normal kidney function	-
Promsonthi and Viseshsindh, 2010 [27]	34/38 (live birth)	Bilateral/Normal	Cryptorchidism, club feet	PBS, normal kidney function	-

GIS: gastrointestinal system, GW: gestational week, PBS: prune-belly syndrome, SUA: single umbilical artery, TOP: termination of pregnancy, VSD: ventricular septal defect.

Table I. Continued.

Publication	GW at diagnosis/ delivery (pregnancy outcome)	Obstructive uropathy/ amniotic fluid index	Prenatal additional findings	Postnatal additional diagnosis/ renal functions	Fetal interventions
Amsalem et al., 2011 [2]	18/38 (live birth, twin)	Bilateral/Normal	Clubfoot	PBS, normal kidney function at 3 year	-
	24/35 (live birth)	Bilateral/Oligohydramnios	Clubfoot	PBS, impaired kidney function at 2 year	Vesicoamniotic shunting, bladder aspiration
	18/- (TOP)	Bilateral/Oligohydramnios	Clubfoot, single umbilical artery (SUA)	Skeletal abnormalities, anal atresia, dextrocardia	-
	20/38 (live birth)	Bilateral/Normal	-	PBS, normal kidney function at 2 year	-
	25/39 (live birth)	Megacystis only/Normal	Clubfoot	Normal kidney function at 2 months	-
	19/ - (TOP)	Bilateral/Normal	-	-	-
	24/38 (live birth)	Bilateral/Normal	-	End stage kidney disease	-
	13/39 (live birth)	Bilateral/Oligohydramnios	-	Rectovesical fistula, imperforate anus	Vesicoamniotic shunting, bladder aspiration
	13/39 (live birth)	Bilateral/Oligohydramnios	-	End stage kidney disease	-
	20/ -(TOP)	Bilateral/Oligohydramnios	Unilateral renal agenesis	Rectovaginal fistula, imperforate anus, SUA	-
Asma & Jumana, 2012 [12]	16/36 (live birth)	Bilateral/Oligohydramnios	-	Renal impairment	Bladder aspiration
Rogers & Sohaey, 2013 [28]	21/term (live birth)	Bilateral/Normal	PBS	-	-
van der Merwe et al., 2013 [29]	27/ - (TOP, both twin)	Bilateral/Normal	Omphalocele, talipes, ventriculomegaly	İmperforate anus, GIS abnormalities, cryptorchidism in one	-
Yamamoto et al., 2013 [30]	17/ - (TOP)	Bilateral/Oligohydramnios	-	Imperforate anus, intestine malrotation	Transabdominal fetoscopy

GIS: gastrointestinal system, GW: gestational week, PBS: prune-belly syndrome, SUA: single umbilical artery, TOP: termination of pregnancy, VSD: ventricular septal defect.

Table I. Continued.

Publication	GW at diagnosis/ delivery (pregnancy outcome)	Obstructive uropathy/ amniotic fluid index	Prenatal additional findings	Postnatal additional diagnosis/ renal functions	Fetal interventions
Di Meglio et al., 2014 [31]	13/ - (TOP)	Megacystis only	Meckel Syndrome	Ambiguous genitalia, anorectal atresia	-
	18/ - (TOP)	Not present/Normal	-	TOP/ pseudohermaphroditism	-
Migliorelli et al., 2015 [32]	21/38 (live birth)	Bilateral/Oligohydramnios	-	Normal kidney function, hydroureter	Fetoscopic laser coagulation of urethral meatus
Moaddab et al., 2015 [5]	20/39 (live birth)	Megacystis only/ Normal	-	Vesicoureteral fistula, VSD, esophageal atresia/normal renal function	-
	23/34 (live birth)	Bilateral/Oligohydramnios	-	Anterior urethral valve, renal impairment	-
	30/39 (live birth)	Not present/Normal	-	Anterior urethral valve normal renal function	-
	22/ - (TOP)	Bilateral/Polyhydramnios	Facial dysmorphia, club feet, clenched hand, renal dysplasia	-	-
	18/ - (TOP)	Bilateral/Normal	-	Anterior urethral valve	-
	20/fetal demise	Bilateral/Normal	-	-	-
Singh et al., 2018 [11]	23/37 (live birth, neonatal death, twin)	Bilateral/Normal	VATER syndrome	Pulmonary hypoplasia	-
Anh et al., 2019 [33]	17/ - (TOP)	Bilateral/Normal	Renal dysplasia	-	-
Chao et al., 2019 [34]	22/ - (TOP)	Bilateral/-	Imperforate anus	-	-

GIS: gastrointestinal system, GW: gestational week, PBS: prune-belly syndrome, SUA: single umbilical artery, TOP: termination of pregnancy, VSD: ventricular septal defect.

microarray analysis were normal. Since urinary dilation was mild and amniotic fluid was normal, it was not necessary to perform any invasive therapeutic procedure. The sonographic findings did not show any significant change during the follow-up scans at 24 and 28 weeks of gestation. At 30th week of gestation, penile urethral dilatation was decreased; and renal pelvic dilatation was resolved.

At the 35th week of gestation, a male newborn, weighing 3000 g and 48 cm in length with Apgar score of 10 at 5 minutes, was delivered by caesarean section due to preeclampsia. The physical examination of neonate was unremarkable, except mildly enlarged penis. Both testes were in scrotal sacs (Fig. 4). Kidney and urinary tract were normal in postnatal sonographic evaluation. Blood urea nitrogen, serum creatinine and urinary protein-to-creatinine ratio were also normal. Under general anesthesia, cystoscopy was performed and the diagnosis of megalourethra was confirmed. The newborn had no urination problems and no intervention was required. At the time of writing this report, the baby was six-month-old and had no problems related to megalourethra and no abnormalities were detected in blood or urine tests during follow-up. He was followed up by the pediatric urology clinic with a plan to evaluate for reconstructive surgery at the age of one.

Written informed consent was obtained from the family for the publication.

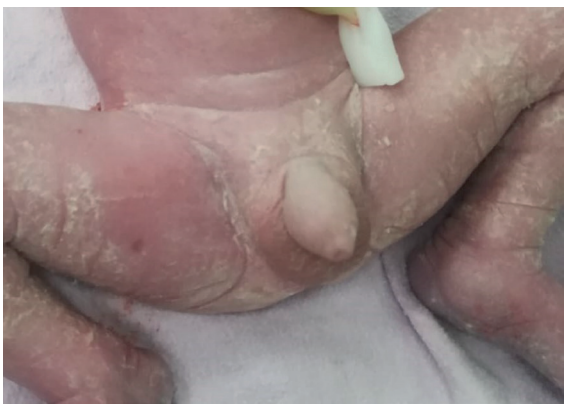

Fig. 4. Postpartum examination of neonate revealing mildly swollen penis.

Discussion

Congenital megalourethra is not inherited and is probably not associated with chromosomal anomalies. Embryological cause is still not well defined. The most commonly presumed causes are differentiation failure of mesenchymal phallic tissues and transient urethral obstruction caused by delayed canalization.[4]

In previous prenatally diagnosed cases, megalourethra was generally detected in the second trimester (40 of 51 reviewed cases; 78.4%). In seven cases, the diagnosis was made in the first trimester. Pregnancy outcomes were as follows: live birth in 32 cases, termination in 17 cases and fetal loss in two cases. Pregnancies were terminated due to the presence of multiple associated anomalies and findings suggesting kidney failure.

Prenatal diagnosis of megalourethra is made by sonographic imaging of fluid filled, cystic, tubular or abnormally shaped dilated penile urethra in a male fetus. Dilated cystic phallus may mimic a loop of umbilical cord between fetal legs. The lack of blood flow on color Doppler may enable to distinguish dilated phallus from the umbilical cord.[7] The keyhole sign, which is specific to lower urinary tract obstruction causes such as posterior urethral valve, can sometimes be seen in megalourethra.[5]

In the vast majority of 51 reviewed cases, genital cystic mass accompanied with varying degrees of urinary tract dilation: bilateral hydroureteronephrosis (39 cases), unilateral hydroureteronephrosis (2 cases) and megacystis (6 cases). In four cases only, there were no sign of urinary tract dilation. In our case, a bilateral mild pelviectasis was observed. As megalourethra has similar consequences such as urinary system dilatation and amniotic fluid reduction, it should be differentiated from other lower urinary tract obstruction causes (e.g. posterior urethral valves, urethral atresia, prune-belly syndrome).[1]

Megalourethra usually occurs together with other congenital anomalies, especially

structural abnormalities of the genitourinary system. In 36 of the 51 reviewed cases (70,5%), other accompanying anomalies were reported. Prune-Belly syndrome, characterized by abdominal wall defect and cryptorchidism, is most frequently associated anomaly with megalourethra (11 cases). Other commonly observed anomalies were imperforate anus, rectal atresia, anterior urethral valve, vesicoureteral fistula, rectovesical fistula, tracheoesophageal fistula, esophageal atresia, gastrochisis, cryptorchidism, VACTERL association, skeletal and cardiac abnormalities (Table I). In our case, no accompanying anomaly was observed.

In mild cases of megalourethra, spontaneous resolution of urethral and urinary tract dilatation in fetus has been reported.[8,9] In our case, we observed some improvement in penile urethral dilatation; and renal pelvic dilatation was resolved.

Fetal intervention has been undertaken in 16 of the 51 reviewed cases. Fetal vesicoamniotic shunting, fetal bladder aspiration and fetal cystoscopy were performed to manage obstructive uropathy and improve renal and pulmonary outcomes. The data was not enough to evaluate the effectiveness of the interventions.

In the postpartum period, anterior urethral valve and urethral diverticula, which have similar clinical presentations such as penile urethra swelling and urinary obstruction complaints, should be considered in the differential diagnosis of congenital megalourethra. Swelling in the megalourethra can be much more severe, and imaging studies such as cystoscopy, retrograde urethrography or voiding cystourethrography may help to diagnose and to differentiate these disorders.[10]

Perinatal and postnatal morbidities caused by megalourethra, if there are no other associated abnormalities, are similar to other causes of lower urinary tract obstruction, and the condition of upper urinary system determines the final result: kidney failure and secondary pulmonary hypoplasia due to oligohydramnios. Like with structural obstructive lesions, the presence of oligo/anhydramnios, bilateral hydroureteronephrosis and renal cysts and echogenity are associated with poor prognosis.[5] Besides these, megalourethra may cause urination problems and sexual dysfunction (e.g. erectile and ejaculatory dysfunction) that can affect patient's quality of life.[4] These complaints tends to be worse in fusiform variant than that of the scaphoid type. In these cases, several reconstructive urosurgical interventions may be required in the postpartum period and later in life, and the long-term prognosis depends on the success of these interventions.[11,12]

In the postnatal period, satisfactory cosmetic and functional results are often provided with penile reconstruction (reduction urethroplasty), especially in scaphoid variants. However, depending on the severity of the defect, especially in the fusiform variants, major interventions such as reassignment of sex may be required.[3]

Congenital megalourethra is a rare cause of fetal lower urinary tract obstruction that is seen mostly in second trimester sonographic examination as a cystic genital mass. In prenatal assessment, extensive systemic evaluation for associated abnormalities and serial follow-up for obstructive uropathy complications are important. Postnatal outcomes may be good as in our case, but there may also be serious disorders such as renal failure, pulmonary hypoplasia, erectile dysfunction and fertility issues. Prior to delivery, the patients should be informed about postnatal outcomes of megalourethra.

