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Original Article

The Role of Systemic Immune-Inflammation Index and Systemic Inflammation Response Index in the Diagnosis of Bell's Palsy

Bell Palsi Tanısında Sistemik İmmün-İnflamasyon İndeksi ve Sistemik İnflamasyon Yanıt İndeksi'nin Rolü

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ABSTRACT

Objective: Recent studies showed that Systemic Immune-Inflammation Index (SII), Systemic Inflammation Response Index (SIRI) can be used as inflammatory markers. The aim of this study was to determine the prognostic value of SII and SIRI in children with Bell's palsy (BP) disease.

Method: This retrospective study included 107 children diagnosed with BP from 2019 to 2022 in institute and an age- and sex-matched 100 healthy control group. A complete blood count was performed for all participants and hemoglobin, erythrocytes, white blood cell (WBC), absolute neutrophil count (ANC), lymphocytes, and platelet counts were measured. The platelet-lymphocyte ratio, neutrophil-to-lymphocyte ratio (NLR), SII and SIRI values were calculated with the formula.

Results: The male-to-female ratio was 54/53 in patient and 42/58 in the control groups. In estimation of BP, area under the curve was 0.78 for WBC [95% confidence interval (CI): 0.72-0.84, p<0.001], 0.80 for ANC [(95% CI: 0.75-0.86), p<0.001], 0.59 for absolute lymphocyte count [(95% CI: 0.51-0.67), p=0.258], 0.70 for NLR [(95% CI: 0.62-0.77), p<0.001], 0.77 for SII [(95% CI: 0.71-0.84), p<0.001] and for SIRI 0.68 [(95% CI: 0.61-0.76), p=0.001]. When the markers were analyzed according to the most appropriate cut-off values in the prediction of BP, the best markers were determined as WBC, ANC, SII and SIRI (p<0.05).

Conclusion: BP has an inflammatory component. The SII and SIRI value can indicate an inflammatory condition in these patients. It may be used as an indicator marker in BP.

Keywords: Bell's palsy, facial paralysis, Systemic Immune-Inflammation Index, Systemic Inflammation Response Index

ÖZ

Amaç: Son çalışmalar, Sistemik İmmün-İnflamasyon İndeksi (SII), Sistemik İnflamasyon Yanıt İndeksi'nin (SIRI) inflamatuvar belirteçler olarak kullanılabileceğini göstermiştir. Bu çalışmanın amacı, Bell felci hastalığı olan çocuklarda SII ve SIRI'nin prognostik değerini belirlemekti.

Yöntem: Bu retrospektif çalışmaya, 2019-2022 yılları arasında pediatrik acil serviste Bell felci tanısı konulan 107 çocuk ve yaş ve cinsiyete göre eşleştirilmiş 100 sağlıklı kontrol grubu dahil edildi. Tüm katılımcılar için tam kan sayımı yapıldı ve hemoglobin, eritrositler, beyaz kan hücresi (WBC), mutlak nötrofil sayısı (ANC), lenfositler ve trombosit sayıları ölçüldü. Trombosit-lenfosit oranı, nötrofil-lenfosit oranı (NLR), SII ve SIRI değerleri formülle hesaplandı.

Bulgular: Erkek-kadın oranı hasta grubunda 54/53, kontrol grubunda ise 42/58 idi. Bell felcinin tahmininde, eğri altında kalan alan WBC için 0,78 [95% güven aralığı (GA): 0,72-0,84, p<0,001], ANC için 0,80 [(95% GA: 0,75-0,86), p<0,001], mutlak lenfosit sayısı için 0,59 [(95% GA: 0,51-0,67), p=0,258], NLR için 0,70 [(95% GA: 0,62-0,77), p<0,001], SII için 0,77 [(95% GA: 0,71-0,84), p<0,001] ve SIRI için 0,68 [(95% GA: 0,61-0,76), p=0,001] idi. Bell felcinin tahmininde en uygun kesme değerlerine göre belirteçler analiz edildiğinde en iyi belirteçler WBC, ANC, SII ve SIRI olarak belirlendi (p<0,05).

Sonuç: Bell felcinin inflamatuvar bir bileşeni vardır. SII ve SIRI değeri bu hastalarda inflamatuvar bir durumu gösterebilir. Bell felcinde bir gösterge belirteci olarak kullanılabilir.

Anahtar kelimeler: Bell felci, yüz felci, Sistemik İmmün-İnflamasyon İndeksi, Sistemik İnflamasyon Yanıtı İndeksi

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INTRODUCTION

Acute peripheral facial paralysis is usually a self-limiting benign disease characterized by paralysis or weakness of the muscles controlled by the facial nerve on one side of the face. Peripheral facial nerve palsy is an uncommon cause of emergency department (ED) access in childhood however, it is a worrying situation for both the clinicians, the child, and the parents. The most common cause is idiopathic which is called Bell's palsy (BP), responsible for 50% to 75% of all cases. Other etiologies include complicated upper respiratory tract infections, herpetic viral infections, and neuroborreliosis[1-4]. Inflammation affecting the facial nerve plays an important role in the pathogenesis of BP. Recent studies have shown that neutrophil-lymphocyte ratio (NLR), platelet-lymphocyte ratio (PLR) and mean platelet volume can be used as inflammatory markers in BP[1,5,6].

Systemic immune-inflammation index (SII) is a novel index calculated by multiplying the thrombocyte count with the NLR and it can be used as an indicator of the relationship between the immune system and the inflammatory situation of the patient. Firstly, it was introduced as a prognostic tool to identify the recurrence of hepatocellular carcinoma in 2014[7]. Recent studies demonstrated the prognostic effect of SII in infective endocarditis, coronavirus-19 disease, Guillain-Barré syndrome, and sinus venous thrombosis[8-11] Kınar et al.[12] demonstrated the prognostic value of SII in adult patients with BP in a recent study. The Systemic Inflammation Response Index (SIRI) is calculated with the formula as; neutrophil count x monocyte/lymphocyte count, which can better reflect the host immune and inflammation balance. SIRI was also reported as a prognostic tool in different malignancies[13]. However, the prognostic value of these indices in children with BP remains unclear.

The aim of this study was to determine the prognostic value of SII and SIRI in children with BP disease.

MATERIALS and METHODS

Study Design and Patient Selection

A retrospective study was conducted in institute after the approval of the University of Health Sciences Turkey, Ankara Dr. Sami Ulus Gynaecology and Paediatrics Trainig and Research Hospital Clinical Research Ethics Committee (approval number: E-22/04-319, date: 06.04.2022). The patients' registry system was scanned from January 1, 2019, to May 30, 2022, for the diagnosis of ICD G51.0 and the inclusion criteria were as follows; age between 1 month to 18 years, had complete blood count (CBC) analysis and treated for BP. A diagnosis of BP is made based on clinical presentation-acute facial nerve weakness or paralysis on one side of the face and by ruling out other possible causes of facial paralysis. Patients were excluded if they had congenital fascial paralysis, central fascial paralysis, otitis media, type 1 diabetes mellitus, congenital adrenal hyperplasia, pregnancy, persistent facial paralysis, stroke, cranial neuritis, and Guillain-Barré syndrome. Finally, there were 107 patients with BP included in this study Figure 1.

Age- and sex-matched control group was conducted from the patients that were admitted to the hospital for routine health control and had a routine blood analysis. There was 100 healthy volunteers (58 girls and 42 boys) in the control group.

Evaluation and Treatment

Patient records were scanned, and the age, gender, time from symptom onset to hospital arrival, time to symptom resolution, the House-Brackmann facial nerve grading system. The House-Brackmann Facial Nerve Grading System was first described at the Annual Meeting of the American Academy of Otolaryngology-Head and Neck Surgery in 1985 and is widely used to characterize the clinical assessment of facial paralysis. This scale ranges from grade I to grade VI, with 1 defining normal function and grade VI representing complete paralysis[14].

The dose and the duration of steroid treatment, and the initial CBC results to calculate the SIRI and SII were recorded. The disease recovery was determined from the face-to-face examination records of patients at the control admittance to pediatric neurology, ear-

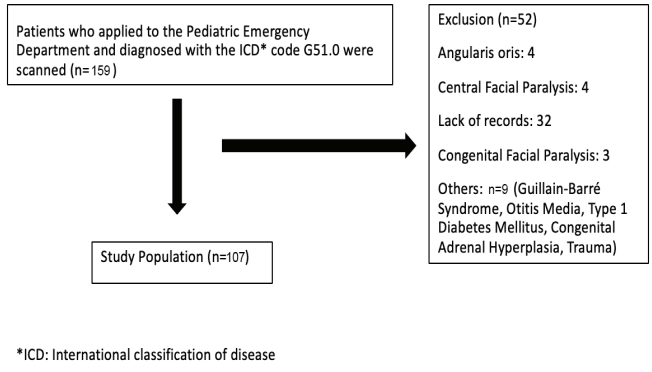

Figure 1. Flow chart of study and inclusion-exclusion criterias

nose-throat, pediatrics, or pediatric EDs. Patients whose symptoms were resolved within the first month were classified as recovery group. We recorded the age, gender, and CBC results of the children in the control group.

Complete blood cell counts were obtained by automatic analysis Advia 2120i (Siemens Healthcare Diagnostics, Eschborn, Germany). The white blood cell (WBC) count, absolute lymphocyte count (ALC), absolute neutrophil count (ANC), NLR, PLR, SII, and SIRI were calculated and recorded.

Statistical Analysis

Statistical analysis of the data obtained in the study was made in IBM SPSS for Windows Version 20.0 package program. Descriptive statistics [percentage, mean, median, standard deviation (SD), range of quarters (IQR)] were used to define the population included in the study. In the comparison of patients and control group, Chi-square or Fisher's exact test and t-test were used for categorical and continuous variables, respectively, and the Mann-Whitney U test was used for variables not suitable for normal distribution. Receiver operating characteristic (ROC) analysis was performed, ROC curves were plotted, and the Youden Index method was used to determine the optimal WBC, ALC, ANC, NLR, SII, and SIRI percent cut-off values to predict BP. The area under the curve (AUC) was calculated to predict BP and compare the performance of each marker. Sensitivity, specificity, positive predictive values (PPV), and negative predictive values (NPV) were calculated for the markers. For all analyses, $p<0.05$ was considered statistically significant.

RESULTS

This study included 107 patients with BP and 100 age- and sex-matched healthy controls. The mean age was 145 ± SD months (2-234 min., max.) in the patient group and 144 ± SD months (54-182 min., max.) in the control group. The male-to-female ratio was 54/53 in the patient group and 42/58 in the control group. The patient and the control groups had similar distributions concerning age and sex (p=0.892). The characteristic features and CBC results of the patients and controls are summarized in Table 1.

The median duration of symptoms in patients with BP was 24 hours (1-192 min., max.). On physical examination, none of the patients had any findings other than facial paralysis. Thirty patients (3 cranial computed tomography and 27 magnetic resonance imagining) underwent further radiological imagining to rule out other causes of fascial paralysis which were all reported as normal. The mean House-Brackmann grade was 3±1 in patients with BP. Of the 107 patients, 91 were treated with 1.02±0.69 mg/kg oral corticosteroids (methylprednisolone). The corticosteroid treatment was tapered and stopped in all patients for mean 8.9±5 days, and no drug-related adverse event was observed.

In estimation of BP, AUC was 0.78 for WBC [95% confidence interval (CI): 0.72-0,84, p<0.001], 0.80 for ANC [(95% CI: 0.75-0,86), p<0.001], 0.59 for ALC [(95% CI: 0.51-0,67), p=0.258], 0.70 for NLR [(95% CI: 0.62-0,77), p<0.001] , 0.77 for SII [(95% CI: 0.71-0,84), p<0.001] and for SIRI 0,68 [(95% CI: 0.61-0,76), p=0.001]. Sensitivity, specificity, PPV, NPV, AUC, and odds ratio (OR) values for each marker are given in Table 2. When the markers were analyzed according to the most appropriate cut-

Table 1. WBC, platelet, ANC, ALC, MPV, NLP, PLR, SII ve SIRI values of participants

	Bell's palsy patients		Control group		p-value
	Median	(Min.-max.)	Median	(Min.-max.)	
WBC (/mm³)	8290	3930-18770	6365	3580-11130	<0.001
Platelet (x10³ cells/mm³)	339	155-683	295	246-552	<0.001
ANC (/mm³)	4450	1670-15090	2895	1430-5670	<0.001
ALC (/mm³)	2630	1590-5950	2495	1450-4590	0.018
MPV(/fl)	8.4	6.1-11.4	10.15	7.2-12.6	<0.001
NLR	1.53	0.62-6.67	1.20	0.51-1.97	<0.001
PLR	120	52.7-558	116.09	40-235.17	0.236
SII (x10⁹/L)	506	140-3338	353	151-793	<0.001
SIRI (x10⁶/L)	933.8	200-517980	585.2	194-1359	<0.001

Mann-Whitney U test, WBC: White blood cell, ANC: Absolute neutrophil count, ALC: Absolute lymphocyte count, MPV: Mean platelet volume, NLR: Neutrophil-to-lymphocyte ratio, PLR: Platelet-to-lymphocyte, SII: Systemic Immune-Inflammation Index, SIRI: Systemic Inflammatory Response Index, Min.-max.: Minimum- maximum

