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Invited Review

Spinal Muscular Atrophy Types, Innovations in Diagnosis and Treatment

Spinal Musküler Atrofi Tipleri, Tanı ve Tedavide Yenilikler

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ABSTRACT

Spinal muscular atrophy (SMA) is a severe condition with recent advancements in diagnosis and treatment. Its prevalence might be higher than anticipated in countries, where consanguineous marriages are common, implying that there could be cases of prenatal deaths. It is crucial for pediatricians to be familiar with the various forms of SMA that exhibit different symptoms and to promptly refer patients to pediatric neurology to ensure timely treatment and prevent complications. Without treatment, decreased levels of survival motor neuron (SMN) protein can result in disability and even death, ranging from functional motor impairments to muscle weakness and respiratory failure. Clinically, the severity of SMA varies significantly depending on the loss of lower motor neurons, ranging from prenatal forms to adult-onset forms, leading to progressive muscle weakness and atrophy. Homozygous deletion of the *SMN1* gene is responsible for approximately 95-98% of SMA cases. A *SMN2* gene closely related to SMA can partially compensate for the loss of *SMN1*, with disease severity correlating with the number of copies. For patients who cannot receive intrathecal treatment with nusinersen, due to spinal deformities like advanced scoliosis, risdiplam may serve as an alternative treatment option. We eagerly await the publication of long-term results of the studies for patients who have received multiple treatments in some way. Future research will potentially identify more cost-effective and easily measurable biomarkers. It is crucial to enhance pediatricians' awareness of this disease, as early treatment can yield promising outcomes.

Keywords: Spinal muscular atrophy, treatment, biomarker, innovation

ÖZ

Spinal musküler atrofi (SMA) son zamanlarda tanı ve tedavisinde gelişmeler olan ciddi bir hastalıktır. Akraba evliliklerinin yüksek olduğu ülkelerde taşıyıcılarının sık olması, bazı tiplerinde prenatal eksitus olan hastalar olduğu için gerçek insidans ve prevelansının tahmin edilenden daha yüksek olabileceğini düşündürmektedir. Pediatri doktorları tarafından bu hastalığın farklı görünümleri olan tiplerini iyi bilmeleri ve zamanında çocuk nörolojisine yönlendirmeleri hastaların komplikasyonlar gelişmeden tedavi şansını kaçırmamaları açısından önemlidir. Klinik olarak alt motor nöronların kaybına bağlı olarak şiddeti prenatal formdan erişkin başlangıçlı forma kadar oldukça değişken olup progresif kas güçsüzlüğü ve atrofi gelişir. Eğer tedavi edilmezse hayatta kalma motor nöron geni (SMN) protein düzeyindeki azalma fonksiyonel motor defisitlerden, kas güçsüzlüğü, solunum yetersizliğine kadar değişebilen sakatlık ve ölüme yol açabilir. *SMN1* geninin homozigot silinmesi, SMA hastalarının yaklaşık %95-98'ini oluşturur. Homolog bir *SMN2*, *SMN1* silinmesini kısmen telafi edebilir ve kopya sayısı, hastalığın ciddiyeti ile ilişkilidir. Nusinersen tedavisini alamayan (ileri skolyoz gibi nedenlerle) hastalar için risdiplam bir başka tedavi seçeneği olabilir. Bir şeklide çoklu tedavi alan hastaların uzun süreli sonuçlarının yayınlanmasını merakla beklemekteyiz. İleride yapılacak çalışmalar daha ucuz ve kolay ölçülebilen biomarkerların belirlenmesine olanak sağlayabilir. Pediatristlerin erken tedavisiyle yüz güldürücü sonuçlar alınabilen bu hastalık hakkında farkındalığını artırmak gerektiğini düşünmekteyiz.

Anahtar kelimeler: Spinal musküler atrofi, tedavi, biyomarker, yenilik

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INTRODUCTION

Spinal muscular atrophy (SMA) is an autosomal recessive neuromuscular disorder, and formation of its most common form, 5q SMA, results from biallelic mutations in the *survival motor neuron-1 (SMN)* gene. However, there are various genetic subtypes of SMA. In the majority of cases (95%), homozygous deletions of the *SMN1* gene, which encodes the vital SMN protein essential for motor neuron survival induces development of SMA[1]. Less frequently, it can arise from missense, frameshift, or heterozygous mutations in one allele paired with point mutations in the other allele[2]. SMA types stemming from mutations in genes other than 5q are referred to as non-5q SMAs. Its reported incidence ranges from 1:6,000 to 1:30,000[3]. Clinically, the loss of lower motor neurons leads to progressive muscle weakness and atrophy, with disease severity varying widely, from prenatal onset to adult-onset forms.

Both *SMN1* and *SMN2* genes encode the SMN protein, but while *SMN1* generates a fully functional protein, only 10% of the protein produced by *SMN2* is functional[4]. This difference arises from a mutation in intron 7 of the *SMN2* gene. As a neurodegenerative disorder, decreased expression of SMN gene and progressive degeneration of motor neurons in the spinal cord and brainstem are characteristic features of SMA[5]. The level of SMN protein produced by *SMN2* copies, when *SMN1* gene is rendered completely functionless, is inversely related to the severity of SMA. The severity and course of the disease range from mild (type 4) to severe (type 1) type depending on the variable number of less stable *SMN2* gene copies[6]. The *SMN2* copy number serves as the primary, though not exclusive, prognostic indicator for SMA types. In SMA type 1, 86% of patients have 2 copies of the *SMN2* gene, 87% of SMA type 2 have 3 copies, 64% of type 3 individuals have 3 copies, and 31% have 4 copies[7].

Types of Spinal Muscular Atrophy

If left untreated, the reduction in SMN protein levels can result in varying degrees of disability and even death, encompassing functional motor impairments, muscle weakness, and respiratory failure[8]. Historically, SMA has been categorized into different types (types 0-4) based on disease severity and the timing of clinical symptoms.

In SMA type 0, symptoms manifest in utero, and there are noticeable signs of profound muscle weakness at birth. SMA type 1, is the most prevalent form that manifests symptoms within the first 6 months of life, and affected individuals are unable to sit independently. Type 2 SMA typically begins to show symptoms after the first 6 months of life, and patients can sit but cannot walk without assistance. Patients with type 3 can initially walk, but they gradually lose their ability to walk with disease progression. In cases where symptoms emerge before the age of 3 (type 3a), roughly half of the individuals lose their ability to walk before reaching adulthood. For type 3b, symptoms appear after 3 years of age, and some individuals can maintain their ability to walk even beyond the age of 40. In the adult-onset type 4 SMA, patients do not lose their ability to walk[9].

In SMA patients, kyphosis, a forward curvature of the spine, typically develops before the age of three, which may later progress to scoliosis or kyphoscoliosis, a combination of forward and lateral spinal curvatures[10]. Consequently, comprehensive patient monitoring, especially for those receiving high-cost medications for the treatment of SMA, and early intervention for spinal deformities through assessments of two-plane radiographic series are crucial for achieving better functional outcomes and prolonged survival.

Treatment of Spinal Muscular Atrophy

Since 2016, the United States Food and Drug Administration has granted approval for three disease-modifying therapies for the treatment of SMA, all of which show their effects by elevating SMN protein levels[11].

Nusinersen (SPINRAZA®), initially marketed as an intrathecal formulation, and recently available in an oral form known as risdiplam (Evrysdi®)[12] are effective through distinct mechanisms to boost *SMN2* mRNA, thus increasing the production of fully functional SMN protein. These therapies are approved for use in pediatric patients, neonates, and adults.

Onasemnogene abeparvovec (ZOLGENSMA®) represents a gene replacement therapy targeting the *SMN1* gene. This therapy involves intravenous administration of an adeno-associated virus 9 (AAV9) and is approved for children under two years of age[13]. Gene therapy, while costly and therefore limited in its indications, entails a one-time injection. It facilitates the transfer of the *SMN1* gene, delivered by the AAV9 vector, through the blood-brain barrier and into motor neuron cells within the central nervous system. Presently, gene therapy is indicated for patients with the diagnosis of 5qSMA type 1 resulting from biallelic *SMN1* gene

mutations and for patients under the age of 2 with a maximum of four copies of the *SMN2* gene[14].

All three drugs have undergone numerous tests in presymptomatic patients younger than 42 days of age. All patients with three copies of the *SMN2* gene achieved independent ambulation before reaching 2 years of age. Half of the patients carrying 2 copies of the *SMN2* gene displayed normal motor development, while the other half experienced mild to moderate developmental delays[15]. These findings underscore the critical importance of early diagnosis and prompt initiation of treatment, particularly for SMA patients carrying two copies of *SMN2* gene[16].

Biomarkers

Responses to nusinersen, onasemnogene abeparvovec, and risdiplam treatments exhibit significant variations among individuals influenced by multiple factors, including *SMN2* copy number, age at the start of treatment, and disease severity[17-21]. Due to this inherent variability, there is an urgent need for SMA biomarkers aiding in treatment decisions, and in the prediction of prognosis (prognostic biomarkers) and treatment outcomes (predictive biomarkers)[22]. Moreover, numerous clinical trials are in progress, focusing on approaches beyond targeting the SMN protein, such as reversing motor neuron loss, enhancing motor function, neuromuscular junction improvement, or enhancing muscle performance[23,24]. Combining SMN-dependent and independent therapeutic modalities may prove to be the most effective strategy for the treatment of SMA[25]. Consequently, the development of novel prognostic, predictive, and pharmacodynamic biomarkers serving as valuable outcome measures in clinical trials and for monitoring responses to evolving treatment regimens over time conveys utmost importance[26,27].

While SMA mRNA and protein levels naturally serve as biomarkers for SMA, their levels in the bloodstream, and central nervous system do not correlate with CNS levels. Recently, extravesicular blood samples obtained from SMA type 2 patients have revealed the presence of full-length SMN transcripts. Additionally, nusinersen treatment has been associated with decreased neurofilament (NF) and SMN transcript levels[28]. Other potential biomarkers encompass genetic factors like *SMN2* copy number, *SMN2* polymorphisms, genetic regulators, transcription and splicing regulators, microRNAs, methylation factors, long non-coding RNAs, NF, tau protein, magnetic resonance imaging, muscle imaging techniques, and electrophysiological parameters, including compound muscle action potential amplitude, motor unit number estimation methodologies, and repetitive nerve stimulation[29-32].

Newborn Screening Programs

The implementation of newborn screening (NBS) for SMA has brought about a profound transformation in the outlook for diagnosed patients. Real-world studies have illuminated the fact that only 34% of individuals possessing 3, 4, or 5 copies of *SMN2* were able to achieve the ability of independent walking[33]. Those who started to receive treatment after the onset of symptoms had notably lower chances of regaining their ambulatory abilities[34]. As implementation of NBS has became widespread, substantial real-world data on the efficacy of early SMA treatment have emerged. A systematic review of the findings of 18 studies by Aragon-Gawinska et al.[35] revealed that early treatment outcomes were contingent upon both the *SMN2* copy number and the initial neurological presentation of the patients. Regrettably, there is no foolproof method to detect early-stage SMA symptoms. In the absence of a positive NBS result, pediatricians may struggle to identify subtle abnormalities unless they possess expertise in the diagnosis of SMA. Functional motor outcome assessment tools like Children's Hospital of Philadelphia Infant Test of Neuromuscular Disorders can facilitate symptom measurement and making comparisons during monitoring of the treatment. In the future, following the conduction of well-designed clinical trials, noninvasive in-utero diagnostic tests followed by prenatal treatment might become a topic of consideration.

Making treatment decisions for patients with 4 *SMN2* copies poses a unique challenge. While adhering to a "wait-and-see" approach is not always straightforward, it has been observed that these patients may exhibit less than four copy numbers in different assessments. More comprehensive data is essential to provide recommendations for these individuals[36,37]. It is critically important to note that current SMA treatments come with substantial costs. Consequently, robust health-related economic analyses are required to assess the value of treating SMA patients identified through NBS programs.

Conclusion

In countries like ours, where consanguinity rates are approximately 30%, early detection of SMA holds immense significance. Identifying high-risk pregnancies for SMA through premarital screening as soon as

possible is also of great economic importance for the nation. Delayed diagnosis and treatment can hinder the achievement of desired favorable outcomes.

Risdiplam could serve as a potential alternative for patients unable to receive nusinersen. This is particularly relevant for cases having advanced scoliosis or other contraindications. We eagerly anticipate the release of long-term results from studies performed with patients who have undergone multiple treatment regimens.

Future research endeavors may pave the way for the discovery of more cost-effective and readily accessible biomarkers. Increasing awareness among pediatricians about this treatable disease is imperative and should be prioritized.

Ethics

Peer-review: Internally peer reviewed.

Author Contributions

Concept: A.Ü., Design: H.T., A.Ü., Data Collection or Processing: A.Ü., Analysis or Interpretation: H.T., A.Ü., Literature Search: A.Ü., Writing: H.T., A.Ü.

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Original Article

The Relationship Between Air Pollution and Suicide Attempts in Children

Çocuklarda Hava Kirliliği ve İntihar Girişimleri Arasındaki İlişki

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ABSTRACT

Objective: In this study we aimed to investigate the relationship between acute exposure to air pollution and suicide attempts in children.

Method: In this study, we retrospectively investigated over a 10-year period the likely relationship between suicide attempts in children, two air pollutants (PM_{10} and SO_2), and meteorological factors affecting their life-endangering behavior.

Results: We have determined that every one-unit increase in air pollution level of PM_{10} increased the risk of suicide attemps 1,002 times in all cases (p=0.016). One unit increase in air pressure, relative humidity, and wind speed increased the risk of suicide attemps in all cases 1,064 (p=0.014), 1,012 (p=0.045), 1,400 (p<0.0001) times, respectively. No statistical significance was found wih respect to air pollution level of SO_2.

Conclusion: This study revealed that even small increases in ambient air pollutant levels increased hospital admissions due to suicide attemps, and that meteorological conditions aggravating air pollution also act as predisposing factors.

Keywords: Air pollution, meteorological factors, suicide attempts

ÖZ

Amaç: Bu çalışmanın amacı, çocuklarda akut hava kirliliği maruziyeti ile intihar girişimleri arasındaki ilişkiyi incelemektir.

Yöntem: Bu çalışmada retrospektif olarak, 10 yıllık bir süre içinde çocuklarda görülen intihar girişimleri ile iki hava kirletici faktör (PM_{10} ve SO_2) ve bunları etkileyen meteorolojik faktörler arasındaki olası ilişki araştırıldı.

Bulgular: PM_{10} hava kirleticisi düzeyinde bir birimlik artışın tüm olgularda intihar girişim riskini 1.002 kat artırdığı saptanmıştır (p=0,016). Hava basıncı, nispi nem ve rüzgar hızında bir birimlik artış, tüm olgularda intihar girişim riskini sırasıyla, 1.064 (p=0,014), 1.012 (p=0,045), 1.400 (p<0,0001), kat artırmıştır. SO_2 hava kirleticisi düzeyinde ise istatistiksel bir anlam saptanmadı.

Sonuç: Bu çalışma, ortamdaki hava kirletici düzeyindeki düşük seviyelerde bile artışların, intihar girişimi nedeniyle artan hastane başvurularıyla ilişkili olduğunu ve hava kirliliğini etkileyen meteorolojik faktörlerin bu sonucu etkilediğini ortaya koymuştur.

Anahtar kelimeler: Hava kirliliği, meteorolojik faktörler, intihar girişimleri

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INTRODUCTION

Suicide (intentional self-harm) is an important global public health problem affecting also both children and adolescents. In the United States, it reportedly continues to be the third leading cause of death among children and adolescents[1]. Most adolescents have suicidal thoughts[2]. Eaton et al.[3] performed a nation wide survey in the USA in 2011, and reported that 12.8% of adolescent students stated that they had seriously considered attempting suicide in the previous 12 months, and 7.8% of them indicated that they had attempted suicide one or more times. According to the Turkish Statistical Institute, suicide attempts were reported at a rate of 1.8% under the age of 15 and at a rate of 9.3% between the ages of 15-19[4]. In the Diyarbakır Province located in the Southeastern Anatolia Region of Turkey with a population of 1,783 million, the total suicide rate between 2006 and 2015 was reported as 4.52 per 100,000 population[5].

Self-harm and suicide have different etiologies including biological psychological, social and cultural factors and genetic predisposition[6]. In recent years; studies have been conducted on the relationship between air pollution, which is an important environmental problem all over the world and suicidal attempts[7]. It is known that polluted air increases morbidity and mortality in cardiovascular and respiratory system diseases[7]. Particulate matter (e.g. $PM_{2.5}$, PM_{10}), gases [e.g. carbon monoxide (CO), nitrogen dioxide (NO_2), ozone (O_3), sulfur dioxide (SO_2)], organic compounds [e.g. polycyclic aromatic hydrocarbon (PAH)] and excessive concentrations of metals (e.g.: lead) in the air are among the causes of air pollution[7]. Air pollution can affect the central nervous system through deleterious effects of neuropathic inflammation, oxidative stress, or damage to blood vessels[7]. Among their other harmful effects, air pollutants pass throughthe blood-brain barrier, and affect the brain[8]. It has been reported that air pollution mainly induces cell cycle arrest and apoptosis in neurons, and degenerative destruction in the brain through oxidative stress and genetic damage[9,10]. Possible impact of air pollution on emotional disorders in children should be also emphaized. Experimental studies have shown that ultrafine particulate matter has a harmful effect on the prenatal development of the central nervous system, resulting in an increased risk of depression[11]. In addition, other deleterious compounds such as PAHs cause anxiety and depression in children[12]. Besides,exposure to air pollution may impair brain development and lead to cognitive disorders in late childhood[13]. To our knowledge, there are few studies in the literature on short-term exposure to air pollution and acute psychiatric outcomes in children and adolescents[14].

The aim of this study is to investigate the relationship between exposure to air pollution and relevant meteorological factors inpediatric patients brought to the pediatric emergency department of a hospital in Diyarbakır Province for suicidal attempts.

MATERIALS and METHODS

Data Collection

The archive data recorded between January 1, 2009 and April 30, 2019 by Diyarbakır Children's Diseases Hospital Data Processing Centerwere used. According to the International Classification of Diseases (ICD-10), suicide cases were determined based on the ICD codes of X60-X69. Since trauma patients were not taken into consideration, cases withassigned ICD codes of X71-X84 were excluded from the study and 1,132 patients were included in the study.

Patients

Patients under the age of 18 who attempted suicide by any method and applied to the emergency department of our hospital were evaluated by psychiatry consultation, and included in the study. All patients were evaluated in consultation with a child psychiatrist.

Patients with a psychiatric disorder (psychotic depression, alcohol/substance abuse, obsessive-compulsive disorder, generalized anxiety disorder, panic disorder, etc.), acute/chronic physical illness, malignancy, acute infection, chronic inflammatory diseases, hematopoietic disease, mental retardation, those receiving anti-inflammatory, immunosuppressive, immunomodulatory drugs, chemotherapy, and steroids, etc., and pregnants were excluded from the study.

Air Pollution and Meteorological Data

In Diyarbakir Province, only air pollutants of PM_{10} (μg/m³) and SO_2 (μg/m³) can be measured. Therefore, the relevant data involving the period between January 1, 2009 and April 30, 2019 have been retrieved from the official website (https://sim.csb.gov.tr) of T.R. Air Quality Monitoring Stations of the Ministry of Environment and Urbanization. The nationwide average limit values stipulated by the Ministry are 50 μg/m³/24 hr for PM_{10}, and 350 μg/m³/24 hr for SO_2. Meteorological data concerning daily average relative humidity (%), wind speed (m/s), temperature (°C), total precipitation (mm), actual air pressure (hPa) were obtained from the

Ministry of Agriculture and Forestry General Directorate of Meteorology of Diyarbakır Region.

For this study, ethics committee approval was obtained from the Ethics Committee of University of Health Sciences Turkey, Diyarbakır Gazi Yaşargil Training and Research Hospital (approval number: 340, date: 27.09.2019).

Statistical Analysis

Patient data collected within the scope of the study were analyzed with SPSS ver.21.0 (IBM Corp. Released 2013. IBM SPSS Statistics for Windows ver. 21.0 Armonk, NY) and R ver.3.6 (R Foundation for Statistical Computing, ver.3.6; Vienna, Austria) package programs. The mean, median, interquartile range (IQR), minimum and maximum descriptive values were given for the total number of suicidesand seasonal variables. Poisson regression analysis was used to determine the seasonal variables affecting the change in the number of suicides. A time-conditioned Poisson regression model was chosen to investigate the relationship between seasonal variables and suicide. The Poisson regression model was classified according to gender groups and the probability of delayed effects of seasonal variables was examined 1-7 days before each observation. The results were considered statistically significant when the p-value was less than 5 percent.

RESULTS

The median age of the study population was 16 years (IQR: 15-18). A total of 340 (30.0%), male, and 792 (70.0%) female patients attempted suicide. There were 292 (25.8%) suicide attempts in spring, 271 (23.9%) in summer, 277 (24.5%) in autumn and 292 (25.8%) in winter. There was no case of death due to suicide.

In Diyarbakir Province, meanconcentrations of PM_{10} (131.4 μg/m^3), and SO_2 (13.9 μg/m^3) between January 1, 2009 and April 30, 2019 were as indicated. Mean values of meteorological variables affecting the incidence rates of suicide attempts were as follows: air pressure 935.4 hPa; humidity 53.8%; wind speed 2.8 m/s; air temperature 16.3°, and precipitation 38.8 mm (Table 1). Air pollution rates according to the seasons are givenin Figure 1.

Everyone-unit increasein the enironmenal concentration of pollutant PM_{10} in the air increased the risk of suicide attempts 1,002 times in all cases (p=0.016). Every one unit increase in relative humidity and wind speed increased the probability of suicide attempts by 1,017 times in the female, and 1,429 times in the male patient group (p=0.020, and p=0.001, respectively). Every one unit increase in air pressure, relative humidity, and wind speed increased the risk of suicide attempts in all cases by 1,064, 1,012, 1,400 times, respectively (p=0.014; p=0.045; p<0.0001, respectively). No statistical significance was found concerning the air pollution level for SO_2 (Table 2).

The Poisson conditional regression models used to examine the delayed effect for each type of pollutant and relevant meteorological factors are shown in Table 3. Although a significant negative difference was observed in the estimation of 3 and 7 days of delay in boys in PM_{10} air pollutant, a significant positive difference was observed in the estimation of 1, 3, 5, 7 days of delay in girls and in the general evaluation. In SO_2 air pollutant, although a significant positive difference was observed

Table 1. Information obtained from the records of daily clinical visits for suicide attempts of children, air pollutants and meteorological factors in Diyarbakır province between January 1, 2009 and April 30, 2019

Variables	Mean	Min[a]	P25[b]	P50[b]	P75	Max[a]	IQR[c]
Suicide attempts	7	1	4	6	10	18	6
Ambient air pollutants							
PM_{10} (μg/m^3)	131.4	55.9	85.0	116.9	170.6	264.2	85.6
SO_2 (μg/m^3)	13.9	1.1	5.8	11.1	17.3	68.1	11.5
Age (year)	16	15	16	16	16	18	0
Ambient meteorological factors							
Air pressure (hPa)	935.4	925.8	932.4	935.8	939.2	943.6	6.8
Humidity (%)	53.8	17.4	30.2	58.1	71.9	90.0	41.7
Wind speed (m/s)	2.8	1.2	2.3	2.8	3.2	4.6	0.9
Temperature (°C)	16.3	-3.5	7.6	15.8	26.1	32.3	18.5
Precipitation (mm)	38.8	0	2.0	21.6	63.8	168.9	61.8

Min: Minimum, Max: Maximum, P25: 25th centile; P50: 50th centile, P75: 75th centile, IQR: Interquartile range

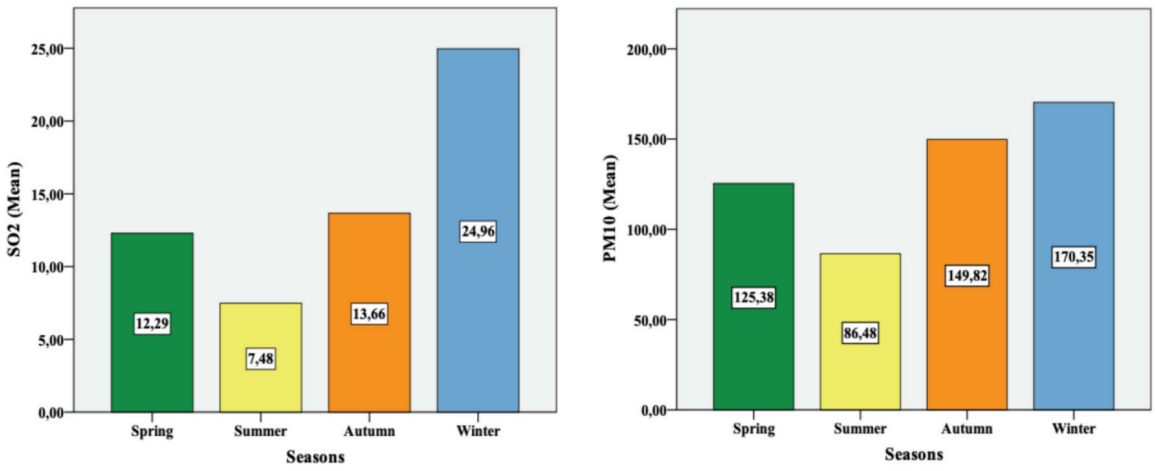

Figure 1. Average PM_{10} and SO_2 levels according to seasons

in the estimation of 5 days delay in boys and a significant negative difference in the estimation of 7 days in delay, a significant positive difference was observed in the estimation of 1, 3, 5, 7 days of delay in girls and in the general evaluation. A significant positive difference was observed in the air pressure factor in girls and in the delay of 1, 3, 5 days in the general evaluation. In the wind speed factor, a significant negative difference was found in the 1, 3, 5 days delay evaluations in girls and in the general evaluation.