Ethical approval

Written informed consent was obtained from the family for the publication.

Author contribution

The authors confirm contribution to the paper as follows: study conception and design: DŞ and FHO; literature review: GGT and FHO; data collection: EE and HTT; draft manuscript preparation: FHO. drafted the manuscript. All authors contributed to critical revision to the paper and approved the final manuscript.

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Conflict of interest

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Case Report

A newborn with anaphylaxis due to vancomycin

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ABSTRACT

Background. All drugs may cause hypersensitivity reactions. Anaphylaxis is a medical emergency that rarely occurs in newborns due to immature immunity. Early diagnosis and treatment are life-saving. Vancomycin, a glycopeptide antibiotic with bactericidal action against Gram-positive bacteria, is commonly used for neonatal nosocomial sepsis.

Case. We hereby present a premature infant (born at the 33rd week of gestation, birth weight: 1745 grams) who was started on vancomycin on postnatal day 7. He had severe circulatory failure and stridor during infusion on day 7 of vancomycin treatment and his tryptase level was elevated to 64.60 micrograms/L

Conclusions. To the best of our knowledge, there is no neonatal case of anaphylaxis due to vancomycin in the literature. Neonatologists should keep in mind that an anaphylactic reaction with a fatal course may develop during vancomycin infusion.

Key words: newborn, anaphylaxis, vancomycin.

Anaphylaxis is a life-threatening systemic hypersensitivity reaction that develops acutely. In epidemiological studies, its lifetime prevalence has been reported to be 0.05-2%.[1] However, the prevalence of anaphylaxis in infants remains unknown.[2] In a European anaphylaxis registry with 1970 patients younger than 18 years, 18 patients were under age one-year (0.9%)[3] The frequency of anaphylaxis under the age of 2 years has been gradually increasing over the years.[2] Common triggers in children are often foods, drugs, and insect venom.[4] While hypersensitivity reactions are common in children, anaphylaxis is very rare in newborns due to immature immunity.[5]

Vancomycin is a glycopeptide antibiotic with bactericidal action against Gram-positive bacteria. It is commonly used in nosocomial neonatal sepsis. To the best of our knowledge, anaphylaxis due to vancomycin has not been reported in newborns as yet. We hereby present a premature infant who had anaphylaxis due to vancomycin.

Case Report

A male baby born to a 24 year - old mother via cesarean section at the 33rd week of gestation, with a birth weight of 1745 grams, with a 1st minute Appearance, Pulse, Grimace, Activity and Respiration (APGAR) score of 7 and 5th minute APGAR score of 8, was admitted to the neonatal intensive care unit (NICU) due to respiratory distress. He was moderately well and his vital signs were, body temperature: 36.6°C, respiratory rate: 60/min heart rate: 141/min. blood pressure: 44 / 22 (32) mmHg, oxygen saturation: 97%. He had bilateral retractions, tachypnea and grunting. He was placed on nasal intermittent positive pressure ventilation (IPPV) and started on 70 mL/kg of fluids and 5 micrograms/kg/min of dopamine. Intravenous ampicillin and gentamicin were started after blood cultures were drawn. Chest X-ray revealed air bronchograms and ground glass appearance,

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so 200 mg/kg of surfactant was administered on day 1. In the follow-up of the patient, there was no need for inotropes, enteral feeding was started. Abdominal distension developed on the 7th day of the patient and his abdominal X-ray and abdominal ultrasound were within normal limits. Enteral feeding was discontinued. An orogastric tube was inserted and placed in free drainage. The patient's white blood cell (WBC) count increased to 9,960/mm^3, platelet (PLT) count increased to 38,000/mm^3, and C-reactive protein (CRP) increased to 10 mg/L, hematocrit was 59%, hemoglobin level was 20 g/dL (Table I). The patient was switched to vancomycin and amikacin. He was weaned off nIPPV and oxygen on day 9. The patient developed stridor, desaturation and circulatory failure within ten minutes of vancomycin (10 mg/kg) infusion on day 14. His blood pressure at the time was 25/9 (14) mmHg, oxygen saturation (SpO2) was 84% and heart rate was 184 beats per minute. Free flow oxygen was provided and vancomycin was stopped immediately. Intravenous adrenaline and methylprednisolone and a 10 mL/kg saline bolus were administered. Serum tryptase level obtained 30 minutes after the episode was 64.6 (0-11.4) μg/L. Vital signs remained normal thereafter and no further vasopressors were needed. Echocardiogram was normal. On the 15th day, blood values were CRP level 2,2 mg/L, hematocrit 54%, hemoglobin level 18.8 g/dL, PLT count 191.000/mm3 and WBC count 12860/mm3 (Table I). Antibiotics were stopped, and the patient remained stable thereafter, tolerating gradually increasing enteral nutrition. His cultures remained negative, and he was discharged on day 30 in good condition. Consent was obtained from the patient's relatives for the publication of this case report.

Discussion

The case presented here is the first newborn who developed anaphylaxis due to vancomycin. In neonatal units, empirical treatment of late-onset sepsis is tailored to common pathogens and antibiotic susceptibility. Vancomycin, a glycopeptide with bactericidal activity against such Gram-positive agents as coagulase-negative staphylococci, methicillin-resistant *Staphylococcus aureus* and enterococci, is a common empirical option.[6,7] Vancomycin was initiated empirically in our patient with a preliminary diagnosis of late onset sepsis, prompted by feeding intolerance, lethargy, thrombocytopenia and increasing CRP levels on postnatal day 7.

Common side effects of vancomycin are nephrotoxicity, ototoxicity, thrombocytopenia, agranulocytosis and phlebitis.[8] Another common side effect is vancomycin flushing syndrome (VFS), a hypersensitivity reaction, also known as "red man syndrome". It is an infusion reaction due to rapid infusion rather than a true allergic reaction. Unlike allergic reactions, it can occur with the first dose. It is characterized by flushing, erythema and itching, especially on the face and neck. Dyspnea and hypotension may also occur.[9,10] VFS is rarely life-threatening.[11] In animal studies, vancomycin was shown to directly activate mast cells, resulting in the release of vasoactive mediators such as histamine.[12] Renz et al.[13] found that when they administered 1000 mg of vancomycin over 10 minutes to 10 presurgical patients, all patients developed VFS, 7 had serious skin reactions and 5 had hypotension. VFS associated with vancomycin use has long been recognized as an adverse drug reaction.

Table I. Laboratory results of the patient.

	1st day	7th day	15th day
Hemoglobin (g/dL)	17.1	20	18.8
Hematocrit (%)	50.3	59	54
Leukocytes (/mm^3)	29560	9960	12860
Thrombocyte (/mm^3)	82.000	38.000	191.000
C-reactive protein (mg/L)	6	10	2.2

However, few systematic investigations have been conducted on pediatric subjects to date. One previous retrospective study evaluated VFS in 650 children who had been exposed to vancomycin, and found a low rate (1.6%) of VFS, which limited their ability to determine risk factors.[14] Deo et al.[15] have recently published a review where they report a total of 11 cases of VFS in the last 8 years, four of which were children. Only a small number of case reports of VFS have been published. One is a report of an infant born at the 32nd week of gestation and had VFS due to vancomycin started for femoral osteomyelitis, one was a newborn who had stridor and VFS after perioperative administration of vancomycin and one was a newborn who had severe hypotension and respiratory distress in addition to VFS while on high-dose vancomycin.[16] In our case, the dosing interval of vancomycin infusion was adjusted according to the gestational age, and it was administered at a dose of 10 mg/kg, diluted to 5 mg/ml concentration, and given as a one hour intravenous infusion. Flushing on the face and neck, which is typical in VFS, was not detected in our case. The main finding in our patient was severe hypotension, tachycardia and respiratory distress. Therefore, VFS was not considered.

Anaphylaxis is a sudden and potentially fatal condition that occurs as a result of exposure to an allergen without any prior symptoms. Therefore, prompt diagnosis and treatment are very important. The prevalence of anaphylaxis in infants remains unknown. One study reported an annual incidence of 0.5/100,000.[17] Anaphylaxis is very rare in newborns due to their immature immunity.[5] There are few case reports about neonatal anaphylaxis in the literature. Kendigelen et al.[18] detected tachypnea, tachycardia, and mild angioedema that developed suddenly during amikacin infusion on the 3rd postnatal day in a premature infant born at 33 weeks of gestational age, and they found that the infant improved rapidly upon the cessation of the amikacin infusion and adrenaline administration. The clinical findings recurred after one hour. The authors suggested that sodium metabisulphite that is present in the amikacin solution was what caused the anaphylaxis. Koklu et al.[19] reported three newborns who had anaphylaxis due to intramuscular vitamin K, intravenous levatiracetam and fluconazole, respectively.

Neonatal anaphylaxis due to vancomycin has not been reported as yet. Patients with anaphylactic reactions due to vancomycin usually have a history of multiple previous exposures. Anaphylaxis is predominantly a clinical diagnosis and involves the skin (itching, urticaria, facial erythema, angioedema), gastrointestinal tract (abdominal pain, vomiting, diarrhea), upper and lower respiratory system (rhinitis, hoarseness, laryngeal edema, stridor, dyspnea, cough, wheezing) and the cardiovascular system (dizziness, hypotension, syncope, shock).[20] Although skin symptoms are predominant in adults in anaphylaxis, the primary presentation in children may be respiratory in some cases.[21] In our case, sudden onset of severe hypotension, tachycardia, stridor and low saturation were detected on day 7 of vancomycin infusion. The findings in neonatal anaphylaxis reported in the literature are cyanosis, tachycardia, hypotension, multiple organ failure with coagulopathy, edema of the eyelids and epiglottis, reduced peripheral perfusion, poor sensorium, flaccidity, apnea, bradypnea and bradycardia, erythematous rashes, urticaria, hypotension and shock. Obviously, neonatal symptoms can easily be confused with sepsis. However, in anaphylaxis, rapid diagnosis and correct treatment are life-saving. In the cases reported in the literature, except for the case who developed anaphylaxis due to cow's milk-based formula, all of these findings were considered to be anaphylaxis, and rapid recovery was observed with adrenaline, steroids, respiratory and circulatory support.