Table 2. Efficiency of inflammation markers in predicting Bell's palsy							
	AUC (%95)	Cut-off	OR (95%, CI)	Sensivity	Specifity	PPV	NPV
WBC	0.78	7155	7.07 (3.8-13)	73.3	72	73.3	72
ALC	0.60	2670	1.4 (0.8-2.4)	46.7	62	56.3	52.5
ANC	0.81	3495	9.1 (4.8-17.1)	75.2	75	76	74.3
NLR	0.70	1.295	3.4 (1.9-6)	65.7	64	65.7	64
SII	0.78	408	2.6 (1.5-4.6)	73.3	73	74	72.3
SIRI	0.68	646	7.4 (4-13.7)	61.9	62	63.1	60.8

p-value <0.05 statistically significant. WBC: White blood cell, ANC: Absolute neutrophil count, ALC: Absolute lymphocyte count, NLR: Neutrophil-to-lymphocyte ratio, SII: Systemic Immune-Inflammation Index, SIRI: Systemic Inflammatory Response Index, AUC: Area under the curve, OR: Odds ratio, CI: Confidence interval, PPV: Positive predictive value, NPV: Negative predictive value

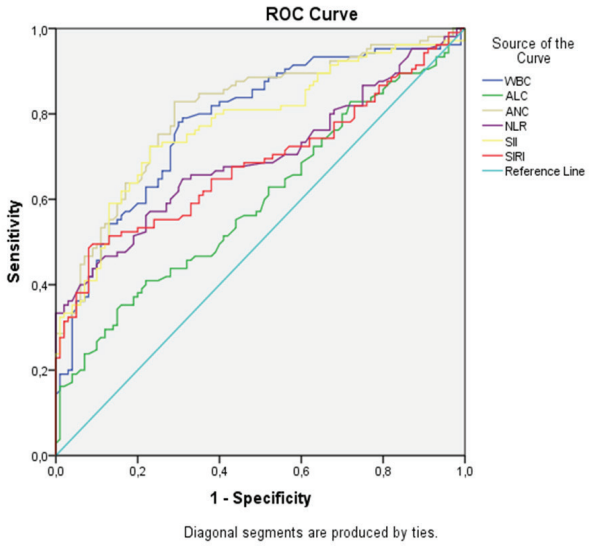

Figure 2. ROC curves of inflammation markers to predict BP

ROC: Receiver operating characteristic, BP: Bell's palsy, WBC: White blood cell, ANC: Absolute neutrophil count, ALC: Absolute lymphocyte count, NLR: Neutrophil-to-lymphocyte ratio, SII: Systemic Immune-Inflammation Index, SIRI: Systemic Inflammatory Response Index

off values in the prediction of BP, the best markers were determined as ANC, SII, and WBC. The ROC curves of the markers for the prediction of BP are shown in Figure 2.

The resolution of the symptoms took less than 1 month in 94 patients (recovery group) while the symptoms persisted in 13 patients after one month (non-recovery group). The mean House-Brackmann grade of these patients in the non-recovery group after one month was 2. There was no difference between the onset of the disease process, the initial House-Brackmann Facial Paralysis Scale, the onset of the treatment period, WBC, PLT, ANC, ALC, NLR, PLR, SII, and SIRI parameters between the recovery and non-recovery patients.

DISCUSSION

This was the first study to evaluate the prognostic value of SII and SIRI in children with BP. This study demonstrated higher SII and SIRI in 107 children with BP compared to the healthy controls. SII is based on platelet counts and NLR (SII is defined as neutrophils x platelets/lymphocytes) and it's a novel inflammation biomarker and can comprehensively reflect the body's inflammatory and immune status[15]. Furthermore, SIRI, which combines the absolute values of neutrophils, monocytes, and lymphocytes (calculated by neutrophil count x monocyte count/lymphocyte count), is a novel inflammatory index and has been widely considered in disease diagnosis and prognosis evaluation in recent years[16]. Based on these findings, we investigated SII and SIRI, a marker that has not yet been studied in children with BP. The cut-off values of SII and SIRI were 408 and 646 respectively and this study demonstrated the prognostic value of SII and SIRI in the pediatric population. Similarly, Kınar et al.[12] reported the prognostic value of SII in adult patients. Compared with SII, the OR ratio of SIRI was higher, and the SIRI is a parameter that is easy to calculate, it can help to identify the patient quickly and make better medical decisions in clinical practice.

Facial nerve palsy is a common disease in children due to congenital or acquired (infectious, neoplastic, traumatic, or idiopathic)[17]. It can affect anyone, regardless of any age and gender. The incidence of peripheral facial nerve palsy in children varies from 5 to 21 in 100,000 annually[18]. The main physical findings of fascial nerve palsy are the inability to fully close the mouth and eye and nasolabial fold on the effected side and causing difficulties in eating and speaking corneal drying and erosion[19]. The diagnosis of BP can made by

physical examination and exclusion of other reasons for facial palsy. In this study, 30 patients needed further imaging to rule out other etiological causes of the disease. The symptoms peak in the first week and then gradually resolve over 3 weeks to 3 months[20]. The first treatment option for pediatric BP is the administration of corticosteroids and for good recovery of facial function, investigators have recommended that physicians initiate steroid therapy within 3 days of symptom onset[21]. Oral prednisolone (1 mg/kg/d) was reported to be highly effective in the treatment of BP in children[22]. The patients in this study were also treated with a similar dose and duration of corticosteroid therapy.

The causes of BP remain unclear, although viral infection, microvascular circulatory impairment, and genetic and immunological diseases have been proposed as possible factors[23]. BP is a diagnosis of exclusion and the initial test required for this is CBC[18]. A CBC provides useful information for evaluating a patient's general state and inflammation status. In this study, the NLR, PLR, SII, and SIRI can be easily calculated from patients' CBC data. The NLR is regarded as a marker of systemic inflammation. It reflects inflammatory status and provides important information about prognosis[24]. Eryilmaz et al.[15] reported that NLR values were higher in children with BP than in the control group and, thus, suggested that NLR is a supportive parameter in the diagnosis of pediatric BP[25]. Similarly, for the prediction of BP, WBC and ANC had the highest AUC and OR in this study. The neutrophil count is the main inflammatory marker, and our study shows a stronger association between increased neutrophils and BP.

The PLR has been used to gauge the risk for microcirculatory thromboembolism. In BP, one group of investigators reported a correlation between high PLR and BP in children[15,26]. Although the platelet count and the PLR was higher in the patients than in the control group, there were no significant difference in this study. Yılmaz et al.[27] reported elevated serum cytokine levels including interleukin-1 (IL-1), IL-6, and tumor necrosis factor-alpha in patients with BP compared to the control group. Accordingly, the authors concluded that BP may more likely be an inflammatory situation than an ischemic disease.

Study Limitations

Limitations of this study include its retrospective design and the small number of patients with BP. Thus, further, multicenter, prospective studies are warranted. The absence of the hearing test due to the conditions of the hospital may be listed as a limitation of this study.

CONCLUSION

In conclusion, pretreatment hematological findings in children with BP provide useful information. Thus, as new and quicker markers with low cost, both the SII and SIRI can be used as diagnostic indicators of BP. More studies are needed to understand the role of SII and SIRI in BP.

Ethics

Ethics Committee Approval: A retrospective study was conducted in institute after the approval of the University of Health Sciences Turkey, Ankara Dr. Sami Ulus Gynaecology and Paediatrics Trainig and Research Hospital Clinical Research Ethics Committee (approval number: E-22/04-319, date: 06.04.2022).

Informed Consent: Retrospective study.

Footnotes

Author Contributions

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

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

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Original Article

Epidemiological and Survival Characteristics of Childhood Lymphomas and Solid Organ Tumors Treated at Our Center

Merkezimizde Tedavi Edilen Çocukluk Çağı Lenfomaları ve Solid Organ Tümörlerinde Epidemiyoloji ve Sağkalım Özellikleri

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ABSTRACT

Objective: Childhood cancers constitute 2% of all cancers seen in the world. Overall 5-year survival rate for childhood cancers is 80% in developed, but it is only 30% in underdeveloped countries. In our study have we aimed to analyze the childhood cancer cases treated in our hospital, and to compare the epidemiological characteristics of the patients followed in our center, the distribution of cancer types in our center and the overall survival rates, with the data obtained from our national, and international literature.

Method: The records of patients aged 0-19 years with lymphomas and malignant solid tumors, who were followed up and treated between December 25,1987 and January 28, 2021 in our department of pediatric oncology were examined. Hospital files of 1326 patient were reviewed retrospectively, and 1175 patients were included in the study. The data were analyzed in SPSS 25.0 package program.

Results: In our study, the male/female ratio was 1.2 among 1175 patients diagnosed with lymphoma and malignant solid organ tumor with a mean age of 7.75 years at the time of diagnosis. Considering the subgroup distribution of childhood cancers other than leukemias; central nervous system tumors (23.6%), lymphomas (19.5%) and neuroblastomas (12%) were found to be the most common malignant diseases. The mean follow-up time of our patients was 62.31±55.3 months, and the mean event-free follow-up period was 50.20±49.681 months. Five- and 10-year overall survival, and event-free survival rates were 74% vs. 68.9%, and 50.5%, vs. 39%, respectively.

Conclusion: In general, survival rates in childhood cancers in our center are close to the average of our region, our country and European countries, but it was found to be lower in some subgroups of our patients compared to developed countries.

Keywords: Childhood cancers, epidemiology, overall survival rate, event-free survival rate

ÖZ

Amaç: Çocukluk çağı kanserleri, dünyada görülen tüm kanserlerin %2'sini oluşturmaktadır. Gelişmiş ülkelerde çocukluk çağı kanserlerinde genel beş yıllık sağkalım oranı %80 iken, az gelişmiş ülkelerde bu oran %30'dur. Bu çalışmanın amacı, merkezimizde takip edilen hastaların epidemiyolojik özelliklerini, kanser türlerinin merkezimizdeki dağılımını ve genel sağkalım oranlarını, dünyadan ve ülkemizden elde edilen verilerle karşılaştırmak ve hastanemizdeki çocukluk çağı kanser vakalarını analiz etmektir.

Yöntem: 25.12.1987 ve 28.01.2021 tarihleri arasında hastanemiz çocuk onkoloji kliniğinde takip ve tedavi edilen, lenfoma ve malign solid tümörleri olan 0-19 yaş arasındaki hastaların kayıtları incelendi. Toplamda 1326 hasta kaydı retrospektif olarak gözden geçirildi. Çalışmaya 1175 hasta dahil edildi. Veriler SPSS 25.0 paket programında analiz edildi.

Bulgular: Çalışmamızda, lenfoma ve malign solid organ tümörü tanısı konulan 1175 hastanın epidemiyolojik cinsiyet dağılımı erkek/kadın: 1,2, tanı anındaki ortalama yaş ise 7,75 yıl olarak bulundu. Lösemiler dışındaki çocukluk çağı kanserlerinin alt grup dağılımları dikkate alındığında, merkezi sinir sistemi tümörleri (%23,6), lenfomalar (%19,5) ve nöroblastom (%12) en yaygın görülen malign hastalıklar olarak tespit edildi. Hastalarımızın ortalama takip süresi 62,31±55,3 ay, ortalama olaysız takip süresi ise 50,20±49,681 ay idi. Beş yıllık genel sağkalım %74 ve 10 yıllık genel sağkalım %68,9; beş yıllık olaysız sağkalım oranı (EFS) %50,5 ve 10 yıllık EFS %39 olarak bulundu.

Sonuç: Genel olarak, merkezimizdeki çocukluk çağı kanserlerinde sağkalım oranları, bölgemiz, ülkemiz ve Avrupa ülkeleri ortalamasına yakın olmakla birlikte, gelişmiş ülkelerle kıyaslandığında bazı alt gruplarda daha düşük bulunmuştur. Daha yüksek yaşam hızları için multidisipliner bir yaklaşıma ihtiyaç duyulmaktadır.

Anahtar kelimeler: Çocukluk çağı kanserleri, epidemiyoloji, genel sağkalım oranı, olaysız sağkalım oranı

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INTRODUCTION

Incidence, mortality, and survival rates of childhood cancers vary by country due to factors related to the local culture, environment, and socioeconomic status of the patients. Major disparities stem from unequal distribution of resourcen between and within countries. Individuals with higher socioeconomic status typically have better health literacy, greater financial resources, and easier access to healthcare services. Consequently, they are more likely to benefit from cancer prevention services, receive earlier diagnoses, and access higher-quality treatment than those with lower socioeconomic status[1].

Childhood cancers account for approximately 2% of all cancers diagnosed worldwide. Cancer remains one of the leading causes of death among children worldwide, with observed incidence rates generally rising over time. There are limited comparative data in the national and international literature on the incidence of childhood cancers diagnosed within the last 20 years[2]. According to 2019 data from the United States, cancer is the leading cause of death among children and ranks among the top four causes of mortality in developing countries like ours[3]. While the incidence of cancer and cancer-related deaths among children is increasing globally, 5-year overall survival (OS) rates have risen up to 80% in developed countries thanks to advances and innovations in its diagnosis and treatment, compared to survival rate of only 30% in developing countries. In the United States, the overall 5-year survival rate for all childhood cancers was reported to be 84.1% in 2015[4].

In the light of these data, the World Health Organization now emphasizes that childhood cancers have become a significant public health issue in the developing world. In our country and other developing nations, understanding the distribution and prevalence of cancer, as well as determining OS rates, is crucial for improving survival rates and living standards of people. Achieving this target is only possible through the regular maintenance of cancer registries and consistent reporting.