DISCUSSION

Suicidal behavior is a complex disorder that is affected by mental disorders, symptoms, inadequate social support, and sociocultural factors. In addition to these factors in suicide risk, it is important to determine the relationship between suicidal behavior and weather conditions and to carry out studies to clarify the true impact of this relationship on suicidal behaviour. In the present study, we have investigated the relationship between the increase in the number of referrals to a single pediatric emergency service in Diyarbakır province for suicide attempts, levels of the pollutants PM_{10} and SO_2 in the environmental air, and meteorological factors. In this study, in compliance with literature findings, we have shown that there is a positive and statistically significant relationship between suicide attempts, air pollution and meteorological conditions[15]. Similar studies on this subject have also reported a positive relationship between air pollution and suicide mortality rates[16].

Studies investigating the relationship between air pollution and suicide, have shown that the number of suicide attempts increases during periods of the highest concentrations of particulate matter in the atmosphere[17]. However, previous studies have indicated that the risk of suicide varies depending on the type of air pollutant and workplace. For example, similar to our study; Casas et al.[18] stated that the increases in PM_{10} levels particularly affect children under 14 years of age, and that there is a seasonal difference and significance in the 6-day delay estimations. A study conducted in 10 cities in Northeast Asia, revealed that the younger age group (10-24 years old) had a higher risk of death due to suicide related to air pollution caused by NO_2, SO_2 and PM_{10} in some cities compared to older age groups[19]. In their study, Kim et al.[20] found a significant relationship between exposure to high concentrations of O_3 and particulate matter and suicide rates, but they could notreveal any relationship between exposure to NO_2, CO, SO_2 and suicide rates. They determined that every one unit increase in PM_{10} levels creates 0.047-fold increases in weekly suicide rates per 10 million people at zero lag time. In a survey study conducted in Japan, Ng et al.[21] could not find a relationship between SO_2 as an air pollutant and suicide risk. Similarly, in our study, a relationship was found between the number of suicide attempts and only PM_{10} O_3 but not between SO_2 levels. However, Yang et al.[22] determined that air pollutants such as SO_2 and O_3 increase the risk of suicide, and indicated that the decreased rate of sunlight is associated with the increased number of suicides. Brokamp et al.[14] found that there was a significant relationship between the increased number of psychiatric emergency unit admissions of children and levels of $PM_{2.5}$ in theenvironmental air. Although Liu et al.[23] found a positive association between psychiatric emergency presentations, including referrals for suicide attempts, and $PM_{2.5}$ levels they could not detect any

seasonal changes in the suicide rates. However, in the present study, as $PM_{2.5}$ levels in the environmental air were not determined in Diyarbakır province; no comparison could be made in this regard. We thought that this inconsistency in the results may be due to the differences in air pollution sources, pollutants, climatic conditions, cultural backgrounds, socioeconomic factors and neuropsychiatric diseases. Additionally, different modeling strategies and results of statistical evaluation make it difficult to make comparisons between studies.

It is a known fact that meteorological factors have a strong effect on increases in suicide rates[24]. Bakian et al.[15] performed a survey study in regions with very different meteorological, geographical and cultural characteristics, and revealed the presence of a positive correlation between air pollution and suicide completion rates. In our study, a significant relationship was found between air pressure, humidity and wind speed in all suicide attempts. In a study conducted in China, Lin et al.[16] investigated the relationship between the confounding effects of three air pollutants (PM_{10}, SO_2 and NO_2) and meteorological factors (daily average temperature, relative humidity, atmospheric pressure, duration of sunshine) and increases in suicide risk, and revealed that the effects of all pollutants were statistically significant in cold seasons. The suicide risk caused by exposure to three air pollutants was found to be positively associated with ambient air pollution levels[16]. In our study, although PM_{10} levels in the ambient air were higher in winter, the reasons for the lack of seasonal differences in the risks of suicide attempts in children have been thought to be related firstly to the increase in time spent indoors during cold periods and the decrease in personal exposure to outdoor air pollution. Secondly, although the composition of PM_{10} changes throughout the year, the highest proinflammatory concentration of PM_{10} is measured in summer[25]. In another study, the authors found a significant positive correlation between the number of suicides and air temperature. They indicated that there was a weak positive correlation between air humidity and the number of suicidal attempts, but a significantly negative correlation between the number of suicides and atmospheric pressure was indicated[26]. Bando et al.[27] reported that the minimum temperature was associated with a 2.28% increase in the total number of suicides with each 1 °C increase in weekly averages; however Gao et al.[28] found that rising temperature had a positive correlationwith increased risk of suicide, especially completed suicide. Although it is not known exactly how the atmospheric temperature affects the human organism, it has been stated that the brown adipose tissue is overactivated in the human body, which produces heat after cold nights and in the early spring and summer seasons, and consequenly, the risk of suicide may increase with the intensification of anxiety and mental activity. Overactivated brown adipose tissue increases tolerance to cold, while tolerance to heat decreases. One hypothesis is that decreased tolerance to heat aggravates anxiety, agitation, and can cause changes in mood (mood-altering effect). As a result, it may facilitate the emergence of suicidal thoughts[29,30]. Another explanation is that high temperature can aggravate impulsive and aggressive behaviorsby increasing serotonin levels or overactivating 5-HT receptors[31]. However, it is clear that a sudden increase in temperature with increasing humidity may cause evaporation of body heat and cause thermoregulational imbalance[29]. In our study, however, no relationship was found between ambient temperature and relative humidity and suicide cases. It was thought that this result may be related to the absence of sudden changes in meteorological factors and the absence of completed suicide cases in our survey.

Falak et al.[32] reported a significant relationship between intentional self-harming behavior, ambient air pressure and wind speed. In our study, a positive relationship was found between air pressure, wind speed, humidity and number of suicide attempts. It is thought that the average lowwind speed of 2.8 m/s in Diyarbakır province is insufficient in the distribution of air pollution in the city, and from time to time it accumulates emissions on the city and causes pollution.

Our findings suggest that people are more likely to commit suicide when air pollution is high. As an extremely important issue the distribution of mental disorders among people who commit suicide should be taken into account, otherwise it would be impossible to predict the effects of pollution on suicide-related outcomes. The exact mechanism why an increase in the levels of air pollutants may be associated with suicide is unknown. It has been stated that high levels of air pollution induce proinflammatory cytokines that may directly or indirectly lead to a neuroinflammatory effect in the brain (for example, dysregulation of the hypothalamic-pituitary-adrenal axis and changes in neurotransmitter levels), and these pathways may be involved in the development of depression, suicidal behavior or both[33]. Results obtained from systematic reviews suggest that there is a statistically significant relationship between air pollution and the risk of depression and suicide[34]. Further clinical and experimental studies are needed to

Table 2. Poisson multivariable conditional regression models for daily suicide attemps among boys and girls in Diyarbakır province between January 1, 2009 and April 30, 2019

Ambient air pollutants and meteorological factors	Boys		Girls		All	
	IRR (95% CI)	p-value	IRR (95% CI)	p-value	IRR (95% CI)	p-value
Air pollutants						
PM_{10} (μg/m³)	1.002 (0.998-1.006)	0.371	1.003 (1.000-1.005)	0.060	**1.002 (1.000-1.005)**	**0.016**
SO_2 (μg/m³)	0.997 (0.983-1.010)	0.625	0.995 (0.985-1.005)	0.312	0.996 (0.998-1.003)	0.270
Meteorological factors						
Air pressure (hPa)	1.088 (0.993-1.192)	0.071	1.057 (0.997-1.119)	0.063	**1.064 (1.013-1.117)**	**0.014**
Humidity (%)	1.003 (0.982-1.025)	0.800	**1.017 (1.003-1.031)**	**0.020**	**1.012 (1.000-1.024)**	**0.045**
Wind speed (m/s)	1.387 (0.974-1.974)	0.069	**1.429 (1.148-1.780)**	**0.001**	**1.400 (1.161-1.687)**	**<0.0001**
Temperature (°C)	1.014 (0.952-1.080)	0.659	1.039 (0.998-1.081)	0.062	1.029 (0.995-1.064)	0.100
Precipitation (mm)	0.997 (0.991-1.002)	0.272	1.001 (0.997-1.004)	0.695	0.999 (0.997-1.002)	0.687

Data were presented with IRR and 95% CI; IRR: Incidence rate ratio, CI: Confidence interval

Table 3. Poisson conditional regression models for lagged PM_{10} and SO_2 effects on daily suicide attempts among boys and girls in Diyarbakır province between January 1, 2009 and April 30, 2019

Ambient air pollutanst and meteorological factors	Lag	Men		Women		All	
		IRR (95% CI)	p-value	IRR (95% CI)	p-value	IRR (95% CI)	p-value
Pollutants							
PM_{10} (μg/m³)	L1	1.009 (0.999-1.020)	0.071	**0.996 (0.994-0.998)**	**<0.0001**	**0.997 (0.995-0.998)**	**<0.0001**
	L2	0.999 (0.995-1.003)	0.520	0.999 (0.997-1.002)	0.651	0.999 (0.997-1.001)	0.398
	L3	**1.008 (1.001-1.014)**	**0.021**	**0.997 (0.995-0.999)**	**0.001**	**0.997 (0.996-0.999)**	**0.004**
	L4	1.002 (0.998-1.006)	0.376	1.001 (0.998-1.003)	0.590	1.001 (0.999-1.003)	0.292
	L5	1.001 (0.996-1.007)	0.603	**0.997 (0.996-0.999)**	**0.007**	**0.998 (0.996-1.000)**	**0.016**
	L6	1.003 (0.999-1.007)	0.136	0.999 (0.996-1.001)	0.227	0.999 (0.997-1.001)	0.481
	L7	**1.008 (1.003-1.013)**	**0.003**	**0.998 (0.996-1.000)**	**0.015**	**0.999 (0.997-1.001)**	**0.020**
SO_2 (μg/m³)	L1	1.006 (0.974-1.039)	0.715	**0.983 (0.974-0.992)**	**<0.0001**	**0.986 (0.977-0.995)**	**0.001**
	L2	0.991 (0.972-1.010)	0.327	0.999 (0.986-1.014)	0.943	0.995 (0.985-1.005)	0.332
	L3	0.977 (0.934-1.022)	0.310	**0.985 (0.976-0.994)**	**0.001**	**0.985 (0.975-0.994)**	**0.001**
	L4	0.985 (0.957-1.013)	0.285	0.990 (0.978-1.002)	0.110	0.992 (0.981-1.004)	0.182
	L5	**0.958 (0.921-0.996)**	**0.033**	**0.977 (0.966-0.987)**	**<0.0001**	**0.976 (0.966-0.986)**	**<0.0001**
	L6	0.997 (0.980-1.015)	0.752	0.991 (0.978-1.003)	0.155	0.992 (0.982-1.002)	0.106
	L7	**1.017 (1.001-1.035)**	**0.042**	**0.985 (0.975-0.994)**	**0.002**	**0.990 (0.982-0.998)**	**0.016**
Meteorological factors							
Air pressure (hPa)	L1	1.013 (0.922-1.112)	0.788	**0.976 (0.957-0.995)**	**0.013**	**0.974 (0.955-0.992)**	**0.006**
	L2	1.006 (0.959-1.055)	0.806	1.012 (0.980-1.046)	0.468	1.013 (0.987-1.040)	0.319
	L3	1.062 (0.984-1.146)	0.123	**0.974 (0.954-0.995)**	**0.015**	**0.976 (0.956-0.995)**	**0.016**
	L4	1.018 (0.969-1.069)	0.475	1.015 (0.984-1.048)	0.346	1.021 (0.995-1.048)	0.117
	L5	1.040 (0.972-1.113)	0.254	**0.975 (0.955-0.995)**	**0.015**	**0.978 (0.958-0.997)**	**0.027**
	L6	1.014 (0.964-1.066)	0.588	1.002 (0.974-1.031)	0.883	0.998 (0.975-1.023)	0.886
	L7	1.069 (0.997-1.146)	0.060	0.986 (0.965-1.007)	0.186	0.990 (0.970-1.010)	0.324

Table 3. Continued

Ambient air pollutanst and meteorological factors	Lag	Men		Women		All	
		IRR (95% CI)	p-value	IRR (95% CI)	p-value	IRR (95% CI)	p-value
Meteorological factors							
Humidity (%)	L1	0.995 (0.972-1.017)	0.634	0.999 (0.995-1.004)	0.801	1,000 (0.996-1.005)	0.911
	L2	1.002 (0.991-1.012)	0.770	1.002 (0.996-1.009)	0.510	1.003 (0.998-1.009)	0.213
	L3	1.011 (0.995-1.028)	0.163	1.000 (0.995-1.004)	0.930	1.001 (0.997-1.005)	0.645
	L4	0.999 (0.988-1.010)	0.865	1.003 (0.997-1.009)	0.365	1.004 (0.999-1.010)	0.115
	L5	1.003 (0.989-1.018)	0.631	1.000 (0.995-1.004)	0.834	0.999 (0.995-1.004)	0.719
	L6	0.993 (0.981-1.004)	0.199	1.004 (0.998-1.010)	0.208	1.002 (0.997-1.007)	0.519
	L7	0.997 (0.983-1.010)	0.619	1.000 (0.995-1.004)	0.934	0.998 (0.994-1.002)	0.350
Wind speed (m/s)	L1	1.028 (0.536-1.972)	0.933	**1.185 (1.034-1.359)**	**0.015**	**1.256 (1.101-1.434)**	**0.001**
	L2	1.026 (0.727-1.448)	0.883	0.893 (0.722-1.103)	0.292	0.924 (0.773-1.105)	0.387
	L3	0.722 (0.442-1.181)	0.194	**1.256 (1.089-1.448)**	**0.002**	**1.237 (1.077-1.420)**	**0.003**
	L4	0.936 (0.669-1.310)	0.701	1.043 (0.842-1.293)	0.700	0.980 (0.822-1.168)	0.819
	L5	0.998 (0.679-1.467)	0.991	**1.201 (1.042-1.384)**	**0.011**	**1.245 (1.092-1.419)**	**0.001**
	L6	1.209 (0.876-1.670)	0.248	1.052 (0.880-1.257)	0.580	1.123 (0.961-1.311)	0.144
	L7	0.807 (0.524-1.242)	0.330	1.210 (1.045-1.401)	0.051	1.144 (0.998-1.312)	0.054
Temperature (°C)	L1	1.001 (0.951-1.052)	0.984	**1.010 (1.001-1.020)**	**0.034**	1.008 (0.999-1.017)	0.092
	L2	1.001 (0.979-1.024)	0.915	0.996 (0.981-1.010)	0.577	0.995 (0.984-1.008)	0.460
	L3	0.978 (0.945-1.012)	0.205	1.009 (0.999-1.019)	0.088	1.007 (0.997-1.016)	0.165
	L4	1.004 (0.979-1.029)	0.781	0.996 (0.983-1.010)	0.615	0.994 (0.982-1.006)	0.300
	L5	0.991 (0.958-1.025)	0.603	1.010 (0.999-1.020)	0.069	1.008 (0.998-1.018)	0.109
	L6	1.000 (0.976-1.026)	0.979	0.995 (0.982-1.008)	0.425	0.997 (0.986-1.009)	0.653
	L7	0.978 (0.946-1.011)	0.196	1.006 (0.996-1.017)	0.216	1.006 (0.997-1.016)	0.194
Precipitation (mm)	L1	0.974 (0.946-1.004)	0.086	**1.003 (1.001-1.006)**	**0.012**	**1.005 (1.002-1.007)**	**0.001**
	L2	0.999 (0.992-1.007)	0.869	0.998 (0.994-1.003)	0.476	1.000 (0.996-1.004)	0.949
	L3	0.991 (0.977-1.005)	0.219	1.002 (0.999-1.005)	0.197	1.002 (0.999-1.005)	0.138
	L4	0.990 (0.980-1.001)	0.065	1.001 (0.997-1.005)	0.566	1.000 (0.997-1.004)	0.909
	L5	0.992 (0.980-1.005)	0.218	1.002 (0.999-1.005)	0.227	1.002 (0.999-1.005)	0.194
	L6	**0.989 (0.980-0.998)**	**0.016**	0.999 (0.994-1.003)	0.529	0.996 (0.993-1.000)	0.057
	L7	0.989 (0.976-1.003)	0.129	1.002 (0.999-1.005)	0.237	1.002 (0.999-1.005)	0.299

Data were presented with IRR and 95% CI; IRR: Incidence rate ratio, CI: Confidence interval

better understand the impact of air pollution on mental health and especially to define its effectson biological systems.

Study Limitations

The first major limitation of our study is the use of information on ambient air pollutants and meteorological factors gathered from monitoring stations, rather than using actual personal exposure data. Therefore, it is necessary to measure the potential impact of air pollution on people with mental disorders in order to investigate whether there is any interaction between the environmental factors and individual sensitivity. Second, due to our limited data we could not assess differences in exposure to air pollutants in urban and rural areas. Therefore, evaluations of these findings in geographical regions with different degrees of pollution could not be made. It is also important to consider the possibility of misclassification and missing suicide data due to coding and diagnostic errors.

We suggest that future studies should include studies on mental health disorders, and it would be appropriate to compare regions with high and low air

pollutant concentrations in these studies. It is important to analyze the relationships with the entire spectrum of suicidal behavior, as suicide attempts may have different risk profiles from completed suicide.

CONCLUSION

Suicide attempts are among increasingly preventable behavioral disorders. The results obtained in this study, in line with the literature, have show that suicidal behavior may be associated with changes in the levels of airborne pollutants and meteorological factors, so further research is required to understand its underlying mechanisms. It is understood that prospective, multicenter studies, including healthy populations and populations with mental health disorders, are necessary in order to evaluate the data more comprehensively and to obtain more accurate data. On the other hand, comparing the ratios of high and low pollutant concentrations could add important information to the relevant literature studies.

Ethics

Ethics Committee Approval: For this study, ethics committee approval was obtained from the Ethics Committee of University of Health Sciences Turkey, Diyarbakır Gazi Yaşargil Training and Research Hospital (approval number: 340, date: 27.09.2019).

Informed Consent: Retrospective study.

Peer-review: Externally peer reviewed.

Author Contributions

Concept: G.Y., Design: A.G., Data Collection or Processing: G.Y., M.A., Analysis or Interpretation: İ.T., Literature Search: G.Y., Writing: G.Y.

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Original Article

The Role of Hematological Parameters in Children with COVID-19, MIS-C, and Other Viral Infections

COVID-19, MIS-C ve Diğer Viral Enfeksiyon Tanılarıyla İzlenen Çocuk Hastalarda Hematolojik Parametrelerin Rolü

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ABSTRACT

Objective: It is known that coronavirus disease-2019 (COVID-19) showed a clinical course with milder symptoms in children than in adults. However, a multisystem inflammatory syndrome in children (MIS-C), which developed 2-4 weeks after COVID-19 infection, emerged in April 2021. Other respiratory viruses such as influenza, respiratory syncytial virus, and parainfluenza spread worldwide after loosening pandemic restrictions. Pediatricians were challenged to distinguish COVID-19, MIS-C, and other viral infections from each other. Herein, we have aimed to determine basic, simple hematological parameters that can predict the prognosis and outcomes of the patients with COVID-19 and MIS-C.

Method: In this study, 300 pediatric inpatients including those with MIS-C, COVID-19, and other respiratory virus infections admitted to Ege University Faculty of Medicine between January 2018 and September 2021, were retrospectively evaluated.

Results: The neutrophil-to-lymphocyte ratio (NLR), neutrophil-to-monocyte ratio (NMR), derived NLR, and the systemic inflammatory index were higher in the MIS-C patients compared to others. The lymphocyte-to-monocyte ratio (LMR) and platelet-to-lymphocyte ratio (PLR) were lower in children with COVID-19 disease than those with MIS-C ($p<0.05$).

Conclusions: In this study, we have shown that commonly used hematological tests, especially higher values of NLR, NMR for children with MIS-C, and lower levels of LMR for children with COVID-19, are significant and can help to determine the possible disease course of children at an early stage.

Keywords: COVID-19, lymphocyte-to-monocyte ratio, multisystem inflammatory syndrome in children (MIS-C), neutrophil-to-lymphocyte ratio, other viruses

ÖZ

Amaç: Koronavirüs hastalığı-2019'un (COVID-19), çocuklarda yetişkinlere göre daha hafif semptomlarla seyrettiği bilinmektedir. Bununla birlikte Nisan 2021'de COVID-19 enfeksiyonundan 2-4 hafta sonra gelişen çocuklarda multisistem enflamatuvar sendrom (MIS-C) görülmeye başlandı. İnfluenza, respiratuvar sinsitiyal virüs ve parainfluenza gibi diğer solunum yolu virüsleri, pandemik kısıtlamaların gevşetilmesinin ardından dünya çapında yayıldı. Çocuk doktorları COVID-19, MIS-C ve diğer viral enfeksiyonları ayırt etmekte zorlandı. Bu çalışmada, COVID-19 ve MIS-C hastalarının prognozunu ve sonuçlarını öngörebilecek temel ve basit hematolojik parametreleri belirlemeyi amaçladık.

Yöntem: Bu çalışmada Ocak 2018-Eylül 2021 tarihleri arasında Ege Üniversitesi Tıp Fakültesi'ne başvuran MIS-C, COVID-19 ve diğer solunum yolu virüs enfeksiyonları ile hastanede yatan 300 çocuk hasta geriye dönük olarak değerlendirildi.

Bulgular: Nötrofil-lenfosit oranı (NLR), nötrofil-monosit oranı (NMR), derived NLR ve sistemik enflamatuvar indeks MIS-C'de diğerlerine göre daha yüksekti. Lenfosit-monosit oranı (LMR) ve trombosit-lenfosit oranı (PLR), COVID-19'lu çocuklarda MIS-C'ye göre daha düşüktü ($p<0,05$).

Sonuç: Bu çalışmada, yaygın olarak kullanılan hematolojik testlerin, özellikle MIS-C'li çocuklar için yüksek NLR, NMR değerlerinin ve COVID-19'lu çocuklar için daha düşük LMR değerlerinin anlamlı olduğunu ve olası erken evrede hastalığı belirlemeye yardımcı olabileceğini gösterdik.

Anahtar kelimeler: COVID-19, lenfosit-monosit oranı, çocuklarda multisistem enflamatuvar sendrom (MIS-C), nötrofil-lenfosit oranı, diğer virüsler

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INTRODUCTION

Pediatric coronavirus disease-2019 (COVID-19) patients showed milder symptoms with a better prognosis than adults. However, it should not be forgotten that severe COVID-19 disease can occur in infants under one year of age and children with chronic diseases[1,2]. In April 2020, pediatricians from the United Kingdom and Italy reported a cluster of patients admitted to pediatric intensive care unit (PICU) with toxic shock syndrome and Kawasaki-like disease. Meanwhile, an epidemiological line with COVID-19 was defined in these patients. The condition characterized by fever and multi-organ involvement seen after COVID-19 has been termed as multisystem inflammatory syndrome in children (MIS-C) and its clinical and laboratory diagnostic criteria have been defined by the Royal College of Paediatrics and Child Health, World Health Organization (WHO), and Centers for Disease Control and Prevention (CDC)[3-5].

Since the emergence of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) pandemic, clinicians have challenged the differential diagnosis of COVID-19 with other respiratory tract viruses and influenza[6]. Symptoms of COVID-19 have significantly overlapped with those of influenza. Therefore, many parameters have been used to differentiate among these infections. However, application of some of these parameters are burdensome and expensive. Simple and more accessible hematological parameters can be used to predict prognosis. The neutrophil-to-lymphocyte ratio (NLR), neutrophil-to-monocyte ratio (NMR), neutrophil-to platelet ratio (NPR), and platelet-to-lymphocyte ratio (PLR) are new biomarkers that provide important data on systemic inflammation and can be easily estimated from routine laboratory studies. NLR, lymphocyte-to-monocyte ratio (LMR), and PLR are thought to reflect physiological stress. Stress causes an increase in circulating cortisol levels which can lead to an increase in circulating neutrophil and a decrease in lymphocyte counts. Higher NLR, LMR, and PLR values are commonly observed in critically ill patients. These indices have not diagnostic value and they are not disease -specific but may guide the prediction of the severity of an inflammatory disease[7]. Increased NLR and PLR values are significantly associated with the mortality of the patients with infectious diseases[8-11]. Therefore, recent studies have suggested that NLR is a good predictor of mortality in COVID-19 patients[12-14].

We have aimed to evaluate the hematological parameters to predict requirement for hospital and PICU admissions of children among COVID-19 patients and compare the hematological parameters in patients with COVID-19 disease, other viral infections and MIS-C.

MATERIALS and METHODS

This retrospective single-center study included COVID-19-associated MIS-C patients, children with COVID-19 disease, and other viral infections admitted to the İzmir Ege University Faculty of Medicine Department of Pediatric Infectious Disease in Turkey, between January 2018 and September 2021. A total of 300 hospitalized children aged ≤18 years were evaluated, including 49 patients with MIS-C, and 147 children with COVID-19. A total of 104 children whose respiratory tract swab samples tested positive for adenovirus, influenza A/B, rhinovirus, parainfluenza, human metapneumonia virus A/B, human bocavirus, or respiratory syncytial virus (RSV) during influenza outbreak period between January 2018 and March 2019 were included in the study.