The fact that our patient deteriorated extremely precipitously prompted us to consider anaphylaxis. Due to the poor general condition of the patient and the open vascular access, adrenaline was administered intravenously. Fortunately, the patient improved rapidly upon

the cessation of vancomycin and administration of adrenaline and corticosteroids.

The main effector cells of systemic anaphylaxis are mast cells and basophils. During an anaphylaxis episode, mast cells release mediators such as tryptase, histamine and platelet activating factor. Measurement of histamine levels and tryptase levels can be used in the diagnosis of anaphylaxis. Blood samples should be drawn after 30-120 min of symptom onset.[22] In a recent study examining tryptase levels in children presenting with anaphylaxis, tryptase levels were found to be high in only 19.2% of children, and only severe reactions were associated with levels of 11.4 micrograms/L or higher.[23] Our patient's tryptase level was 64.6 micrograms/L in the serum sample obtained 30 minutes after the onset of anaphylaxis. Severe elevation in serum tryptase level supports that our patient had anaphylaxis due to vancomycin.

In conclusion, anaphylaxis is a clinical emergency. Early diagnosis and treatment are lifesaving. It should be kept in mind that anaphylaxis may occur during vancomycin infusion which is frequently used in neonatal intensive care units.

Ethical approval

Informed consent was obtained from the family for the publication of this case report.

Author contribution

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

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Conflict of interest

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Case Report

Duodenal Dieulafoy lesion: a rare pathogeny of gastrointestinal bleeding in children

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ABSTRACT

Background. Dieulafoy lesion is a calibre persistent submucosal artery associated with a minuter mucosal defect. Dieulafoy lesion has been reported to account for 1-5.8% of acute nonvariceal upper gastrointestinal bleeding in adults, but it is rarely reported in children.

Case. Here we report a case of duodenal Dieulafoy lesion in a 13-year-old boy. After endoscopy and laparotomy, he still had no definite diagnosis and effective treatment. The duodenal Dieulafoy lesion was finally identified by selective angiography and was effectively treated by intravascular embolization.

Conclusions. For unexplained upper gastrointestinal bleeding, the possibility of duodenal Dieulafoy lesion should be considered. A combination of multiple diagnosis and treatment methods can improve the success rate of diagnosis and treatment when a single test or treatment method cannot provide definitive diagnosis or effective treatment.

Key words: duodenal, Dieulafoy lesion, gastrointestinal bleeding, children.

Dieulafoy lesion (DL) is a calibre persistent submucosal artery associated with a miniature mucosal defect, which was first described by Paul Georges Dieulafoy in 1897.[1] Most of DL is located in the stomach, within 6 cm of the gastro-esophageal junction, and rarely occur further along the gastrointestinal tract including duodenum, jejunum, ileum, cecum, appendix, colon and anal canal.[2] DL in children is rarely reported which is difficult to timely diagnose and can be fatal without appropriate treatment. Here we report a case of DL in a 13-year-old boy. Through the diagnosis and treatment of this case, we hope to provide experience for gastrointestinal bleeding of unknown pathogeny in children.

Case Report

A 13-year-old boy was admitted to pediatric surgery clinic for hematochezia and dizziness. The patient had been hospitalized in another hospital with the same symptoms 3 months prior. No definite bleeding was found on abdominal contrast-enhanced computerized tomography (CT), and no abnormal lesions in the esophagus, stomach and duodenum were found by gastroscopy. The patient denied any surgical history and was not taking any medication. The family history was negative for colorectal cancer, *Helicobacter pylori* infection and intestinal polyps.

Physical examination showed hypotension (blood pressure 92/50 mmHg), pallor and normally capillary filling test. The initial laboratory testing showed severe anemia (white blood cell count 7.6×10^9/L, hemoglobin 5.6 g/dL, hematocrit 18.5%, platelet count 322×10^9/L). Coagulation tests showed normal liver function, kidney function, cardiac enzymes and electrolytes were normal.

On the first day of hospitalization, the treatment regimen of blood transfusion (10 units of packed red blood cells) and application of hemostatic

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drugs were adopted, but the laboratory testing still showed severe anemia (white blood cell count 8.9×10^9/L, hemoglobin 6.5 g/dL, hematocrit 21.0%, platelet count 255×10^9/L). The first endoscopy revealed no abnormalities in the esophagus and stomach, but showed suspicious duodenal polyp without active bleeding (Fig. 1). Because the patient had always presented with black tarry stool, we considered that it might be small intestinal bleeding, so colonoscopy was not given priority. The exploratory laparotomy was taken immediately to identify the reason of hematochezia. No malformations of intestines and blood vessels were found during the operation. No jejunum abnormality was detected by enteroscopy. However, there are many convex nodules and fresh blood on the wall of the distal ileum. The distal ileum was resected, but histopathological analysis showed no abnormalities such as granuloma. On the fifth day after surgery, the patient had hematochezia again.

During the second endoscopy, we found a suspected actively bleeding protruding vessel at the suspicious duodenal polyp found in the first endoscopy and fresh blood in the stomach and duodenum (Fig. 1). Endoscopic ligation failed due to rapid bleeding and poor visual field exposure.

We urgently performed selective angiography of the celiac trunk and mesenteric artery. The angiography of each branch of celiac trunk

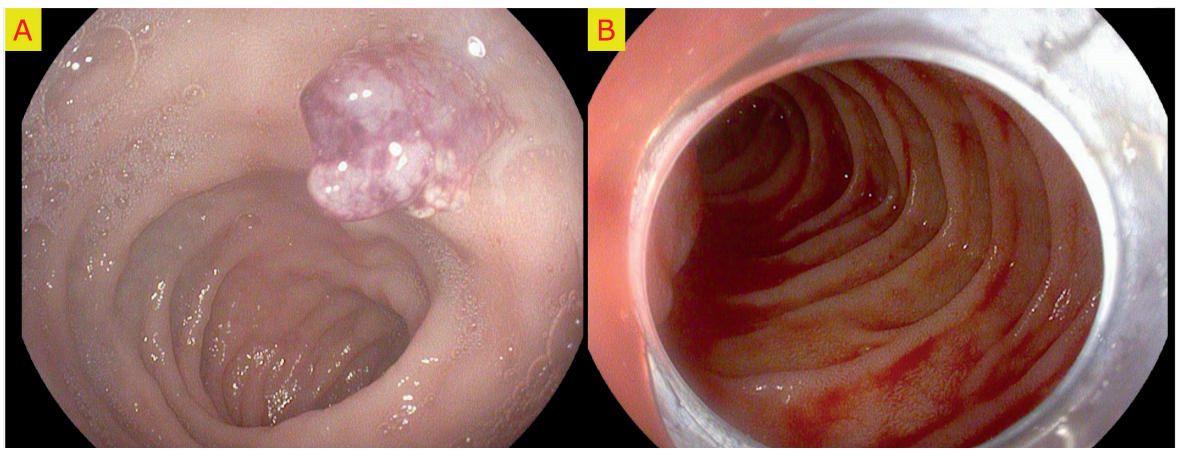

Fig. 1. Endoscopic features. (A) The first endoscopy showed suspicious duodenal polyp without active bleeding. (B) The second endoscopy showed fresh blood in the duodenum.

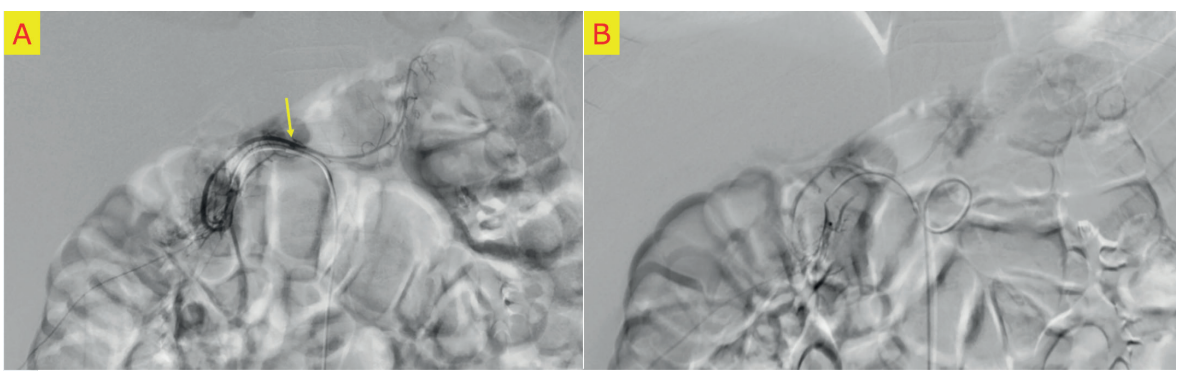

Fig. 2. Image of the selective angiography. (A) The selective angiography showed that the distal branch of gastroduodenal artery had a constant diameter, and there was a leak of contrast agent. (B) Distal embolization of the bleeding branch was performed utilizing gelatin sponge particles, and post-embolization arteriograms showed complete cessation of bleeding.

artery was performed successively. The distal branch of gastroduodenal artery had a constant diameter, and there was a leak of contrast agent (Fig. 2). The diagnosis of duodenal Dieulafoy lesion (DL) was confirmed. Distal embolization of the bleeding branch was performed utilizing gelatin sponge particles (700-1000 μm, 1000-1400 μm, 1400-2000 μm, Hangzhou Alicon Pharmaceutical Co., Ltd), and post-embolization arteriograms showed complete cessation of bleeding (Fig. 2).

The patient recovered steadily and was discharged 10 days after vascular embolization. No further hematochezia occurred after 10 months of follow-up. Informed consent was obtained from his parents for publication and photographs.

Discussion

Pediatric DL is rarely reported.[3] Hematochezia in children is usually caused by intestinal malformation such as Meckel's diverticulum, intestinal duplication. The reason for DL is often overlooked by pediatricians. In this case we report, although suspicious lesions in the duodenal bulb were found under the first endoscopy, the possible presence of duodenal DL was ignored, which was a profound lesson for us.

There were many deficiencies in the diagnosis of this case. Firstly, we had insufficient understanding of the pathogenies of gastrointestinal bleeding in children, especially for some rare pathogenies. Secondly, for unexplained gastrointestinal bleeding, it was necessary to perform enhanced CT or conventional angiography after endoscopy was negative, even if these tests might be negative during the bleeding interval of DL. Because the enhanced CT examination of the patient was negative 3 months ago, we ignored the necessity of reexamination of enhanced CT or conventional angiography.