The aim of this study is to evaluate the epidemiological characteristics, distribution of cancer types, and OS rates of patients diagnosed with lymphoma and childhood solid organ tumors, treated at our pediatric oncology clinic, between December 25, 1987, and January 28, 2021. We aim to compare these findings with data obtained from both global and national sources, thereby assessing the status of pediatric cancer cases in our hospital.

MATERIALS and METHODS

This research was conducted after obtaining ethical approval from the Clinical and Laboratory Research Ethics Committee of the Dokuz Eylül University (approval number: 2021/24-13, dated: 25.08.2021). The epidemiological data, diagnostic methods used, treatments provided, and OS status of all patients aged 0-19 years who were followed up and treated for lymphoma and malignant solid organ tumors in our pediatric oncology clinic, between December 25, 1987, and January 28, 2021, were retrospectively recorded.

Hospital records of a total of 1,326 patients were retrospectively reviewed. Patients with missing data (n=130) and those who applied to our clinic only once or for consultation purposes (n=21) were excluded from the analysis. The remaining 1,175 patients were included in the study. The study population consisted of 54 patients who applied to our center after they experienced relapse while being treated at another center, 383 patients who were diagnosed at another centers, and referred to our department for follow-up and treatment, and the remaining 738 patients applied firstly to our center, received their histopathological diagnosis, and then treated at our center. The last date of patient follow-up was set as December 31, 2021.

Event-free survival (EFS) and OS curves for the patients were also drawn. The OS of the patients was calculated by subtracting the date of diagnosis from the last follow-up date, while EFS was estimated by subtracting the date of diagnosis from the date of the first event experienced. Patients who were lost to follow-up or unreachable were classified as "lost to follow-up", those who transferred to another center for subsequent follow-up were labeled as "transferred to another center", and cases refractory to treatment or presence of progressive disease, recurrence, death, or secondary cancer development were defined as "events".Cancer types were classified according to the International Classification of Childhood Cancer (ICCC-3, 2005)[5].

Statistical Analysis

The data were analyzed using the SPSS 25.0 software package program. Categorical variables were analyzed using the chi-square test, while continuous variables were analyzed using the t-test. Correlation analyses were conducted with multivariate logistic regression test. Survival analyses were performed using the chi-square test and Kaplan-Meier analysis, and survival curves were compared using the log-rank test. A p-value of less than 0.05 was considered statistically significant.

RESULTS

In this study, hospital files of a total of 1,175 cases including 536 (45.6%) female, and 639 (54.4%) male (Male/Female:1.2:1) patients who were followed up in our pediatric oncology clinic, between December 25, 1987, and January 28, 2021, were reviewed. The mean age of our patients at the time of diagnosis was 7.75 years. Our study population consisted of patients (n=738:63%) who received their histopathological diagnosis at our center or at other centers and referred to our department (n=383, 32%). While 54 (5%) patients were under follow-up at other centers before presenting to us with relapsed disease. Metastasis was detected in 246 (20.9%) patients, and the site of metastasis was identified in 237 of these patients. Specifically, metastatic lesions were observed in the skeleton in 6.9% (n=81), in the lung in 6.6% (n=77), in the bone marrow in 3.2% (n=37), in the liver in 2% (n=23), in the central nervous system in 0.6% (n=7), and in distant lymph nodes in 0.4% (n=5) of the patients.

The mean duration of follow-up for the patients included in this study was 62.31±55.327 months, while the mean duration of event-free follow-up was 50.20±49.681 months. A pathological event occurred in 50.9% of the patients. Considering the final medical outcome of the study population, patients had died (n=300; 25.5%), were lost to follow-up (n=193; 16.4%) livied with (n=68: 5.8%) and without the disease (n=565; 48.1%), and had been transferred to another center (n=49: 4.2%). The most common cause of mortality was cardiopulmonary arrest due to disease progression.

The information regarding the types of cancer diagnosed in our patients was evaluated, and the results are presented in Table 1.

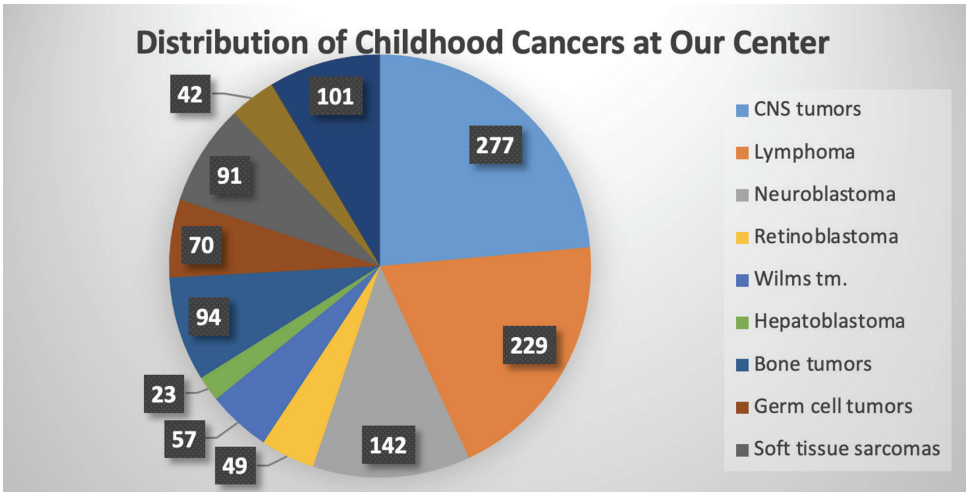
The five-year and 10-year OS rates for patients diagnosed with lymphoma and solid organ tumors followed at our center were found to be 74%, and 68.9%, respectively (Figure 1). The five-year, and 10-year EFS rates for our patients were 50.5%, and 39%, respectively (Figure 2).

We investigated the average follow-up duration, five-year OS, and five-year EFS rates according to tumor subgroups in our patients. In patients diagnosed with central nervous system tumors, the five-year OS rate was found to be 65%, while specific 5-year OS rates for astrocytomas (72%), medulloblastomas (64%) and ependymomas (51%) were also determined. The respective average follow-up times, 5-year OS, and EFS rates for patients diagnosed with Hodgkin lymphoma (86 mos, 90%, and 76.6%). non-Hodgkin lymphoma (63 mos, 75%, and 52%), neuroblastoma (60 mos, 70%, and 48%), hepatoblastoma (68 mos, 75%, and 70%), osteosarcoma (44.8 mos, 56.9%, and 41.9%), Ewing sarcoma (54.7 mos, 60.7%, and 37.8%), rhabdomyosarcoma (65.4 mos, 57.8%, and 42%), and Wilms tumor (65.9 mos, 77%, and 54%) were as indicated (Figures 3-5).

DISCUSSION

The average age of 1,175 patients diagnosed with malignant childhood cancers who presented to our hospital was found to be 7.75 years, with a male-to-female (M/F) ratio of 1.19. This distribution was found to be similar to the data collected from across Turkey by the Turkish Pediatric Oncology Group (TPOG) between 2009 and 2021. In the TPOG 2009-2021 report, the median age of the patients was reported as 6.7 years, with a M/F ratio of 1.27[6]. According to an alliance of

Table 1. Distribution of non-leukemic pediatric tumor subgroups followed at our center, 1987-2021



non-governmental and public health organisations with member organisations across European countries (EUROCARE) and the Automated Childhood Cancer Information System data from 2010 in Europe, the median age of patients was reported as 5.8 years, with a M/F ratio of 1.2[3]. It was determined that the epidemiological analysis of our patients in terms of age and gender is consistent with the literature data.

In the study conducted in our center, excluding leukemias, the most common childhood cancers in decreasing frequency were found to be central nervous system tumors (23.6%), lymphomas (19.5%), and neuroblastoma (12%), respectively.

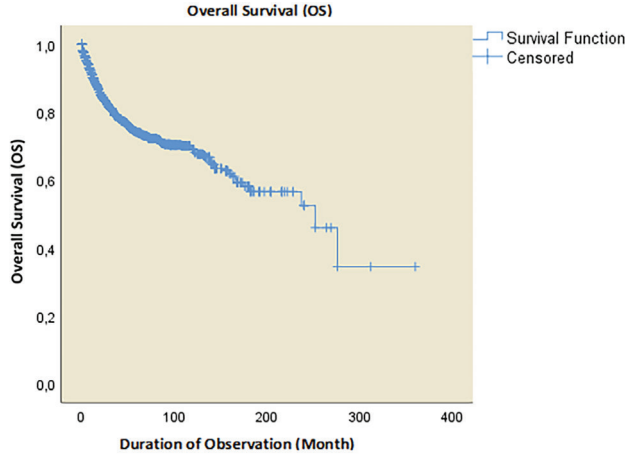


Figure 1. OS rates in childhood cancers
OS: Overall survival

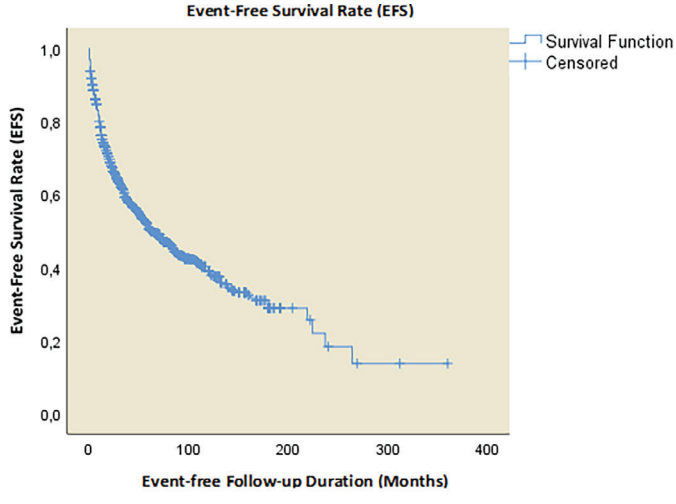


Figure 2. EFS rates in childhood cancers
EFS: Event-free survival

According to the 2021 TPOG report, the most common subgroups of childhood cancers in our country were lymphomas (18.8%), central nervous system tumors (15.0%), and neuroblastoma (8.2%)[6]. In contrast, at our center, the most common cancers are central nervous system tumors (23.6%), followed by lymphomas (19.5%) and neuroblastoma (12%). This discrepancy may be attributed to our center's status as a referral center for Neurosurgery and Radiation Oncology, particularly in our region.

Additionally, the incidence of neuroblastoma at our center (12%) was found to be higher than that reported in both national and European literatüre (7-8%). This

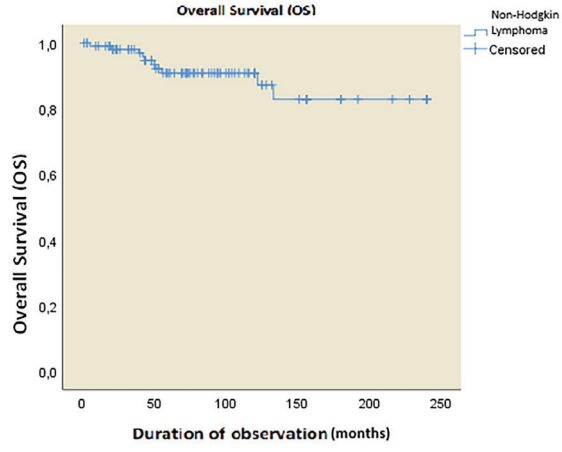


Figure 3. OS rates in Hodgkin lymphoma patients
OS: Overall survival

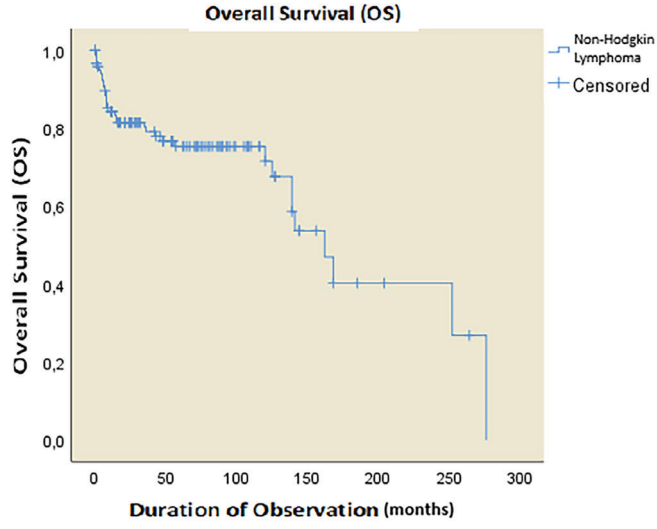


Figure 4. OS rates in non-Hodgkin lymphoma patients
OS: Overall survival

difference in incidence rates may be related to our center becoming a referral center for neuroblastoma cases coming from the Aegean region and even across Turkey, particularly following the implementation of the TPOG-NB-2003 protocol and the initiation of molecular and cytogenetic studies at our center. While the incidence of retinoblastoma and bone tumors was comparatively higher at our center, the incidence rates of kidney tumors, liver tumors, soft tissue sarcomas, and germ cell tumors were comparable to those reported in the literature.