Demographic characteristics, comorbid conditions, and duration of hospital stay were recorded on a standardized form. Laboratory analysis on admission, including complete blood count (CBC), NLR, LMR, NMR, PLR, NPR, monocyte-to-platelet ratio (MPR), ferritin, D-dimer, C-reactive protein (CRP), and procalcitonin values were recorded. Thrombocytopenia was defined as a blood platelet count less than $150x10^9$/L, neutropenia as absolute neutrophil count (ANC) less than 1500/mm^3, and lymphopenia as an absolute lymphocyte count (ALC) less than 1500/mm^3. For analysis, inflammatory hematological indexes including NLR (NLR: ANC/ALC); LMR [LMR: ALC/absolute monocyte count (AMC)]; NMR (NMR: ANC/AMC); NPR (NPR: ANC/platelet count); MPR (MPR: AMC/platelet count), derived-NLR [ANC/(total white blood cell (WBC) count-ANC)]; and PLR (platelet count/ALC). Were calculated with values obtained from CBCs,

Systemic inflammatory index (SII) was calculated as follows: (SII) = platelet count × neutrophil count/ lymphocyte count.

According to the COVID-19 Guideline released by Turkish Ministry of Health, confirmed cases with COVID-19 disease were defined as those in whom SARS-CoV-2 virus was demonstrated in their nasal and throat swabs by molecular methods[15]. Diagnostic criteria of MIS-C have been defined by the WHO and the CDC in May 2020[3,5].

The ethics committees of Ege University Faculty of Medicine Medical Research Ethics Committee approved

the conduction of this study (approval no: 21-6T/66, date: 11.06.2021).

Microbiological Methods

After admission, nasopharyngeal swab specimens for polymerase chain reaction (PCR) analysis were obtained by a physician. All nasopharyngeal and oropharyngeal swab specimens were collected in a viral transport medium (vNat® Bioeksen, Turkey). All samples were tested using the Bio-speedy® SARS-CoV-2 Double Gene RT-qPCR kit (Bioeksen, Turkey). This same kit can differentiate among respiratory tract pathogens [influenza A (H3N2 and H1N1), influenza B, human metapneumonia virus A/B, human bocavirus, RSV A/B, adenovirus, enterovirus]. Anti-spike immunoglobulin G (IgG) and IgM antibodies were detected in serum samples using rapid lateral flow immunoassay (Colloidal Gold-Hotgen, Germany).

Statistical Analysis

Statistical analyses were performed using Statistical Package for the Social Sciences version 25 program. Continuous variables were expressed, if appropriate, as means and standard deviations or medians and interquartile ranges. Categorical variables were summarized as blood cell counts and respective percentages for each category. Categorical variables were compared between MIS-C, COVID-19, and other viral infection groups using the chi-square test. Non-parametric data were compared using the t-test for independent samples or Mann-Whitney U test. The Spearman's rank correlation test was used to analyze the association between laboratory markers. A p-value of less than 0.05 was set as the level of statistical significance, within corresponding 95% confidence intervals (CIs). After binary logistic regression analysis, we displayed the receiver operating characteristic (ROC) curve of statistically significant variables. We calculated the area under the curve (AUC) to evaluate the sensitivity and specificity of each parameter/model to predict PICU admissions and to differentiate COVID-19 from other viruses.

RESULTS

The mean ages of the COVID-19 group (95.7±75 months), MIS-C group (101.5±53.5 months), and other viral infection groups (49.9±55.7 months) were as indicated, while these corresponding groups contained 81 (55.1%), 24 (49%), and 63 (60.6%) male children, respectively. The mean age was significantly lower in the other viral infection group ($p<0.001$). There was no significant difference between the groups regarding gender of the patients ($p>0.05$). The baseline characteristics of all patients are shown in Table 1.

Influenza A/B, RSV, rhinoviruses, parainfluenza viruses, and adenoviruses were detected in 49 (47.1%), 15 (14.4%), 11 (10.6%), 4 (3.8%), and 3 (2.9%) patients, respectively.

Indicated number of patients with COVID-19 (n=77; 52.4%), MIS-C (n=7; 14.3%), and other viral infections (n=53; 51%) had at least one underlying medical condition. As is seen, underlying medical conditions were observed at significantly lower rate in the MIS-C group ($p<0.001$; Table 2).

When laboratory findings were evaluated, MIS-C patients had significantly higher mean values for parameters of WBC and eosinophil counts, ANC, mean platelet volume (MPV), derived NLR, median NLR, NMR, PLR, NPR, SII, and lower mean levels for ALC, monocyte, and platelet counts, median MPR than patients with COVID-19 or other viral infections ($p<0.05$). Median LMR was significantly lower in the COVID-19 group ($p=0.002$) (Table 1). Patients with influenza A/B had significantly lower median eosinophil counts, mean monocyte, and platelet counts than those with COVID-19 disease. The median values of NLR, LMR, NMR, PLR, NPR, MPR, derived NLR, and SII were not significantly different between influenza and COVID-19 groups ($p>0.05$) (Table 3).

The patients with MIS-C required invasive mechanical ventilation at a lower rate, and the duration of mechanical ventilation was shorter relative to the other groups ($p=0.001$). Children with MIS-C were more likely to need intensive care, and the mean length of stay in PICU for COVID-19 patients was more prolonged than in other groups (mean 17.5±19 days for COVID-19, 3.4±2.5 days for MIS-C, and 4.3±2.5 days for groups with other viral infections). Two patients with other viral infections died. No deaths were observed among patients with MIS-C and COVID-19 ($p>0.05$).

The mean age of the group of patients with other viral infections was 60.2±64.3 months, and 11 (50%) male patients from this group were admitted to the PICU. Among patients hospitalized in the PICU, the mean age of the children with other viral infections was significantly lower compared to the children with COVID-19 or MIS-C ($p<0.001$) (Table 4). Mean NMR, derived NLR indices, CRP, D-dimer values, platelet, and eosinophil counts, and median NLR were higher in the

patient group with MIS-C hospitalized in the PICU than in other groups (p<0.05) (Table 4). Mean SII was lower in patients with other viral infections admitted to the PICU without any significant intergroup difference (p>0.05).

The positive correlation between NMR and NLR, LMR, NPR, WBC, CRP, procalcitonin, D-dimer, MPV, use of inotropes, length of PICU stay, and negative correlation between NMR and MPR, lymphocyte count are shown in Figure 1. We detected a positive correlation between NPR and CRP, MPV, length of hospitalization, and PICU stay. This study have shown the presence of positive correlations between NLR, CRP, procalcitonin, and D-dimer, and also between PLR, CRP, MPR, MPV and length of hospitalization.

The ROC analysis was performed to determine the cut-off values of NLR, NMR, NPR, derived NLR, SII to predict the requirement for hospitalization of the patients with the MIS-C in PICU. Respective diagnostic sensitivities, specificities and AUC values of indicated cut-off values of NLR, NMR, NPR, derived NLR, and SII for the MIS-C group of patients were as follows: NLR: >2.62 [87.8%, 66.5%, 0.802 (95% CI 0.752-0.846), p<0.0001];

Table 1. Baseline characteristics, and laboratory data of children presenting with MIS-C, COVID-19, and other viral infections

	Group of patients with other viral infections, n=104	Group of patients with COVID-19 disease, n=147	Group of patients with MIS-C, n=49	p-value
Gender Male (n, %)	63 (60.6)	81 (55.1)	24 (49)	0.384
Age, months, (Mean ± SD)	49.9±55.7	95.7±75	101.5±53.5	<0.001
Underlying disease (n, %)	53 (51)	77 (52.4)	7 (14.3)	<0.001
WBC/(Mean ± SD)/mm^3	9128±5431	9014.6±7375	11884.9±6718.9	0.002
ANC/(Mean ± SD)/mm^3	5115±4217	5523.9.6±6410	9510.6±6298.6	<0.001
ALC/(Mean ± SD)/mm^3	3027±2291	2479.5±2001.1	1638±1344.9	<0.001
Hb (Mean ± SD, g/dL)	10.8±1.9	12.5±6.5	11±1.1	<0.001
PLT/(Mean ± SD)/mm^3	266836±129108	271768±123839	22040±120416	0.017
MPV (Mean ± SD)/FL	9.8±1.71	10±1.04	10.5±1.2	0.001
Eosinophil/(Median-IQR)/mm^3	10 (67.5)	20 (100)	100 (235)	<0.001
Monocytes/(Mean ± SD)/mm^3	817±808	878.8±689.8	516±403	<0.001
Leucopenia (n, %)	13 (12.5)	21 (14.3)	4 (8.2)	0.535
Neutropenia, (n, %) (<1,500/µL)	13 (12.5)	23 (15.6)	5 (10.2)	0.575
Lymphopenia (n, %) (<1,500/µL)	25 (24)	57 (38.8)	33 (67.3)	<0.001
Thrombocytopenia (n, %) (<150,000 µL)	21 (20.2)	22 (15)	16 (32.7)	0.026
NLR (Median, IQR)	1.4 (2.67)	1.8 (3.46)	6.3 (9.5)	<0.001
LMR (Median, IQR)	4.04 (5.12)	2.9 (3.03)	3.54 (5.59)	0.002
NMR (Median, IQR)	6.34 (7.44)	5.43 (6.33)	21.6 (25.8)	<0.001
PLR (Median, IQR)	103.7 (106.05)	127.3 (114.2)	142 (132.6)	0.001
NPR (Median, IQR)	0.016 (0.02)	0.015 (0.018)	0.041 (0.033)	<0.001
MPR (Median, IQR)	0.0024 (0.002)	0.0027 (0.002)	0.0019 (0.002)	0.022
Derived NLR (Mean ± SD)	1.7±2.2	2.1±2.4	5.5±4.9	<0.001
SII (Median, IQR)	347 (566)	433 (933)	1228 (2078)	<0.001
The total hospital length of stay, days, (Mean ± SD)	11.9±9.9	9.3±12.2	10.9±5.4	<0.001
PICU admission, n, (%)	21 (20.2)	22 (15)	25 (51)	<0.001

COVID-19: Coronavirus disease-2019, SD: Standard deviation, IQR: Interquartile range, WBC: White blood cell count, ANC: Absolute neutrophil count, ALC: Absolute lymphocyte count, Hb: Hemoglobin, PLT: Platelet count, MPV: Mean platelet volume, NLR: Neutrophil/lymphocyte rate, LMR: Lymphocyte/monocyte ratio, NMR: Neutrophil/monocyte ratio, PLR: Platelet/lymphocyte ratio, NPR: Neutrophil/platelet ratio, MPR: Monocyte/platelet ratio, SII: Systemic inflammatory index, PICU: Pediatric intensive care unit

NMR: >9.93 [79.6%, 77.2%, 0.812 (95% CI 0.763-0.855), p<0.0001]; NPR; >0.024 [81.6%, 73.7%, 0.797 (95% CI 0.746-0.841), p<0.0001]; derived NLR: >1.63 [91.8%, 63.3%, 0.819 (95% CI 0.771-0.861), p<0.0001]; SII: >570,263 [81.6%, 60.2%, 0.744 (95% CI 0.691-0.793), p<0.0001].

DISCUSSION

Several inflammatory markers have been evaluated as predictors of severity in hospitalized patients with severe and non-severe COVID-19 disease[16-18]. Circulating cytokine levels and inflammatory biomarkers have been shown to successfully predict disease severity and mortality; however, these are not readily available outside tertiary medical centers[19]. For this reason, cheap and simpler parameters that can be easily accessible have been evaluated in the studies. We have shown the presence of higher NLR, NMR, PLR, NPR, derived NLR, SII values and lower MPR levels in MIS-C group, and lower LMR ln COVID-19 group. We have also demonstrated that higher levels of NLR, NMR, and derived NLR are associated with PICU stay in the MIS-C group.

The parameters of NLR, LMR, and PLR are thought to reflect physiological stress. Stress causes an increase in circulating cortisol levels, which triggers an increase

Table 2. Underlying conditions in patient groups

Underlying conditions	Group of patients with other viral infections n=53	Group of patients with COVID-19 disease n=77	Group of patients with MIS-C n=7
Respiratory conditions (n, %)	5 (4.8)	8 (5.4)	1 (2)
Neurologic conditions (n, %)	6 (5.8)	14 (9.5)	0 (0)
Obesity (n, %)	0 (0)	7 (4.8)	4 (8.2)
Cardiac problems (n, %)	3 (2.9)	6 (4.1)	0 (0)
Hematological problems (n, %)	9 (8.7)	7 (4.8)	0 (0)
Transplantation (n, %)	4 (3.8)	5 (3.4)	0 (0)
Other (n, %)	26 (25)	30 (20.4)	2 (4.1)

COVID-19: Coronavirus disease-2019, MIS-C: Multisystem inflammatory syndrome in children

Table 3. Laboratory data of influenza A/B and COVID-19 groups

	Influenza A/B (n=49)	COVID-19 (n=147)	p-value
Gender Male (n, %)	26 (53.1)	81 (55.1)	0.804
Age, months, (Mean ± SD)	68.3±63.7	95.7±75	0.113
WBC/(Mean ± SD)/mm^3	7405±4292	9014.6±7375	0.351
ANC/(Mean ± SD)/mm^3	4372±3209	5523.9.6±6410	0.848
ALC/(Mean ± SD)/mm^3	2201±1420	2479.5±2001.1	0.724
Hb (Mean ± SD, g/dL)	11.4±1.95	12.5±6.5	0.099
PLT/(Mean ± SD)/mm^3	216708±102627	271768±123839	0.050
MPV (Mean ± SD)/FL	10.4±1.2	10±1.04	0.056
Eosinophil/(Median, IQR)/mm^3	10 (20)	20 (100)	0.004
Monocytes/(Mean ± SD)/mm^3	697±659	878.8±689.8	0.017
NLR (Median, IQR)	1.7 (3.03)	1.8 (3.46)	0.526
LMR (Median, IQR	3.5 (5.97)	2.9 (3.03)	0.062
NMR (Median, IQR)	6.5 (6.87)	5.43 (6.33)	0.189
NPR (Median, IQR)	0.016 (0.024)	0.015 (0.018)	0.472
PLR (Median, IQR)	108.9 (109.2)	127.3 (114.2)	0.127
MPR (Median, IQR)	0.002 (0.003)	0.0027 (0.002)	0.272
Derived NLR (Mean ± SD)	1.75±1.67	2.1±2.4	0.700
SII (Median, IQR)	342 (612)	433 (933)	0.113

COVID-19: Coronavirus disease-2019SD: Standard deviation, IQR: Interquartile range, WBC: White blood cell count, ANC: Absolute neutrophil count, ALC: Absolute lymphocyte count, Hb: Hemoglobin, PLT: Platelet count, MPV: Mean platelet volume, NLR: Neutrophil/lymphocyte ratio, LMR: Lymphocyte/monocyte ratio, NMR: Neutrophil/monocyte ratio, NPR: Neutrophil/platelet ratio, PLR: Platelet/lymphocyte ratio, MPR: Monocyte/platelet ratio, SII: Systemic inflammatory index

Table 4. Laboratory parameters and management of PICU and non-PICU group				
	Requirement for PICU stay in group of patients with other viral infections (n=22)	**Requirement for PICU stay in patients with COVID-19 disease (n=22)**	**Requirement for PICU stay in patients with MIS-C (n=25)**	**p-value**
Age, months, (Mean ± SD)	60.2±64.3	136.3±63.4	115.8±46.2	<0.001
Gender Male (n, %)	11 (50)	8 (36.4)	12 (48)	0.614
Underlying disease (n, %)	12 (54.5)	12 (54.5)	2 (8)	0.001
WBC/(Mean ± SD)/mm^3	8394.5±5363	11254±8993	12272±7406	0.251
ANC/(Mean ± SD)/mm^3	5574±4260.5	9191±8399	10540±7353	0.056
ALC/(Mean ± SD)/mm^3	2036±2328	1415±1008	1158±821	0.354
Hb (Mean ± SD, g/dL)	10.4±1.5	11.9±3.2	10.7±1.2	0.072
PLT/(Mean ± SD)/mm^3	269500±138172	268227±99507	180920±86986	0.008
MPV (Mean ± SD)/FL	9.2±1.93	10.1±1.1	11.3±1.2	<0.001
Eosinophil/(Mean ± SD)/mm^3	18.6±37.5	23±78	154±131	<0.001
Monocytes/(Mean ± SD)/mm^3	616±804	616±460	380±334	0.182
Leucopenia (n, %)	5 (22.7)	3 (13.6)	2 (8)	0.357
Neutropenia, (n, %) (<1,500/µL)	5 (22.7)	4 (18.2)	2 (8)	0.340
Lymphopenia (n, %) (<1,500/µL)	11 (50)	13 (59.1)	20 (80)	0.088
Thrombocytopenia (n, %) (<150,000 µL)	5 (22.7)	3 (13.6)	12 (48)	0.026
NLR (Median, IQR)	2.8 (5.3)	3.7 (10.7)	7.6 (12.9)	0.004
LMR (Median, IQR)	3.5 (8)	2.5 (3.2)	3.6 (5.9)	0.380
NMR (Mean ± SD)	16.3±16.7	17±11.9	40.1±33.8	0.002
PLR (Mean ± SD)	388±626	290.4±256.3	215.5±171	0.421
NPR (Mean ± SD)	0.033±0.045	0.063±0.140	0.062±0.05	0.001
MPR (Mean ± SD)	0.008±0.03	0.002±0.001	0.002±0.001	0.519
Derived NLR (Mean ± SD)	3.6±4.3	4.9±4.1	7.5±5.9	0.001
SII (Median, IQR)	636 (1827)	1478 (1782)	1228 (2789)	0.075
C-reactive protein (Mean ± SD, mg/L)	111.5±138	43±46	195±71	<0.001
D-dimer (Mean ± SD, µG/L FEU)	2357±1562	1968±1407	3152±1283	0.024
Ferritin (Mean ± SD, µG/L)	-	668±1038	653±432	0.076
Procalcitonin (Median, IQR)/µg/L	1.2 (9.07)	0.87 (3.09)	2.39 (7.13)	0.084
Tracheostomy (n, %)	0 (0)	7 (31.8)	0 (0)	<0.001
Use of Inotropes (n, %)	6 (27.3)	9 (40.9)	19 (76)	0.002
Oxygen support (n, %)	21 (95.5)	19 (86.4)	17 (68)	0.034
Nasal oxygen	16 (72.7)	11 (50)	18 (72)	0.192
BIPAP	4 (18.2)	4 (18.2)	5 (20)	0.983
Mechanical ventilation	11 (50)	10 (45.5)	1 (4)	0.001
Duration of using inotropes (Mean ± SD)	1.14±2.1	2.5±4.5	1.7±1.5	0.078
Duration of mechanical ventilation (Mean ± SD)	1.8±2.5	9±12	0.1±0.6	0.001
Duration of BIPAP (Mean ± SD)	0.41±1.1	0.54±1.3	0.3±0.6	0.990
Length of stay in PICU (Mean ± SD)	4.3±2.5	17.5±19	3.4±2.5	0.023
Length of hospital stay (Mean ± SD)	15±12	27±23	13±12	0.099
Mortality (n, %)	2 (9.1)	0 (0)	0 (0)	0.095

SD: Standard deviation, IQR: Interquartile range, WBC: White blood cell, ANC: Absolute neutrophil count, ALC: Absolute lymphocyte count, Hb: Hemoglobin, PLT: Platelet count, MPV: Mean platelet volume, NLR: Neutrophil/lymphocyte ratio, LMR: Lymphocyte/monocyte ratio, NMR: Neutrophil/monocyte ratio, PLR: Platelet/lymphocyte ratio, NPR: Neutrophil/platelet ratio, MPR: Monocyte/platelet ratio, BIPAP: Bilevel positive airway pressure PICU: Pediatric intensive care unit, MIS-C: Multisystem inflammatory syndrome in children

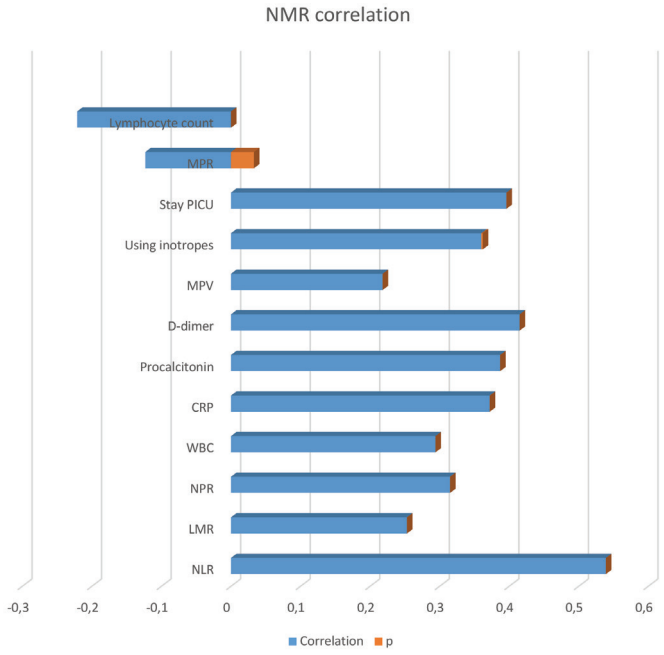

Figure 1. Correlations between NMR and other laboratory markers

MPR: Monocyte/platelet rate, PICU: Pediatric intensive care unit, MPV: Mean platelet volume, CRP: C-reactive protein, WBC: White blood cell, NPR: Neutrophil/platelet ratio, LMR: Lymphocyte/monocyte ratio, NLR: Neutrophil/lymphocyte ratio, Stay PICU: Stay in PICU, Using inotropes: Use of inotropes, NMR: Neutrophil/monocyte ratio

in circulating neutrophil and a decrease in lymphocyte counts. NLR not only reflects the increased number of neutrophils in infection but also indicates the decrease in the number of lymphocytes *in vivo*[20]. Higher NLR, LMR, and PLR values have been shown to associate with the disease severity in critically ill patients. Normal NLR values between 0.78 and 3.53 have been reported in adults excluding the geriatric period[21]. In a study by Zhang et al.[22], on 237 patients from China, NLR was demonstrated as an independent risk factor for mortality of the patients infected with influenza viruses. The same study determined that the H7N9-infected patients with NLR >19.94 had a higher mortality rate than those with lower levels of NLR[23]. Aktürk et al.[24] showed that NLR was significantly higher in patients who were hospitalized for respiratory tract infections than those hospitalized for other indications (mean NLR value 2.05 vs. 3.27). In Liao et al.'s[23] study, NLR showed a certain degree of diagnostic accuracy at optimal cut-off value of 1,478 in children with influenza A and the diagnostic value of NLR was well established in this patient population. Storch-de-Gracia et al.[25] evaluated 39 children with a median age of 9 years who were positive for SARS-CoV-2 PCR and determined that the higher values of NLR were associated with complicated COVID-19 disease. In a study by Yildiz et al.[26] on 79 children, NLR levels were found to be significantly higher in symptomatic children. Yang et al.[27] demonstrated that the increase in NLR values could be used as an independent prognostic biomarker in patients with COVID-19 disease and showed that NLR, LMR, PLR, and CRP levels were significantly higher in severely diseased patients. Feldstein et al.[28] showed higher NLR values were more common in MIS-C patients than in patients with severe COVID-19 disease. Prozan et al.[29] demonstrated lower NRL values in COVID-19 patients than in RSV infection and influenza patients, whereas higher NLR values were associated with poor clinical outcomes only in the COVID-19 group. They suggested that NLR was a more valuable prognostic marker of COVID-19 infection rather than influenza and RSV infection[29]. We have shown that the values of NLR, NMR, and derived NLR were higher in PICU-admitted MIS-C patients. NLR values were not significantly different between influenza and COVID-19 groups.

Studies support LMR as a good predictor of inflammatory events. A comparative assessment of LMR values in outpatients diagnosed with H1N1 influenza or pneumonia caused by culture-proven *Streptococcus pneumonia* demonstrated that LMR values below 2 was significantly associated with influenza[30]. Cunha et al.[31] demonstrated that the LMR <2 was more frequently seen in the human parainfluenza virus infections compared to human metapneumovirus, coronaviruses, HRV, human parainfluenza virus, and RSV infections. Temel et al.[32] showed that the mean LMR value was significantly lower, and NLR values were significantly higher in patients with influenza A relative to non-influenza A patients. Fei et al.[33] found that LMR in the influenza A-positive and influenza A-negative patients were significantly lower, while NLR was higher compared to healthy children. We found that LMR value was significantly lower in children with COVID-19 disease. The LMR values were lower in children with COVID-19 disease who were admitted to PICU, without any significant difference in LMR values between those who weren't.

In a recent study, NLR, PLR, SII, and derived NLR were shown to be helpful in the diagnosis and evaluation of disease severity in COVID-19 patients[27]. Bg et al.[34] showed that the derived NLR was not a significant predictor of mortality in adult patients with COVID-19

disease. Núñez et al.[7] demonstrated that the value of derived NLR was higher in COVID-19 patients with primary outcomes (requirement for mechanical ventilation; admission to a critical care unit or death). We have shown that derived NLR was significantly higher in MIS-C patients and higher NLR was significantly associated with PICU admission rate of MIS-C patients.

Several studies have reported that higher NMR values were associated with mortality rates related to COVID-19 disease[35]. A previous study demonstrated that the levels of LNR <0.088 and NMR >17.75 at admission could accurately predict in-hospital mortality rates from severe COVID-19 disease in Mexican adults, and NMR was suggested to be more sensitive and specific than LNR to predict the mortality risk[36]. This is the first study that evaluated NMR in MIS-C patients, and we have shown that NMR was significantly higher in the MIS-C group than in other groups.

PLR is related to immune-inflammatory reactions and indicates the severity of infection[36]. Gong et al.[37] showed higher PLR levels in severely ill patients compared to patients with non-severe COVID-19 disease. Qu et al.[38] reported that the increase in PLR was correlated with the poor prognosis of COVID-19 disease and patients with higher PLR had longer hospital stays. Nalbant et al.[39] showed that PLR values were significantly higher in patients with COVID-19 disease than those without.