The diagnosis and treatment of DL mainly include endoscopy, selective angiography, and surgery. Endoscopy is the preferred treatment for DL, and the success rate can reach 90%.[4] Endoscopic injection, hemostatic clamping, ligation, and electrocoagulation are all common treatments, but there are still risks of hemostatic failure or bleeding recurrence.[5,6] Due to rapid bleeding and poor visual field, hemostasis under endoscopy failed in our case. Selective angiography can be used as a second-line treatment for DL.[7] It is suitable for patients who have failed endoscopic treatment and cannot tolerate the surgery. Interventional vascular embolization can achieve precise treatment of bleeding, but it also has risks of gastrointestinal necrosis and bleeding recurrence, etc. Surgery used to be the preferred treatment for DL, and surgical methods included electrocoagulation, suture hemostasis, and subtotal gastrectomy, etc. Although electrocoagulation and suture hemostasis were simple, the risk of postoperative recurrence was high. With the continuous development of endoscopic and interventional techniques, surgery is mainly used as the choice after endoscopy or interventional treatment fails, while laparoscopic exploration can be used as the preferred surgical method.[8,9] The surgical exploration of this case failed, which was a profound lesson for us. We hope to provide experiences for other researchers with the solution of the appropriate problem and the addition of the lessons learned.

We searched previous literature with "duodenum", "Dieulafoy" and "children" as keywords, and a total of 9 cases related to the diagnosis and treatment of DL were retrieved (Table I).[10-18] 3 cases were successfully diagnosed and effectively treated under endoscopy, 1 case was successfully treated with duodenotomy, 1 case died 4 hours after surgery, 4 cases were successfully treated with other methods after failed diagnosis or treatment under endoscopy (angiographic embolization in 2 cases, exploratory laparotomy in 2 cases). The case we reported was effectively diagnosed and treated after receiving endoscopy, surgical exploration, endoscopy, and selective angiography, and has been followed up for 10 months without recurrence.

Table I. Case reports of duodenum Dieulafoy lesion (DL) in children.

Authors	Age (sex)	Diagnostic tool	Treatment measures	Follow-up	Recurrence
Komissarov I A, et al.[10]	13 m (M)	Endoscopy; Selective angiography	3 coils of Trufill	5 y	No
Alomari A I, et al.[11]	14 y (F)	Endoscopy; Selective angiography	50% NBCA glue	8 m	No
McClave SA, et al.[12]	16 y (M)	Endoscopy; Laparotomy	A "figure eight" suture	3 y	No
Bilal M, et al.[13]	18 y (M)	Esophagogastroduodenoscopy	Band ligation	6 m	No
Rao S, et al.[14]	3 y (M)	Colonoscopy; Endoscopy; Duodenotomy	Duodenotomy.	_	_
Akira Hokama, et al.[15]	10 y (M)	Endoscopy	MD-850 hemoclips	1 y	No
Shi SJ, et al.[16]	9 y (M)	Laparotomy	Gastroduodenectomy	3 y	No
Wang CL, et al.[17]	11 m (F)	Endoscopy; Laparotomy	Ligasure	_	_
Jadhav DV, et al.[18]	1 m (M)	Endoscopy	Epinephrine and sporadic argon plasma coagulation	1 w	No

Because duodenal DL is clinically rare, the possibility of duodenal DL should be considered for unexplained upper gastrointestinal bleeding.[19] Suspicious gastrointestinal lesions found by endoscopy without bleeding should also be actively treated, which may be the bleeding interval of DL or submucosal thrombosis. A combination of multiple diagnosis and treatment methods can improve the success rate of diagnosis and treatment when a single test or treatment method cannot provide definitive diagnosis or effective treatment, and angiography should be tried primarily before laparotomy.

Ethical approval

This research is supported by the Ethics Committee of Shandong University Qilu Hospital (Qingdao) [KYLL-KS-2021030].

Author contribution

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

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Conflict of interest

The authors declare that there is no conflict of interest.

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Case Report

Celiac crisis with thrombocytopenia and coagulopathy in a child

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ABSTRACT

Background. Celiac disease rarely presents with edema, hypoalbuminemia, acute metabolic deterioration, and electrolyte imbalances. This life-threatening condition is defined as a celiac crisis and may mimic disorders with metabolic derangement and sepsis. The crisis may present at onset or develop in celiac disease patients with poor compliance to a gluten-free diet. The fluid resuscitation and replacement of electrolyte deficits are life-saving modalities.

Case. A 14-month-old girl was admitted with fever, lethargy, severe dehydration, edema, hypotension, and commenced sepsis therapy. However, the patient had a growth delay and loss of weight with diarrhea and delayed motor skills. On admission, laboratory evaluation showed anemia, coagulopathy, hypoalbuminemia, electrolyte disturbances, and metabolic acidosis and developed thrombocytopenia during follow-up. The celiac serological tests and upper gastrointestinal endoscopic duodenal mucosa appearance, and duodenum histopathology findings suggested celiac disease.

Conclusions. This case highlights that a celiac patient may present with a severe illness like sepsis and may be associated with cytopenia and coagulopathy in the celiac crisis.

Key words: celiac crisis, thrombocytopenia, coagulopathy.

Celiac disease is an autoimmune and inflammatory disease that develops in genetically susceptible individuals against the gluten protein in wheat, barley, and rye.[1] The prevalence is 0.7-1.4 %, and is common in Europe.[1] The estimated prevalence of celiac disease is 0.47% in Turkish school children.[2] The most severe and rare complication is the celiac crisis.[3] The crisis may present at onset or develop in celiac disease patients with poor compliance to a gluten-free diet. The mortality rate has been reported as 9% in the crisis.[4] A celiac crisis is presented with edema, hypoalbuminemia, fluid and electrolyte imbalances, and metabolic acidosis following diarrhea requiring hospitalization and parenteral nutrition (Table I).[3] The diagnostic criteria are not clarified in children.

To the best of knowledge, the celiac crisis was accompanied by thrombocytopenia in one child reported in the literature, while the celiac crisis accompanied by coagulopathy was not reported in children.[5] We present a 14-month-old girl admitted with fever, lethargy, severe dehydration, edema, hypotension, metabolic acidosis mimicking sepsis, accompanying anemia, thrombocytopenia, and coagulopathy who was diagnosed with the celiac crisis.

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Table I. Celiac crisis criteria.[3]

1. Acute onset or rapid progression of gastrointestinal symptoms attributable to celiac disease requiring hospitalization and /or parenteral nutrition along with at least 2 of the following:
2. Signs of severe dehydration including hemodynamic instability and/or orthostatic changes
3. Neurologic dysfunction
4. Renal dysfunction, creatinine level, >2.0 g/dL
5. Metabolic acidosis, pH <7.35
6. Hypoproteinemia (albumin level, <3.0 g/dL)
7. Abnormal electrolyte levels including hypernatremia/hyponatremia, hypocalcemia, hypokalemia, or hypomagnesemia
8. Weight loss, >4.5 kg

Case Report

A 14-month-old girl was admitted with irritability, poor feeding, diarrhea for the last two days, and a 38.6 °C fever. She had lethargy, and moderate to severe dehydration findings. Blood pressure (BP) was 58/38 mmHg (systolic BP below 5th percentile, diastolic BP at 75th percentile), and the heart rate was 136/minute, the respiratory rate was 42/minute and capillary refill time was 3 seconds. She had reduced skin turgor and pretibial edema. Her weight was 6.5 kg (z score -3.46), and her height was 69 cm (z score -3.03). She had no cough, dyspnea, and rhinorrhea. COVID-19 was excluded in the patient by nasal swab polymerase chain reaction and chest radiography. She had severe metabolic acidosis in her blood gas analysis. The laboratory values are shown in Table II. The blood smear test showed hypochromia, microcytosis except for schistocytes and helmet cells, which were not compatible with hemolysis.

Fluid replacement containing potassium phosphate and bicarbonate therapy commenced. The fever did not recur again. C-reactive protein was 0.005 g/L (N: <0.005). The patient had a growth delay and weight loss in the preceding two months with diarrhea, steatorrhea, and delayed motor skills. The parents are consanguineous, and there was no celiac disease in her family. The stool microbiological and microscopic examinations and the rotavirus and adenovirus antigen tests were negative. The platelet level decreased to 83.000 $x10^9$/L, which was confirmed by peripheral smear, and recovered to >150.000 $x10^9$/L in a week. The Coombs test was negative. No bleeding was observed. The international normalized ratio (INR) was not corrected with vitamin K treatment, possibly due to disseminated intravascular coagulation (DIC). The DIC score (including platelet count, d-dimer, PT, fibrinogen level) was three. A score lower than five is not suggestive of overt DIC. The blood, urine, and fecal bacterial cultures were negative. The metabolic screening tests, including tandem mass spectrometry, urine, and blood amino acid levels, were normal. The tissue transglutaminase immunoglobulin (Ig) A and endomysium IgA were positive, >200 RU/mL (N: ≤20), 1/100 titers, respectively. The upper gastrointestinal endoscopy revealed a cracked-mud appearance in duodenum mucosa, accompanied by Marsh 3C duodenal histopathological changes (severe villous flattening, crypt hyperplasia, increased intraepithelial lymphocytes, and lymphoplasmacytic inflammation in lamina propria) suggesting celiac disease (Fig. 1). After that, a gluten-free diet and enteral feeding formulas were initiated. Vitamin A, vitamin E, folic acid and vitamin B_{12} serum levels were normal. The serum 25-OH-vitamin D level was 16 ng/mL (<20: deficiency), and the serum zinc level was 67 mg/dL (N: 70-120), so vitamin D 600 IU/day and zinc 5 mg/day were supplemented. The serum glucose levels and thyroid function tests were normal. Genetic testing for HLA-DQ2 was positive. She gained

Table II. Laboratory results of the patient.