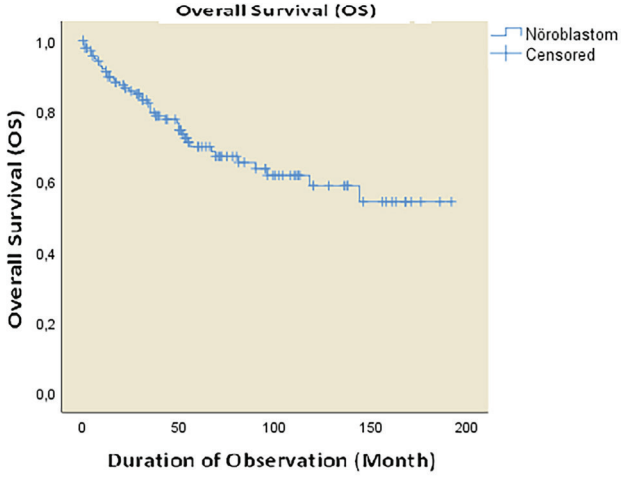


Figure 5. OS rates in Neuroblastoma patients
OS: Overall survival

The 5-, and 10-year OS rates for patients diagnosed with lymphoma and solid organ tumors followed at our center were 74%, and 68.9%, respectively. Whereas, 5-, and 10-year EFS rates for these patients were 50.5%, and 39%, respectively. In our study, 20% of the patients had metastasis at the time of diagnosis. The five-year OS rate for patients with metastatic disease at diagnosis was 52.3%, compared to 79.8% for those without. Statistically, a significant difference in survival rates was observed based on the presence of metastasis at diagnosis. This result is a critical indicator of the importance of early diagnosis and treatment in improving survival rates.

In a study conducted by Ege University Medical Faculty Hospital, which analyzed childhood cancers between 1992 and 2017, 5-year OS rate was found to be 74%[7]. According to data from the Oncology Institute of İstanbul University, 5-year OS rate for childhood cancers treated between 1990 and 2012 was reported to be 74.4%[8]. According to TPOG data, 5-year OS rate for childhood cancers increased from 65% between 2002 and 2008 to 72% between 2009 and 2020[6,9]. According to the data from the Middle East Cancer Consortium whose member countries are Jordan, Iraq, Egypt, Israel, Lebanon, Palestine, Pakistan, and Turkey, leukemias are the most common childhood cancers, followed by central nervous system tumors and lymphomas. The five-year OS rates were reported to be the lowest in Morocco at 30%, followed by at an ever-increasing rate by Pakistan (55%), Egypt (40-60%), Iraq (62%), Jordan,

Table 2. Five-year OS rates by tumor subgroups

Five-year OS rates							
	Our pediatric oncology clinic (%)	**EUMF* pediatric oncology (7) (%)**	**İstanbul Oncology Institute (8) (%)**	**TPOG-TPHS** (9) (%)**	**ACCIS (12) (%)**	**EUROCARE-5 (3) (%)**	**SEER (11) (%)**
All patients	74	74	74.4	72	72	78	83
Hodgkin lymphoma	91	95	98	78	93	-	97
Non-Hodgkin lymphoma	75	83.5*	92		79	-	85
CNS tumors	65	67	63	47	76		75
Neuroblastoma	70	60	70	55.6	59	70	79
Osteosarcoma	56	57	-	45		64	71
Ewing sarcoma	61	54	-			66	72
Rabdomyosarcoma	58	62	70	52	65	68	64
Wilms tumor	77	76	92	74	84	90	90
Liver tumors	75		48	53	-	-	74

*EUMF: Ege University Faculty of Medicine, **TPOG: Turkish Pediatric Oncology Group, CNS: Central nervous system, TPHS: Turkish Pediatric Hematology Society, ACCIS: The Automated Childhood Cancer Information System, SEER: The Surveillance, Epidemiology, and End Results, EUROCARE: An alliance of non-governmental and public health organisations with member organisations across European countries, OS: Overall survival

Lebanon (75%), and Israel (84%)[10]. Between 1999 and 2007, the EUROCARE-5 study analyzed approximately 58,000 pediatric cancer patients from 29 countries, finding a 5-year OS rate of 77.9%[3]. According to data from the United States, the National Cancer Institute's Surveillance, Epidemiology, and End Results program, 5-year OS rate for pediatric cancers increased from 63% between 1975 and 1979 to 83% between 2003 and 2009[11]. According to these data, although the survival rates for childhood cancers at our center are comparable to the averages in our region, country, and European countries, they are lower than those reported in developed countries like the United States.

When compared to the literature data, 5-year OS rates of central nervous system tumors in our region were found to be consistent with local data. Although these rates were higher than those reported by TPOG, they remained at lower levels when compared to developed countries. The five-year survival rates for Hodgkin lymphoma and non-Hodgkin lymphoma at our center were found to be lower than those reported in other studies conducted in our country and in developed countries. The survival rates for neuroblastoma, hepatoblastoma, and Wilms tumor were found to be consistent with those reported in studies from both developed countries and our country. For the subgroups of osteosarcoma, Ewing sarcoma, and rhabdomyosarcoma, the survival rates of patients treated at our center were comparable to those indicated in our national registry, but lower compared to data reported from developed countries. Five-year OS rates of institutions by tumor subgroups are presented in Table 2.

CONCLUSION

With the exception of certain tumor groups, the survival rates of the patients treated at our center were found to be at comparable levels with those reported in national and international literature. These results are not limited to a single department; rather, they represent a collective outcome of a multidisciplinary team, including the intensive care units involved in the diagnosis, follow-up, and treatment of pediatric oncology patients at our hospital, as well as all branches of pediatric health and diseases, pediatric surgery, radiology, pathology, radiation oncology, nuclear medicine, laboratory services, nursing services, child and adolescent mental health, physical therapy and rehabilitation, social services, and management units. Multidisciplinary approaches to childhood cancers, adherence to specific treatment protocols, and good supportive care are crucial factors for achieving therapeutic success. In order to attain higher survival rates, there is a need for targeted antineoplastic agents developed alongside advancing technology, new treatment protocols, and effective supportive care within a multidisciplinary approach[13].

Ethics

Ethics Committee Approval: This research was conducted after obtaining ethical approval from the Clinical and Laboratory Research Ethics Committee of the Dokuz Eylül University (approval number: 2021/24-13, dated: 25.08.2021).

Informed Consent: Retrospective study.

Footnotes

Author Contributions

Surgical and Medical Practices: E.Ç., Concept: N.O., Design: N.O., Data Collection or Processing: B.A, Analysis or Interpretation: D.İ., Literature Search: B.A., D.K., Writing: B.A.

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

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Original Article

Does Exposure to General Anesthesia Have Worsening Effects on ADHD Treatment Efficiency?

Genel Anesteziye Maruziyetin DEHB Tedavi Etkinliği Üzerinde Olumsuz Etkileri Var mıdır?

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ABSTRACT

Objective: This study aimed to examine whether exposure to general anesthesia (GA) has impairing effects on the pharmacological treatment efficiency in Attention-Deficit/Hyperactivity Disorder (ADHD), and to compare symptoms of inattention (IN), hyperactivity/impulsivity (HI), Oppositional Defiant Disorder (ODD) and Conduct Disorder (CD) between those exposed, and non-exposed to GA.

Method: A total of 106 children with ADHD, aged 7 to 12 years who received pharmacological treatment with methylphenidate or atomoxetine for ADHD and followed up for 3 months were included in the study. An appropriate and standardized dose titration process was applied to all cases. Parents completed Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition, Disruptive Behavior Disorders Rating Scale questionnaire items at the beginning and at the end of the follow-up period. Information about the children's exposure to GA, frequency of exposures, and age they received GA was obtained from their parents.

Results: Both at the beginning and at the end of the follow-up period, exposure to GA, the age at the onset of exposure and the number of exposures were detected to have no significant effect on the decreases in any dimensional symptom counts (IN, HI, ODD and CD) (all $p>0.05$). However, the symptom counts of HI were found to be significantly higher in children with a history of exposure to GA, those with multiple exposures to GA and younger than 3 years of age than patients not exposed to GA (all $p<0.006$).

Conclusion: Although exposure to GA is associated with ADHD, neither exposure to GA itself, exposures at earlier ages and multiple exposures do not seem to weaken the response to pharmacological treatment of ADHD. However, particularly symptoms of HI may be more vulnerable to adverse effects of GA and related factors. These preliminary findings need to be confirmed by future studies.

Keywords: Exposure to general anesthesia, ADHD, treatment efficiency, children, environmental factors

ÖZ

Amaç: Bu çalışmanın amacı, genel anestezi (GA) maruziyetinin Dikkat Eksikliği Hiperaktivite Bozukluğu (DEHB) farmakolojik tedavisi etkinliği üzerinde olumsuz etkilerinin olup olmadığını incelemek ve GA maruziyeti olan ve olmayan olgular arasında Dikkat Eksikliği (DE), Hiperaktivite/Impulsivite (HI), Karşıt Olma Karşı Gelme Bozukluğu (KOKGB) ve Davranış Bozukluğu (DB) semptomlarını karşılaştırmaktır.

Yöntem: Yedi ila 12 yaşları arasındaki 106 DEHB'li çocuk DEHB tedavisi (metilfenidat veya atomoksetin) ile tedavi edilmiş ve 3 ay boyunca takip edilmiştir. Tüm olgulara uygun ve standart bir doz titrasyonu uygulanmıştır. Hem takibin başında hem de sonunda ebeveynler Ruhsal Bozuklukların Tanısal ve İstatistiksel El Kitabı, Dördüncü Baskı, Yıkıcı Davranış Bozuklukları Derecelendirme Ölçeği'ni doldurmuştur. Olguların GA alma durumu, kaç kez ve hangi yaşta GA aldıkları hakkında bilgiler ebeveynlerden alınmıştır.

Bulgular: İki dönem arasında, GA maruziyeti durumu, GA maruziyeti yaşı ve GA maruziyeti sayısının herhangi bir alt ölçek semptom sayısındaki (DE, HI, KOKGB, DB) azalmalar üzerinde anlamlı bir etkisi olmadığı tespit edilmiştir (tüm $p>0,05$). Ancak, HI semptom sayısının, GA'ya birden fazla maruz kalan ve üç yaşın altında GA'ya maruz çocuklarda, GA'ya maruz kalmayanlara göre anlamlı derecede daha yüksek olduğu saptanmıştır (tüm $p<0,006$).

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Sonuç: GA maruziyetinin DEHB ile ilişkisi olmasına rağmen, GA'ya maruz kalmanın kendisi, erken yaşlarda maruz kalma ve de birden fazla kez maruz kalma DEHB farmakolojik tedavi yanıtını zayıflatıyor gibi görünmemektedir. Ancak, özellikle HI semptomları GA'ya ve ilişkili faktörlere karşı daha duyarlı olabilir. Bu ön bulgular gelecekteki çalışmalarla mutlaka tekrarlanmalı ve doğrulanmalıdır.

Anahtar kelimeler: Genel anestezi maruziyeti, DEHB, tedavi etkinliği, çocuklar, çevresel faktörler

INTRODUCTION

Attention-Deficit/Hyperactivity Disorder (ADHD) is a childhood-onset neurodevelopmental disorder with the symptoms of Inattention (IN), hyperactivity/impulsivity (HI),[1]. The etiology of ADHD has always been an interesting field of research. Although ADHD has a high level of heritability and multiple genes play a substantial role on its pathogenesis, growing evidence suggests that, environmental factors have also a non-negligible role on its etiopathogenesis. It has been reported that environmental factors exert their effects either independently of genetic factors, or through gene-environment interaction or epigenetic mechanisms[2]. Despite the existing evidence indicating associations with the development of ADHD, and an environmental factor ie. Exposure to general anesthesia (GA), its developmental process at early ages is still debatable.

GA is described as a state of unconsciousness and painlessness maintained during unpleasant and painful surgical and invasive interventions. Experimental animal studiessuggestthatanestheticagents,especiallyN-methyl D-aspartate (NMDA) antagonists and gamma-amino butyric acid (GABA) agonists exert long-term adverse effects on developing brain by provoking widespread apoptotic neurodegeneration and emergence of deficits in hippocampal synaptic function[3]. Growing evidence claims that multiple rather than a single exposure to GA before 2 or 3 years of age may facilitate the development of behavioral-learning difficulties and also ADHD[4,5]. On the other hand, some studies have not detected a possible association between exposure to GA and later development of ADHD[6,7]. Supportively, an animal study suggests that early exposure to sevoflurane does not cause impairments in attentional processes in rats[8]. In fact, the literature findings are contradictory and do not indicate the presence of an explicit relationship between exposure to GA and ADHD. Indeed, a recent meta-analysis of cohort studies documents that the degree of association between exposure to GA and ADHD depends on the dose of the general anesthetic agent and duration of GA[9].

Given the hypothesis that general anesthetic agents contribute to the development of ADHD in the long term by damaging neural structures, the question of whether exposures to GA at an early age complicate the pharmacological treatment of ADHD conveys critical importance. A recent study has investigated the association between exposure to GA and subsequent use of medications for the treatment of ADHD and found that children only exposed to GA were 37% times more likely to need subsequent and persistent drug treatment for ADHD when compared to non-exposed children[10]. Although this study revealed that children exposed to GA persistently require drug treatment for ADHD, the dilemma whether GA exerts adverse effects on the pharmacological treatment process of ADHD has not been clearly elucidated yet. Moreover, does early exposure to GA have a negative effect on psychotropic treatment efficiency in terms of oppositional defiant disorder (ODD) and conduct disorder (CD) symptoms-that often accompany ADHD- as well as ADHD symptoms? To our knowledge,these conflicting issues have not been resolved yet.