Fei et al.[33] reported that patients in influenza A positive group had significantly higher PLR values than the negative group. We have demonstrated that PLR values were higher in the MIS-C group, however, they were not significantly correlated with PICU admissions in MIS-C patients.

SII is an index that describes instability in the inflammatory response, based on platelet, neutrophil, and lymphocyte counts. SII is recommended as a prognostic indicator in the follow-up of patients with sepsis[40]. Usul et al.[41] showed that SII was significantly lower for COVID-19-positive patients. SII was significantly associated with survival in a study including 119 adults with COVID-19 disease[42]. We have shown that SII was lower in children with other viral infections.

Study Limitations

There were several notable limitations to this study. Firstly data were not obtained from multiple centers but from a single center using a retrospective design, Secondly, the experimental data were limited. Our conclusions based on the findings of this study may differ from those of other researchers, and they must be elaborated further in clinical studies.

CONCLUSION

In conclusion, we have shown that higher NLR, NMR, derived NLR values for children with MIS-C and lower LMR values for children with COVID-19 disease could be used to predict the course of the disease. However, predictive diagnostic hematological parameters have not been specified for the COVID-19 disease so far.

Ethics

Ethics Committee Approval: The ethics committees of Ege University Faculty of Medicine Medical Research Ethics Committee approved the conduction of this study (approval no: 21-6T/66, date: 11.06.2021).

Informed Consent: Retrospective study.

Peer-review: Externally peer reviewed.

Author Contributions

Surgical and Medical Practices: S.Y.A., Z.Ş.B., Concept: S.Y.A., Z.Ş.B., G.G.Ö., N.M.B., P.Y.Ö., F.Ö., B.K., C.Ç., Z.K., Design: S.Y.A., Z.Ş.B., G.G.Ö., N.M.B., P.Y.Ö., F.Ö., B.K., C.Ç., Z.K., Data Collection or Processing: S.Y.A., Z.Ş.B., G.G.Ö., N.M.B., P.Y.Ö., F.Ö., B.K., C.Ç., Z.K., Analysis or Interpretation: S.Y.A., Z.Ş.B., G.G.Ö., N.M.B., P.Y.Ö., F.Ö., B.K., C.Ç., Z.K., Literature Search: S.Y.A., Z.Ş.B., N.M.B., Writing: S.Y.A., Z.Ş.B., F.Ö.

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Original Article

Short and Medium Term Neurological Outcomes of Children with Antenatal or Neonatal Exposure to Severe Acute Respiratory Syndrome Coronavirus 2

Antenatal veya Yenidoğan Döneminde Şiddetli Akut Solunum Yolu Enfeksiyonu Sendromu Koronavirüs 2'ye Maruz Kalan Çocukların Kısa ve Orta Dönem Nörolojik Sonuçları

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ABSTRACT

Objective: Neurological complications are among the main causes of mortality and morbidity in antenatal infections. Data on the long-term outcomes of infants exposed to severe acute respiratory syndrome-coronavirus-2 (SARS-CoV-2) infection in the antenatal or neonatal period are limited. This study aimed to investigate potential neurological complications in children with antenatal or neonatal exposure to SARS-CoV-2.

Method: In this prospective cross-sectional study, infants and toddlers with a history of antenatal or neonatal SARS-CoV-2 exposure underwent neurological evaluation by a pediatric neurologist.

Results: Of 31 children (19 males, median age 9.3 months) included in the study, maternal coronavirus disease-2019 (COVID-19) diagnosis was made in the first trimester of pregnancy in 1, the second trimester in 3, and the third trimester in 25 children. Two children were diagnosed with COVID-19 in the neonatal period, and 3 children with maternal COVID-19 diagnosis during pregnancy were also diagnosed with COVID-19 neonatally. On neurological examination, hypotonia and motor/social delays were observed in 1, microcephaly in 2, and macrocephaly in 1 child. Of the 8 children evaluated with magnetic resonance imaging, 1 had findings consistent with Joubert syndrome and the others were normal. All infants passed the standard auditory brainstem response test. The only ocular abnormalities detected were retinopathy of prematurity (stage 3) in 1 infant and poor eye contact and object tracking in the child with Joubert syndrome.

Conclusion: Our study suggests that neurological development is mostly favorable in infants and toddlers exposed to SARS-CoV-2 in the antenatal or neonatal period.

Keywords: COVID-19, newborns, pregnancy, prognosis

ÖZ

Amaç: Gebelikte geçirilen enfeksiyonlarda mortalite ve morbiditenin en önemli nedenlerinden biri nörolojik komplikasyonlardır. Şiddetli akut solunum yolu enfeksiyonu sendromu-koronavirüs-2 (SARS-CoV-2) enfeksiyonuna antenatal veya yenidoğan döneminde maruz kalan bebeklerin uzun vadeli sonuçları ile ilgili veriler kısıtlıdır. Bu çalışmanın amacı antenatal veya yenidoğan döneminde SARS-CoV-2'ye maruz kalan bebeklerde olası nörolojik komplikasyonların araştırılmasıdır.

Yöntem: Bu kesitsel, prospektif araştırmada antenatal veya yenidoğan döneminde SARS-CoV-2'ye maruz kalan bebeklere çocuk nöroloji uzmanı tarafından nörolojik değerlendirme yapıldı.

Bulgular: Çalışmaya alınan 19'u erkek toplam 31 bebeğin annelerinin 1'i ilk, 3'ü ikinci ve 25'i üçüncü trimesterde koronavirüs hastalığı-2019 (COVID-19) tanısı almıştı. İki bebek yenidoğan döneminde COVID-19 tanısı almış, 3 bebek ise annesi gebelikte COVID-19 geçirmekle birlikte yenidoğan döneminde COVID-19 tanısı almıştı. Nörolojik bakıda bebeklerin birinde hipotoni, motor ve kişisel/sosyal, ikisinde mikrosefali, birinde makrosefali saptandı. Manyetik rezonans görüntüleme ile değerlendirilen 8 bebeğin 1'inde Joubert sendromu ile uyumlu bulgular mevcut olup diğerleri normaldi. Tüm bebekler standart işitsel beyinsapı yanıtı testinden geçmişti. Bir bebekte prematüre retinopatisi (evre 3) saptanması, ayrıca Joubert sendrom tanısı alan 1 hastamızda göz teması ve obje takibinin zayıf olması dışında göz anomalisi saptanmadı.

Sonuç: Çalışmamız, antenatal veya yenidoğan döneminde SARS-CoV-2'ye maruz kalan bebeklerde nörolojik gelişimin çoğunlukla pozitif olduğunu göstermektedir.

Anahtar kelimeler: COVID-19, yenidoğan, gebelik, prognoz

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INTRODUCTION

It is not clear what effects coronavirus disease-2019 (COVID-19) infection in mothers during pregnancy or in infants during the neonatal period may have in early childhood. Several studies have shown that antenatal vertical transmission is possible but rare, and most perinatal infections are asymptomatic or progress with mild symptoms[1-7]. The available data increase our knowledge about the outcomes of infants born to mothers with COVID-19 and enable better treatment of the mother-neonate dyad (in most cases allowing room entry and breastfeeding) while also highlighting the lack of knowledge about the possible medium- and long-term effects of perinatal or transplacental severe acute respiratory syndrome-coronavirus-2 (SARS-CoV-2) neonatal infection[8,9].

In the face of this ambiguity, it might be helpful to refer to other congenital infections such as cytomegalovirus (CMV) to make an educated guess about how best to monitor infants born to mothers infected with SARS-CoV-2 and infants with COVID-19 during the neonatal period[10]. Studies conducted in the last decade have linked congenital CMV infection to sensorineural hearing loss (SNHL) and neuropsychiatric involvement, even in completely asymptomatic newborns[11]. Recent studies on the long-term effects of the newly identified Zika virus also demonstrated clinical sequelae in children[12].

The neurotropism of SARS-CoV-2 could result in a broad spectrum of neuropathic effects, including potentially affecting hearing[13]. Other coronavirus infections were shown to cause neurologic invasion via retrograde neuronal pathways or the blood, and the nervous system has a recognized receptor for SARS-CoV-2[14]. Evidence also suggests that COVID-19 infection may have a negative impact on the function of cochlear hair cells in asymptomatic adult patients, potentially affecting their hearing abilities[15]. In addition, a recent study in Turkey showed that the only presenting signs of COVID-19 infection may be non-specific symptoms such as sudden SNHL[16]. These studies showed that prenatal or neonatal exposure to SARS-CoV-2 may cause neurodevelopmental harm in infants. Another study showed that the placentas of SARS-CoV-2-infected women exhibited strong immune responses such as increased expression of interferon-associated genes and increased natural killer and T-cell activation[17]. Placental changes resulting from antenatal SARS-CoV-2 infection may create a pro-inflammatory environment that exposes the fetus to potential neurological sequelae. This is due to both the inflammatory responses that occur during the infection and direct viral infection, which can lead to pathological effects in vulnerable organs such as the eyes.

The present study investigated the short- and medium-term neurological characteristics of children with intrauterine or neonatal exposure to SARS-CoV-2 infection.

MATERIALS and METHODS

This observational, prospective, cross-sectional study included children born to women who were diagnosed with SARS-CoV-2 infection during pregnancy or at the time of delivery via molecular polymerase chain reaction (PCR) test of a nasopharyngeal swab and children with a history of COVID-19 infection confirmed by nasopharyngeal swab PCR during the neonatal period since the start of the pandemic in Turkey. A total of 107 families of women who were antenatally diagnosed as having SARS-CoV-2 infection and/or children infected with COVID-19 in the neonatal period in our hospital were contacted by phone. The study was explained, and 31 infants whose families provided informed consent to participate in the study underwent neurological and developmental assessments performed by a pediatric neurologist between May and August 2022. The developmental assessment evaluated fine motor, gross motor, personal-social, and language delays and was conducted by a pediatric neurologist based on history and neurological examination[18]. Developmental tests such as the Denver or Bayley tests were not routinely administered to all patients within the scope of the study.

The children's current age, sex, self and family history, head circumference, neurological examination findings, neuroimaging [e.g., brain magnetic resonance imaging (MRI), brain computed tomography (CT), transfontanel ultrasonography], electroencephalography (EEG), blood and urine analyses (biochemistry, complete blood count, thyroxine, thyroid-stimulating hormone, infectious, nutritional, and metabolic examinations), eye examination findings, and hearing test results (Ministry of Health routine neonatal screening) were recorded in the patient follow-up form. In the presence of clinical indications, brain CT (e.g., head trauma history), brain MRI (e.g., focal seizure, prematurity, risky procedures such as mechanical ventilation, abnormal physical examination findings such as toe walking, developmental delay, microcephaly, paresis), EEG (e.g., seizure or suspected seizure) were planned.

Inclusion Criteria

- Aged 0-3 years,

- History of PCR-confirmed neonatal SARS-CoV-2 infection in child or antenatal SARS-CoV-2 in mother who had antenatal follow-up and/or delivery at our hospital,

- Informed consent obtained from a parent/legal guardian for study inclusion.

Exclusion Criteria

- Suspected but unconfirmed neonatal or maternal antenatal SARS-CoV-2 infection,

- Lack of informed consent from parent/legal guardian.

Approval for the study was received by Necmettin Erbakan University Pharmaceutical and Medical Device Research Ethics Committee [date: 13.05.2022, decision no: 2022/3785:(9696)].

The parents/legal guardians of the children provided informed consent.

Statistical Analysis

Quantitative data were analyzed using IBM SPSS Statistics version 23 and presented as mean ± standard deviation or median and range. Categorical data were presented as frequency and percentage.

RESULTS

A total of 31 infants, 19 (61.3%) of which were males, were included in the study. The clinical characteristics of the infants and mothers are summarized in Tables 1 and 2.

Of the infants included in the study, 1 of the mothers was diagnosed with COVID-19 in the first trimester, 3 were diagnosed in the second trimester, and 25 were diagnosed in the third trimester. Two infants had COVID-19 during the neonatal period without a maternal history of COVID-19 during pregnancy, and 3 infants whose mothers were diagnosed with COVID-19 during pregnancy were also diagnosed with COVID-19 during the neonatal period. On neurological examination, motor and personal-social delays and hypotonia were observed in 1 child, microcephaly in 2, and macrocephaly in 1 child.

Transfontanel ultrasonography was performed in 18 children (58%) and brain CT was performed in 1 child (3.2%), and all were found to be normal. Brain MRI was performed on 8 (25.8%) of the infants; 1 had findings consistent with Joubert syndrome and the others were normal. EEG was performed in 4 children (12.9%) and was normal in all cases. All infants passed the hearing screening test of standard auditory brainstem response (ABR). The only ocular abnormalities detected were retinopathy of prematurity (stage 3) in 1 infant and poor eye contact and object tracking in the child with Joubert syndrome.

DISCUSSION

Managing pregnant women and their babies during a pandemic is challenging. The first case series from

Table 1. Clinical characteristics of children with antenatal or neonatal exposure to SARS-CoV-2

Age (months), median (range)	9.3 (1-15.9)
Male sex, n (%)	19 (61.3)
Cesarean delivery, n (%)	9 (70.9)
Neonatal COVID-19 transmission, n (%)	5 (16.1)
Symptomatic COVID-19 transmission, n (%)	4 (12.9)
Received medical treatment for COVID-19, n (%)	4 (12.9)
Preterm birth, n (%)	12 (38.7)
Concomitant infection, n (%)	15 (48.4)
Presence of symptoms at birth/in the neonatal period, n (%)	20 (64.5)
Meconium aspiration, n (%)	3 (9.7)
Neonatal pneumonia, n (%)	14 (45.2)
Transient tachypnea of the newborn, n (%)	4 (12.9)
Seizures, n (%)	2 (6.5)
Dysmorphia, n (%)	1 (3.2)
Microcephaly/macrocephaly, n (%)	2 (6.5) / 1 (3.2)
Developmental delay: motor and social/adaptive, n (%)	1 (3.2)
Hypotonia, n (%)	1 (3.2)
Anemia/polycythemia, n (%)	9 (29) / 1 (3.2)
Thrombocytopenia/thrombocytosis, n (%)	1 (3.2) / 12 (38.7)
Leukocytosis/neutropenia, n (%)	16 (51.6) / 3 (9.6)
Jaundice, n (%)	9 (29)
Ocular findings: retinopathy of prematurity, n (%)	1 (3.2)
Sensorineural hearing loss, n (%)	0 (0)
Epilepsy, n (%)	1 (3.2)

SARS-CoV-2: Severe acute respiratory syndrome-coronavirus-2, COVID-19: Coronavirus disease-2019

China provided some promising results about COVID-19 pneumonia in pregnant women, which had similar clinical features to non-pregnant adult patients and did not show vertical transmission. However, most centers adopted a cautious approach in terms of isolation during the first wave of the pandemic[19-22].

Not many studies have been done regarding the long-term effects of COVID-19 during pregnancy or its effect on infants. Previous studies on congenital infectious diseases have shown that even those without symptoms of the disease may be at risk of long-term sequelae such as vision, hearing, and neuropsychological problems [11,23,24]. Results showing strong placental inflammation during maternal SARS-CoV-2 infection strengthen this hypothesis[17].

Our study showed that neurological development is generally normal in infants exposed to SARS-CoV-2 in the antenatal or neonatal period. In comparison to a study that reported developmental delay in 13.2%, microcephaly in 5.3%, and SNHL in 5.3% of infants with in-utero Zika virus exposure over 3-year follow-up, the shorter follow-up of our patients (whose median age was 9.3 months) is a limitation in terms of monitoring language and cognitive development, but our findings are encouraging in terms of neurodevelopment[12].

Table 2. Clinical characteristics of the mothers of children with antenatal or neonatal exposure to SARS-CoV-2

Age (years), median (range)	27 (18-44)
Weeks of gestation at birth, median (range)	38 (22-40)
Advanced maternal age at birth (>35 years), n (%)	6 (19.4)
Parity-primigravida, n (%)	6 (19.4)
Consanguinity, n (%)	7 (22.6)
Antenatal fever, n (%)	11 (35.5)
Antenatal coinfection, n (%)	6 (19.4)
Multiple pregnancy, n (%)	2 (6.5)
COVID-19 vaccination status (BNT162b2), n (%)	3 (9.7)
COVID-19 vaccination timing: before pregnancy/first trimester, n (%)	2 (6.5) / 1 (3.2)
Weeks of gestation at the time of COVID-19 diagnosis, median (range)	32 (8-40)
Trimester at time of COVID-19 diagnosis, median (range)	3 (1-3)
Symptomatic COVID-19 infection, n (%)	25 (86.2)
Required medical treatment for COVID-19, n (%)	9 (29)
Radiologically documented pneumonia, n (%)	2 (6.5)

SARS-CoV-2: Severe acute respiratory syndrome-coronavirus-2, COVID-19: Coronavirus disease-2019

In a study evaluating infants with intrauterine COVID-19 exposure early in the pandemic, brain MRI findings of delayed myelination with brain hypoplasia, abnormal white matter signals, and bilateral periventricular abnormal signals suggestive of hypoxic effects were reported[2]. Neuroimaging in our study consisted of transfontanel ultrasonography in 18, brain CT in 1, and brain MRI in 8 children, and the only abnormal result in all of these examinations was MRI findings consistent with Joubert syndrome in 1 child. In the literature, Joubert syndrome in a patient with congenital rubella was reported as a cerebellar anomaly believed to possibly be associated with congenital infections[25]. However, we think Joubert syndrome was coincidental in our patient rather than related to COVID-19 exposure because the mother had COVID-19 at 36 weeks of gestation (third trimester), and the formation of the cerebellum is expected to be completed before this stage.

As antenatal COVID-19 exposure happened in the third trimester in most of the patients in our study (n=25, 80.6%), a statistically significant comparison could not be made with COVID-19 exposure in the first two trimesters or during the neonatal period. In a study by Rosen et al.[26] evaluating 55 pregnant women (similar in age to our maternal population) who had COVID-19 in the first 2 trimesters, no abnormalities in central nervous system development were detected by fetal ultrasound or brain MRI. The preterm birth rate was 3.4% in that study and 38.7% in our study, suggesting maternal COVID-19 in the third trimester could be a risk factor for preterm birth compared to infection in the first 2 trimesters. A study conducted on 388 pregnant women who were confirmed to have SARS-CoV-2 infection found that most cases were diagnosed in the third trimester and 26.3% gave birth before term[27].

In the study by Rosen et al.[26] cited above, all but 1 of the 55 infants were reported to be asymptomatic or mildly symptomatic during the neonatal period. In contrast, approximately 65% of the patients in the present study were symptomatic in the neonatal period, 16% were diagnosed with COVID-19 in the neonatal period (13% of which were symptomatic), 10% had meconium aspiration syndrome, 45% had neonatal pneumonia, 13% had transient tachypnea of the newborn,

29% had neonatal jaundice, and 1 patient developed *Streptococcus agalactiae* meningitis and associated focal seizure (based on the ILAE 2017 classification: focal impaired awareness with motor onset) and epilepsy (based on the ILAE 2017 classification: focal epilepsy, etiology: infectious) during the neonatal period[28]. However, except for the patient with Joubert syndrome, all children showed age-appropriate neurological development, and the patient with neonatal meningitis exhibited no seizures after the neonatal period. Compared to the results reported by Rosen et al.[26], these data suggest a risk of neonatal complications in third-trimester maternal COVID-19 infection compared to the first 2 trimesters but make no difference in terms of longer-term neurological outcomes. The patient samples in their study and the present study were similar in terms of the rate of symptomatic COVID-19 (90.4% and 86.2%, respectively) and prevalence of antenatal maternal fever during COVID-19 (31.4% and 35.5%, respectively)[26]. However, while Rosen et al.[26] reported a 10.3% rate of cesarean delivery in their study, this rate was 70.9% in our study. This may be related to the possible negative impacts of COVID-19 on fetal and maternal well-being in the last trimester, as well as physicians and families being worried about COVID-19 vertical transmission. Rosen et al.[26] showed that COVID-19 in the first two trimesters was not associated with vertical transition.

In our study, apart from our patient who developed symptomatic seizures due to neonatal meningitis (*S. agalactiae*), one child with neonatal COVID-19 infection had febrile seizures (focal impaired awareness with motor onset according to the ILAE 2017 classification) three times, at 7, 10, and 12 months of age[28]. Both of these patients had normal neurological examination findings during follow-up and normal EEG and brain MRI. An epileptic seizure is a frequent symptom in COVID-19. In a study using EEG in critical COVID-19, seizures were documented in 63.6% of patients[29]. Epileptiform activity is reported to be mainly focal (most commonly frontal) in the EEGs of COVID-19 patients[29,30]. Few studies have focused on the relationship between antenatal and neonatal COVID-19 and the occurrence of seizures and epilepsy development in children. However, our patient with antenatal COVID-19 exposure and meningitis-related seizures in the neonatal period and our patient with neonatal COVID-19 and febrile seizures both exhibited normal neurological development during follow-up, with normal EEG and brain MRI findings.

In our study, ABR screening at birth demonstrated normal hearing in all children. These outcomes were more favorable than those in a previous study on infants with intrauterine SARS-CoV-2 exposure, which reported defects in the medial olivocochlear efferent system[31]. However, this discrepancy may be due to methodological differences, as that study used otoacoustic emission tests, and our study used ABR. A retrospective study suggested that antenatal COVID-19 exposure caused transient abnormalities in ABR test results[32]. Considering the different results reported for audiological outcomes in adults with SARS-CoV-2 infection, we recommend longer-term studies including more patients to clarify this issue[33].

In a study in which 20 infants with intrauterine and perinatal COVID-19 exposure underwent ophthalmological assessments using fundus fluorescence angiography (FFA), optical coherence tomography (OCT), and behavioral assessment of visual functions at 3-7 months, OCT in all infants was normal, while abnormal FFA findings such as choroidal perfusion abnormalities, peripheral choroidal hypofluorescence, mild obliteration of the capillary bed, and vascular tortuosity were observed in up to 15% of the infants[1]. In a visual function study, although most infants showed normal behavioral assessments, 30% of them had reduced attention at a distance, and 15% had reduced contrast sensitivity[1]. Compared to that study, the lack of OCT and FFA data is a limitation of our study. However, eye examinations were normal in our patients except for the detection of stage 3 retinopathy of prematurity in a preterm infant (gestational age of 29 weeks) and poor eye contact and object tracking in a patient diagnosed with Joubert syndrome. In another study in the literature, ophthalmological assessment of 165 neonates with intrauterine COVID-19 exposure revealed venous engorgement and vascular tortuosity in 1, intraretinal hemorrhage in 7, and retinopathy of prematurity in 2 of the infants[34]. Again, more insight could be gained by evaluating more patients over a longer follow-up period.

Study Limitations

The strength of our study stems from its prospective, cross-sectional design. Limitations of our study are the cross-sectional evaluation of a limited number of patients, the fact that some patients were too young to assess cognitive and language development, that hearing and vision assessments were made by retrieving screening data and the lack of a control group. Also, most (86.2%) of the 29 infants with intrauterine COVID-19 exposure were born to mothers with COVID-19 in the third trimester. For this reason, our results cannot be

generalized to infants born to mothers infected at earlier stages of pregnancy, when the risk of malformations is higher in theory. Nevertheless, because of the insufficient data in this area, our observations can be considered preliminary results in this regard.

CONCLUSION

In summary, although our study showed that neurodevelopment was mostly normal in infants with antenatal or neonatal SARS-CoV-2 exposure, we believe that studies with more patients, longer follow-up periods, and control groups should be conducted to better understand these infants' long-term outcomes.

Ethics

Ethics Committee Approval: Approval for the study was received by Necmettin Erbakan University Pharmaceutical and Medical Device Research Ethics Committee [date: 13.05.2022, decision no: 2022/3785:(9696)].

Informed Consent: The parents/legal guardians of the children provided informed consent.

Peer-review: Externally peer reviewed.

Author Contributions:

Surgical and Medical Practices: F.M.A.Ö., F.H.Y., Concept: F.M.A.Ö., F.H.Y., Design: F.M.A.Ö., F.H.Y., Data Collection or Processing: F.M.A.Ö., F.H.Y., Analysis or Interpretation: F.M.A.Ö., F.H.Y., Literature Search: F.M.A.Ö., F.H.Y., Writing: F.M.A.Ö., F.H.Y.

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Original Article

Effect of Mobile Phone Usage During Pregnancy on Total Oxidant and Antioxidant Levels in Cord Blood

Gebelikte Cep Telefonu Kullanımının Kord Kanında Total Oksidan ve Antioksidan Madde Düzeyleri Üzerine Etkisi

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ABSTRACT

Objective: Although cell phones are considered to have non-ionizing radiation, they have many adverse health effect. Non-ionizing radiation causes oxidant stress when the balance between the production of free oxygen radicals and their elimination by antioxidants is disrupted. The effects of mobile phone usage during pregnancy on the growing fetus is an important problem that needs to be resolved. We aimed to investigate the effects of using mobile phone during pregnancy on cord blood oxidant-antioxidant levels.

Method: Cell phone usage features of 67 healthy pregnant women without additional risk factors were recorded. Total antioxidant status, total oxidant status (TOS), ischemia modified albumin (IMA), total thiol, native thiol, disulfide levels and disulfide/total thiol, disulfide/native thiol, native thiol/total thiol ratios evaluated in umblical cord blood.

Results: A negative correlation was found between daily talking duration by mobile phone and IMA levels; a positive correlation was found between daily talking duration and native thiol, total thiol levels ($p<0.05$). TOS, native thiol and total thiol levels were higher in the mothers who have another mobile phone in their bedroom at night ($p<0.05$).

Conclusion: Our study is the first clinical study that investigates the effects of using mobile phone during pregnancy on cord blood oxidant and antioxidant levels. Mobile phone exposure during pregnancy could have an important potential to cause oxidative stress in cord blood. Therefore, we think that it is important for pregnant women to protect themselves and the fetus by staying away from mobile phones as much as possible during pregnancy.