Laboratory Test; Normal range	Day 1	Day 4
Hemoglobin, 11-13 g/dL	8.7	9.8
MCV; 70-85 fL	74	86.5
RDW; 11-16 %	19	20
WBC; 5.4-13.6 x10^9/L	11.36 x10^9	6.49 x10^9
Platelets; 160-385 x10^9/L	373 x10^9	90 x10^9
pH; 7.37-7.45	6.8	7.3
HCO_3; 18-26 mmol/L	6	24.4
Base Excess; ±2 mmol/L	-26	0.8
Creatinine; 0.3-0.6 mg/dL	1.11	0.05
Albumin; 32-48 g/L	27	29
Sodium; 132-149 mEq/L	139	141
Potassium; 3.5-5.5 mEq/L	1.7	5.0
Chloride; 99-109 mEq/L	121	118
Calcium; 9.1-10.3 mg/dL	8.2	8.9
Phosphorus; 2.9-4.8 mg/dL	2.6	4.1
Magnesium; 1.3-2.7 mg/dL	2.2	1.6
Fibrinogen; 1.7-4.2 g/L	1.2	1.5
PT; 9.8-14 seconds	17.6	17.7
aPTT; 21-32 seconds	28.9	27
INR	1.52	1.54

MCV: mean corpuscular volume, RDW: red cell distribution width, WBC: white blood cell, PT: prothrombin time; aPTT: activated partial thromboplastin time, INR: International normalized ratio

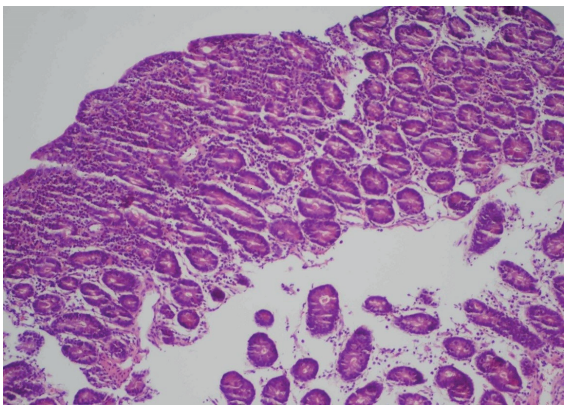

Fig. 1. Severe villous flattening, crypt hyperplasia, increased intraepithelial lymphocytes, and lymphoplasmacytic inflammation in lamina propria (HE, x100).

weight at an outpatient clinic visit after 30 days of beginning the gluten-free diet and vitamin and zinc supplements. The patient caught up on developmental milestones.

Informed consent was received from the parents for the publication of this case report.

Discussion

Celiac disease's pathophysiology involves autoimmune and innate immune responses affected by environmental, chemical, and infectious factors based on genetic susceptibility, including HLA DQ2 and DQ8. It is characterized by a loss of gluten tolerance. Patients may present with intestinal and/or extraintestinal findings such as arthritis, cardiomyopathy, ataxia, peripheral neuropathy, encephalopathy, dermatitis, and hematological involvement or celiac crisis.[6] The crisis findings, including dehydration, electrolyte imbalances, hypoalbuminemia, metabolic acidosis, lethargy, and increase of creatinine, occurred in our case. The fluid resuscitation and replacement of electrolyte deficits are life-saving modalities for the crisis. Corticosteroids may be used in refractory cases, but their efficiency is controversial.[4] The crisis should be considered in patients with acute metabolic deterioration accompanied by chronic fatty diarrhea, growth, and developmental delay. Switching to a gluten-free diet as soon as the patient is diagnosed is the first step to avoiding this fatal crisis.

Anemias in celiac disease are common due to the malabsorption of iron, vitamin B_{12}, folate, and micronutrients such as zinc and copper.[7] A meta-analysis revealed that 3.2% of the patients with iron deficiency anemia had celiac disease.[8] Also, elevated cytokines due to intestinal mucosa inflammation may stimulate hepcidin production, which eventually decreases iron absorption and leads to iron accumulation in the reticuloendothelial system contributing to chronic disease anemia.[9] The more severe mucosal histopathological findings correlate with more severe anemia.[9] Our patient had anemia and coagulopathy attributed to iron, zinc, and vitamin K deficiency due to malabsorption. The literature suggests that vitamin B_{12} deficiency anemia is more common than iron deficiency anemia.[10] However, our

patient had iron deficiency anemia, while serum vitamin B_{12} was normal. Although the patient's histopathology was severe, she had moderate anemia. Thrombocytopenia, leukopenia, and aplastic anemia are rarely associated with celiac disease.[11,12] It has been suggested that the intestinal mucosa and hematopoietic stem cells in the bone marrow are damaged by autoreactive T cells.[13] Thrombocytopenia is rare compared to other hematological manifestations, such as anemia, thrombocytosis, and hypersplenism.[14] As in our case, thrombocytopenia that occurs with the crisis may also result from auto-inflammatory processes. Celiac disease may also be accompanied by autoimmune disorders, including Evans syndrome and immune thrombocytopenic purpura (ITP).[15,16] Karunakaran et al.[17] reported that celiac disease in adult patients with ITP was significantly higher than in the healthy population. Although this study did not report an increase in platelets with a gluten-free diet, other studies report an increase in platelet count with introducing a gluten-free diet.[17,18] They speculated that gluten-related inflammatory mechanisms of celiac disease might lead to autoimmune hematologic manifestations.[17] In our case, chronic disease anemia was associated with celiac disease, nevertheless mild thrombocytopenia was associated with the celiac crisis. The absence of ongoing fever, lack of viral symptoms, and examination findings did not support the infection. A 14-year-old girl diagnosed with a celiac crisis accompanied by severe thrombocytopenia showed gastrointestinal bleeding and died of pulmonary hemorrhage.[5] Another hematologic manifestation that causes bleeding diathesis in celiac disease is coagulopathy, often caused by vitamin K deficiency due to malabsorption. Also, DIC may lead to anemia and thrombocytopenia. However, the DIC score and peripheral smear findings did not suggest overt DIC in the presented case. On the other hand, the coagulopathy or thrombotic condition that can be seen in celiac disease is attributed to causes such as chronic inflammation, endothelial damage, the presence of phospholipid Ig A autoantibodies, and hyperhomocysteinemia due to protein loss in the intestinal wall or related to folate deficiency.[19-21]

In children, while thrombocytopenia and coagulopathy are rarely seen in the course of celiac disease, this association has been described for the first time in the celiac crisis. This case highlights that a celiac patient may present with a severe illness like sepsis and may be associated with thrombocytopenia and coagulopathy in the crisis. Early diagnosis and commencing a gluten-free diet as soon as possible can be life-saving.

Ethical approval

Written informed consent was obtained from the parents for the publication of this case report.

Author contribution

The authors confirm contribution to the paper as follows: study conception and design: ŞH, SD, AÜA; data collection: GH, BBA; analysis and interpretation of results: HTD, AMD; draft manuscript preparation: AÜA, DİM. All authors reviewed the results and approved the final version of the manuscript.

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Conflict of interest

The authors declare that there is no conflict of interest.

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Case Report

The coexistence of two rare diseases thought to use the same pathologic pathway: cystic fibrosis and Niemann-Pick disease

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ABSTRACT

Background. Cystic fibrosis (CF) is a multisystemic, autosomal recessive disease, which is caused by a mutation in the transmembrane conduction regulator protein (CFTR) gene. We present a patient who was diagnosed with CF and later diagnosed with Niemann-Pick type-A (NPA) disease, which is an autosomal recessive lysosomal lipid storage disease.

Case. A 2-month-old Syrian refugee patient was diagnosed with CF due to a high sweat test and two homozygous CFTR-related pathogenic gene mutations in our pediatric pulmonology clinic, where she was referred due to a high immunoreactive trypsinogen (IRT) value as a result of newborn screening. As the patient had neurological symptoms and hepatosplenomegaly that could not be explained by CF in the clinical follow-up, the patient was diagnosed with NPA was made with a cherry red spot on eye examination, foam cells in the bone marrow, and low sphingomyelinase activity, in addition to CF.

Conclusions. Although CF and NP have common systems of involvement in both diseases, pathological symptoms have different origins. If a patient with CF has simultaneous neuromotor delay, other autosomal recessive diseases that may accompany it should be suspected. In studies, similar pathological pathways related to abnormal cholesterol accumulation in the cell were detected between NP type C and CF. But our case was NPA. As case reports on the coexistence of the two diseases increase, we believe that a better understanding of similar pathological pathways may lead to new therapeutic targets for both diseases.

Key words: cystic fibrosis, pathological pathway, Niemann-Pick, autosomal recessive diseases.

Cystic fibrosis (CF) is an autosomal recessive disease caused by mutations in the gene encoding the cystic fibrosis transmembrane conductivity regulator (CFTR). CFTR is a chloride channel located mainly in the apical membrane of epithelial cells, affecting salt and fluid transport.[1] Niemann-Pick (NP) is an autosomal recessive lysosomal lipid storage disease and organomegaly and neurological retardation are seen due to lipid storage.[2]

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In animal experimental studies, although CF and NP type-C (NPC) diseases contain different genetic defects, similar cholesterol processing defects and abnormal accumulation of free cholesterol in the perinuclear membrane compartments have been shown. Cholesterol storage affects many cellular functions and signaling pathways. It was hypothesized that both CFTR activation and expression and NPC protein expression were regulated by the cyclic adenosine monophosphate (cAMP) pathway, and a feedback response involving this pathway might play a role in the cholesterol accumulation phenotype.[3,4]

In our patient, CF and Niemann-Pick type-A (NPA) coexistence were detected.

The aim of this case report is to increase the number of CF and NP case reports, to better elucidate the pathological pathway and thus to reach common therapeutic goals.

Case Report

A 2-month-old male Syrian refugee patient was referred to the pediatric pulmonology clinic due to an immune reactive trypsinogen (IRT) level of 135.1 ng/mL at the first measurement and 93.7 ng/mL at the second measurement. His general condition was good, his body weight was 4000 g (< 3p) standard deviation score (SDS): -1.9, his height was 53 cm (10-25 p) SDS: -1.76, and his head circumference was 38.5 cm (3-10p) SDS: -0.86. His parents were second-degree relatives, and the mother lost her first pregnancy due to anencephaly. There was no family history of disease suggestive of CF or metabolic disease. The patient's history was unremarkable other than a one-day hospitalization and phototherapy due to jaundice. His complete blood count was as follows: white blood cell (WBC): 13,300/µL, absolute neutrophil count (ANC): 3400/µL, absolute lymphocyte count (ALC): 8200 /µL, hemoglobin (Hgb): 11 g/dL, platelet count (PLT): 247,000/µL, alanine aminotransferase (ALT): 208 U/L, aspartate aminotransferase (AST): 137 U/L, sodium (Na): 133 mmol/L, potassium (K): 4.9 mmol/L, chloride (Cl): 99 mmol/L, gamma-glutamyl transferase (GGT): 142 U/L, direct bilirubin: 0.5 mg/dL, indirect bilirubin: 0.61 mg/dL, total protein: 56.5 g/dL, albumin: 4.2 g/dL, total cholesterol: 169.4 mg/dL, and triglyceride: 335 mg/dL. His fecal fat test was positive, his sweat chloride test result was 89 mEq/L, and a genetic examination revealed compound heterozygosity for c.328G>C (D110H mutation)/c.274-8T>C, leading to a diagnosis of CF for which treatment was started. Hypotonicity and hepatomegaly developed in the second month and so a further evaluation was planned. A neurologic examination revealed a lack of head control, and an abdominal examination revealed the liver to be palpable 3 cm below the costal margin. An eye examination revealed bilateral cherry-red spots. Abdominal ultrasonography revealed grade-I increased echogenicity in the liver and diffuse hepatosplenomegaly, and a bone marrow aspirate showed NP foam cells (Fig. 1). Cranial magnetic resonance (MR) revealed a thin corpus callosum and a slightly increased subarachnoid distance. The sphingomyelinase (SM) activity measured from bone marrow aspiration findings was found as 1.7 nm/hr/mg protein (normal: 52-173 nm/hr/mg protein), and the patient was reported as homozygous c.1430C>T. Based on these clinical and examination findings, the patient was diagnosed as having NPA in addition to CF. At follow-up, the patient's liver and spleen continued to increase in size. The patient experienced increased respiratory distress due to diaphragmatic compression and developed a recurrent pulmonary infection, so a tracheostomy was performed. Respiratory support was given to the patient with a home-type mechanical ventilator. Over a year, the patient's throat cultures revealed the growth of *Pseudomonas aeruginosa* eight times, thus the patient was started on treatment for Pseudomonas colonization; he did not have CF-related pulmonary parenchyma involvement. In the pediatric intensive care unit (PICU) follow-up, the patient had ascites and required frequent paracentesis. The patient, who was followed up by the departments of Pediatric