To fulfill these gaps, we primarily aimed to investigate if exposure to GA per se , the age of exposure to GA and the number of exposures have complicating effects on drug treatment efficiency of ADHD, ODD and CD symptoms. We secondarily aimed to compare ADHD, ODD and CD symptoms of children with ADHD by categorizing them in terms of exposure to GA (if any) , age of exposure to GA and the number of exposures.

MATERIALS and METHODS

Participants

This was a multi-centered study conducted in child and adolescent psychiatry outpatient clinics of Ege University and University of Health Sciences Turkey, Dr. Behçet Uz Training and Research Hospital of Pediatrics. The sample was derived from medical files of both hospitals which are located in the third-largest Turkish city of İzmir. The ethics committee approval for this study was obtained from University of Health Sciences Turkey, Dr. Behçet Uz Training and Research Hospital of Pediatrics (approval number: 405, dated: 18.06.2020). Before recruiting to the study, the study participants and their parents were informed of the study protocol , and written informed consent was obtained from the parents/guardians of the children.

According to the power analysis performed for the study, the minimum sample size was calculated to be 100 children, with a 12,4% frequency of ADHD, a 4% variance level, and a 95% confidence level. Initially, there were 110 registered participants. Four participants dropped out during the study period, primarily due to scheduling conflicts faced by their families and their unwillingness to participate in the study. These drop-outs were random and unrelated to clinical or demographic variables, minimizing the risk of selection bias. Therefore, final sample consisted of 106 participants. Participants from each center were selected from among patients who met the study inclusion criteria, and had a designated outpatient clinic application order on the specified days.

Among 7-12 year-old patients not receiving any medication for at least one year before their first admissions to the clinic, those having a clinically determined normal cognitive capacity with a diagnosis of ADHD without any comorbid bipolar disorder, psychotic disorder, or autism spectrum disorder were included in the study.

Procedures and Materials

Participants for the current study were determined at their first admissions to the child and adolescent psychiatry outpatient clinic. At the first admission, clinicians gathered information regarding children's demographic profile, psychopathologies, their previous exposures to GA (if any), the age at which they had received GA, and surgeries they had undergone. The clinicians performed a mental status examination to make an accurate diagnosis based on the criteria established by both Fifth Edition of the Diagnostic and Statistical Manual of Mental Disorders (DSM-V) and the Schedule for Affective Disorders and Schizophrenia for School-Age Children Present and Lifetime Version, which is a commonly used, and conducted a semi-structured diagnostic interview to scan present and previous psychiatric diagnoses[11]. The validity and reliability study of its Turkish version was realized in 2004[12].

Patients were followed up for 3 months. Both at the beginning (T1) and at the end (T2), of the follow-up period, parents completed Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition (DSM-IV) Disruptive Behavior Disorders Rating Scale-IV (ADHD-RS-IV). ADHD-RS-IV is an assessment tool using the DSM-IV diagnostic criteria for symptoms of disruptive behavior disorders[13]. The scale is comprised of 41 items. Nine items inquire about IN; but also contains items inquiring HI (n=9); ODD (n=8), and CD (n=15). The scale is rated by a 4-point Likert-type scale with scores ranging from 0 to 3 (ie. 0= not at all; 1= just a little; 2= much; and 3= very much). If a case gets 2 or 3 points on any symptom item, it is considered that the symptom is present in the case. In 2001, the study on the reliability and validity of the Turkish version of the scale was performed[14]. In this study, pre- and post-treatment sub-dimensional symptom counts of the samples were compared.

To provide optimal standardization in pharmacological treatment procedures, psychostimulant treatment was initiated at doses appropriate for the age and weight of the participants, as indicated in the guidelines[15]. For immediate release-methylphenidate (MPH) users, MPH dose was started at 5-10 mg/d and increased by 5-10 mg/d every 2 weeks, whereas for extended release-MPH users initial daily MPH dose of 10-18 mg was increased up to 30-36 mg within 3 months. For atomoxetine (ATX) users, initial ATX dose of 0.5 mg/kg/d was increased to 1.2 mg/kg/d every 2 weeks.

Statistical Analysis

The resulting data were transferred into 26th version of the SPSS. A p-value below 0.05 was considered as statistically significant. To compare categorical variables, Pearson's chi-square test was performed. Fitness of variables to normal distribution was evaluated via Kolmogorov-Smirnov test for continuous variables. For intergroup comparisons of continuous variables with normal, and non-normal distribution independent samples t-test, and Mann-Whitney U test were used, respectively.

The repeated measures analysis of variance (ANOVA) test was used to compare the scale scores of the same sample estimated at two different time periods. IN, HI, ODD and CD dimensional symptom counts at both T1 and T2 time periods were determined as within-subject factors. Exposure to GA (if any), the number of exposures to GA and the age at which GA was received were determined as between-subject factors in separate models. After potentially confounding factors that may affect dimensional symptom counts were eliminated, gender was determined as a covariate in the models in which 'IN symptom count was determined as a within-subject factor. In each between-subject model, the main effects of between-subject factors and (if present) covariates were analyzed. Type III sum of squares were used for between-subject tests. If the between-subject factor is a categorical variable consisting of more than two categories, pairwise main effect comparisons among the categories were performed using Bonferonni

correction. When sphericity assumption could not be provided in Mauchly's test of sphericity, Greenhouse-Geisser test, which measures within-subject effects, was taken into consideration.

RESULTS

The final sample was comprised 106 cases, including 82 (77.4%) boys, and 24 (22.6%) girls. The mean age of the study participants was 9.67±1.67 years and 41.5% (n=44) of them had at least one comorbidity in addition to ADHD, while 58 (54.6%) patients had previously received GA. All the cases used stimulant and/or non-stimulant medications for the treatment of ADHD (Table 1).

Our male study population received GA at significantly higher proportion than girls (χ^2=11.057; df=1; p=0.001). The number of exposures to GA were significantly higher in boys than in girls (χ^2=12.784; df=2; p=0.002). Boys also received GA at an significantly earlier age than girls (χ^2=13.069; df=2; p=0.001). Among the cases who had received GA, the most common surgery types were circumcision (n=37; 63.7%) and adenoidectomy (n=18; 31.0%). Besides, age did not significantly differ between the cases with and without exposure to GA (p=0.124).

Mean IN symptom counts at T1 were significantly different between male and female (t=-2.271, p=0.025) participants, however other dimensional symptom counts estimated at T1 and T2 were not significantly different between both genders (all p>0.05). Any dimensional symptom counts were not significantly associated with age (all p>0.05). Thus, gender was determined as a confounding factor for IN symptom counts.

The Effect of GA Exposure Status

Symptom counts related to the sub-dimensions of IN, HI, ODD and CD at T1 and T2 time periods were compared. In all ADHD sub-dimensions and ODD and CD dimensions, symptom counts of the sample significantly reduced within 3 months [all p<0.01; (Table 2)]. However, GA exposure status had no significant effect on the decreases in any dimensional symptom counts [all p>0.05; (Table 2)]. At both T1 and T2 periods, IN and HI symptom counts of cases exposed to GA were significantly greater than those of the non-exposed cases (F=4.289, p=0.041; F=9.537, p=0.003, respectively). However, after making adjustments for gender, significant difference regarding IN symptom counts between the cases with and without exposure to GA was eliminated.

Table 1. Descriptive statistics of participants

	n	%
Gender		
Female	24	22.6
Male	82	77.4
Diagnoses		
ADHD	62	58.5
ADHD + SLD	11	10.3
ADHD + ODD	21	19.8
ADHD + AD	1	0.9
ADHD + SD	1	0.9
ADHD + SLD + ODD	2	1.9
ADHD + SLD + SD	2	1.9
ADHD + ODD + CD	2	1.9
ADHD + ODD + MDD	4	3.8
Medications		
MPH	76	71.7
ATX	6	5.7
MPH + AP	16	15.1
MPH + SSRI	2	1.9
MPH + ATX	2	1.9
ATX + AP	1	0.9
MPH + AP + SSRI	3	2.8
GAE status		
No	48	45.3
Yes	58	54.6
The age at exposure to GA		
< 3 years	21	19.8
> 3 years	37	34.9
Not exposed to GA	48	45.3
The number of exposures to GA		
None	48	45.3
Once	39	36.8
>2	19	17.9
Types of surgeries performed		
Circumcision	37	63.7
Adenoidectomy	18	31.0
Tonsillectomy	7	12.0
Inguinal hernia	6	10.3
Others	15	25.5
	M	SD
Age	9.67	1.67

Values are shown as number (n) and percentage (%) or mean (M) and standard deviation (SD). ADHD: Attention-Deficit/Hyperactivity Disorder, SLD: Specific Learning Disability, ODD: Oppositional Defiant Disorder, AD: Anxiety Disorder, SD: Speech Disorder, CD: Conduct Disorder, MDD: Major Depressive Disorder, MPH: Methylphenidate, ATX: Atomoxetine, AP: Antipsychotics, SSRI: Serotonin-specific reuptake inhibitor, GA: General anesthesia

Table 2. The effects of exposure to general anesthesia on changes in symptom coumts in IN, HI, ODD and CD dimensions within two periods

Dimension	Exposure to GA	Symptom counts				Tests of within-subjects effects				Tests of between subjects effects	
		T1		T2		Time		Time * GAE status			
		M	SD	M	SD	F	p*	F	p*	F	p
IN	No	4.89	2.56	2.16	2.65	8.158	**0.005**†	0.189	0.664†	2.077	0.153†
	Yes	5.70	2.82	3.10	3.04						
HI	No	3.62	2.95	1.70	2.27	31.138	**<0.001**	0.007	0.936	9.537	**0.003**
	Yes	4.93	3.10	3.06	2.78						
ODD	No	2.10	2.40	0.89	1.43	15.886	**<0.001**	0.641	0.425	2.918	0.091
	Yes	2.49	2.36	1.68	2.26						
CD	No	0.32	0.92	0.06	0.32	11.109	**<0.001**	0.120	0.730	0.106	0.745
	Yes	0.39	0.98	0.07	0.32						

Bold values mark statistically significant differences. Values are shown as mean (M) and standard deviation (SD).

*Repeated measures test was performed. Since sphericity criteria were not met, the assessments were made according to Greenhouse-Geisser test. †The effects were calculated after controlling for gender. IN: Inattention, HI: Hyperactivity-Impulsivity, ODD: Oppositional Defiant Disorder, CD: Conduct Disorder, GA: General anesthesia

The Effect of the Age at Exposure to GA

When the interaction of the age at exposure to GA and time interval between T1 and T2 was applied to the repeated measures ANOVA model, patient's age at exposure to GA had no significant effect on the reductions in any dimensional symptom count [all p>0.05; (Table 3)]. The symptom counts of almost all ADHD, ODD and CD dimensions in both T1 and T2 periods were found to be highest in patients exposed to GA under 3 years of age when compared with older patients, and lowest in patients who did not receive any GA. However, the only statistically significant change was detected in the symptom counts of HI dimension [F=5.738, p=0.004; see (Table 3)]. The children exposed to GA under 3 years of age had significantly higher HI symptom counts relative to the non-exposed children (p=0.005).

The Effect of the Number of Exposures to GA

When the interaction of the number of exposures to GA and time interval between T1 and T2 was applied to the repeated measures ANOVA model, the number of exposures had no significant effect on the decreases in any dimensional symptom count [all p>0.05; (Table 4)]. Participants exposed to GA for two or more times had the highest symptom counts, compared to those with single exposures, and patients without exposure to GA had the lowest symptom counts on nearly all ADHD, ODD and CD dimensions in both T1 and T2 time periods. Similarly the only statistically significant difference was observed in HI dimension (F=5.995, p=0.003). The cases with multiple exposures to GA had significantly higher HI symptom counts than those without [p=0.004; (Table 4)].

DISCUSSION

The present study has documented that neither exposure to GA itself, nor the age at exposure to GA or the number of exposures to GA had significantly worsening effects on efficiency of the drug treatment for ADHD, ODD and CD symptoms. It was also found that, among all the symptom dimensions, particularly hyperactive-impulsive symptoms were significantly more frequently detected in those who categorically had been exposed to GA, those who had exposures to GA more than 2 times, and those who had received GA before the age of 3 years compared to those who had not.