Keywords: Pregnancy, mobile phone, cord blood, oxidant, antioxidant

ÖZ

Amaç: Cep telefonlarının non-iyonize radyasyona sebep olduğu kabul edilse de, sağlık üzerine birçok olumsuz etkileri vardır. Non-iyonize radyasyon, serbest oksijen radikallerinin üretimi ile bunların antioksidanlar tarafından eliminasyonu arasındaki dengeyi bozarak oksidan strese neden olur. Gebelikte cep telefonu kullanımının oksidan denge ve dolayısıyla fetüs üzerindeki etkileri aydınlatılması gereken önemli bir sorundur. Bu çalışmada gebelikte cep telefonu kullanımının kord kanında oksidan ve antioksidan madde düzeyleri üzerindeki etkisini araştırmayı amaçladık.

Yöntem: Herhangi bir risk faktörü olmayan 67 sağlıklı gebenin gebelik süresince cep telefonu kullanım özellikleri kaydedildi. Umblikal kord kanında total antioksidan düzeyi, total oksidan düzeyi (TOS), iskemi modifiye albümin (IMA), total tiyol, nativ tiyol, disülfit düzeyleri ve disülfit/total tiyol, disülfit/nativ tiyol, nativ tiyol/total tiyol oranları değerlendirildi.

Bulgular: Cep telefonu ile günlük konuşma süresi ile IMA düzeyleri arasında negatif korelasyon; nativ tiyol, total tiyol düzeyleri arasında pozitif korelasyon bulundu ($p<0,05$). Geceleri yatak odalarında kendi telefonlarına ek olarak başka bir cep telefonu daha olan annelerde TOS, nativ tiyol ve total tiyol düzeyleri daha yüksekti ($p<0,05$).

Sonuç: Çalışmamız gebelikte cep telefonu kullanımının kord kanında oksidan ve antioksidan düzeylerine etkisini araştıran ilk klinik çalışmadır. Gebelik sırasında cep telefonu maruziyeti, kord kanında oksidan madde düzeylerinin artması, antioksidan madde düzeylerinin ise azalmasına sebep olarak oksidatif strese neden olabilecek önemli bir potansiyele sahip olabilir. Bu nedenle gebelerin gebelik süresince cep telefonlarından mümkün olduğunca uzak durarak kendilerini ve gelişmekte olan fetüsü korumalarının önemli olduğunu düşünüyoruz.

Anahtar kelimeler: Gebelik, cep telefonu, kord kanı, oksidan, antioksidan

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INTRODUCTION

Today, mobile phones have become an undetachable part of modern life. Especially in recent years, the use of mobile phones has increased rapidly with the ease of affordability, the increase in its popularity, and the fact that communication technologies are one of the fastest developing technologies in the world. The rate of mobile phone usage, which was 57% with 4.15 billion mobile phone users in the world in 2015, reached 62% with 4.68 billion in 2019[1]. A small increase in the adverse health effects that may occur due to the increase in use may cause serious effects on public health in the long term[1].

Cell phones emit low radiofrequency (RF) energy (450-2700 MHz; peak power=0.1-2 watt), a form of non-ionizing electromagnetic radiation that can be absorbed by tissues that are close to the phone. Mobile phone technology creates an electromagnetic field (EMF) in two ways: 1. Via base stations and 2. Via the phones themselves[2].

The amount of RF energy a mobile phone user is exposed to depends on many factors such as the technology of the phone, the distance between the phone and the user, duration of use, the extent of mobile phone usage, and the users distance from the base stations[2].

The International Agency for Research on Cancer classifies radio-frequency EMFs as possible carcinogens[2]. In studies on the side effects of EMFs on health, it has been shown EMF has been associated with many diseases such as Alzheimer's disease, autism, blood-brain barrier damage, brain tumors, depression, suicide, DNA damage, fatigue, headache and migraine, heart diseases, hormonal imbalance, joint pain, upper respiratory tract infections, immune system disorders, high blood pressure, learning difficulties, leukemia, loss of concentration, decreased sperm count, miscarriages, Parkinson's disease, sleep disorders and insomnia[3-12].

Specific absorption rate (SAR) value is a measure of the maximum energy absorbed by a unit of mass of exposed tissue of a person using a mobile phone, over a given time or more simply the power absorbed per unit mass. The rate of energy transfer measured from an EMF to a specific point is expressed in SAR.

In all organisms, the production of free oxygen radicals and the antioxidant defense system formed against it are in balance. Oxidative stress occurs when the balance between the production of free oxygen radicals and their elimination by antioxidants is disrupted. Oxygen free radicals are toxic biological substances that cause lipid, protein, carbohydrate oxidation, and DNA damage[13].

There are several parameters used to detect oxidative stress and evaluate its severity. One of these is the thiol/disulfide balance. Thiol an important antioxidant that interacts with almost all physiological oxidants, thus preventing tissue and cellular damage, are oxidized by reactive oxygen species (ROS) and transformed into reversible disulfide bonds. The resulting disulfide bond structures can be reduced back to thiol groups and thus the thiol-disulfide balance continues[14,15]. Thiol/disulfide balance is impaired in situations of oxidative stress. This imbalance can impair the function of proteins containing the thiol group, and this leads to increased sensitivity of cysteine-rich proteins to oxidation[14].

Pregnancy is a physiological process, tissue oxygen demand and metabolic demand increase in this process. An increase in oxygen demand causes an increase in the production of free oxygen radicals. Therefore, oxidative stress is seen at an increased rate in a pregnant woman without any problem compared to a non-pregnant woman of the same age[16]. Because of these increased levels of oxidative stress, the effects of mobile phone use during pregnancy on the growing fetus may be an important problem that needs to be resolved.

Since newborns, especially premature babies, are frequently exposed to procedures such as resuscitation and mechanical ventilation, the production of oxygen-free radicals is higher. On the other hand, antioxidant systems are not sufficiently developed in newborn babies therefore newborns are more likely to experience their toxic effects[17,18].

This study aims to evaluate the effects of mobile phone usage during pregnancy on cord blood oxidative and antioxidative systems. Our study is the first clinical study in the English literature investigating the effect of mobile phone use in mothers during pregnancy on oxidant and antioxidant levels in umbilical cord blood.

MATERIALS and METHODS

This prospective, cross-sectional study was carried out between November 2019 and February 2020 in our hospital, departments of the neonatal intensive care unit (NICU) and Gynecology-Obstetrics Clinic. Our center has an average of 1500 births per year and the level III NICU treats approximately 380 newborns annually.

The study was approved by the University of Health Sciences Turkey, Ankara Training and Research Hospital Ethics Committee (decision number: E-19, date: 18.11.2019) and was conducted according to the Declaration of Helsinki.

Sixty-seven mothers and baby pairs who gave birth by normal spontaneous vaginal way in the gynecology and obstetrics clinic were included in this study. Mothers who met the including criteria were hospitalized and prepared for delivery in the last trimester of pregnancy in the obstetric-gynecology service and were informed about the study before delivery. Informed consent was obtained from all individuals who agreed to participate in the study. These participants were included in the study by taking blood samples from the umbilical cord at the time of delivery.

Brands and models were recorded to evaluate the radiation emitted by the mobile phones used by patients and; the head and body SAR value of mobile phones. In addition, how long they have used mobile phones during pregnancy and in their life, the duration of daily phone call time, daily internet usage times, where they leave their mobile phones during the day and night (the place of mobile phones such as in the bed, near the bed, far from the bed in the same room or in a different room) how many mobile phones there are at home asked to evaluate the rate of radiation exposure related to mobile phones.

Prolonged labor, premature rupture of membranes, obstetric intervention, cesarean section delivery, having a concomitant disease (hyper-hypothyroidism, diabetes mellitus, epilepsy, rheumatological diseases, cancer, cirrhosis, hepatitis, kidney diseases, active infection, etc.), small for gestational age, large for gestational age, intrauterine growth restriction, smoking, alcohol and drug use, perinatal asphyxia, neonatal meconium aspiration, multiple pregnancy were excluded from the study, because it may affect oxidant and antioxidant substance levels of cord blood. We evaluated total antioxidant status (TAS), total oxidant status (TOS), ischemia modified albumin (IMA), native thiol, total thiol, disulfide, disulfide/native thiol, disulfide/total thiol levels in cord blood.

After delivery umbilical cord was clamped, 2 mL of blood sample was taken from the umbilical cord vein immediately and put into a serum seperator tube and centrifugated at 1200 rpm for 15 minutes. Serum specimens were stored at -80 ºC until analysis. TAS and TOS levels were measured with a spectrophotometer called Roche Cobas C501 automatic analyzer and a new automated colorimetric method developed by Erel[19]. IMA level measurement was done with the colorimetric method defined by Bar-Or et al.[20]. Thiol-disulfide balance tests were measured using the automatic colorimetric "modified Ellman method" defined by Erel and Neselioglu[21].

Statistical Analysis

Statistical analysis was performed using IBM SPSS Statistics 22 (Statistical Package for Social Sciences, IBM Inc., Chicago, IL, USA). Histogram, Skewness, and Kurtosis values were used in addition to the Kolmogorov-Smirnov test for normality distribution. Chi-square was used to compare categorical groups. In correlation evaluation, Pearson correlation for normal distribution values and Spearman correlation for those without normal distribution values were performed. Independent samples t-test was used to compare the averages of two independent groups with normal distribution and Mann-Whitney U test was used to compare the median of two independent groups with no normal distribution. The significance level was accepted if the p-value was less than 0.05 ($p<0.05$).

RESULTS

All babies were born at term and by normal spontaneous vaginal delivery. The mean birth weight was 3182±345 (minimum=2515, maximum=4490) grams, all of whom were appropriate for gestational age (Table 1). The sociodemographic characteristics of mothers and babies are presented in Table 1.

Table 1. Sociodemographic characteristics of study group	
Gestational age (week), median (min-max)	39 (38-41)
Birth weight (gram) mean ± SD	3182±345
Gender n (%)	
Female	35 (52.2)
Male	32 (47.8)
Maternal age (year) median (min-max)	25 (18-41)
Occupation, n (%)	
Housewife	61 (91)
Employed	6 (9)
Mother education n (%)	
Lower primary-primary school	15 (22.4)
Middle school	21 (31.3)
High school-university	31 (46.3)
Gravidity (n) median (min-max)	2 (1-5)
min-max: Minimum-maximum, SD: Standard deviation	

The average mobile phone usage time of mothers was 7 years, daily phone talk time was 30 minutes, and daily internet usage time was 60 minutes. In addition, the average head and body SAR values of their mobile phones and the body SAR values of another mobile phone in the bedrooms at night are shown in Table 2.

TAS, TOS, IMA, native thiol, total thiol, and disulfide levels were evaluated in cord blood at the time of delivery. The results are shown in Table 3.

Table 2. Characteristics of mobile phone usage of study group

Mobile phone usage time (year)*	7 (0.25-20)
Daily talk time (minute)*	30 (1-240)
Daily internet usage time (minute)*	60 (0-420)
Head SAR value of mobile phone (watt/kg) *	0.64 (0.20-1.29)
Body SAR value of mobile phone (watt/kg)*	0.64 (0.22-1.68)
Body SAR value of other mobile phone in the bedroom (watt/kg)*	0.64 (0.30-1.72)

*Median (minimum-maximum), SAR: Specific absorption rate

Table 3. Oxidant and antioxidans substance levels in umblical cord blood

TAS (mmol/L)*	1.45 (1.04-2.97)
TOS (umol/L)*	7.22 (2.11-17.05)
IMA (ABSU)**	0.68±0.11
Native thiol (umol/L)*	391.6 (293.36-586.48)
Total thiol (umol/L)**	440.57±64.65
Albumin (gram/dL)**	3.48±0.81
Disulfide (µmol/L)**	23.13±7.62

*Median (minimum-maximum), **Mean ± standard deviation, TAS: Total antioxidant status, TOS: Total oxidant status, IMA: Ischemia modified albumin, ABSU: Absorbance units

There was no statistically significant relation between the working status-educational status of the mothers, birth weight, gestational age, and oxidant-antioxidant levels (TAS, TOS, albumin, IMA, native thiol, total thiol, disulfide, disulfide/native thiol, disulfide/total thiol, native thiol/total thiol level) (p>0.05).

TOS, native thiol, and total thiol levels were found significantly higher in male babies (p=0.001; 0.002; 0.007, respectively).

A negative correlation was found between maternal age and TAS level of umbilical cord blood (p=0.020, r=-0.283). No significant difference was found between maternal age and other oxidant and antioxidant levels (p>0.05).

When daily mobile phone usage time was evaluated, a positive correlation was found with total thiol, and native thiol (p=0.015, r=0.296; p=0.017, r=0.291). There was no significant relationship between TAS, TOS, disulfide, disulfide/native thiol, disulfide/total thiol, native thiol/total thiol levels, and phone usage time (p>0.05).

The median daily mobile phone use time was 30 minutes. When we compared the oxidant and antioxidant levels between the mothers who use mobile phone more than 30 minutes and less than 30 minutes in a day, a significant relationship was found on TOS, IMA, native thiol, and total thiol levels (p=0.047; 0.006; 0.003; 0.025, respectively); TOS and IMA levels were found higher and total thiol, native thiol levels were found lower with longer daily phone usage time (Table 4).

There wasn't any significant relation between the location of mothers' mobile phones and TAS TOS levels (p>0.05). However; disulfide, disulfide/native thiol, and disulfide/total thiol were higher and IMA, native

Table 4. Oxidant and antioxidant statues accoring to daily phone usage time

Daily phone usage time parameters	≤30 min	>30 min	p-value
TAS*	1.37 (1.12-2.89)	1.52 (1.04-2.97)	0.109
TOS*	6.01 (2.11-17.05)	8.26 (3.64-11.86)	0.047
IMA*	0.63±0.10	0.71±0.09	0.006
Native thiol**	407.20 (343.92-512.24)	373.28 (293.36-586.48)	0.003
Total thiol*	462.68±56.95	426.55±65.95	0.025
Disulfide**	21.96±7.21	24.97±8.02	0.115
Disulfide/native thiol**	5.67±1.52	5.97±1.57	0.451
Disulfide/total thiol**	5.06±1.23	5.30±1.25	0.454

*Median (minimum-maximum), **Mean ± standard deviation, TAS: Total antioxidant status, TOS: Total oxidant status, IMA: Ischemia modified albumin

thiol/total thiol levels were statistically lower in the group whose phone was in the same room concerning the whose phone was generally in another room in the daytime (p=0.024; 0.033; 0.033; 0.038; 0.033, respectively) (Table 5).

TAS, TOS, native thiol, and total thiol levels were statistically higher in the group who had more than one mobile phone in the bedroom at night (p=0.003; 0.024; 0.019; 0.044, respectively) (Table 5).

Body SAR values of the phones used by mothers and IMA, native thiol/total thiol were in negative correlation (p=0.043, r=-0.248; p=0.041, r=-0.250, respectively), disulfide/native thiol, disulfide/total thiol levels were in positive correlation (p=0.044, r=0.247; p=0.041, r=0.250, respectively); no significant relationship was found with TAS, TOS, native thiol, total thiol, and disulfide levels (p>0.05).

DISCUSSION

The health effects of non-ionizing radiation from mobile phones are a common topic of many studies, but there are many questions about the underlying mechanism. The basic interaction mechanisms of RF energy and the human body are thermal and non-thermal effects[22]. In the frequency range in which mobile phones are used, most of the energy is absorbed by the skin, brain, and other tissues, and these organs experience an increase in temperature. Studies are showing that this warming of tissues has negative effects on the functions of the body, but these studies do not provide definitive evidence that exposure to EMF below the level that will cause tissue heating has any negative effect on health[1].

Other effects of radio frequency energy on the human body are non-thermal effects seen in energy levels that cannot increase the temperature of the whole body or tissue to a destructive level. It has been reported that free radicals increase in the affected tissue at these energy levels[22]. Reactive free radicals that increase in the body with non-ionized RF energy are mostly oxygen-sourced and are called free oxygen radicals.

The increase in the use of mobile phones in societies and the acceleration of the prevalence of certain diseases suggested that there may be a relationship between these two conditions, and many studies have been conducted on this for many years[3-12,23].

There are many studies on the effects of mobile phone-derived RF energy on pregnant animals; however, human experiments are limited. In two studies by Tomruk et al.[24], it was shown that there was DNA damage in the liver and brain due to the increase of oxidative stress in rabbits and their babies after 1800 MHz RF exposure[25].

There was no significant relationship between birth weight, gestational week of newborns, and the levels of oxidant-antioxidant substances. Premature babies were excluded not only due to having a marked increase in oxidant stress and exposure to exaggerated reactive oxygen/reactive nitrogen products but also a deficiency in antioxidant defense mechanisms responsible for the removal of oxidant stress products in premature. Excluding the newborns with prematurity, postmaturity, and large or small for gestational age, which may affect the levels of these substances, prevented a significant difference between these values.

Table 5. Oxidant and antioxidant substance levels according to mobile phone exposure

	Locations of mobile phone in daytime		p-value	Existence of another mobile phone in bedroom at nights		p-value
	Same room (n=23)	Different room (n=44)		Existence (n=42)	None (n=25)	
TAS*	1.51 (1.13-2.89)	1.41 (1.04-2.97)	0.362	1.52 (1.04-2.97)	1.34 (1.13-1.70)	**0.003**
TOS*	6.97 (3.64-11.86)	7.54 (2.11-17.05)	0.526	8.14 (2.11-17.05)	5.86 (3.21-11.31)	**0.024**
IMA *	0.64±0.11	0.70±0.10	**0.038**	0.68±0.11	0.68±0.10	0.951
Native thiol **	400.16 (293.68- 512.26)	382.88 (293.36-586.48)	0.103	399.28 (293.68-586.48)	368.24 (293.36-488.08)	**0.019**
Total thiol *	457.18±64.53	431.89±63.72	0.129	452.79±67.53	420.04±54.80	**0.044**
Disulfide **	26.02±7.82	21.61±7.14	**0.024**	23.98±7.86	21.69±7.11	0.237
Disulfide/native thiol**	6.34±1.46	5.50±1.5	**0.033**	5.84±1.52	5.69±1.58	0.704
Disulfide/total thiol**	5.60±1.14	4.92±1.23	**0.033**	5.20±1.22	5.08±1.28	0.699
Native thiol/total thiol **	88.80±2.29	90.14±2.46	**0.033**	89.59±2.45	89.83±2.56	0.699

*Median (minimum-maximum), **Mean ± standard deviation, TAS: Total antioxidant status, TOS: Total oxidant status, IMA: Ischemia modified albümin

TOS level was higher in male babies compared to females. In a study evaluating the levels of oxidative stress markers by gender, it was shown that their levels were higher in young men than in women of the same age[26]. Similarly, in another study, SOR production in vascular cells was reported to be at higher levels in men than in women[27]. Under healthy conditions, cellular respiration in the mitochondria is the main source of SORs. It is thought that oxidant stress in men may be higher than in women due to the faster basal metabolism in men compared to women and the antioxidant effect of the higher level of estrogen in women[28,29]. Consistent with this, in our study, in addition to the increase of TOS levels in male babies' native thiol, total thiol levels were also found to be higher compared to female babies.

It was observed that as the mothers' age increased, the level of TAS in cord blood decreased. The oxidative stress theory of aging is based on the hypothesis that age-related functional losses occur due to the damage of macromolecules (DNA, lipids, proteins) by reactive oxygen and nitrogen derivatives. As oxidative stress increases, antioxidant levels decrease, and age-related morbidity and mortality increase[30]. Antioxidant capacity, which decreases with advancing age, explains the low level of TAS in older mothers.

IMA levels were found statistically higher in mothers with longer daily mobile phone talking time. Oxidative stress causes a decrease in the binding affinity of albumin due to free radical damage at the n-terminal end of the albumin molecule. This chemically altered albumin molecule is called IMA. It is a sensitive biochemical marker of ischemia and oxidative stress as a result of tissue hypoxia[31]. In a study investigating the relationship between IMA levels and morbidity-mortality in preeclamptic mothers and their babies, IMA levels in venous blood in preeclamptic pregnant women and their babies were found to be significantly higher than in the control group[32]. In our study, in addition to the increased IMA levels, the higher levels of TOS and lower levels of Native thiol and Total thiol in these mothers support that the increased exposure to radiation due to longer time of phone calls increases the oxidant capacity of the organism.

Disulfide levels and disulfide/native thiol and disulfide/total thiol ratios were found higher in those whose mobile phones were in the same room during the day compared to those in different rooms. It is difficult to measure the levels of SORs, which are formed as a result of RF energy and play an important role in cell regulation, in cells, tissues, and body fluids due to their short half-life and low concentrations. Therefore, other lipid peroxidation, DNA, and protein damage biomarkers are used to measure the potential and the level of oxidative stress. Cysteine and methionine amino acids contained in thiol groups are the primary targets of ROSs. The reversible transformation of thiol groups to disulfide form causes a decrease in thiol levels. This thiol/disulfide balance plays a critical role in detoxification, signal transfer, apoptosis, and enzyme activity regulation[33,34]. In a study on rats exposed to whole-body radiation, conducted by Deniz et al.,[35] antioxidant-effective native thiol and native thiol/total thiol ratios were found lower, while disulfide/native thiol and disulfide/total thiol ratios were found to be higher compared to the control group. We think that RF exposure, which increases with the shortening of the distance between mothers and the mobile phone, increases the free oxidant radicals. As a result of the increased SORs, conversion of thiol groups to disulfide is increased and the disulfide levels were found in higher titers.

In addition to a high level of TOS; TAS, native thiol, and total thiol levels were also found to be high in the umbilical cord blood of mothers who had one other mobile phone in their bedrooms at night. However, the result was not considered meaningful because the SAR values of all those other phones, and the exposure times and patterns of the mothers to those phones were not known well.

Native thiol/total thiol ratio was lower and disulfide/native thiol, and disulfide/ total thiol ratios were significantly higher in cord blood in participants with higher body SAR value of their mobile phones. Limit SAR values permitted for the sale of mobile phones have been determined in the USA and EU. The SAR value allowed by the American Federal Communications Commission in the USA is 1.6 W/kg[36]. In the USA, measurements are made based on values per 1 gram of tissue, while in Europe, measurements are made on 10 grams of tissue. With the increase in the measured tissue, the limit SAR value also increases. In Europe, smartphones with a SAR value of up to 2 W/kg are allowed to be sold by following IEC standards[36]. Therefore, phone use with a high SAR value increases radiation exposure, and these rates, which indicate increased oxidant levels, are in line with the increased exposure to higher energy levels of radiation.

Study Limitations

This study has some limitations. Although the conditions such as the presence of comorbid disorders, being under medical treatment, smoking, alcohol and drug use, premature rupture of membranes, prolonged labor, intervened birth, perinatal asphyxia, delivery with meconium, prematurity or postmaturity, large or small for gestational age, cesarean delivery, multiple pregnancy and ethnicity that may affect oxidant and antioxidant substance levels were carefully excluded, it was not possible to exclude all possible factors that could cause oxidant stress. In addition, searching the effects of oxidant stress on the fetus in mothers exposed to oxidant stress has several difficulties in this and similar studies. These difficulties are the need for long-term follow-up to investigate the effects of long time and low doses of exposure to environmental factors, the long latent period of the diseases that may be seen due to RF, the need to be repeated to evaluate the results in terms of accuracy but this is difficult because it requires manpower, equipment and sufficient time. It is difficult the ethical issues of the study and to identify the early stages of the health impact being investigated. Today, the number of young women who do not use mobile phones is very low due to developing communication technologies and easier access to this technology. For this reason, a control group that does not use mobile phones could not be formed in our study and the comparison was made according to the exposure time and characteristics. In addition, the number of cases is limited because it is the first human study conducted on this subject.

CONCLUSION

Best of our knowledge, it was the first study in English literature that investigates the effect of mobile phone use during pregnancy on oxidant and antioxidant levels in cord blood, the exclusion of many personal and environmental factors that could negatively affect these substance levels, and guide further studies on this subject.

The lack of certainty about the health problems caused by the use of mobile phones and related radiation does not necessarily indicate the absence of risks. The principle of "avoidance" should be adopted for known risky situations and "precautionary" for suspicious and unknown situations. The need for large-scale and long-term epidemiological studies within the community is clear for more precise results.

Ethics

Ethics Committee Approval: The study was approved by the University of Health Sciences Turkey, Ankara Training and Research Hospital Ethics Committee (decision number: E-19, date: 18.11.2019) and was conducted according to the Declaration of Helsinki.

Informed Consent: Informed consent was obtained from all individuals who agreed to participate in the study.

Peer-review: Externally and internally peer reviewed.

Author Contributions

Surgical and Medical Practices: G.Ö., D.K., Y.Ü., M.A.T., Concept: G.Ö., İ.B., Ö.E., M.A.T., Design: G.Ö., S.N., Y.Ü., M.A.T., Data Collection or Processing: G.Ö., D.K., Y.Ü., M.A.T., Analysis or Interpretation: G.Ö., M.A.T., Literature Search: G.Ö., D.K., İ.B., Ö.E., S.N., Y.Ü., M.A.T., Writing: G.Ö., D.K., Ö.E., S.N., M.A.T.

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Original Article

Clinical, Molecular, Immunological Properties and Our Clinical Experiences in Patients Diagnosed with X-linked Agamaglobulinemia

X'e Bağlı Agamaglobulinemi Tanısı Alan Hastalarda Klinik, Moleküler, İmmünolojik Özellikler ve Klinik Deneyimlerimiz

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ABSTRACT

Objective: As a primary immunodeficiency X-linked agammaglobulinemia (XLA) that develops due to Bruton tyrosine kinase signal transduction protein deficiency which progresses with antibody deficiency was firstly described by an American pediatrician Ogden Bruton. In our study, we have aimed to evaluate the demographic, clinical, immunological, genetic characteristics and follow-up findings of patients diagnosed with XLA in our tertiary care Pediatric Immunology Clinic.