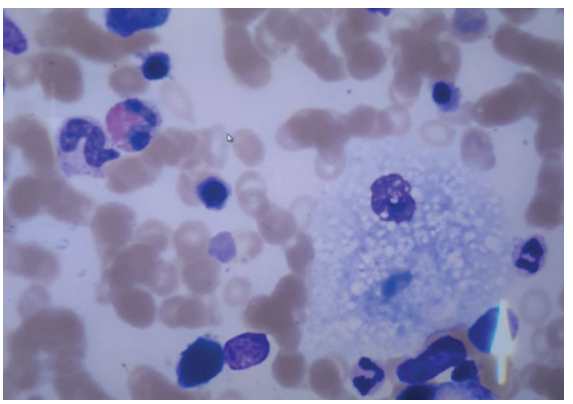

Fig. 1. Typical foam cell (arrow) from the marrow aspiration of the patient.

Pulmonology and Gastroenterology for CF and NP, died during PICU follow-up for respiratory distress and sepsis. Written informed consent was obtained from the family for this case report.

Discussion

Cystic fibrosis is an autosomal recessive disorder that presents with different clinical symptoms due to the involvement of multiple systems. Early diagnosis and appropriate treatment are important for the reduction of long-term morbidity, for which IRT newborn screening programs have been developed and are used in our country.[1] Pulmonary symptoms are the most common of all symptoms in all age groups, followed by gastrointestinal symptoms. This leads to such manifestations as fat-soluble vitamin deficiencies, calorie starvation, and growth and developmental retardation. The typical lesions in liver disease that develop in one-third of patients with CF include biliary obstruction, focal biliary cirrhosis due to progressive periportal fibrosis, and hypersplenism.[5] Our patient was referred to our clinic with a high IRT identified during newborn screening and was found to have fat malabsorption, growth retardation, elevated liver enzymes, hepatosplenomegaly, and hypotonicity at follow-up. The diagnosis of CF was confirmed through a sweat chloride test and from the identification of a genetic mutation, but the patient had unexplained neurological symptoms and organomegaly.

NP disease is a rare, autosomal recessive lysosomal lipid storage disorder that is characterized by visceromegaly and neurologic changes that occur due to the excessive storage of lipids, sphingomyelin, and cholesterol. It often presents with neurologic symptoms, gastrointestinal symptoms such as hypersplenism and cirrhosis, growth retardation-developmental delay, ocular symptoms, and pulmonary involvement. There are six clinical subgroups of NP, among which types A, B, and C are the most common.[2] Type A is the most common acute form with neurologic involvement, the course of which is fatal. In this form, symptoms start within the first 5 months of life, with the most prominent symptoms in infancy being hepatosplenomegaly, feeding difficulties, abdominal distension, frequent pulmonary infections, progressive retardation of early motor functions, macular degeneration, and convulsions. Patients rarely survive beyond the age of 2 years. Sphingomyelinase activity is almost totally reduced or absent. In type B, which occurs in infancy or early childhood, symptoms progress slowly, and patients often survive into adulthood.[2,6] The clinical spectrum of type C ranges from a neonatal rapidly fatal disorder to an adult-onset chronic neurodegenerative disease. The most characteristic symptom is vertical supranuclear gaze palsy.[7] Our patient was evaluated for neurologic diseases because he had hepatosplenomegaly and neurologic findings that could not be explained by CF alone, which was detected at the age of 2 months. An eye examination and cranial MR imaging were performed. The eye examination revealed bilateral cherry-red spots. The patient was diagnosed with NPA because of the marked decrease in SM activity, genetic mutation, and foam cells on the bone marrow aspiration material.

Significant differences in NP compared with CF are excessive lipid deposition in the liver and spleen leading to hepatosplenomegaly and progressive liver failure, in addition to neurologic symptoms.

Cystic fibrosis and NP disease have many common signs, such as gastrointestinal symptoms growth retardation-developmental delay, and pulmonary involvement. In the literature, this co-existants has been explained through the similar pathological pathways between NPC and CF. In a study by Kelley et al.[3] NPC and CF cell models shared a series of cell regulatory changes, including reduced nitric oxide synthase 2 (NOS2), high sterol-sensing domain (STAT1), Ras Homology Family Member A (RhoA) expression, and they reported that NPC-fibroblasts were similar to CF cells in

terms of altered expression of various signaling proteins. In another study, it was shown that both CFTR activation and expression were regulated by the cAMP pathway, and a feedback response involving this pathway played a role in the cholesterol accumulation phenotype in CF cells. The similarity between the two diseases is that cholesterol accumulation in dysfunctional NPC cells occurs with the same cAMP-mediated response. The diagnosis of our patient was NPA. To the best of our knowledge, there is no report on the similarity between NPA and CF in the literature. Considering the similar metabolic pathways between NPC and CF, and the co-occurrence of NPA and CF in our patient, it suggests that a similar pathway may also cause NPA and CF.[4] It is also believed that the F508del mutation, which is the most common cause of CF, causes misfolding of the CFTR protein by impairing the efficient exit signal of the CFTR protein from the endoplasmic reticulum (ER), and NPC1 mutations are believed to cause the same defective protein response from the ER by causing protein misfolding. Our patient did not have a F508del mutation, but the coexistence of NPA and CF suggests that other CFTR mutations should also be investigated in this respect.[3]

In conclusion, if a patient's presentation cannot be explained by CF alone, other rare autosomal recessive diseases such as storage disorders should also be considered. There are articles about the similarity of pathologic metabolic pathways between NPC and CF, but our case was NPA. We believe that as the relevant case reports in the literature increase, the similar pathologic pathway relationship between them will be better understood.

Ethical approval

Written informed consent was obtained from the family for this case report.

Author contribution

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

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Conflict of interest

The authors declare that there is no conflict of interest.

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High-flow nasal cannula failure in the Pediatric Emergency Department: Remarks and questions to explore the predictive factors

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To editor,

We have read with great interest this study "Predictive factors of high-flow nasal cannula oxygen therapy failure in children with respiratory distress treated in a Pediatric Emergency Department (ED)".[1] We congratulate Aydın et al. for their contribution to predictive factors of high-flow nasal cannula (HFNC) oxygen therapy failure in children. However, we think some points need to be taken into account for an adequate evaluation of the results.

Firstly, there are some points which need to be clarified regarding the population enrolled in this study. Medical history was coded into 4 binary variables defined by previous history which makes this group very diverse. We do not think that an atopic patient and a patient with muscular dystrophy will have the same clinical response to HFNC. Additionally, there may be some complications in ailments causing respiratory distress with HFNC.[2,3] Some undesirable effects like septic shock, arrhythmia, and cardiopulmonary arrest may occur. We don't know what complications happened in the HFNC treated group.[1]

Secondly, the patients admitted immediately to the intensive care unit (ICU), patients with cyanotic heart disease, craniofacial anomaly, skull base fractures, upper airway obstruction, and the patients who received oxygen therapy at home were excluded. These factors may cause changes in the results.[2]

Thirdly, patients with pneumonia received salbutamol and steroids which are the main medication given to patients with bronchiolitis and are controversial in patients with pneumonia.[2,3] This may raise questions about the effectiveness of HFNC therapy by putting these two different diseases into the same group as this may have influenced the results.[1]

It would have also been interesting to know the duration of non-invasive and invasive mechanical ventilation in the successful or unsuccessful treatment groups, length of stay in the hospital and mortality.[1]

Lastly, it would have been noteworthy to know the HFNC escalation or des escalation options[2,3,4] such as was a reduction in respiratory rate and heart rate evaluated, when was oxygen flow and the percentage increased for patients with successful HFNC therapy and was the oxygen saturation above 94 percent in blood gas taken as the only threshold for success. In those who started on HFNC treatment, the markers at the 2nd hour were evaluated it would have been interesting to know what the status of those who improved in the second hour and then became worse after a few hours. It is unclear what the outcome was for those who improved with HFNC treatment and those who did not.[1,4]

Further clinical trials need to confirm the impact of HFNC and these predictive factors in the emergency department.

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Key words: high flow nasal cannula oxygen, predictive factors, pediatric, emergency department.

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Response to "High-flow nasal cannula failure in Pediatric Emergency Department: Remarks and questions to explore the predictive factors"

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Dear Editor,

This letter is a reply to the correspondence entitled "High-flow nasal cannula failure in Pediatric Emergency Department: *Remarks and questions to explore the predictive factors*". We would like to thank the authors for their interest in our article and for giving us the opportunity to further explain. In their letter, they comment on our original article entitled "Predictive factors of high-flow nasal cannula oxygen therapy failure in children with respiratory distress treated in a Pediatric Emergency Department".[1]

Their first comment is about the classification of past medical history and its diversity. In our study, an underlying disease was present in 65.8% of the patients, and a history of atopy including eczema, asthma, reactive airway disease, or allergic rhinitis was present in 31 patients.[1] We agree with the concern about the classification of medical history. However, because there is no generally accepted standard classification for the underlying diseases we preferred to classify the diseases that may affect the course of the lower respiratory tract infection of the patient.