Deficits in prefrontal cortex (PFC) which regulates attention, executive functions, behaviors, and emotions play a substantial role in the neurobiology of ADHD[16]. Psychostimulants (MPH and amphetamine) work as reuptake inhibitors by inhibiting dopamine and norepinephrine transporters and increasing neurotransmission in the PFC and corpus striatum[17] while ATX inhibits norepinephrine reuptake in all brain regions and dopamine reuptake selectively in the PFC[18]. Whereas the histopathological changes caused by GA in the animal brain are listed as apoptosis, pathological neurogenesis, and dendritic formation[19]. The findings of our study indicate that even earlier exposure to GA and receiving GA multiple times might

Table 3. The effects of the age of the patients at the time of exposure to general anesthesia on symptom counts in IN, HI, ODD and CD dimensions within two periods

Dimension	The age of GA	Symptom counts				Pairwise comparisons among age categories (p-values**)			Tests of within-subjects effects				Tests of between subjects effects	
		T1		T2					Time		Time* The age of GA			
		M	SD	M	SD	1 vs. 2	1 vs. 3	2 vs. 3	F	p*	F	p*	F	p
IN	Below 3 years	5.80	2.97	3.28	3.30	1.000	0.230	0.294	6.249	**0.014**†	0.125	0.883†	1.038	0.358†
	Above 3 years	5.64	2.77	3.00	2.93									
	Not exposed to GA	4.89	2.56	2.16	2.65									
HI	< 3 years	6.09	3.19	2.95	3.04	0.527	**0.005**	0.102	34.538	**<0.001**	2.324	0.103	5.738	**0.004**
	>3 years	4.27	2.89	3.13	2.66									
	Not exposed to GA	3.62	2.95	1.70	2.27									
ODD	<3 years	2.95	2.56	2.10	2.78	0.497	0.088	1.000	12.339	**0.001**	0.322	0.726	2.447	0.092
	> 3 years	2.24	2.24	1.45	1.92									
	Not exposed to GA	2.10	2.40	0.89	1.43									
CD	<3 years	0.35	0.81	0.05	0.22	0.946	1.000	0.902	10.321	**0.002**	0.069	0.934	0.103	0.902
	>3 years	0.41	1.07	0.08	0.36									
	Not exposed to GA	0.32	0.92	0.06	0.32									

Bold values mark statistically significant differences. Numbers indicated in the columns of pairwise comparisons refer to the paiens that did not receive general anesthesia (1), or received anesthesia twice (2) or more than two times (3), respectively. *Repeated measures test was performed. Since sphericity criteria were not met, the assessments were made acclording to Greenhouse-Geisser test results. **Adjustment for multiple comparisons was performed via Bonferonni correction. †The effects were calculated after adjustments were made for the gender of the patients. M: Mean, SD: Standard deviation, IN: Inattention, HI: Hyperactivity-Impulsivity, ODD: Oppositional Defiant Disorder, CD: Conduct Disorder, GA: General anesthesia

not cause a significant attenuation in response to drug treatment of ADHD.

This condition reveals that despite the micro and macro morphological changes in the brain caused by GA, psychostimulants and ATX might not be affected by these structural deficits and continue to exert their effects mostly through the dopamine/norepinephrine transporter system The general anesthetic agents, and usually GABA agonists (e.g., volatile anesthetics, midazolam, and propofol) or NMDA antagonists (e.g., ketamine, isoflurane, and nitrous oxide), -which affect the brain through glutamate/GABA system supposed to have associations with behavioral deficits and cognitive abnormalities by leading to the development of neurotoxicity[19]. However, the targets for psychostimulants and ATX are dopamine/norepinephrine reuptake systems. The differences in target systems might explain the mechanism by which ADHD drugs might continue to show their own mechanism of action without being adversely affected by the neurotoxicity of general anesthetics.

Experimental animal studies also support the fact that psychostimulants ameliorate hyperactivity symptoms of the animals whose brains had been exposed to neural injury by general anesthetics. A study reported that hyperactivity symptoms of neonatal rodents exposed to NMDA antagonists were reversed with the use of dextroamphetamine[20]. Another study has documented that 6-hydroxydopamine-induced hyperactivity in neonates was improved by the acute use of dextroamphetamine[21]. Although animal studies cannot be extrapolated to human beings, these findings indicate that exposure to GA does not irreversibly impair efficiency of psychostimulant treatment.

Another reason for non-significant effects of GA on treatment efficiency may be related to the higher effectiveness of drugs used for the treatment of ADHD. There is a wide consensus that psychostimulants have the best treatment efficiency in treating ADHD. A network meta-analysis has indicated that the estimated effect sizes of MPH and amphetamine are greater than 0.8, while of ATX is between 0.5 and 0.8[22]. Given the high effect sizes and considering the relationship between ADHD and exposure to GA

Table 4. The effects of the number of exposures to general anesthesia on symptom counts in IN, HI, ODD and CD dimensions both at the beginning and at the end of the follow-up period

Dimension	The number of exposures to general anesthesia	Symptom counts				Pairwise comparisons among age categories (p values**)			Tests of within-subjects effects				Tests of between subjects effects	
		T1		T2					Time		Time* The number of exposures to GA			
		M	SD	M	SD	1 vs. 2	1 vs. 3	2 vs. 3	F	p*	F	p*	F	p
IN	None	4.89	2.56	2.16	2.65	0.432	0.103	1.000	7.038	**0.009**†	0.177	0.838†	1.303	0.276†
	Once	5.43	2.92	3.00	2.75									
	≥2	6.26	2.57	3.31	3.65									
HI	None	3.62	2.95	1.70	2.27	0.098	**0.004**	0.389	31.308	**<0.001**	0.908	0.407	5.995	**0.003**
	Once	4.41	3.15	2.97	2.55									
	≥2	6.00	2.76	3.26	3.26									
ODD	None	2.10	2.40	0.89	1.43	0.797	0.161	0.891	12.565	**0.001**	0.391	0.677	2.010	0.139
	Once	2.28	2.37	1.56	2.17									
	≥2	2.94	2.33	1.94	2.48									
CD	None	0.32	0.92	0.06	0.32	1.000	1.000	1.000	13.324	**<0.001**	1.012	0.367	0.139	0.870
	Once	0.31	0.87	0.10	0.38									
	≥2	0.55	1.19	0.00	0.00									

Bold numbers mark statistically significant differences. Numbers indicated in the columns of pairwise comparisons refer to the paiens that did not receive general anesthesia (1), or received anesthesia twice (2) or more than two times (3), respectively. *Repeated measures test was performed. Since sphericity criteria were not met, the assessments were made acclording to Greenhouse-Geisser test results. **Adjustment for multiple comparisons was performed via Bonferonni correction test. †The effects were calculated after adjustments were made for the gender of the patients. M: Mean, SD: Standard deviation, IN: Inattention, HI: Hyperactivity-Impulsivity, ODD: Oppositional Defiant Disorder, CD: Conduct Disorder

is dose-, developmental stage-, duration- and repetition-dependent[4,9], it is not surprising that a negative effect of receiving GA on the ADHD treatment response has not been determined in our study.

The findings also indicate that the improvements in the symptomatology of ODD, CD and also ADHD provided by ADHD medication were not adversely affected by generasl anesthesia-related factors. The etiological roots of disruptive behavioral disorders such as ODD and CD more likely stem from psychological, social issues and intra-familial conflicts[23] and less likely depend on neurobiological underpinnings when compared to ADHD. This might be a reason why the anesthetic agents had not adversely affected improvements in the symptomatology of ODD/CD. To our knowledge, these are the first estimates documenting that exposure to GA and related features have no significant effect leading to restrictions in both ADHD treatment response and improvements in the symptomatology of ODD/CD.

Another important advantage of our study is the comparison of HI symptoms in pediatric patients with ADHD. Existing studies are usually case-control studies and aim to comparatively evaluate the risk of ADHD in later life in children that had been exposed and not exposed to GA. Tsai et al.[4] concluded that children exposed to GA on more than one occasion or below 3 years of age had an increased risk for the development of ADHD. Sprung et al.[5] also found an association between repeated procedures requiring GA performed before 2 years of age and a later development of ADHD. However, the current study sample consisted of ADHD subjects, not of controls. Since the methodology was determined in this way, it was concluded that HI symptoms were more frequently detected in children with ADHD who had been exposed to GA categorically, who had multiple exposures to GA or received GA before 3 years of age compared to those with ADHD not exposed to GA. Although these outcomes are consistent with the literature, they

also expand literature knowledge by suggesting that exposure to GA at earlier ages and multiple exposures might increase especially the severity of HI symptoms in children with ADHD even in comparisons among themselves. A study documented that inguinal hernia repair had a significant association with ADHD. Supportive of our study results, it was suggested that this relationship may arise since inguinal hernia repair, which requires GA, is usually performed at very early ages[24].

In our study, in addition to HI symptoms, IN, ODD and CD symptoms were also highly, but not statistically significantly more frequent in those who were exposed to GA before the age of 3 and those experienced multiple exposeures to GA. HI symptoms might be more vulnerable to environmental factors such as GA compared to other symptom dimensions. In a study, elevated HI symptoms but not IN symptoms were associated with surgical history of the patients[24]. Although the effects of GA on ADHD symptoms were not directly measured in that study, the positive association between surgery and increased frequency of HI symptoms are in line with the current findings. Supportively, it was established that propofol induces hyperactivity in adolescent rats through its neurotoxic effects on the neurons of the corpus striatum, thalamus and medial PFC[25].

Strengths and Limitations of the Study

As one of the strengths of our research, this study has focused on the possible effects of GA and related factors on the efficiency of drug treatment of ADHD which has been investigated for the first time in the literature. Besides, whether or not GA has adversely affected the treatment efficiency against ODD, CD as well as ADHD symptoms has been evaluated for the first time. Apart from that, symptomatological changes at the beginning, and end of the follow-up period could be observed accurately and objectively.

Study Limitations

However, some limitations of our study must be taken into consideration. Small sample size restricted the generalizability of the findings to the community. Besides, the sample had heterogenous characteristics in terms of different comorbidities and medication regimens. It is important to consider the influence of comorbidities and diverse medication regimens when interpreting the findings. For instance, comorbid ODD or CD may exacerbate symptom severity, potentially impacting the treatment response. A Turkish study suggested that the parents, and the teachers of the pediatric patients with ADHD + ODD reported IN and HI symptoms at a significantly higher rate when compared to those with only ADHD[26]. Therefore, comorbid conditions may constitute a handicap in terms of the reliability of the findings. In addition, although administration of medication for each participant was tried to be standardized as much as possible, additional medications other than psychostimulants and ATX and additional comorbidities other than ADHD might have prevented us from observing the unique effects of exposure to GA on ADHD treatment efficiency. For instance, it was reported that atypical antipsychotics such as risperidone had improved HI symptoms in ADHD + ODD/CD patients[27]. Furthermore, certain selective serotonin re-uptake inhibitors were also shown to control IN or HI symptoms of ADHD[28]. Hence, the outcomes of this study might not reflect the unique effects of exposure to GA on pharmacological efficiency of specific drugs used for the treatment of ADHD in children using multiple psychotropic drugs. Finally, since we could not know the duration of exposure to GA for each participant, the effect of duration of exposure to GA ob treatment efficiency could not be estimated.

CONCLUSION

Although the association between exposure to GA and ADHD has not been fully clarified, growing evidence indicates the presence of such a relationship. However, neither exposure to GA itself, nor earlier ages to exposures or multiple exposures do not seem to attenuate pharmacological treatment response to ADHD. Although general anesthetics cause neurodegeneration in the developing brain, the pharmacological effects of psychostimulants, and ATX might not be altered by these structural deficits and these drugs continue to show their own mechanism of action without being adversely affected by the neurotoxicity of general anesthetic agents. Besides, exposure to GA before 3 years of age and more than one exposure might even comparatively increase especially the severity of HI symptoms in children with ADHD. This condition indicates that HI symptoms may be more vulnerable to the adverse effects of GA and related factors. As a clinical implication, fortunately exposure to GA and anesthesia-related factors had not complicated the treatment process of ADHD. Nonetheless, this study reveals preliminary findings and larger scale future studies performed with homogenous samples should replicate the current findings.

Ethics

Ethics Committee Approval: The ethics committee approval for this study was obtained from University of Health Sciences Turkey, Dr. Behçet Uz Training and Research Hospital of Pediatrics (approval number: 405, dated: 18.06.2020).

Informed Consent: The participants and their parents were informed of the study and written inform consent was obtained from the parents/guardians of the children.

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Footnotes

Author Contributions

Surgical and Medical Practices: Concept: A.E.Y., S.F.D., S.G.S., E.S.E., Design: A.E.Y., S.F.D., S.G.S., E.S.E., Data Collection or Processing: Z.İ.E., A.T., S.F.D., Analysis or Interpretation: A.T., S.F.D., Literature Search: A.E.Y., A.T., S.G.S., Writing: A.E.Y., A.T., E.S.E.

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

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Original Article

The Effect of Adipocyte-Derived Stem Cell Media on Stem Cell Markers in Neuroblastoma Cells

Adiposit Kaynaklı Kök Hücre Ortamının Nöroblastom Hücrelerindeki Kök Hücre Belirteçleri Üzerindeki Etkisi

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ABSTRACT

Objective: Different effects of stem cell media have been reported in different types of cancer. Neuroblastoma (NB) is the most common extracranial tumour of childhood. To be knowledgeable about pathophysiology of the disease is crucial for its effective treatment. The study aimed to determine the efficacy of human adipocyte-derived stem cell media (ADSC-M) on stem cell markers in NB cells.

Method: In the study, SH-SY5Y/N-MYC(-) and KELLY/N-MYC(+) cells were grown and adipocyte-derived stem cell conditioned medium was applied. Flow cytometry was used to identify changes in the expressions of stem cell markers in cells' own proliferative, and normal conditioned medium (NCM) and ADSC-M.

Results: While CD14, CD33, CD44, CD73 expressions disappeared in KELLY cells, CD34 levels in SH-SY5Y neuroblastoma cells decreased in ADSC-M rather than NCM. CD90 and CD106 expressions increased in KELLY and decreased in SH-SHY5Y when incubated in ADSC-M. Expression levels of HLA-ABC decreased twofold in SH-SY5Y when incubated in ADSC-M rather than NCM. Again, incubation of SH-SY5Y cells in ADSC-M decreased the expression of HLA-DR and CD45 down to undetectable levels compared to NCM.