Method: Twelve patients diagnosed with XLA between 2003-2022 in our pediatric immunology clinic we're included in our study. The patient's age, sex, age at symptom onset and diagnosis, family history, laboratory findings at the time of diagnosis, complications observed during clinical follow-up and treatment modalities used were evaluated retrospectively.

Results: The median age of the patients at diagnosis was 36 [interquartile range (IQR) 10.2-69.0] months. While the median age of the patients without a family history at the time of diagnosis was 66 (IQR 41.2-66.0) months which was found to be significantly higher compared to the patients with a family history [11.5 (IQR 2.5-30.0) months] (p=0.004). Recurrent respiratory tract infections were the most common indications for admission. Agammaglobulinemia was detected in all patients except two cases. A significant decrease in B cells was detected by flow cytometry in all patients. The diagnoses were confirmed by genetic analysis for nine patients. Bronchiectasis was observed in four, arthritis in three, and inflammatory bowel disease in one case. In one patient, metaplasia was detected in the cytologic examination of the biopsy specimen obtained during endoscopy performed for the diagnosis of inflammatory bowel disease.

Conclusion: Early diagnosis, treatment and regular follow-up convey critical importance in terms of preventing complications in patients with XLA.

Keywords: X-linked agammaglobulinemia, Bruton tyrosine kinase, primary immunodeficiencies

ÖZ

Amaç: Bruton tirozin kinaz sinyal transdüksiyon proteini eksikliğine bağlı gelişen, antikor eksikliği ile seyreden X'e bağlı agammaglobulinemi (XLA), ilk kez Amerikalı çocuk doktoru Ogden Bruton tarafından tanımlanan primer bir immün yetmezliktir. Çalışmamızda merkezimizde XLA tanısı alan hastaların demografik, klinik, immünolojik, genetik özellikleri ve takip bulgularının değerlendirilmesi amaçlanmıştır.

Yöntem: Çalışmamıza 2003-2022 yılları arasında üçüncü basamak çocuk immünoloji kliniği olan merkezimizde XLA tanısıyla takip edilen 12 hasta dahil edildi. Hastaların yaşı, cinsiyeti, semptom başlangıç yaşı, tanı yaşı, aile öyküsü, tanı anındaki laboratuvar bulguları, klinik takipteki komplikasyonları ve tedavileri retrospektif olarak değerlendirildi.

Bulgular: Ortanca tanı yaşı 36 [interquartile range (IQR) 10.2-69.0] [minimum (min) 1.27- maksimum (max) 84] aydı. Aile öyküsü olmayan hastaların tanı anında ortanca yaşı 66 (IQR 41,2-66,0) ay olup, aile öyküsü olan hastalara göre anlamlı derecede yüksek bulunmuştur [11,5 (IQR 2,5-30,0) ay] (p=0,004). Hastalarımızda en sık başvuru nedeni tekrarlayan solunum yolu enfeksiyonları idi. İki olgu dışında hastaların tamamında agamaglobulinemi saptandı.

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Tüm hastalarda akım sitometride B hücrelerde anlamlı düşüklük izlendi. Dokuz hastada tanılar genetik analiz ile doğrulandı. Dört olguda bronşektazi, üç olguda artrit, bir olguda enflamatuvar bağırsak hastalığı gelişti. Bir olguda inflamatuvar bağırsak hastalığı nedeniyle yapılan endoskopi materyalinde metaplazi saptandı.

Sonuç: XLA'lı hastalarda erken tanı, tedavi ve düzenli takip komplikasyonları önleyerek hastaların yaşam kalitelerini artırması yönünden önemlidir.

Anahtar kelimeler: X'e bağlı agammaglobulinemi, Bruton tirozin kinaz, primer immün yetmezlikler

INTRODUCTION

As one of the firstly discovered genetically monogenic immunological disorders, X-linked agammaglobulinemia (XLA) is a primary immunodeficiency associated with antibody deficiency due to lack of Bruton tyrosine kinase (BTK) protein[1-3]. The disease was termed after pediatrician Ogden Carr Bruton who first described it in 1952 in an 8-year-old male patient with recurrent pneumococcal sepsis and agammaglobulinemia[4]. However, the genetic defect underlying XLA was only described simultaneously in 1994 by both Sideras et al.[5] and Ohta et al.[6]. It was found that the BTK gene, a member of the Tec kinase family, was located in the Xq21.3-Xq22 region and mostly mutated in male patients presenting with agammaglobulinemia[5,6]. Currently, 2152 different *BTK* gene mutations have been reported in the international mutation database[7]. BTK is a signal transduction protein expressed in the entire hematopoietic system excepting T and NK cells[2]. This disease develops due to a mutation in this gene, which is mapped on the long arm of the X chromosome[2], and is characterized by a significant decrease (less than 2% of total lymphocytes) or absence of mature B lymphocytes as a result of early arrest in the differentiation and maturation of B cells at the Pro B cell stage due to the deficiency of BTK protein (Figure 1)[8,9].

Since XLA is inherited as an X-linked recessive disease, males are primarily affected, and women are usually passive carriers[11]. Symptoms usually manifest after six months of life at the time when maternal IgG is lost[7]. However, rare cases diagnosed in adulthood have been reported[12-14]. Hypoplasia or absence of lymphoid tissue, normal-sized spleen and liver, significant decrease in serum immunoglobulin (Ig) levels, absence of antibody response to antigenic stimuli, and very few (<2%) or lack of B lymphocytes in the peripheral blood aid in making the clinical diagnosis. However; the importance of various factors in the clinical phenotype of the disease, such as age at diagnosis, serum B cell percentage, Ig concentrations, and polymorphic changes in Tec gene should be considered[15-17]. Recurrent bacterial infections associated with capsular, extracellular pyogenic pathogens such as *Streptococcus pneumoniae* and *Haemophilus influenzae* are common in affected individuals[18]. Susceptibility to severe and recurrent bacterial infections such as lower and upper respiratory system infections, skin infections, otitis media, conjunctivitis and meningoencephalitis due to existing hypogammaglobulinemia has increased to a great extent[19,20]. Other clinical presentations include diarrhea caused by common pathogens such as *Campylobacter jejuni* and *Giardia lamblia*[24]; skin involvement such as pyoderma gangrenosum, drug-induced Stevens-Johnson syndrome and eczematous dermatitis[21,22]. Although rare, purulent/non-purulent arthritis, osteomyelitis, sepsis, hepatitis, vaccine-associated polio, neutropenia and autoimmune diseases may develop[20,23]. While the cellular immune response to viral infections is normal in patients, sensitivity to the

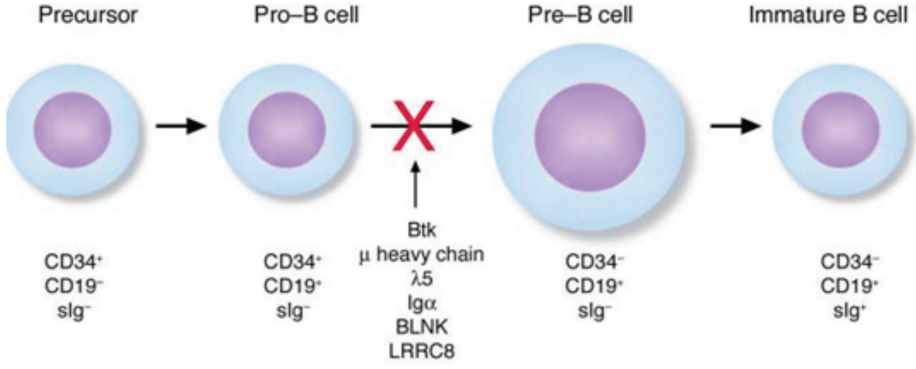

Figure 1. Early stages of B-cell differentiation can be identified by the status of the immunoglobulin genes using cell markers CD34, CD19 and surface immunoglobulin (10)

enterovirus family, in which secretory IgA is an important innate defense mechanism, increases. Therefore, disseminated infections can be observed secondary to the administration of a live virus vaccine such as oral poliovirus vaccine. Persistent enteroviral infections may rarely cause fatal encephalitis and dermatomyositis-meningoencephalitis syndromes. Bronchiectasis due to recurrent sinopulmonary infections is one of the most important complications of the disease[24]. Lifelong Ig replacement therapy is required to reduce the frequency of infections and he risk of mortality in patients with XLA[7].

The aim of this study which was performed in our pediatric immunology clinic, was to evaluate the clinical, molecular, immunological features of the disease and accompanying complications in 12 cases diagnosed with XLA, also known as Bruton's disease.

MATERIALS and METHODS

This study was carried out with 12 patients diagnosed with XLA between 2003-2022 in our tertiary care pediatric immunology clinic. The patient's age, sex, age at symptom onset and diagnosis, follow-up period, parental consanguinity and family history, laboratory findings at the time of diagnosis, complications developed during clinical follow-up and treatments they received were evaluated retrospectively. Peripheral B and T lymphocytes were identified by flow cytometry. In nine patients with suspected XLA, the diagnosis was confirmed by *BTK* gene mutation analysis. The study was approved by the University of Health Sciences Turkey, Dr. Behçet Uz Pediatric Diseases and Surgery Training and Research Hospital Ethics Committee (decision number: 2022/22-02, date: 22.12.2022) and was conducted according to the Declaration of Helsinki.

Statistical Analysis

Descriptive Analysis of the data was carried out by using IBM SPPS Statistics 22.0 program (version 22; SPSS, Chicago, IL, USA). We compared categorical variables using Fisher's exact and Pearson's chi-square tests. The Mann-Whitney U test or the t-test was used to compare numerical variables with and without normal distribution, respectively, and p-values less than 0.05 were considered statistically significant.

RESULTS

Twelve male patients followed up with XLA were included in the study. The mean age of the patients was 14.9±7.88 [minimum (min): 1.75-maximum (max): 24.75] years, and the median age at diagnosis was 36 (IQR 10.2-69.0) (min: 1.27-max: 84) months. The median age at diagnosis of patients without a family history was significantly higher when compared to patients with a family history [66 (IQR 41.2-66.0; min: 21-max: 84) months vs. 11.5 (IQR 2.5-30.0; min: 1.27-max: 48) months] (p=0.004). Respiratory tract infections were the most common indication for admission. One patient had a history of meningitis and the other patient had a history of poliomyelitis before the diagnosis of XLA was made. One of our patients presented with parechovirus encephalitis when he was one year old and diagnosis of XLA was revealed during the follow-up period. In the follow-up, bronchiectasis was diagnosed in four and arthritis in three cases. Inflammatory bowel disease was observed in a patient, and intestinal metaplasia was detected in the endoscopy material. *Helicobacter pylori* was identified as the causative pathogen in the patient who developed gastrointestinal symptoms during follow-up. Deep tissue infection leading to joint contracture developed in one patient. Physical examination disclosed absence of tonsillar tissue in eight and tonsillar hypoplasia in four patients (Table 1).

Agammaglobulinemia was detected in all but two patients aged 38 days, and 3 months who were screened in the early asymptomatic period because of the family history of XLA, in their siblings and cousins. Thanks to the presence of maternal IgG antibodies in newborns, agammaglobulinemia may not be detected in infancy especially during the first six months of life. A significant decrease in B cells was detected in the evaluation of lymphocyte subgroups by flow cytometry in all patients. The diagnoses were confirmed by genetic analysis in nine patients. However, genetic analysis was not required for three patients with confirmed family history of XLA (Table 2). Intravenous immunoglobulin (IVIG) replacement was performed for all cases. Excluding patients who received IVIG at an another center before admission, the median IgG levels of the patients were 147.5 (IQR 137.5-346.0) mg/dL before treatment and 795.50±197.99 mg/dL after six months of regular IVIG replacement. Besides the regular IVIG replacement, antibiotic prophylaxis was performed during the follow-up for nine patients.

DISCUSSION

Twelve patients with XLA were evaluated cumulatively throughout their routine treatment and follow-up periods since 2003. Although the mean age at diagnosis was similar between sporadic and

familial cases in a study coordinated by Italian Primary Immunodeficiency Network Centers so as to better define the natural history of XLA[12], in our study, the median age at diagnosis was significantly higher [66 (IQR 41.2-66.0) years] in cases without a family history, when compared with those with a family history of XLA (p=0.004). We believe that genetic and laboratory analyses performed in our clinic in some patients with a positive family history of XLA led to the diagnosis of this disease at an early stage in other words at a younger age. A similar delay in diagnosis for familial cases was observed in another cohort study in which only one-third of the patients with a positive family history were diagnosed before the disease became symptomatic[20].

Table 1. Conditions and complications that developed in our patients with XLA

	Patient no											
	P_1	P_2	P_3	P_4	P_5	P_6	P_7	P_8	P_9	P_{10}	P_{11}	P_{12}
Age at onset (m)	18	7	12	-*	12	6	8	-*	60	4	8	4
Age at diagnosis (m)	48	24	72	3	72	21	84	1.2	60	48	14	9
Present age (y)	17.9	23.5	18.5	8.91	14.3	24.7	12	5.33	21.3	23.5	1.75	6.91
Family history	+	+	-	+	-	-	-	+	-	-	+	+
Tonsils	a	a	a	a	a	a	a	h	h	h	a	h
Respiratory tract infection	+	+	+	+	+	+	+	+	+	+	+	+
Sinusitis	+	+	+	-	+	+	-	+	+	-	-	-
Otitis media	-	-	+	-	+	+	+	+	+	-	-	-
Pneumonia	-	+	+	+	+	+	+	+	+	+	-	+
Bronchiectasis	+	+	+	-	-	+	-	-	-	-	-	-
Arthritis	+	+	-	-	-	+	-	-	-	-	-	-
Menengitis/encephalitis	-	+	-	-	-	-	-	-	-	-	+	-
Poliomyelitis	+	-	-	-	-	-	-	-	-	-	-	-
Recurrent diarrhea	-	+	-	-	+	-	-	+	-	-	-	-
Malignancy	-	+	-	-	-	-	-	-	-	-	-	-
Deep tissue infection	+	-	-	-	-	-	-	-	-	-	-	-

*Asymptomatic, a: Absent, h: Hypoplastic

Table 2. Immunological evaluation of the patients on admission

	Age at diagnosis (months)	IgG (mg/dL)	IgM (mg/dL)	IgA (mg/dL)	B cell (%-#)	Family history	BTK mutation
P_1	48	33	12.6	6	0.5	+	c.1581_1584delTTTG mutation
P_2*	24	664	0.0	24.5	0.0	+	p.His454 Arg homozygous missense mutation
P_3*	72	680	17.3	25	0.3	-	p.Trp124Ser homozygous missense mutation
P_4	3	388	4	12	0.5	+	homozygous p.Arg255x nonsense mutation
P_5	72	134	16.9	26.4	0.9	-	homozygous p.Arg255x nonsense mutation
P_6	21	<142	3.99	5.9	0.0	-	p.Gln459X homozygous mutation
P_7	84	139	8.91	6.66	0.0	-	homozygous c.1775 C>T (p.ser592Phe) hemizygous mutation
P_8	1.2	596	16.8	26.6	1.5	+	-
P_9	60	317	14.2	0.0	0.7	+	-
P_{10}	48	<140	17	24.9	0.1	-	c.1581_1584delTTTG homozygous mutation
P_{11}	14	153	4.79	6.69	0.0	+	hemizygous c.1888A>G (p.Met630Val)
P_{12}	9	332	12.3	26	0.5	+	-

*Patients who received IVIG at another center before admission

These findings underline the fact that physicians should pay attention to positive family history to ensure early diagnosis of XLA.

In our follow-up, immunological parameters of most patients met the diagnostic criteria of the European Society of Immunodeficiencies[25]. The diagnosis was made in two patients by integrating data obtained from analyzes of all five primary classes of Igs, peripheral B cell percentages, genetic analyzes and family history. It was predicted that with the use of these three parameters in combination, XLA can be diagnosed and treated earlier, thus preventing the symptomatic onset of systemic findings of the disease.

In a large-scale study of 168 patients followed for XLA, the most common clinical manifestations recorded during follow-up were respiratory tract infections[12]. In the same study, the respective percentages of patients had developed bronchiectasis (51.8%), gastrointestinal involvement (52.4%), skin infections (30.5%), arthritis (10.4%), sepsis (2.4%), and meningitis (0.6%) during follow-up[12]. In our patients, the most common indication for hospital admission was similarly recurrent respiratory tract infections. One-third of the patients developed bronchiectasis and 25% arthritis during our follow-up. Before the diagnosis, a patient had a history of meningitis and another patient had poliomiyelitis. A patient had parechovirus encephalitis and XLA was diagnosed during follow-up period. In the cranial magnetic resonance imaging of the patient, a hyperintense nodular lesion and increased signal intensity in the thalamus, and numerous millimeter-sized calcifications at the interthalamic level were observed. Parechovirus encephalitis is a rare condition in healthy pediatric populations[26]. Asis stated in the literature parechovirus encephalitis should be considered in the differential diagnosis in patients with deep and periventricular white matter damage, increased signal intensity, bilateral thalamus damage, especially in patients with comorbid disorders or immunosuppression[27].

Lougaris et al.[12] diagnosed malignancies in their 6 (3.7%) patients with XLA including 4 cases with gastrointestinal tract malignancies. Inflammatory bowel disease was observed in one of our patients, and intestinal metaplasia was detected in the endoscopy material of the same patient. Joint contracture developed in another patient following deep tissue infection. Although our incidence rates were significantly lower than those reported in their large-scale study due to our small patient population, symptoms of our patients with Bruton's disease at admission and the complications observed in the follow-up were comparable.

There are known cases of poliovirus paralysis due to administration of attenuated oral Sabin vaccine in patients with XLA[12], and polio sequelae developed in one of our patient. During follow-up of our patients, respiratory tract infections such as pneumonia, otitis media and sinusitis persisted despite routine Ig replacement. This finding reinforces the fact that polyspecific IgG replacement therapy can not adequately compensate for the mucosal IgA deficiency in patients with XLA. As a result of mucosal IgA deficiency, we observed development of bronchiectasis in four patients. Considering the impact of chronic lung disease on the patient's daily life and especially on long-term outcomes, clinicians should pay more attention to the progressive course of lung morbidity in XLA[28], and individual Ig replacement, respiratory physiotherapy program, and antibiotic prophylaxis should be initiated in the early stage of the disease in affected cases[29].

Agammaglobulinemia was detected in all cases, except for two cases aged 38 days and 3 months, who were screened in the early asymptomatic period due to the family history of XLA in their siblings and cousins. Because of the maintenance of maternal IgG levels in newborns, agammaglobulinemia may not be detected in infancy especially during the first six months of life. A significant decrease in B cells was detected in the evaluation of lymphocyte subgroups by flow cytometry in all patients.

The diagnosis was confirmed by genetic analysis in nine patients. In similar studies, the most common mutation types were missense mutations (49%), followed by indels, nonsense mutations and deletions[12]. In our study, missense mutations were found in three, nonsense mutations in two, deletions in two, and indels in two patients. Genetic analysis was not performed in three patients because they met the clinical and laboratory diagnostic criteria of XLA, and a *BTK* gene mutation was detected in a family member (Table 2).

In affected individuals XLA is usually accompanied by severe hypogammaglobulinemia[19]. However, up to 10% of XLA cases have atypical presentations and exhibit normal or almost normal serum Ig levels that usually leads to diagnosis at an older age in these patients with milder phenotypes[12,30]. The ratio of CD19+ B cell remains less than 1% similar to classical XLA, but certain "leaky"

B cells mature with higher Ig levels in these unusual cases[31]. Mutations that cause atypical XLA are similar to those that induce classical XLA, but it is thought that other genetic and environmental factors might cause the diversity of phenotypes[30].

Lifelong Ig replacement therapy is required to reduce the frequency of infection and the risk of mortality in patients with XLA[7]. IVIG replacement was performed for all cases. Excluding patients who received IVIG at another center before admission, the median IgG levels of the patients were 147.5 (IQR 137.5-346.0) mg/dL before treatment and 795.50±197.99 mg/dL after six months of regular IVIG replacement. Achieving normal serum IgG concentrations with early diagnosis, antibiotic prophylaxis and appropriately administered Ig replacement therapy in individuals diagnosed with XLA has significantly improved the prognosis and quality of life of patients in the last 25 years[7,21].

Study Limitation

This study has several limitations due to the retrospective collection of data. Additionally, our sample size is small preventing generalization of our findings; however, given the limited number of studies focusing on Bruton's disease, our study provides additional useful data to assist clinicians in early identification of the patients who need further investigation and treatment.

CONCLUSION

As a conclusion, treatment of XLA includes regular Ig replacement as well as appropriate antibiotic prophylaxis. It is important to evaluate the relevant family history during the diagnostic process and not to overlook tonsillar hypoplasia in the physical examination. To improve survival and increase quality of life in affected patients, the focus should be on the prevention and prompt treatment of associated complications, particularly chronic lung disease. By raising awareness, early diagnosis, treatment and regular follow-up will improve the quality of life of the patients with XLA by preventing development of complications.

Ethics

Ethics Committee Approval: The study was approved by the University of Health Sciences Turkey, Dr. Behçet Uz Pediatric Diseases and Surgery Training and Research Hospital Ethics Committee (decision number: 2022/22-02, date: 22.12.2022) and was conducted according to the Declaration of Helsinki.

Informed Consent: Retrospective study.

Peer-review: Externally and internally peer reviewed.

Author Contributions

Surgical and Medical Practices: E.B., İ.T., İ.A.H., N.G., F.G., Concept: E.B., Ö.A., N.G., F.G., Design: E.B., S.Ö.B., Ö.A., N.G., F.G., Data Collection or Processing: E.B., N.G., F.G., Analysis or Interpretation: E.B., S.Ö.B., Ö.A., N.G., F.G., Literature Search: E.B., N.G., F.G., Writing: E.B., S.Ö.B., Ö.A., N.G., F.G.

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Original Article

Hazardous Effects of Electrocautery on Penile Arteries: An Experimental Study

Elektrokoterin Penil Arterler Üzerindeki Zararlı Etkisi: Deneysel Çalışma

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ABSTRACT

Objective: Urethral arteries may be affected during electrocauterization The present study aims to investigate whether urethral artery spasm induced by electrocauterization during penile surgery causes pudendal nerve injury.

Method: Eighteen male New Zealand rabbits were allocated into control (G-I, n=5), penile surgery without electrocauterization (SHAM group, G-II, n=6) and monopolar cauterization under general anesthesia (study group, G-III, n=9) groups. The animals were followed up for three weeks and then sacrificed. Vasospasm index values (VSI: wall ring surface value/lumen surface value) of urethral arteries and degenerated neuron densities (DNDs) of pudendal nerve dorsal root ganglia at sacral-3 level (S3) were examined using stereological methods. Results were compared using the one-way ANOVA test.

Results: Neuronal angulation, cytoplasmic condensation, nuclear shrinkage, and condensed nuclei were detected in the pudendal nerve ganglia. The mean VSI values of urethral arteries and DNDs of pudendal ganglia (n/mm^3) were estimated as 1.012±0.024 vs. 4±1 in GI; 1.082±0.323 vs. 28±7 in GII and 2.54±0.0621 vs. 137±14 in GIII, respectively. Statistical significance values (p-values) in terms of VSI, and DND for the differences between GI and GII (p<0.01 vs. p<0.005), GII and GIII (p<0.001 vs. p<0.0001)- and GI and GIII (p<0.0001 vs. p<0.0001) were as indicated.

Conclusion: Electrocautery during penile surgery should not be used because of retrograde degeneration of the pudendal nerve and ganglia secondary to the injury to urethral taste bud-like structures.

Keywords: Urethral arteries, pudendal ganglia, electrocautery

ÖZ

Amaç: Üretral arterler elektrokoter kullanımından etkilenebilir. Bu çalışma penis cerrahisi sırasında elektrokoter ile pudendal sinir hasarının üretral arter spazmı olup olmadığını araştırmayı amaçlamaktadır.

Yöntem: On sekiz erkek Yeni Zelanda tavşanı incelendi: beşi kontrol (G-I, n=5), beşi elektrokotersiz penil cerrahi (SHAM grubu, G-II, n=6) ve dokuzu genel anestezi altında monopolar koter ile opere edilen grup (çalışma grubu, G-III, n=9). Hayvanlar üç hafta boyunca izlendi ve sonrasında sakrifiye edildi. Üretral arterlerin vazospazm indeks değerleri (VSI: Wall ring yüzey değeri/lümen yüzey değeri) ve sakral-3 seviyesindeki (S3) pudendal sinir dorsal kök ganglionlarının dejenere nöron yoğunlukları (DND) stereolojik yöntemlerle incelendi. Sonuçlar one-way ANOVA testi kullanılarak karşılaştırıldı.

Bulgular: Pudendal sinir ganglionlarında nöronal açılanma, sitoplazmik yoğunlaşma, nükleer büzülme ve yoğunlaşmış çekirdekler saptandı. Üretral arterlerin ortalama VSI değerleri ve pudendal ganglionların dejenere nöron yoğunlukları (n/mm^3) GI'de 1,012±0,024/4±1; GII'de 1,082±0,323/28±7 ve 2,54±0,0621/137±14 olarak hesaplandı. GIII. VSI için istatistiksel değerler: (p<0,01)-GI/GII; (p<0,001)-GII/GIII ve (p<0,0001)-GI/GIII; ve DND için ise (p<0,005)-GI/GII; (p<0,005)-GII/GIII ve (p<0,0001)-GI/GIII olarak bulundu.