In the literature, underlying diseases or past medical history as predictors of high-flow nasal cannula (HFNC) therapy failure are less described. In a study designed by D'Alessandro et al.[2], patient characteristics associated with HFNC failure in bronchiolitis were evaluated. The authors categorized the past medical history of the patients such as congenital cardiac disease, chronic lung disease, neuromuscular disease, genetic diagnosis, previous intubation, home oxygen, atopy, and others. Two hundred-eight patients were included in the study and fifty-eight patients (27.8%) had a significant past medical history. An underlying disorder was found in 31.2% and 19.6% of HFNC responder and nonresponder groups, respectively (p=0.089). However, they did not investigate separately whether each medical history affected the response to HFNC. Kelly et al.[3] reported no correlation between medical history and treatment outcome, which is similarly reflected in the results of our study. On the other hand, Betters et al.[4] showed that HFNC failure is more likely in children with a history of cardiac disease. However, their study included patients with only cardiac disease and respiratory chronic illnesses such as asthma and bronchiolitis. Although the correspondents highlighted the diversity of medical history and differences in response to HFNC in patients with atopic dermatitis or muscular dystrophy, HFNC can be used easily regardless of the underlying disease or the patient's diagnosis. With our current knowledge, it seems that HFNC failure is associated with multiple factors including patient age, HFNC duration, respiratory rate, initial venous pCO2, initial venous pH, history of intubation, and underlying cardiac disease.[2-4] To clear this question, studies including a larger number of patients who are evaluated according to the underlying disease in different study populations such as bronchiolitis, pneumonia, or other diagnoses should be conducted.

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The second comment raised concerned the complications during the HFNC therapy. Generally, HFNC therapy is well tolerated in children and complications are not common during the treatment.[5,6] In our study population, pneumothorax or any other adverse events were not observed. Kelly et al.[3] reported an immediate complication in an infant who had a superficial burn from the heated tubing connected to the apparatus. This complication was managed with burn wound care easily. However, subcutaneous scalp emphysema, pneumo-orbit, and pneumocephalus were rarely reported in neonates as complications of HFNC.[6]

In our study, patients who need an escalation of respiratory support were transferred to the pediatric intensive care unit (PICU) and patients who need follow-up for at least two hours in the Pediatric Emergency Department (PED) were included in the study. Patients aged 28 days or under were excluded from the study. Therefore, we conducted the study with patients followed in the PED. Contraindications of HFNC therapy are upper airway obstruction, central apnea, blocked nasal passages/choanal atresia, trauma/surgery to the nasopharynx, pneumothorax, and requiring an immediate higher level of respiratory support like noninvasive ventilation (NIV) or invasive ventilation. However, some of these contraindications may be accepted as relative contraindications. None of the patients in our study had any contraindications.

The third comment is the evaluation of the effectiveness of HFNC therapy in patients with diagnosed bronchiolitis or pneumonia in the same study. The role of HFNC therapy has been studied in selected populations such as acute bronchiolitis, pneumonia, or asthma.[2] There are a few studies investigating the failure of HFNC therapy in all causes of respiratory distress in children presenting with the PED.[3] Therefore, our study included not only patients with bronchiolitis (75.3% of patients) but also those with other causes of respiratory distress. Additionally, the effects of salbutamol and steroid therapies are controversial in patients with bronchiolitis and it was not possible to exclude these therapies.

Lastly, we investigated early predictors for HFNC therapy failure which was determined as escalation to another ventilation support treatment. Therefore, we did not determine mortality, the duration of non-invasive and invasive mechanical ventilation and hospital stay as potential predictors in the study. Similarly, oxygen concentration and oxygen flow rate were not evaluated, although vital signs were evaluated at the admission and the second hour of the follow-up period to follow the clinical improvement of patients.

We hope that this additional information helps to further clarify some aspects of our study and thank the authors again for their correspondence.

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SARS-CoV-2 related encephalitis requires documentation of the virus in the cerebrospinal fluid

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I read with interest the article by Yimenicioglu et al.[1] about a 15-year-old male who developed headache, malaise, vomiting, and loss of appetite one week prior to admission and additionally vertigo, clumsiness, and drop attacks three days prior to admission. Because he developed a coma one day after admission, he was intubated and mechanically ventilated[1]. The patient was diagnosed with encephalitis upon the clinical presentation and cerebral MRI, which showed cytotoxic edema in the fronto-temporal regions bilaterally[1]. Despite extensive work-up the cause of encephalitis could not be clarified and empiric treatment did not result in resolution of the abnormalities and the patient died[1]. The study is appealing but raises concerns that should be discussed.

The main limitation of the study is that a causal relationship was established without providing evidence for it. The patient had a SARS-CoV-2 infection five months prior to the onset of the neurological compromise, a latency too long to establish a causal relationship. Furthermore, a SARS-CoV-2 infection was not ruled out on admission[1] and it is not mentioned if the index patient had undergone anti-SARS-CoV-2 vaccination or not. It is unclear if mentioning a positive PCR for SARS-CoV-2 in the discussion refers to the infection five months before admission or the current admission.

A further strong limitation of the study is that no autopsy had been carried out to prove or disprove SARS-CoV-2 associated encephalitis.

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A third limitation is that no cerebrospinal fluid (CSF) investigations had been carried out to prove or disprove the diagnosis of encephalitis. Encephalitis cannot be diagnosed upon imaging alone but requires documentation of the infectious agents causing encephalitis in the CSF. Imaging is only a supportive diagnostic tool.

Furthermore, differential diagnoses of cytotoxic edema were not sufficiently considered. The most common differential diagnosis characterized by a cytotoxic edema is ischemic stroke. Because ischemic stroke can be a complication of venous sinus thrombosis (VST), because the D-dimer was elevated, and because VST can be a complication of SARS-CoV-2 infections[2], it is crucial that VST had been appropriately ruled out by MR venography (MRV).

Missing are the levels of autoantibodies associated with immune encephalitis. There are an increasing number of reports showing that SARS-CoV-2 infections can trigger the development of antibody mediated autoimmune encephalitis.[3]

I disagree with the notion that a Glasgow Coma Scale (GCS) of 3 is a contraindication for lumbar puncture.[1] On the contrary, a coma is an indication for lumbar puncture if imaging or electroencephalography (EGG) do not sufficiently explain the cause of unconsciousness.

It is not comprehensible how the D-dimer could be elevated on hospital day three, although the patient had already died 48 hours after admission. This discrepancy should be explained.

No reference limits for blood tests were provided, which is why it is difficult to assess which parameters were truly elevated or normal.

Overall, the interesting study has some limitations that call the results and their interpretation into question. Clarifying these weaknesses would strengthen the conclusions and improve the study. Diagnosing encephalitis requires CSF investigations and either confirmation of an infectious agent or documentation of elevated antibodies associated with immune encephalitis.

Key words: COVID-19, SARS-CoV-2, encephalitis, complication, brain, MRI.

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Response to "SARS-CoV-2 related encephalitis requires documentation of the virus in the cerebrospinal fluid"

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Dear Editor,

We appreciate your interest in our report[1] and your insightful comments. We would also like to thank you for the opportunity to respond to the issues addressed in your letter and to clarify aspects of our audit in relation to these concerns.

If we start with the laboratory results, the patient had normal laboratory values except for the positive viral PCR at current admission. All laboratory data were provided when the publication was first submitted but during revision it was advised that values within normal range were not necessary. The diagnosis of COVID-19 at the second admission was removed by mistake and an erratum has also been published.[2]

The patient's laboratory and reference values upon admission were as follows: pH: 7.39 (7.35-7.45); partial pressure of carbon dioxide (pCO_2): 34 (35-45) mmHg; O_2 saturation: 80%; bicarbonate (HCO_3): 21 mmol/L; fasting blood glucose (FBG): 128 (70-100) mg/dL; creatinine: 0.66 (0.7-1.1) mg/dL; ferritin: 12 (11-190) ng/mL, triglyceride: 34 (40-150) mg/dL, procalcitonın: 0.04 ng/mL, CK-MB: 0.6 (0-5.2) IU/L, D-Dimer:0.19 (0-0.55) ng/mL, Troponin I: 1.1 (0-34.2) ng/mL , Na: 142 mEq/L; K: 4.2 mEq/L; Mg: 1.9 mEq/L; Ca: 8.1 mg/dL; phosphorus: 3 mg/dL; aspartate aminotransferase (AST): 14 U/L; alanine aminotransferase (ALT): 29 IU/L ; white blood cell count (WBC): 15,540 (4,100-10,500)/μL; lymphocytes: 7.8%; Hemoglobin (Hb): 12.7 (11.8-16.5) g/dL; platelet count: 256,000 (145,000-400,000)/μL; albumin: 5.0 g/L, and C-reactive protein (CRP)= 0.1 mg/L, COVID-19 (SARS-CoV-2) reverse transcriptase PCR: positive, brain natriuretic peptide (BNP): 10 (0-100) pg/mL. On the third day of admission, D-dimer (1.14 mg/L), Troponin I (150 pg/mL), and BNP (38.2 pg/mL) increased .

The patient had a history of a COVID-19 infection five months ago. This was his second infection with COVID-19. We assume that the present COVID-19 infection caused the neurological damage, but the quick decline may have been brought on by the previous SARS-CoV-2 infection he had five months prior. It is possible for the adaptive immune system to become engaged, which would stimulate immunological memory and cause significant central nervous system (CNS) damage. The patient was diagnosed with encephalitis due to the clinical presentation and cerebral magnetic resonance imaging (MRI) which showed cytotoxic edema in the fronto-temporo-parietal regions bilaterally. As we previously indicated, our patient initially displayed neurological symptoms such as headache, dizziness, and finally loss of consciousness. He did not exhibit severe respiratory symptoms at this time. We also agree with you that a limitation was that no other viral or causal agents were ruled. This was because our of laboratory insufficiencies. At the time this patient was admitted the national vaccination program for children in Turkey had not yet introduced the COVID-19 vaccine.

Although there is not a widespread agreement, an autopsy may be done when a death is suspicious or when there is a suspicion that an

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unidentified infectious agent, a condition that has not been properly diagnosed, or another factor caused the death. In our setting, an autopsy was not a recommended as the death was brought on by COVID-19.

In spite of the fact that virus isolation is necessary for a certain diagnosis of viral encephalitis, COVID-19 is a challenge because SARS-CoV-2 transmission is transient and the CSF titer may be extremely low or even negative in some cases.[3,4]

Cytotoxic edema is characterized by intracellular fluid accumulation that results in cell swelling. Minutes after acute CNS trauma, cytotoxic edema, also known as cellular swelling, becomes apparent. Following cytotoxic edema, ionic edema, an extracellular edema that develops in the presence of an intact blood brain barrier, emerges. Along with acute ischemic stroke, traumatic brain injury, subarachnoid hemorrhage, fulminant liver failure, global ischemia, infections (encephalitis, menegitis, or abscess), and postsurgical edema are other conditions that can cause cytotoxic edema. Vasogenic edema may also be brought on by these situations.[5] After the patient was intubated, his health and the imaging equipment were not good enough for an MR venogram.