Conclusion: The study found that ADSC-M negatively impacted mesenchymal and adipocyte-derived stem cell markers, especially in N-MYC- positive KELLY neuroblastoma cells, indicating poor prognosis.

Keywords: Neuroblastoma, adipocyte-derived stem cell media, stem cell markers, normal conditioned media

ÖZ

Amaç: Farklı kök hücre ortamlarının farklı kanserlerde çeşitli etkileri olduğu bildirilmiştir. Nöroblastom, çocukluk çağının en yaygın ekstrakraniyal tümörüdür. Hastalığın patofizyolojisinin anlaşılması, etkili tedavi için kritik öneme sahiptir. Bu çalışma, adiposit türevli kök hücre ortamının (ADSC-M) nöroblastom hücrelerindeki kök hücre belirteçleri üzerindeki etkinliğini belirlemeyi amaçlamıştır.

Yöntem: Çalışmada, SH-SY5Y/N-MYC(-) ve KELLY/N-MYC(+) hücreleri üretilmiş ve bu hücrelere ADSC-M uygulanmıştır. Hücrelerin kendi proliferatif ortamı (NCM) ve ADSC-M ile oluşan kök hücre belirteçlerindeki değişiklikler, akış sitometrisi kullanılarak değerlendirilmiştir.

Bulgular: ADSC-M uygulaması sonrasında KELLY hücrelerinde CD14, CD33, CD44, CD73 ekspresyonlarının kaybolduğu, SH-SY5Y nöroblastom hücrelerinde ise CD34 seviyelerinin NCM'ye kıyasla azaldığı görülmüştür. ADSC-M uygulandığında, CD90 ve CD106 ekspresyonları KELLY hücrelerinde artış, SH-SY5Y hücrelerinde ise azalış göstermiştir. HLA-ABC ekspresyon seviyesi, SH-SY5Y hücrelerinde ADSC-M uygulandığında NCM'ye kıyasla iki kat azalmıştır. Yine SH-SY5Y hücrelerinde, ADSC-M tedavisi, HLA-DR ve CD45 ekspresyonlarının NCM'ye kıyasla tespit edilemez hale gelmesine neden olmuştur.

Sonuç: Çalışma, adiposit türevli kök hücre ortamının, özellikle N-MYC pozitif KELLY nöroblastom hücrelerinde, mezenkimal ve adiposit türevli kök hücre belirteçlerini olumsuz etkilediğini ve bunun kötü prognozla ilişkili olabileceğini ortaya koymuştur.

Anahtar kelimeler: Nöroblastom, adiposit kök hücre ortamı, kök hücre belirteçleri, normal şartlandırılmış ortam

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INTRODUCTION

Neuroblastoma (NB) is the most common extracranial solid tumour in children, accounting for 8-10% of all pediatric cancers. It accounts for 15% of cancer deaths in children[1]. According to the National Pediatric Cancer Registry (Turkish Pediatric Oncology Group/ Turkish Pediatric Hematology Association Pediatric Cancer Registry) data, 1243 (7.9%) out of 15713 cases registered between 2009 and 2018, were diagnosed with sympathetic system tumours[1]. In modern protocols including induction chemotherapy, surgical resection, high-dose chemotherapy with autologous stem cell rescue, external beam radiotherapy and immunotherapy or treatment with different antitumoral agents, three-year survival rates currently exceed 60%, demonstrating achievement of better outcomes[2]. Cisplatin is the main chemotherapeutic agent used in the treatment of NB which greatly improves survival in pediatric patients with NB. However, it causes serious side effects such as nephrotoxicity, ototoxicity and peripheral neurotoxicity[3,4]. The biggest therapeutic challenge in the advanced disease stage of NB is the presence of minimal residual disease. Mesenchymal stem cells (MSCs) can be defined as non-hematopoietic multipotent stem cells capable of differentiating into mesodermal (adipocytes, osteocytes and chondrocytes), ectodermal (neurocytes) and endodermal lineages (hepatocytes). MSCs may be derived from many sources including adipose tissue, bone marrow, peripheral blood and neonatal tissues[5].

Human MSCs are defined by three main criteria: expression of CD73, CD90 and CD105 and lack of expression of hematopoietic markers such as CD11b, CD34, CD45, CD79, CD19 and human leukocyte antigen complex (HLA-DR), *in vitro* differentiation into osteoblasts, chondrocytes and adipocytes and plastic adhesion[6]. It is well established that the source of MSCs has a significant impact on their yield, the range of surface markers they express, and their cytokine profiles. The best source of adipose tissue-derived MSCs is adipose tissue, among other MSC sources[5].

Adipocyte-derived stem cells (ADSCs) are self-renewing and can differentiate along various mesenchymal tissue lineages, including adipocytes, osteoblasts, myocytes, chondrocytes, endothelial cells and cardiomyocytes[7,8]. ADSCs can differentiate into neuronal and vascular structures both *in vitro* and *in vivo* settings[9,10]. Therefore, ADSCs are considered promising agents for regenerating tissues and organs damaged by injury and disease. It has been currently revealed that the environment of stem cells has different effects in different diseases[11,12]. ADSC-derived media (ADSC-M) may be protective for neuronal tissue by causing a dose-dependent decrease in infarct volume both *in vitro* and *in vivo* cerebrovascular infarct models[11]. In another study, ADSC-M was found to increase proliferation, migration and invasion in melanoma and colorectal cancer cells[12]. However, direct injection of MSCs into the tumour in a subcutaneous animal NB tumor model has been shown to reduce tumor growth by inhibiting proliferation and causing apoptosis[13]. It was found that human ADSCs can transform into neuronal phenotype and can be positive for glial acidic fibrillary protein (GFAP), nestin and neuronal nuclei (NeuN)[10]. Olfactory ensheathing cells and B104 NB cells showed neuronal cell properties after treatment with ADSCs which expressed markers of both progenitor and mature neurons such as nestin, PGP 9.5 and MAP2[14]. In a recent study, the use of dedifferentiated adipose cells conditioned medium and PI3K inhibitors together has been shown to reduce proliferation and differentiation rates of NB cells[15]. Despite all these studies, the effect of ADSC environment on MSC markers in NB cells with different prognosis has not yet been investigated. ADSC-M alters the expression of stem cell markers in NB cells, with more pronounced changes in N-MYC-positive (KELLY) cells compared to N-MYC-negative (SH-SY5Y) cells, potentially indicatig a worse prognosis for N-MYC-positive NB. This hypothesis clearly states the expected effect of the ADSC-M on different NB cell lines and disease prognosis.

The aim of this study is to determine whether ADSC-M could alter the surface markers of these cells in the presence of N-MYC expression, a key prognostic factor for NB.

MATERIALS and METHODS

Cell Culture

Human SH-SY5Y (N-MYC-) (DSMZ) and KELLY (N-MYC+) (DSMZ) NB cell lines were grown in 5% CO_2 and 37 °C humid conditions. For human SH-SY5Y, KELLY cell lines, DMEM (Gibco), RPMI (Gibco), ADSC basal media (Gibco), 10% fetal bovine serum (FBS), 1% penicillin/streptomycin and 1% L-glutamine were added on the medium to be used for the evaluation of cell proliferation.

ADSC Conditioned Media Applications

ADSC basal medium (Gibco) prepared with stem cell quality FBS 10%, L-glutamine and pencillin/ streptomycin 1% was applied to KELLY and SH-SY5Y cells for 48 hours. The effects of cells on adipose-tissue

stem cell surface markers and whether they differed from normal conditioned medium (NCM) medium were evaluated.

Flow Cytometry Analysis of Stem Cell Markers

Stem cells were collected after 48 hours of incubation with adipose tissue-derived stem cell conditioned medium and also with their own specific medium. For this purpose, cells were removed from their flasks by trypsinization, counted and homogenized in phosphate buffered saline (PBS) solution to approximately $8x10^6$ cells.

The stem cells were then incubated for 45 minutes in the dark with fluorescein isothiocyanate- and phycoerythrin-conjugated monoclonal antibodies (BD™ Biosciences) (Table 1) specific for the identified cell surface markers and 10 µL of appropriate isotype controls. After incubation, PBS containing 0.1% sodium azide was added to the cell suspension, washed and resuspended. The prepared cell suspension was read at the relevant excitation and emission wavelengths in FACS Calibur flow cytometry device (BD). Flow cytometric analyses were performed using BD Cell Quest TM software program[16].

Statistical Analysis

Data were evaluated using the non-parametric Mann-Whitney U test. Mean values were evaluated by Student's t-test using SPSS 15.0 program. Stem cell surface protein expressions were evaluated as at least ≥2 fold increases/decreases compared to the control group. $p<0.05$ was considered statistically significant.

RESULTS

In this study, changes both in the number of both stem cell surface markers representing good and poor prognosis in NB cells and also in the number of stem cell surface markers in N-MYC expressing and non-expressing cells in the adipocyte-derived conditioned medium prepared from stem cells were revealed for the first time.

Flow Cytometry Results

CD29 MSC markers were expressed at rates of 48.20% with NCM and 57.30% with ADSC-M in KELLY cells. In SH-SY5Y cell line, the expression level of 78.40% with NCM decreased to 56% with ADSC-M (Figures 1 and 2).

CD44 MSC marker is involved in a wide range of cellular functions, including lymphocyte activation, circulation and targeting, hematopoiesis and tumor metastasis. In KELLY cells, they were expressed at a rate of 15.70% with NCM, whereas expression (0.90%) was abolished with ADSC-M. Again, in SH-SY5Y cell line, the expression level, which was 35.30% with NCM, decreased to 20.30% with ADSC-M (Figures 1 and 2).

CD73 lymphocyte differentiation marker is normally a positive marker in mesenchymal and adipocyte stem cells. In KELLY cells, it was expressed at 10.70% with NCM, whereas expression disappeared in ADS-CM medium (1.10%). In SH-SY5Y cell line, the expression level of NCM at 11.20% decreased to 8.50% in ADSC-M (Figures 1 and 2).

Table 1. Antibodies used in flow cytometry
CD10 (N-cadherin/common leukocyte lymphocytic leukemia antigen-CALLA; PE)
CD14 (monocyte differentiation antigen/LPS receptor; FITC)
CD29 (integrin b1 chain; PE)
CD33 (sialic acid-binding immunoglobulin-like lectin 3; SIGLEC3; a surface marker for very early bone marrow-derived hematopoietic stem cells; PE)
CD34 (hematopoietic progenitor cell antigen; PE)
CD44 (hyaluronate/lymphocyte homing-associated cell adhesionmolecule-HCAM; PE)
CD45 (protein tyrosine phosphatase, receptor type, C/PTPRC/leukocyte common antigen/cell marker of hematopoietic origin; FITC)
CD73 (5'-nucleotidase, ecto;NT5E/integrin b5; PE)
CD90 (Thy-1/Thy-1.1-FITC)
CD106 (vascular cell adhesion molecule, VCAM-1- FITC)
CD166 (activated leukocyte cell adhesion molecule; ALCAM/integrin a3;mesenchymal stem cell marker; PE)
HLA-DR (major histocompatibility complex, MHC class II, cell surface receptor; FITC)
HLA-ABC[Q3] (major histocompatibility class I antigen reseptor; PE)
Monoclonal antibodies and isotype controls used to identify cell surface markers. FITC: Fluorescein isothiocyanate, LPC: Lipopolysaccharid, PE: Phycoerythrin, MHC: Major histocompatibility complex

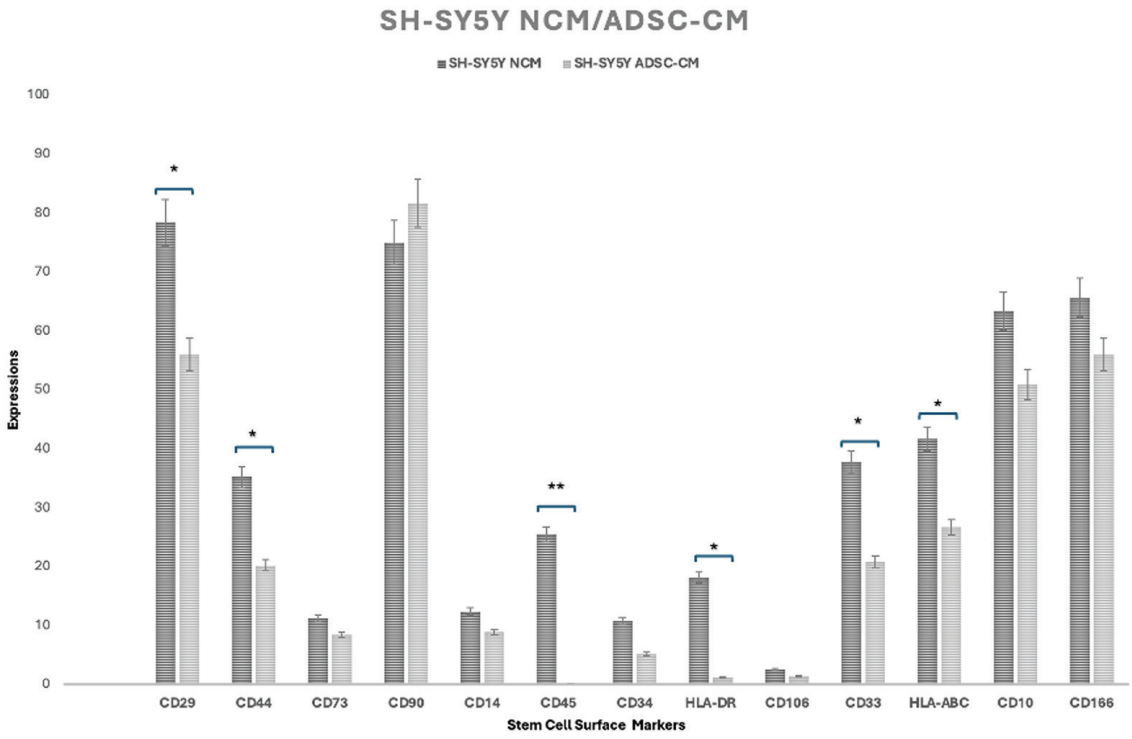

Figure 1. Evaluation of the expression levels of stem cell surface markers in SH-SY5Y cells compared to ADSC-M and NCM. Assessment of the expression levels of stem cell surface markers in SH-SY5Y cells following treatment with ADSC-M and NCM is shown