Sonuç: Tat tomurcuğuna benzer yapıların yaralanmasına sekonder pudendal sinir ve ganglionların retrograd dejenerasyonu nedeniyle penis cerrahisi sırasında elektrokoter kullanılmamalıdır.

Anahtar kelimeler: Üretral arterler, pudendal ganglia, elektrokoter

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INTRODUCTION

Various somatosensitive, parasympathetic, and sympathetic nerves are responsible for the control of penile tissues. Penile tissue is mainly innervated by parasympathetic nerves that come from the Onuf's nucleus located in the parasympathetic region of the spinal cord, sympathetic nerves arise from the sympathetic region of the spinal cord from L1-L2 and fibers of somatosensitive neurons stem from the dorsal root ganglia (DRG) L6-S1[1]. Healthy sexual functioning in human beings requires an intact spinal cord[2], sacral parasympathetic and thoracolumbar sympathetic networks together with taste bud-like structures innervated by them[3].

Since normal functioning of above-mentioned anatomical structures also requires an intact interaction between pudendal network, Onuf's nucleus[4] and vascular supply, an electrocautery knife should be used with a lower voltage current or it should not be used unless absolutely necessary[5]. If orgasmic pleasures are regulated by taste bud-like structures in penile tissues and the pudendal nerve network, urethral artery spasm induced penile injuries may also be responsible for sexual dysfunctions and sexual anhedonia. This paper emphasizes the concerns about using electrosurgical devices such as monopolar electrocautery in penile surgeries.

MATERIALS and METHODS

Our study was initiated with the approval of the Atatürk University Animal Experiments Local Ethics Committee (decision no: 177, date: 17.09.2018). We preferred the rabbit model for our experiment. Five of 18 male New Zealand rabbits were allocated into control (G-I,) group. Penile incision was performed without using electrocautery in five rabbits (SHAM group, G-II) while monopolar cautery was used under general anesthesia in eight rabbits (study group, G-III).

The rabbit's penises were sterilized using a povidone-iodine solution. Within the SHAM group, solely an anteroposterior midline incision was performed, and hemostasis was accomplished without the utilization of cautery. A urethral catheter was introduced into the rabbits' urethra. The procedure in the SHAM group was conducted without employing electrocautery, whereas cautery was utilized in the research group. In accordance with the study conducted by some authors, a 15-W energy level was chosen for cautery. This energy level was administered for a duration of 5 seconds on an approximate region of 2 mm on both the ventral and dorsal sides of the apex of the penile shaft[6]. The cauterization was performed following the incision made on the penis. Subsequently, the surgical cut was sealed using uninterrupted stitches made of Vicryl (7/0) threads. Antibiotics and analgesics were administered during the postoperative phase. The animals were housed in cages under typical laboratory room conditions for a duration of three weeks following operation. The animals were sedated by administering a combination of ketamine hydrochloride (25 mg/kg), lidocaine hydrochloride (15 mg/kg), and acepromazine (1 mg/kg) through a subcutaneous injection. The circumcision procedure involved the use of surgical scissors and monopolar cautery (20W/400 kHz/Petas-Petkot 600) after sterilizing the operative area with local antiseptics. Following the administration of general anesthesia, all animals were euthanized. The pudendal nerves were revealed by dissecting between the gluteus maximus and medius muscles.

Subsequently, a laminectomy was performed on the S1-S3 vertebrae to extract the spinal cord at the S2 level and the pudendal ganglia. In addition, the edges of the cut ventral urethra were carefully separated from the surrounding tissue and removed together with its associated nerve and blood vessel structures. Following fixation in a 10% formalin solution for one week, all collected tissues were subsequently embedded in paraffin blocks. The blocks were sliced into slices measuring five micrometers and then treated with hematoxylin and eosin stain. The preparations were observed using a microscope at magnifications of 4x and 40x. The density of neurons in pudendal ganglia and taste bud-like structures was estimated using the stereological method outlined in the work by Caglar et al.[4], together with the taste bud-like structures estimation method employed in our most recent investigation.

Statistical Analysis

Statistical analysis was performed using one-way ANOVA test in SPSS 20.0 for Windows ($p<0.005$).

Statistical significance values (p-values) in terms of vasospasm index values (VSI), and DND for the differences between Gi and GII ($p<0.01$ vs. $p<0.005$), GII and GIII ($p<0.001$ vs. $p<0.0001$)- and GI and GIII ($p<0.0001$ vs. $p<0.0001$) were as indicated.

RESULTS

All DNDs of pudendal nerves of S3 were evaluated by stereologic analysis. The control and SHAM groups

shows urethral and penile arteries with normal and nearly normal endothelium and smooth muscle cells (Figure 1 and 2, respectively). Severe vasospasm of urethral arteries, axonal degeneration in the pudendal nerve roots, and neuronal degeneration and apoptosis in the S2 DRG were observed in the electrocautery group (Figure 3-5). Electrocautery might adversely affect urethral and pudendal nerves with resultant damage to centers related to sexual function. Pudendal nerve injury induced by an electrocautery knife may accompany urethral artery spasm following penile surgery (Figure 6). The degree of DND and VSI values were analyzed. Histopathological examinations were performed by a pathologist (R.A.) blinded to the allocated study groups using a light microscope.

Neuronal angulation, cytoplasmic condensation, nuclear shrinkage, and condensed nuclei were detected in the pudendal nerve ganglia. The mean VSI values of urethral arteries and degenerated neuron densities of pudendal ganglia (n/mm^3) were estimated as 1.012±0.024 and 4±1 in GI; 1.082±0.323 and 28±7 in GII and 2.54±0.0621 and 137±14 in GIII, respectively.

DISCUSSION

Pelvic visceral tissues innervated by pelvic plexus which is composed of somatosensitive and autonomic networks. Penile tissues innervated by neural web which contains parasympathetic fibers originated from Onuf's nucleus and sympathetic nerves arising from L1-L2 levels of the spinal cord and travel with pelvic nerves somatosensitive neurons coming from DRG L6-S1[1].

Intact spinal cord, sacral parasympathetic and thoracolumbar sympathetic networks are required for the maintenance of normal sexual function[2,3]. Sympathetic nerves cause vasospasm and

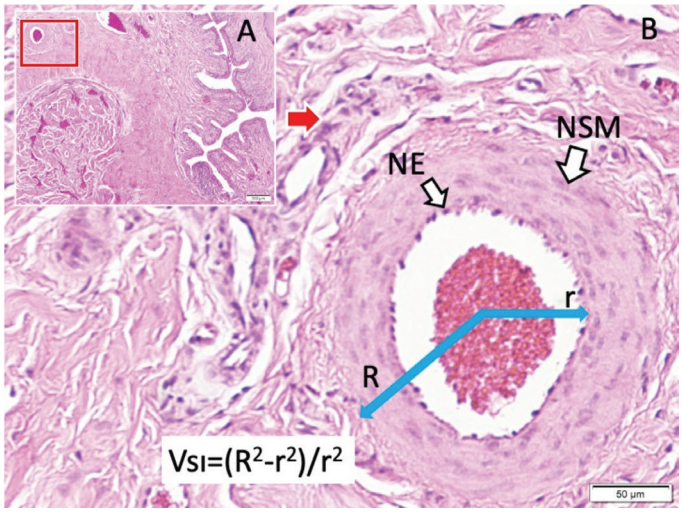

Figure 1. Urethral and penile arteries with normal endothelium (NE) and normal smooth muscle cells (NMC) of an intact rabbit penis seen under low (LM, H&E, x4/A) and higher magnification (LM, H&E, 10/B) and display of VSI estimation formula

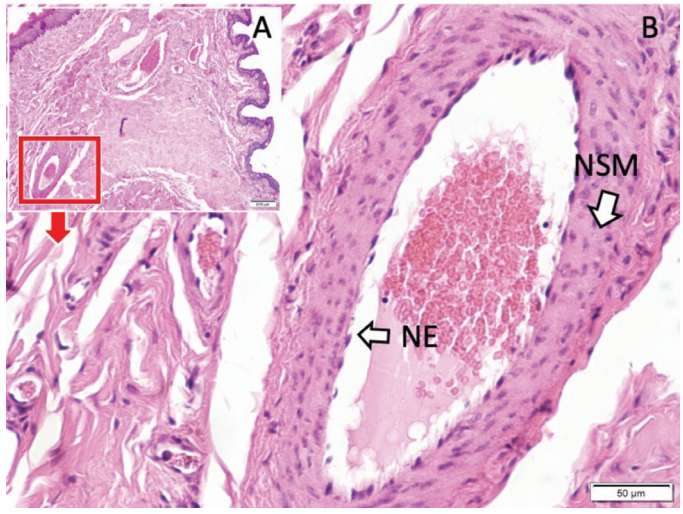

Figure 2. Urethral and penile arteries with nearly normal endothelium (NE) and normal smooth muscle cells (NMC) in a simple circumcison applied rabbit penis seen under low (LM, H&E, x4/A) and higher magnification (LM, H&E, 10/B)

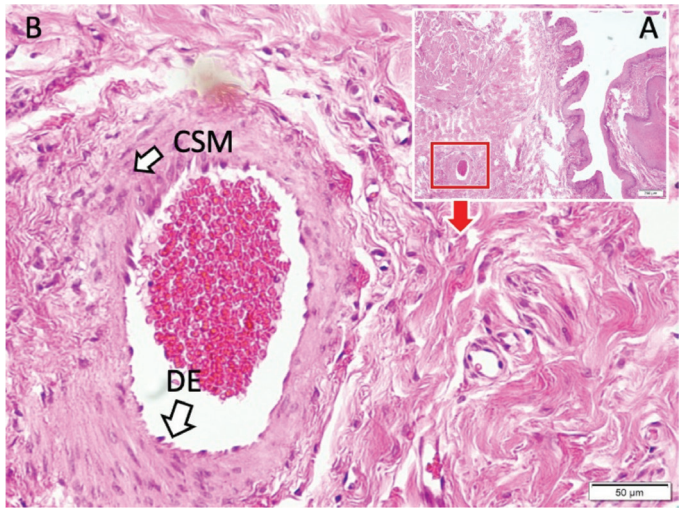

Figure 3. Urethral and penile arteries with minimally degenerated endothelium (DE) and contracted smooth muscle cells (CMC) seen in MEC electrocautery blade applied in rabbit penile tissue (images seen under low (LM, H&E, x4/A) and higher magnification (LM, H&E, 10/B)

parasympathetic nerves dilation of penile arteries[1]. We think that electrocautery may be more hazardous for parasympathetic than sympathetic nerves, because parasympathetic fibers are more densely populated in penile tissue[1].

As orgasmic pleasure is modulated by taste bud-like structures and pudendal nerves, the disruption of that network following penile surgery may cause sexual dissatisfaction and also infertility[4]. In the circumcision procedure, a ritual which has been used for centuries, currently applied monopolar electrocautery may damage very delicate penile tissues. Indeed electrocauterization induces development of neural/extraneural injuries with its thermal or electricity effects[7]. These hazardous effects can cause neuroma formation, vascular injury, degeneration of DRG and phimosis[8].

Neuronal degeneration criteria such as neuronal shrinkage, angulation of cells, cytoplasmic condensation and cytoplasmic halo formation were more prominent in the electrocautery group than in the non-electrocautery group. Therefore, electrocautery used during spine surgery may be injurious to spinal ganglia and should be used with a lower voltage current[9]. The animals that underwent facet denervation exhibited vascular wall injury, endothelial necrosis, muscle lesions, and thrombus development in the spinal radicular arteries. Facet denervation by monopolar electrocautery can lead to arterial lesions and the formation of blood clots in the radicular arteries. Therefore, it should only be used when absolutely necessary[5]. Electrocautery-related mechanism of electrical injury can cause neuroma[8] and scar formation[10]. Since electrocauterization causes neurodegeneration[9], endothelial-muscular insults and formation of thrombi in the radicular arteries it should not be used unless absolutely necessary[5].

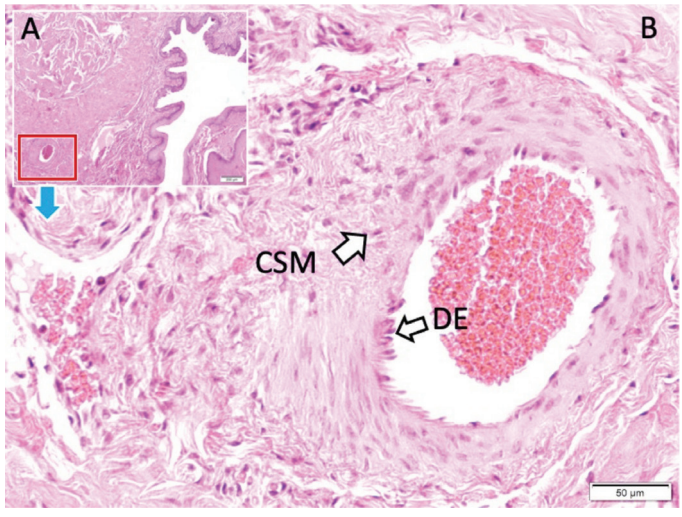

Figure 4. Urethral and penile arteries with severely degenerated/desquamated endothelium (DE) and prominently contracted smooth muscle cells (CMC) seen in MEC electrocautery blade applied rabbit penile tissue [images seen under low (LM, H&E, x4/A) and higher magnification (LM, H&E, 10/B)]

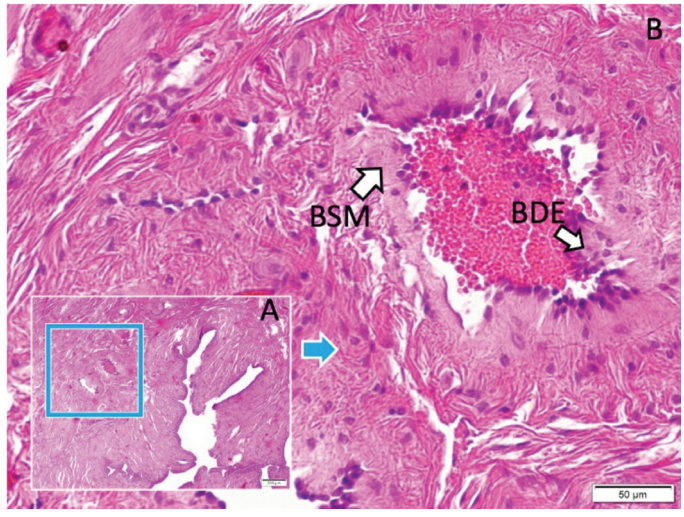

Figure 5. Urethral and penile arteries with moderately boiled/ degenerated endothelium (DE) and boiled/ contracted/degenerated smooth muscle cells (BMC) seen in thermocoagulation applied rabbit penile tissue [images seen under low (LM, H&E, x4/A) and higher magnification (LM, H&E, 10/B)]

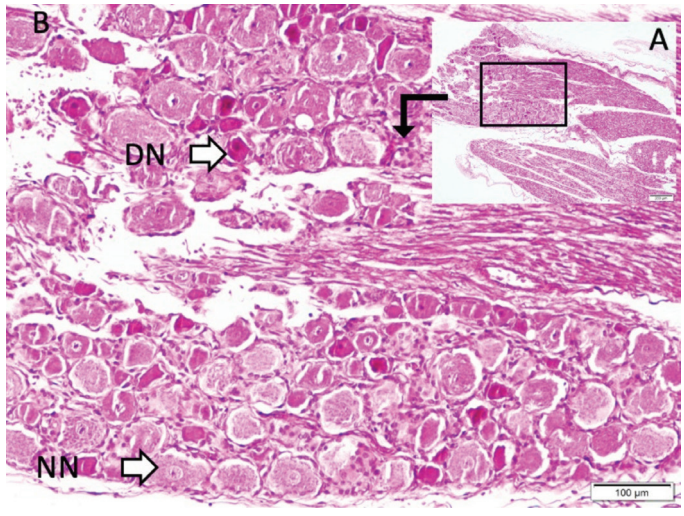

Figure 6. Histopathological view of pudendal dorsal root ganglia (DRG) with normal (NN) and degenerated neurons (DN) seen under lower and higher magnification (LM, H&E, x4/A; x10/B) in an animal study

Since electrocauterization can transmit electric current to neurovascular tissues, target tissue damage occurs because electric energy is converted to thermal energy or heat[7]. The damage that occurs depends on the tissue resistance, sensitivity and type of the tissue, as well as the duration, targeted direction, type, intensity and strength of the electric current[8].

In the present study; angulation of cells, cytoplasmic condensation, neuronal shrinkage and cytoplasmic halo formation accepted as cellular degeneration criteria were more prominent in the electrocautery group than in the non-electrocautery group. We recommend that electrocautery should be used with lower voltage current[9]. For example, Aydin et al.[5] have shown that application of high voltage electrocautery causes vascular injury and thrombus formation in the radicular arteries. Our study has also shown that application of electrocautery during penile surgery can be hazardous for penile arteries and pudendal nerve networks which has not been mentioned so far. Surgical circumcision is a common procedure frequently performed, and the related complications that develop can cause irreversible problems. It should be noted that the first of these complications is the damage due to the use of electric current, because electrocauterization and surgical cutting are widely used during circumcision.

This study shows that electrocautery used during surgery of apex of penis leads to degeneration of urethral arteries and pudendal nerve ganglia. Although we did not discuss it in this article, we have determined by the analysis of testicular tissues that this procedure may also have negative effects on spermatogenesis.

Study Limitations

This type of study should be performed in the future again using more advanced techniques, methods, and adequate number of experimental animal subjects. Besides, this study does not represent the human model. If a researcher opts to use small number of experimental animals for the conduction of this type of experiment, misleading conclusions may be drawn.

CONCLUSION

We would like to emphasize that either low-voltage or bipolar cautery should be used or preferably not applied in penile surgery, since the use of electrocautery causes damage to Onuf's nucleus-pudendal nerve-orgasmic sensation-sensing taste bud network and urethral arteries.

It is now certain that the amputation of the naturally occurring preputium causes medical problems. We anticipate that this harmful application will cause significant legal problems in the future.

Acknowledgement

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Ethics

Ethics Committee Approval: Our study was initiated with the approval of the Atatürk University Animal Experiments Local Ethics Committee (decision no: 177, date: 17.09.2018).

Informed Consent: Informed consent is not required.

Peer-review: Externally and internally peer reviewed.

Author Contributions

Surgical and Medical Practices: B.F., Concept: M.D.A., Design: Ö.Ç., Data Collection or Processing: A.A., Analysis or Interpretation: R.A., Literature Search: R.A., Writing: B.F.

Conflict of Interest: The authors have no conflict of interest to declare.

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J Behcet Uz Child Hosp 2023;13(3):198-202

Case Report

A Childhood Inflammatory Myopathy with Cytochrome Oxidase Deficiency: Which Came First, the Chicken or the Egg?

Sitokrom Oksidaz Eksikliği Olan Çocukluk Çağı Enflamatuvar Miyopatisi: Yumurta mı Tavuktan Çıkar, Yoksa Tavuk mu Yumurtadan?

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ABSTRACT

Inflammatory myopathies are autoimmune disorders rarely seen in childhood. Normally high-dose corticosteroid is the current treatment for inflammatory myopathies. For a specific subgroup of patients with inflammatory myopathy with cytochrome oxidase (COX)-negative myofibers that do not typically respond to corticosteroid treatment, and methotrexate (MTX) is used for therapy. Herein we present a 10-year-old girl who initially received clinical diagnosis of juvenile inflammatory myopathy which did not respond to corticosteroid treatment. Examination of her muscle biopsy specimen showed the presence of COX-negative muscle fibers which are very rarely seen in childhood inflammatory myopathies, and she was diagnosed as inflammatory myopathy characterized with COX-negative myofibers. The patient, who recovered with MTX therapy underwent genetic examination 3 years after the treatment was terminated. The sequence analyses of mitochondrial DNA (mtDNA) identified 19 variants in the rRNA, *ND2, ND4, ND5, COX1, COX3,* and *CytB* genes of the mtDNA of the patient and her mother. These mutations generally induce the production of synonym amino acids. However, four missense mutations on the *ND4, ATP6,* and *CytB* genes have caused structural changes in amino acids. None of these mutations have been previously reported as pathogenic variants. We have thought that these variations in such essential genes might destabilize mtDNA and could probably affect the ATP synthesis in our patient. Our final diagnosis was established based on abnormal inflammatory response induced by a hereditary mtDNA defect in a child with mitochondrial myopathy, rather than an inflammatory myopathy with COX deficiency.

Keywords: Childhood inflammatory myopathy, polymyositis with COX-negative myofibers, *ATP6* synthase gene, *ND4* gene, *CytB* gene, mitochondrial myopathy

ÖZ

Enflamatuvar miyopatiler, çocukluk çağında nadiren görülen otoimmün bozukluklardır. Normalde yüksek doz kortikosteroid, enflamatuvar miyopatiler için güncel tedavidir. Sitokrom oksidaz (COX) negatif miyofiberleri olan enflamatuvar miyopatili bir hasta alt grubu tipik olarak kortikosteroid tedavisine yanıt vermez. Bu durumda tedavi için metotreksat kullanılır. Burada başlangıçta klinik olarak juvenil enflamatuvar miyopati tanısı konulan ve kortikosteroid tedavisine yanıt vermeyen 10 yaşında bir kız çocuğu sunulmaktadır. Kas biyopsisinde çocukluk çağı enflamatuvar miyopatilerinde çok nadir görülen COX-negatif kas lifleri görüldü ve COX-negatif miyofiberli enflamatuvar miyopati tanısı aldı. Metotreksat tedavisi ile iyileşen hastada, tedavi kesildikten 3 yıl sonra genetik inceleme yapılabildi. Mitokondriyal DNA dizi analizlerinde hastanın ve annesinin mitokondrial DNA'sının rRNA, *ND2, ND4, ND5, COX1, COX3* ve *CytB* genlerinde 19 varyant tespit edildi. Bu mutasyonlar genellikle aynı amino asitlerin üretimine neden olmaktaydı. Ancak *ND4, ATP6* ve *CytB* genlerindeki dört missens mutasyon, amino asitlerin değişmesine neden olmuştu. Bu mutasyonların hiçbiri daha önce patojenik varyantlar olarak bildirilmemişti. Bu temel genlerdeki varyasyonların, mitokondrial DNA'nın kararsızlığına neden olabileceğini ve sunulan hastada ATP sentezini etkileyebileceğini düşündük. Genetik inceleme sonrası hastayı COX enzim defekti olan enflamatuvar bir miyopatiden çok, kalıtsal mitokondrial DNA kusurunun neden olduğu anormal enflamatuvar yanıt gözlenen mitokondrial miyopati olarak değerlendirdik.

Anahtar kelimeler: Çocukluk çağı enflamatuvar miyopatisi, COX-negatif miyofiberli polimiyozit, *ATP6* sentez geni, *ND4* geni, *CytB* geni, mitokondriyal miyopati

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***This case was presented as a poster at the 19th international congress of the World Muscle Society in Berlin on 7-11 October 2014 before performing genetic analyses with the title "A Rare Inflammatory Myopathy Patient with COX-negative Muscle Fiber Which Presents in Childhood".**

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INTRODUCTION

Inflammatory myopathies (IMs) with an autoimmune etiology are rarely seen in childhood[1,2]. Juvenile dermatomyositis, juvenile polymyositis and overlap myositis are the most frequent types of IMs. IMs are characterized with proximal muscle weakness, increased serum creatinine kinase (CK) levels, myopathic-patterns on electromyogram (EMG) and myopathy with inflammation detected during the histopathological examination of muscle biopsy specimens[2,3]. High-dose corticosteroids and methotrexate (MTX) are the current treatment modalities of IMs[2]. A subgroup of patients with IM with cytochrome oxidase (COX)-negative fibers typically do not respond to corticosteroid treatment. Furthermore, COX-negative patients can be misdiagnosed as a polymyositis due to lack of knowledge about this subgroup and lesser usage of COX- staining technique for biopsy specimens which delays the onset of preferred treatment with MTX[4,5].

COX is an enzyme encoded from mitochondrial DNA (mtDNA) in mitochondria that is responsible for electron transport chain. MtDNA deletions have been showed in 90% of patients with polymyositis which is characterized by the presence of COX-negative muscle fibers[4-6]. Contrary to the nuclear genome, the circular 16.6 kb (16,569 bp) mtDNA does not contain introns. The mtDNA has two strands. The heavy strand (H), which encodes for two rRNAs (12S rRNA and 16S rRNA), fourteen tRNAs, and twelve polypeptides, all of which are subunits of the respiratory chain complexes, and contains the majority of the information as follows: cytochrome b is composed of one complex III subunit, six complex I subunits (*ND1*, *ND2*, *ND3*, *ND4*, *ND4L*, and *ND5*), three complex IV subunits (*COI*, *COII*, and *COIII*), and two complex V subunits (ATPase 6 and ATPase 8). Nearly all of four subunits of complex II subunits are encoded by the nucleus[7-9].

Herein, we present a 10-year-old girl who was initially diagnosed as juvenile polymyositis which did not respond to corticosteroid treatment. Enzyme histochemical staining of muscle biopsy specimen demonstrated the presence of COX-negative muscle fibers that are very rarely detected in childhood. We also argue the dilemma: "Which came first: the chicken or the egg?"