One of the tests performed in patients to find thrombosis is a D-dimer level. D-dimer and fibrinogen concentrations have been seen to rise in the early stages of COVID-19 disease, according to studies. D-dimer levels that increase by 3 to 4 fold are associated with a poor prognosis. When COVID-19 infection is in its first stages, fibrinogen and D-dimer levels usually rise. These individuals' elevated D-dimer levels may be brought on by thrombin production-inducing endothelial cell malfunction or inflammatory reactions to COVID-19 infection.[6] In our patient, D-dimer started out normal, but on the third day of hospitalization, it had increased six times. The 6-fold increase in our patient's D-dimer might have contributed to the disastrous outcome since a D-dimer increase of 3–4 fold is linked to a poor prognosis.[6]

Encephalitis in children has a wide range of potential diagnoses, and etiology examination is frequently inconclusive. A developing cause of non-infective encephalitis with a wide range of symptoms is autoimmune encephalitis (AE). The traditional method for diagnosing AE is the finding of antibodies in a patient who has a clinical profile suggestive of the disease. Children with autoimmune encephalitis typically present with a variety of subacute symptoms. The appearance of oligoclonal bands, lymphocytic pleocytosis, and increased protein levels in the cerebrospinal fluid (CSF), among other concurrent inflammatory signs, may be present but are often nonspecific. Particularly on fluid-attenuated inversion recovery (FLAIR) or T2-weighted images, MRI of the CNS may potentially reveal anomalies that offer diagnostic hints.[7,8] It was reasonable for us to treat the patient as having acute encephalitis in light of the patient's clinical deterioration and ongoing COVID-19 infection. Findings from the radiology and clinical fields did not support an autoimmune encephalitis.

When the patient was admitted, the brain scan results were normal. After six hours of admission, he experienced status epilepticus. A non-enhanced CT we acquired showed cortical sulci effacement, which is brain edema. A pressure differential between the supratentorial and infratentorial compartments is supported by the available data. Elevated pressure both above and below the tentorium cerebelli may contribute to the development of such a pressure gradient.[9] In these conditions, a lumbar puncture might have resulted in bilateral uncal herniation.

The authors questioned how the D-dimer increased on the 3rd day if the patient died 48 hours after admission but this was when brain death occurred. The patient passed away on the eighth day of admission. The expected laboratory finding of D-dimer increase in COVID-19 infection is significant for prognosis.[6]

To fully comprehend SARS-CoV-2's neurologic involvement, more cases are required. We

attempted to make patient-specific decisions and schedule the course of the treatment in accordance with the clinical course. Considering the patient's COVID-19-related acute encephalitis, especially given the positive COVID PCR on the day of arrival, was a consideration. We did not take AE into account. In reality, the recommendations for the diagnosis of AE advise performing a full antibody panel in a case of suspected AE, which includes antibodies against intracellular, surface antigens, and ion channels. The overlap in diagnostic and therapeutic aspects has grown so large in recent years as our understanding of this disease has increased that the antibody-based diagnosis has gradually given way to a diagnosis primarily based on clinical symptomatology.[8]

We believe that this case report will emphasize the deteriorating prognosis of acute COVID-19 encephalitis with an elevated D-dimer. Initial findings from the diffusion-weighted series may be apparent on brain MRI. The other MRI series could be normal. To rule out any further sources of neuronal injury, we advise using MRI with diffusion-weighted images early on in the neurologic problem.

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Erratum to "Lethal encephalitis in a pediatric patient with SARS-CoV-2" [Turk J Pediatr 2022; 64: 571-575]

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When the above paper was published laboratory results and their normal ranges were not fully written. We apologize for any inconvenience or confusion caused by this error.

The patient's laboratory and reference values upon admission were as follows: hemoglobin 12.7 g/dl (11.8-16.5), white blood cell count 15,540/µL (4,100-10,500), lymphocytes 7.8%, platelet count 256,000/µL (145,000-400,000), pH 7.39 (7.35-7.45), partial pressure of carbon dioxide (pCO_2) 34 mmHg (35-45), O_2 saturation 80%, bicarbonate 21 mmol/L, fasting blood glucose 128 mg/dl (70-100), creatinine 0.66 mg/dl (0.7-1.1), ferritin 12 ng/ml (11-190), triglyceride 34 mg/dl (40-150), procalcitonin 0.04 ng/ml (0.05-0.5), C-reactive protein (CRP) 0.1 mg/L (1-5), CK-MB 0.6 IU/L (0-5.2), D-dimer 0.19 ng/ml (0-0.55), troponin-I 1.1 pg/ml (0-34.2), Na 142 mEq/L, K 4.2 Eq/L, Mg 1.9 Eq/L, Ca 8.1 mg/dl, phosphorus 3 mg/dl, aspartate aminotransferase 14 U/L (18-40), alanine aminotransferase 29 IU/L (10-33), albumin 5.0 g/dl (4-5), and COVID-19 (SARS-CoV-2) reverse transcriptase-PCR positive, brain natriuretic peptide (BNP) 10 pg/ml (0-100). On the third day of admission, D-dimer (1.14 ng/ml), troponin-I (150 pg/ml), and BNP (38.2 pg/ml) increased.

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Erratum to "Recommendations on phenylketonuria in Turkey" [Turk J Pediatr 2022; 64: 413-434]

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After publication of the article the authors wished to clarify the safety profile of pegvaliase. In Statement #12, the sentence "Current information about PegPAL indicates that it is too early to be used in pediatric patients, and there are many issues to be resolved beore it is accepted as a safe and reliable treatment option." is now replaced by "In Europe, pegvaliase (rAvPAL-PEG) is indicated for the treatment of patients with phenylketonuria aged 16 years or older who have inadequate blood phenylalanine control (>600 μmol/L) despite prior management with the available treatment options. Studies on children younger than 16 years are ongoing. At the time of writing, clinical experience on the use of pegvaliase in Turkey is lacking." The authors would like to apologize for this and any inconvenience it may have caused.

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NOTICE TO AUTHORS

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Original Article: Original articles should report scientific findings within the pediatric field of interest. They must include a structured abstract of less than 300 words, with the following headings: background, methods, results and conclusions to appear after the title page. The manuscript should be structured with the following headings: introduction, materials and methods, results, and conclusions. The introduction should put the study in context with the current literature and reflect the purpose of the study. The materials and methods should include the study methodology, the setting for the study (although affiliation must be covered), the subjects (number and type), the treatment or intervention, principal outcomes measured, and the type of statistical analysis. The results section should include the outcome of the study and statistical significance, if appropriate. The conclusion(s) states the significance of the results and limitations of the study. The manuscript should be no longer than 3,500 words.

Case Reports: Case reports should contain accounts of rare syndromes, new diagnostic tools and methods, new kinds of treatment and laboratory research with foreseeable practical application. Case reports should consist of a structured abstract with the following headings: background, case and conclusions that summarizes the case(s), a brief introduction, a section that details patient presentation, initial diagnosis and outcome, as well as a discussion that includes a brief review of the relevant literature and describes how this case brings new understanding to the disease process. The number of authors is limited to 6 for case reports. If more than 6 authors have had a significant contribution defined by the International Committee of Medical Journal Editors (ICMJE) then the authors may contact the editorial office to request an increase.

Review Articles: Review articles should contain an objective account of different points of view and an internationally representative bibliography. Authors considering submission of review articles of medical progress are encouraged to consult with the Editor before submission.

Structured manuscript for systematic reviews are recommended. Headings should include: Structured abstract with the following headings: background and objectives, material and methods results and conclusions, Introduction, Data Sources, Study Selection, Data Extraction, Results, Limitations, and Conclusions. The general instructions regarding submission (including cover letter, title page requirements, journal style guidance, and conflict of interest statements) also apply to Review Articles.

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Clinicopathological exercises: Authors can submit for consideration an clinicopathological exercises that is interesting, instructive, and visually attractive, along with a few lines of explanatory text and references. No abstract, discussion or conclusion are required but the general submission instructions (including cover letter, title page, journal style guidance, and conflict of interest statements) also apply to clinicopathological exercises.

Image of the month: Authors can submit for consideration a striking image that is interesting, instructive, and visually attractive, along with a few lines of explanatory text and references. Images can be either clinical, pathologic (gross or microscopic), endoscopic, or radiographic. They must be of high quality (300 PPI resolution) and illustrate the diagnosis well. No abstract, discussion or conclusion are required but the general submission instructions (including cover letter, title page, journal style guidance, and conflict of interest statements) also apply to the Image of the month.

Letters to the Editor: Letters to the Editor should pertain to papers published in The Turkish Journal of Pediatrics within the past year. Provide a unique title for the Letter on the title page with complete contact information for the author(s). References, including reference to the pertinent article(s) in The Journal, should conform to style for manuscripts. No abstract is needed for a letter to editor. Letters to the Editor may be sent to the authors of the original article to which the Letter was written for review and/or Reply. If the Editors choose to publish the Reply, it will be published in the same issue as the Letter to the Editor.

Summary / Abstract and key words: An informative structured abstract not exceeding 300 words must accompany each manuscript, Unexplained abbreviations and references should not be given. The abstract should state the objective (the purpose of the study and hypothesis) and summarize the rationale for the study. It is critically important that all significant findings should be presented in the abstract. The abstracts should be structured with the following headings for an original article: Background, Methods, Results and Conclusions and Background, Case Report, Conclusion for a case report.

The authors should list three to five key words or phrases taken from Index Medicus

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The Turkish Journal of Pediatrics takes issues of publication ethics very seriously and subscribes to its principles on how to deal with acts of misconduct thereby committing to investigate allegations of misconduct in order to ensure the integrity of research. Scientific articles sent to journals should take into account the recommendations of the International Committee of Medical Journal Editors (ICMJE) and the International Standards for Editors and Authors of COPE (Committee on Publication Ethics).

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References to books:

Example: Strasburger VC, Brown RT, Braverman PK, Rogers PD, Holland-Hall C, Coupey SM. Adolescent Medicine: A Handbook for Primary Care. Philadelphia: Lippincott Williams & Wilkins, 2006: 13-21.

References to chapters in books:

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  - Title of article
  - Short running title
  - Name(s), academic degrees, and affiliations of author(s)
  - Name, address, and business and home telephone numbers of corresponding author
  - Word count and number of tables and figures
  - Ethical approval
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CASE REPORTS

LETTERS TO THE EDITOR

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