CASE REPORT

In October 2010, a 10-year-old girl was presented to our outpatient clinic with complaints of pain in arms, shoulders, legs, knees, gait disorder and weakness in the arms and legs for 6 months which gradually worsened in the previous month. She was the second born baby of non-consanguineous parents, delivered at term with no significant perinatal problems. There is history of celiac disease and diabetes in her 14-year-old sister, and goiter in her mother. On physical examination, her body weight (59.5 kg: 97 p), height (149.5 cm: 90-97 p), body temperature (36 °C), pulse rate (90 bpm), respiratory rate (16/min), and blood pressure (125/80 mm/Hg: 50-90 p) were as indicated. She was conscious, oriented, and cooperated. Neurological examination revealed that bilateral proximal upper and lower extremity muscle strengths were 3/5, 4/5, respectively, bilateral deep tendon reflexes were symmetrically normal with flexor plantar responses. Rest of the physical examination findings were unremarkable. Laboratory test results were as follows: white blood cells: 6.600/mm^3, red blood cells: 4.000.000/mm^3, hemoglobin: 10.9 gr/dL, platelets: 213.000/mm^3, uric acid: 5.2 mg/dL (N: 3.8-5.8 mg/dL), aspartate aminotransferase: 240 U/L (10-40), alanine aminotransferase: 90 U/L (5-45), lactate dehydrogenase: 826 U/L (120-330), creatine kinase: 4694 U/L (5-130), creatine kinase MB: 106 U/L (0-20), C-reactive protein: 1.46 mg/dL (0.0-0.8), erythrocyte sedimentation rate: 32 mm/h (0-20), serum protein: 5.7 gr/dL (6.6-8.2), and serum albumin: 3.4 mg/dL (3.5-5.6). Patient was considered to have myopathy with increased serum muscle enzymes and proximal muscle weakness. Electromyographic findings were also compatible with a myopathic pattern. Magnetic resonance imaging revealed inflammatory process around proximal muscles of lower extremities. Patient was diagnosed as juvenile polymyositis, thereafter oral prednisolone (2 mg/kg/day) treatment was started. Muscle biopsy could not be performed priorly, due to the objection of her parents. Patient did not respond to steroid treatment. After second week of prednisolone treatment, pneumonia, urinary tract infection, multisystemic infectious disease and oral candidiasis were observed as complications of immune suppression. Since the patient did not respond to 18 days of prednisolone treatment, pulse steroid therapy was administered for 3 days, still without any clinical response. Thereafter, muscle biopsy was considered in consultation with her family and performed uneventfully. Histological examination of the biopsy specimen revealed only mild inflammatory infiltration that may be suppressed with corticosteroid treatment. In addition, inconspicuous areas of perifascicular atrophy (Figure 1) and numerous COX- negative muscle fibers visible with combined COX/succinate dehydrogenase (SDH) enzyme

histochemical staining were noted (Figure 2). Prednisolone dose was decreased, and MTX treatment (250 mcg/kg/week) was initiated. With MTX therapy her complaints decreased over several months and follow-up continued in outpatient clinics of pediatric immunology. She has been in good health for 7 years since the establishment of the diagnosis of COX-negative myopathy. The mtDNA sequence analyses revealed 19 variants in the rRNA, *ND2*, *ND4*, *ND5*, *COX1*, *COX3*, and *CytB* genes of the mtDNA of both patient and her mother. The variants of *m.8860A>G, Thr112Ala* and *m.9070T>G, Ser182Ala* variations at the *ATP6* gene, *m.10907T>G, Phe50Leu* at the *ND4* gene and *m.15326A>G, Thr194Ala* at the *CytB* gene were identified. None of these mutations have been reported as pathogenic variants previously[6-9]. Informed consent was obtained from the patient's parents.

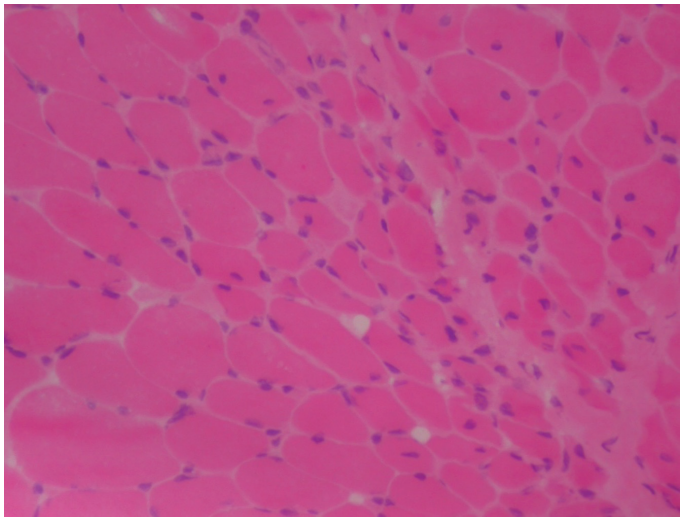

Figure 1. Presence of perifascicular atrophy which is often described in dermatomyositis (HEx100)

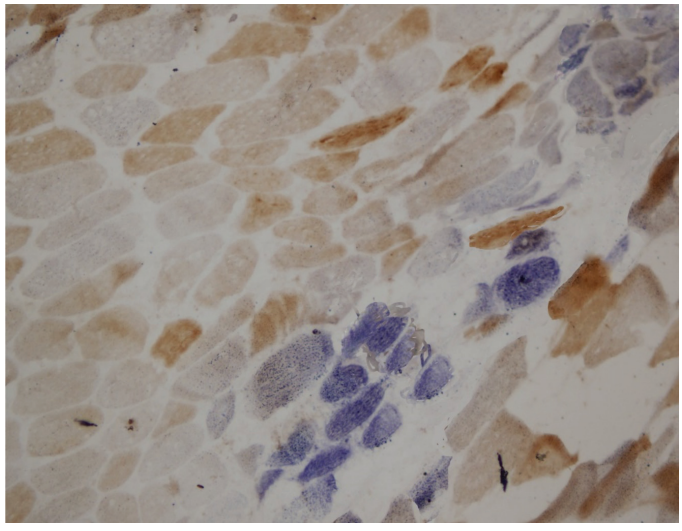

Figure 2. The "pathological blue myofibers" representing the presence of dysfunctional mitochondria (Combined COX/SDH stain x200)

COX: Cytochrome oxidase, SDH: Succinate dehydrogenase

DISCUSSION

The first report of polymyositis characterized with COX-deficient fibers, also known as PM with mitochondrial pathology (PM-Mito), was published in 1997[4]. It is a rare form of IM that shares clinical and pathological characteristics of sporadic inclusion body myopathy, such as a delayed age of onset, slow progression of quadriceps weakness, poor response to corticosteroids, endomysial inflammation with focal invasion of intact muscle fibers[4]. More precisely, the muscle biopsy should show diffuse HLA-1 upregulation, presence of >1% COX-negative fibers, lymphocytic endomysial infiltrates, particularly of CD8+ T-cells and/or macrophages invading non-necrotic fibers in this type of IM. But cytoplasmic inclusions and rimmed vacuoles ought to be absent[5]. IM with COX- negative fibers is usually recognized in adult patients who did not respond to long-term steroid therapy. Up to date, based on literature data, the patients with IM characterized by COX- negative muscle fibers were generally older than 50 years of age, and it has been rarely reported in children before[4-7]. All patients presented with proximal muscle weakness, abnormal EMG findings, and elevated serum CK values. Patients who typically had no clinical improvement with corticosteroid treatment, may be diagnosed as polymyositis with COX- negative muscle fibers based only on muscle biopsy findings[3,4]. Our patient was also presented with proximal muscle weakness, abnormal EMG findings, elevated serum CK values and did not show clinical response to steroid treatment which forced us to perform a muscle biopsy and then she was diagnosed as having inflammatory myositis with COX-negative muscle fibers. Clinical findings of our case were also compatible with the relevant literature data.

Examination of muscle biopsy specimens of these cases reveals inflammatory cell infiltrates, myopathic changes, complete absence of muscle fibers containing rimmed vacuoles and an excess of muscle fibers with deficient COX activity[3]. Myopathy characterized with muscle fibers of different sizes, focal invasion of muscle fibers by inflammatory cells, endomysial foci of CD4 and CD8 lymphocytes, excluding CD20 lymphocytes, positive cells and diffuse overexpression of MHC Class

I on the surfaces of muscle fibers identified throughout the muscle biopsy specimens are commonly seen during histopathological examination[5]. Inconspicuous inflammatory infiltration, excessive number of COX-negative muscle fibers and numerous "blue fibers" visualized with combined COX/SDH staining due to non-functioning mitochondria have been demonstrated during histopathological examination of the biopsy sample of our patient. There were no ragged red fibers, and rimmed vacuoles. Increased HLA class I expression was also observed.

Immunosuppressive therapy is the principal treatment in IMs. Corticosteroids are strongly recommended as the first-line immunosuppressive agents because of favorable treatment response achieved in IMs[1]. Unresponsiveness to steroid treatment has suggested that histochemical examination of muscle biopsy specimen might be useful to identify COX-negative IM patients as in our case. MTX is a treatment option for this group of patients, and this regimen is administered once weekly[3]. We initiated treatment with MTX (250 mcg/kg/week) for our patient and she responded over several months to this therapy. However, very often, the presence of COX-negative muscle fibers predicts a poor prognosis, even in cases treated with immunosuppressive treatment using MTX[3].

The nuclear genome is highly robust, while the typical mutation rate for the mitochondrial genome is 10-20 times greater. There are several explanations for this phenomenon, including the presence of unprotected histone proteins, a less effective mtDNA repair system relative to the nuclear repair system, and the close proximity of the respiratory chain to the mitochondria, which produces large amounts of reactive oxygen species, and exposes the mitochondrial genome to oxidative damage. This phenomenon has crucial importance during replication process because mtDNA is found in a single-stranded form for a prolonged period of time, making it more vulnerable to the assaults of radical oxygen species[5,6]. Numerous investigations have also demonstrated that mitochondrial mutations increase with ageing[6-9]. It has been also reported that mtDNA deletions occurred in 90% of the patients with IM characterized by COX-negative myofibers[4]. This finding aroused some suspicions indicating that mitochondrial dysfunction may induce pathologic inflammatory response, or an abnormal inflammatory response may be a contributory factor in mitochondrial dysfunction[6-9]. Due to the instability of mtDNA, the development of IMs characterized by COX-negative muscle fibers generally observed in elderly patients suggests possibly a coincidental association in some cases. The presence of multiple mutations in mtDNA in the presented case suggested that genetic examination is essential to elucidate the pathogenesis, especially in childhood IMs with COX-negative myofibers[6-9].

In conclusion, IM with COX-negative muscle fibers is a rare subtype of IMs in childhood[5]. COX-negative myopathy is a histopathologically proven myopathy. Pediatric patients with COX- negative muscle fibers are generally, and priorly receive corticosteroid therapy as the patients with polymyositis. However, in the presence of unresponsiveness to steroid therapy, muscle biopsy specimens should be obtained for the histopathologic diagnosis of COX-negative myopathy and genetic testing should be performed to disclose mtDNA mutations. Early initiation of MTX therapy should be kept in mind as a critical clinical decision to ensure a favorable treatment outcome in children with COX-negative myopathy. In the present case, genetic analyses have revealed different variants of several genes in the mtDNA. Same variants were also identified in her mother. These finding reminded us of the dilemma: "Which came first: the chicken or the egg?" Our final diagnosis was myositis due to an abnormal inflammatory response induced by hereditary mtDNA defects, rather than an IM with mitochondrial dysfunction.

Ethics

Informed Consent: Informed consent was obtained from the patient's parents.

Peer-review: Externally and internally peer reviewed.

Author Contributions

Surgical and Medical Practices: G.D., Ö.Y., Ü.B.Ş., Z.Y., C.A., C.Ö., A.B., Concept: G.D., Ö.Y., Ü.B.Ş., Z.Y., C.A., C.Ö., A.B., Design: G.D., Data Collection or Processing: G.D., Ö.Y., C.A., A.B., Analysis or Interpretation: G.D., A.B., Literature Search: G.D., Writing: G.D., Ö.Y., C.A., C.Ö.

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Case Report

Pediatric Pseudotumor Cerebri Syndrome Secondary to Superior Sagittal Sinus Thrombosis Associated with Severe Acute Respiratory Syndrome Coronavirus 2 Infection and Brief Literature Review

Şiddetli Akut Solunum Yolu Enfeksiyonu Sendromu Koronavirüs 2 ile İlişkili Süperior Sagittal Sinüs Trombozuna Sekonder Pediatrik Psödotumor Cerebri Sendromu ve Literatür İncelenmesi

Mehmet Can Yeşilmen[1], Çağatay Günay[1], Gamze Sarıkaya Uzan[1], Özlem Özsoy[1], Semra Hız Kurul[1], Elif Yaşar[2], Uluç Yiş[1]

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ABSTRACT

Pseudotumor cerebri syndrome (PTCS) is characterized by the presence of elevated intracranial pressure in the environment of intact brain parenchyma and cerebrospinal fluid (CSF). PTCS can occur in pediatric populations and cause permanent vision loss if left untreated. It is known that evere acute respiratory syndrome-coronavirus-2 (SARS-CoV-2) infection predisposes patients to adverse arterial and venous thromboembolic events. Several reports from the literature suggest that cerebral venous sinus thrombosis (CVST) may be a direct complication of SARS-CoV-2 infection. Herein, we have aimed to report a case of PTCS secondary to superior sagittal sinus thrombosis associated with the first episode of pediatric SARS-CoV-2 infection. A previously healthy 13-year-old boy presented to the emergency department in February 2022 with complaints of headache, tinnitus and double vision persisting for four days. His mental status and cranial nerve examination results were normal as revealed during neurological examination performed at admission, but bilateral papilledema was detected in fundoscopic examination. SARS-CoV-2 polymerase chain reaction was negative, while anti-SARS-CoV-2 antibody test was positive. There was no variant study in the case. Contrast-enhanced brain magnetic resonance imaging showed signs of intracranial hypertension and magnetic resonance venography demonstrated the presence of superior sagittal sinus thrombosis. CSF opening pressure was elevated (73 cm H_2O). As measured during lumbar puncture Our patient was accepted as a case of PTCS secondary to CVST associated with SARS-CoV-2 infection. PTCS secondary to CVST associated with SARS-CoV-2 infection was diagnosed and the child was treated with oral topiramate and low-molecular weight heparin. After the treatment, his headache and visual functions improved and then the child was included in our follow-up protocol. Clinicians should consider the risk of acute CVST in SARS-CoV-2 positive patients, especially if neurological symptoms develop. Prompt diagnosis and treatment can prevent vision loss.

Keywords: SARS-CoV-2, pseudotumor cerebri, papilledema, venous thrombosis, headache

ÖZ

Psödotümör serebri sendromu (PTSS), normal beyin parankimi ve beyin omurilik sıvısı (BOS) ortamında artmış kafa içi basıncın varlığı ile tanımlanır. PTSS pediatrik popülasyonda ortaya çıkabilir ve tedavi edilmezse kalıcı görme kaybına neden olabilir. Şiddetli akut solunum yolu enfeksiyonu sendromu-koronavirüs-2 (SARS-CoV-2) enfeksiyonunun hastaları arteriyel ve venöz tromboembolik olaylara yatkınlaştırdığı bilinmektedir. Literatürden çeşitli raporlar, serebral venöz sinüs trombozunun (SVST) SARS-CoV-2'nin 10 doğrudan bir komplikasyonu olabileceğini düşündürmektedir. İlk pediatrik SARS-CoV-2 enfeksiyonu ile ilişkili süperior sagittal sinüs trombozuna sekonder bir PTSS olgusunu sunmayı amaçladık. Daha önce sağlıklı olan 13 yaşında erkek hasta 2022 Şubat ayında son dört gündür baş ağrısı, kulak çınlaması ve çift görme şikayetleri ile acil servise başvurdu. Başvuru sırasında yapılan nörolojik muayenesinde mental durumu ve kraniyal sinir muayenesi normaldi. Fundus muayenesinde bilateral 15 papilödem saptandı. SARS-CoV-2 polimeraz zincir reaksiyonu negatif çıkarken, anti-SARS-CoV-2 antikoru pozitif çıktı. Kontrastlı beyin manyetik rezonans görüntüleme intrakraniyal hipertansiyon belirtileri gösterdi ve manyetik rezonans venografi süperior sagittal sinüs trombozu gösterdi. Lomber ponksiyon BOS 73 cm H_2O ölçüldü. Hastamız SARS-CoV-2 enfeksiyonu ile ilişkili SVST'ye sekonder PTSS olarak kabul edildi. SARS-CoV-2 enfeksiyonu ile ilişkili SVST'ye sekonder PTSS tanısı konulan çocuğa oral topiramat 20 ve düşük molekül ağırlıklı heparin tedavisi başlandı. Tedavi sonrasında baş ağrısı ve görme fonksiyonları düzelen çocuk takibe alındı. Klinisyenler SARS-CoV-2 pozitif hastalarda özellikle nörolojik semptomlar gelişirse akut SVST riskini göz önünde bulundurmalıdır. Hızlı tanı ve tedavi görme kaybını önleyebilir

Anahtar kelimeler: SARS-CoV-2, psödotümor serebri, papilödem, venöz trombozu, baş ağrısı

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INTRODUCTION

Pseudotumor cerebri syndrome (PTCS) is characterized by a constellation of symptoms due to elevated intracranial pressure with an unclear etiology absolutely in the presence of normal brain parenchyma and cerebrospinal fluid (CSF) constituents[1]. PTCS can be classified as primary and secondary depending on whether the etiologic agent is identified or not[1]. Intracranial venous thrombosis has been implicated as a cause for intracranial hypertension secondary to CSF outflow obstruction[2].

Since the beginning of the coronavirus disease-2019 pandemic, a diverse spectrum of neurological manifestations associated with severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection, including headache, seizures, altered mental status, aseptic meningitis, and also intracranial hypertension has been identified[3]. Furthermore, SARS-CoV-2 infection deteriorates coagulation pathways, predisposing infected individuals to venous thromboembolism[4]. Underlying pathomechanisms include endothelial dysfunction with increased levels of von Willebrand factor, systemic inflammation induced by the activation of Toll-like receptors (TLRs), and a procoagulatory state established via activation of tissue factor pathway[4]. We report a pediatric patient with secondary PTCS associated with sagittal sinus vein thrombosis emerged after SARS-CoV-2 infection, which to our knowledge a similar case has not been cited in the literature so far.

CASE REPORT

A previously healthy 13-year-old boy presented to the emergency department in February 2022 with complaints of headache, tinnitus and diplopia persisting for four days. His mental status and cranial nerve examination results were normal as revealed during neurological examination performed at admission, but bilateral papilledema was detected in fundoscopic examination detailed ophthalmologic examination revealed stage 3 papilledema, minimal dilation of the blind spot, bilateral enlargement of optic nerve sheaths up to a diameter of 6 mm, and increased peripapillary retinal nerve fiber layer thickness. During visual acuity test he could count fingers from a distance of 4 meters. SARS-CoV-2 polymerase chain reaction test from a nasopharyngeal swab was negative but SARS-CoV-2 immunoglobulin (Ig) G IgM rapid test was positive for SARS-CoV-2 infection in the patient who had been exposed to SARS-CoV-2 infection 10 days previously. There was no variant study in the case. Routine blood test results were their normal limits. Brain magnetic resonance imaging revealed partially empty sella turcica, enlargement of the bilateral perioptic nerve in the subarachnoid space, and optic nerve tortuosity. Magnetic resonance venography revealed subacute dural venous sinus thrombosis in the short segment at the level of the superior sagittal sinus vertex (Figure 1). Echocardiograms, results of Doppler ultrasonography examinations, genetic thrombophilia panel, hemostasis and rheumatological tests were within normal limits. The opening pressure at the lumbar puncture was as high as 73 cm H_2O. Biochemical parameters of CSF were not remarkable. Results of detailed viral and bacterial serologic tests were not pathologic. Acetazolamide and low-molecular weight heparin were started in the patient who was diagnosed with PTCS secondary to sagittal sinus vein thrombosis associated with SARS-CoV-2 infection. Due to metabolic acidosis and taste disturbance, acetazolamide was replaced with topiramate. On the third day of treatment, his headache and ocular complaints improved significantly. Control ophthalmological examination revealed normal visual acuity and peripapillary retinal nerve fiber layer thickness. Papilledema was regressed completely and bilateral optic nerve sheath diameters were reduced to 4 mm. The patient is still being followed up with

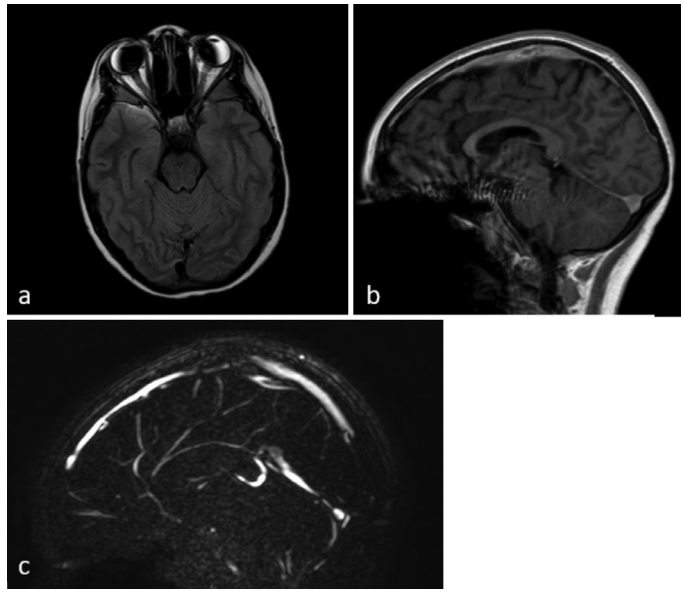

Figure 1. a) Axial FLAIR image showing protrusion of the optic nerve head and optic nerve tortuosity. **b)** Sagittal contrast-enhanced T1 image demonstrating focal hypointensity in superior sagittal sinus due to partial thrombosis. **c)** Sagittal TOF magnetic resonance venography displaying lack of flow in the short segment of the superior sagittal sinus

topiramate and low molecular weight heparin. Informed consent was received from the family.

DISCUSSION

Although children and adults had comparabie PTCS symptoms, demographic characteristics differ, and children are reported to have a higher incidence of secondary PTCS[5]. In the pediatric population, the most common causes of secondary PTCS are treatment with tetracycline antibiotics, and synthetic growth hormone, withdrawal from chronic corticosteroid therapy, and cerebral venous sinus thrombosis (CSVT)[6]. Since the beginning of the SARS-CoV-2 pandemic, many entities have been defined in the clinical course of the infection, one of which is an increase in the risk of thrombosis by disrupting the coagulation system leading to CSVT[7]. To date, in the literature, CSVT secondary to SARS-CoV-2 infection has been reported in 41 cases with a female predominance (53.7%) and a mean age of 50.1±16.5 years. In these patients, CVST was localized in the transverse and superior sagittal sinus in 63.4% and 46.3% of the cases, respectively[8].

Our case will contribute to the literature due to being the first pediatric case with PTCS associated with superior sagittal sinus vein thrombosis secondary to SARS-COV-2 infection. Despite the female predominance in the literature, our case was male. Again, in terms of thrombosis localization, contrary to the literature, superior sagittal sinus venous thrombosis was demonstrated in our case.

In patients with CVST secondary to SARS-COV-2 infections, headache, low-grade fever, and gastrointestinal symptoms are the most common initial symptoms. However, clinicians should be alerted for a wide range of symptoms such as seizures, signs of intracranial hypertension, decreased consciousness, altered sensorium, and typical stroke symptoms, all of which can occur within hours, days, or weeks without any specific time frame, complicating the already challenging diagnostic decision-making process for CVST even further[8,9]. Our patient had headache, a symptom that can be seen separately in both CVST and PTCS. However, tinnitus and diplopia, which are not typical for CVST, but classical symptoms for PTCS, were present in our patient.

Swelling of optic disc in the setting of SARS-CoV-2 infection was proposed to have many possible etiologies including ischemic optic neuropathy, papillophlebitis, optic neuritis, and retinal vein occlusion. The presence of normal visual acuity and color vision along with normal pupillary responses and the absence of associated retinal hemorrhages or venous tortuosity in our patient made us exclude these above-mentioned etiologies.

The etiology of neurologic symptoms in SARS-CoV-2 infection and multisystem inflammatory syndrome in children (MIS-C) have not been well described. Neurological symptoms in MIS-C are rarely seen compared to other organ system involvements. MIS-C was not considered in the differential diagnosis because clinical and laboratory parameters of our patient did not comply with the definition of MIS-C determined by Centers for Disease Control and Prevention/the World Health Organization[10].

The main goals of treatment for PTCS are to prevent vision loss and to relieve symptoms of elevated intracranial pressure. As an acceptable first-line therapy, carbonic anhydrase inhibitors provide effective management of PTCS by decreasing the production of CSF[11]. The treatment process of our patient was initiated with acetazolamide and maintained with topiramate due to the side effects of acetazolamide. In addition, as classical treatment for CSVT, low-molecular weight heparin was used. This combination was found to be well tolerated and beneficial without relevant side effects.

Although conduction of further studies is needed to establish a definitive cause-effect relationship, inflammation triggered by SARS-CoV-2 infection in association with the state of hyperviscosity and hypercoagulability may induce intracranial hypertension in some infected individuals.

Therefore, we suggest that in patients with confirmed SARS-COV-2 infection presenting with unexplained neurological symptoms including severe, persistent headache or papilledema, a high degree of suspicion for CSVT should be always kept in mind.

Ethics

Informed Consent: Informed consent was received from the family.

Peer-review: Externally peer reviewed.

Author Contributions

Concept: M.C.Y., Ç.G., Design: M.C.Y., Ç.G., G.S.U., Ö.Ö., S.H.K., E.Y., U.Y., Data Collection or Processing: M.C.Y., Ç.G., G.S.U., Ö.Ö., S.H.K., E.Y., U.Y., Analysis or Interpretation: M.C.Y., U.Y., Literature Search: M.C.Y., Ç.G., U.Y., Writing: M.C.Y.

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Anıl Er
Aslı Memişoğlu
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Aycan Ünalp
Aykut Çağlar
Ayşenur Celayir
Binali Fırıncı
Cem Paketçi
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