NCM: Normal conditioned medium, ADSC-M: Adipocyte-derived stem cell media

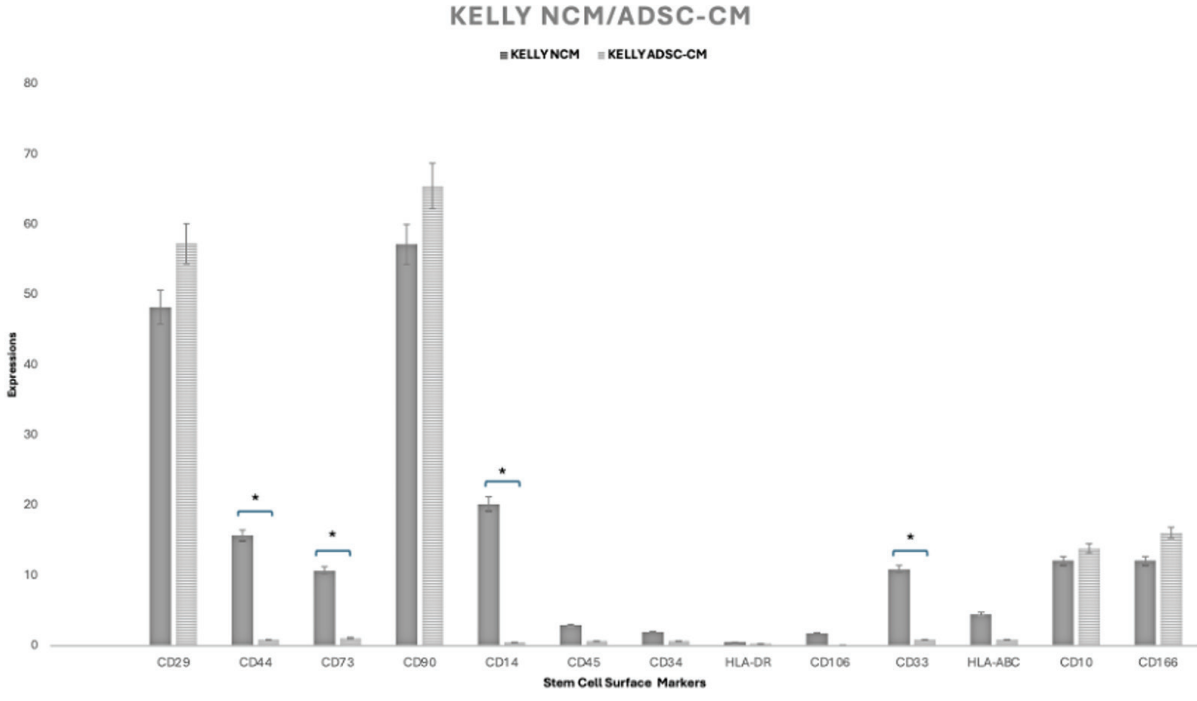

Figure 2. Evaluation of the expression levels of stem cell surface markers in KELLY cells compared to ADSC-M and NCM. Following treatment with ADSC-M and NCM, the expression levels of stem cell surface markers in KELLY cells are evaluated and shown

NCM: Normal conditioned medium, ADSC-M: Adipocyte-derived stem cell media

CD90 adipocyte-derived MSC marker showed an expression in the same direction in KELLY and SH-SY5Y cell lines. While the expression level was 57.20% with NCM in KELLY cells, the expression level increased to 65.50% in ADSC-M. In SH-SY5Y cell line, the expression level of NCM was 75.00%, while the expression level increased to 81.70% in ADSC-M (Figures 1 and 2).

CD14 monocyte differentiating marker is not normally expressed in MSCs and therefore shows negative expression in mesenchymal and adipose tissue. Accordingly, while NCM was expressed at 20.20% in KELLY cells, expression was lost with ADSC-M (0.50%). In SH-SY5Y cell line, the expression level of NCM at 12.40% decreased to 8.90% with ADSC-M (Figures 1 and 2).

CD45 leukocyte surface marker; not expressed in adipose cells. In KELLY cells, no expression was detected with NCM (2.90%) and 0.70% with ADSC-M. In SH-SY5Y cell line, the expression level of NCM was 25.50%, while the expression was abolished with ADSC-M (Figures 1 and 2).

CD34 hematopoietic stem cell marker is expressed on endothelial progenitor cells and endothelial cells. It is also an important adhesion molecule. No expression was detected in KELLY cells with NCM (2%) and ADSC-M (0.70%). In SH-SY5Y cell line, NCM expression level was 10.90% and decreased to 5.20% with ADSC-M (Figures 1 and 2).

HLA-DR is an MHC class II cell surface receptor, and one of the key cell surface molecules expressed on antigen presenting cells (monocytes, macrophages and dendritic cells). It is responsible for presentation of antigens to T cells and initiation of the inflammatory cascade during infection. While it is expressed in MSCs, but not in mesenchymal cells of adipocyte origin. In KELLY cells, there is no expression when incubated in either NCM (0.50%) or ADSC-M (0.30%). In SH-SY5Y cell line, NCM expression level was 18.20%, while its expression level in ADSC-M decreased (1.20%) (Figures 1 and 2).

CD106 MSC marker; also known as vascular cell adhesion molecule-1. It mediates adhesion of lymphocytes, monocytes, eosinophils and basophils to vascular endothelium. It was not expressed with NCM (1.80%; 2.60%) and ADSC-M (0.10%; 1.40%) in KELLY and SH-SY5Y cells (Figures 1 and 2).

CD33 myeloid leukemia (AML) marker is expressed on blast cells and represents a tumour-associated target antigen suitable for antibody-based therapies. Accordingly, in KELLY cells, it showed 10.90% expression with NCM, whereas expression was lost with ADSC-M (0.90%).

In SH-SY5Y cell line, the expression level was 37.80% with NCM and decreased to 20.80% with ADSC-M (Figures 1 and 2).

HLA-ABC, MHC I cell surface receptor: While NCM expression level was 4.50% in KELLY cells, no expression level (0.90%) was detected with ADSC-M application. In SH-SY5Y cell line, while its expression level was 41.70% with NCM, it decreased to 26.70% with ADSC-M (Figures 1 and 2).

CD10 acute lymphoblastic leukemia antigen is one of the endopeptidases which are widely expressed cell surface proteins. In KELLY cells, its expression levels with NCM and ADSC-M was 12.10% and 13.90%, respectively and did not change. In SH-SY5Y cell line. Its expression level decreased from 63.40% with NCM to 50.90% with ADSC-M (Figures 1 and 2).

CD166 leukocyte cell adhesion molecule is incorporated into neurite extensions by neurons through heterophilic and homophilic interactions. In KELLY cells, it was expressed at a rate of 12.10% with NCM, while its expression was slightly increased as 16.10% with ADSC-M. In SH-SY5Y cell line. Its expression level was 65.70% with NCM, but it decreased to 56% with ADSC-M (Figures 1 and 2). All numerical data of the flow cytometry results are shown in (Table 2).

According to these data, the expression levels of MSC markers CD14, CD33, CD34, CD44, CD45 and CD73 disappeared in KELLY NB cells with ADSC-M application, while expression levels of SH-SY5Y cells decreased almost by 50 percent. Expression level of CD90, an ADSC marker, showed an 10% increase with ADSC-M, although not significant in both cells. CD45 and HLA-DR, which were not expressed in adipocytes and mesenchymal-derived cells, were found to be expressed only in SH-SY5Y with NCM application, whereas they were eliminated with ADSCM-M. Again, expression levels of HLA-ABC decreased 2-fold in SH-SY5Y cells with ADSC-M, whereas it was eliminated in KELLY. CD106 did not show expression and change in both NB cells with both media. While CD10 and CD166 expression did not show a significant change in KELLY cells, their expression decreased in SH-SY5Y cells with ADSC-M compared to NCM. CD29 expression was slightly increased by ADSC-M in KELLY, but decreased in SH-SY5Y.

Table 2. Stem cell surface marker flow analyses of KELLY and SH-SY5Y cell lines

SH-SY5Y NCM													
Marker	**CD44**	**CD34**	**CD14**	**CD73**	**CD106**	**CD33**	**HLA-DR**	**HLA-ABC**	**CD90**	**CD10**	**CD45**	**CD29**	**CD166**
Percent	35.30%	10.90%	12.40%	11.20%	2.60%	37.80%	18.20%	41.70%	75.00%	63.40%	25.50%	78.40%	65.70%
SH-SY5Y ADSC-M													
Marker	**CD44**	**CD34**	**CD14**	**CD73**	**CD106**	**CD33**	**HLA-DR**	**HLA-ABC**	**CD90**	**CD10**	**CD45**	**CD29**	**CD166**
Percent	20.30%	5.20%	8.90%	8.50%	1.40%	20.80%	1.20%	26.70%	81.70%	50.90%	0.20%	56.00%	56.00%
KELLY NCM													
Marker	**CD44**	**CD34**	**CD14**	**CD73**	**CD106**	**CD33**	**HLA-DR**	**HLA-ABC**	**CD90**	**CD10**	**CD45**	**CD29**	**CD166**
Percent	15.70%	2.00%	20.20%	10.70%	1.80%	10.90%	0.50%	4.50%	57.20%	12.10%	2.90%	48.20%	12.10%
KELLY ADSC-M													
Marker	**CD44**	**CD34**	**CD14**	**CD73**	**CD106**	**CD33**	**HLA-DR**	**HLA-ABC**	**CD90**	**CD10**	**CD45**	**CD29**	**CD166**
Percent	0.90%	0.70%	0.50%	1.10%	0.10%	0.90%	0.30%	0.90%	65.50%	13.90%	0.70%	57.30%	16.10%

NCM: Normal conditioned medium, ADSC-M: Adipocyte-derived stem cell media

DISCUSSION

Adult adipose tissue is now recognised as a useful source of adult MSCs[17,18]. ADSCs can differentiate into neuronal and vascular structures both *in vitro* and *in vivo* settings[9,10].

It has been shown that transplantation of ADSCs into the brain could improve neurological problems in rats with ischemia[19]. Direct intratumoral injection of MSCs in NB animal tumor model decelerates tumour development by driving tumour cells to apoptosis[13]. Conventional cell growth media such as Dulbecco's Modified Eagle's Medium and RPMI, which are used for the *in vitro* cultivation of NB cells in a culture media provide the necessary ingredients for the continuous proliferation of these cancer cells. These media usually lack metabolites normally found in human fluids. However, substances such as glucose, glutamine or pyruvate are usually contained in higher concentrations in these media[20]. A liquid solution called "stem cell-derived conditioned medium" (CM) is made up of several bioactive substances that are released by stem cells. These substances, which are thought to be involved in tissue regeneration and repair, can include growth factors, cytokines, and extracellular matrix constituents[21,22]. It is possible to obtain the stem cells needed to create the conditioned media from adipose tissue, bone marrow, or umbilical cord blood, among other sources.

Since, they are cultivated in a controlled setting, these stem cells are able to release advantageous chemicals into the medium[22]. ADSC-M is a stem cell culture multiplication medium derived from human adipose tissue and is rich in growth factors such as epithelial growth factor, fibroblast growth factor, transforming growth factor-β1 and bioactive substances such as various cytokines, enzymes and extracellular matrix structural protein. Thus, these factors help to activate the cells by stimulating aging, damaged and inactive cells. A study has shown that ADSCs can secrete various biologically active molecules that affect the microenvironment through a specific paracrine mechanism[23]. To utilize the paracrine effects of ASCs without utilizing the cells themselves, conditioned media is frequently employed in wound healing, regenerative medicine, and other research fields.

The processes of anti-inflammation, tissue healing, angiogenesis, and immunomodulation are thought to be enhanced by the substances produced in the conditioned media[24]. The major goals of ASC's own medium also known as basal medium, are to maintain, develop, and multiply the stem cells produced from adipocytes in culture. It provides the cells with vital nutrition and environmental circumstances they need to survive and operate[25]. Bioactive substances, including growth factors and cytokines, are present in ADScs, whereas basal media comprise vital minerals, vitamins, salts, serum, and supplements. Although ASC's own medium is designed to sustain the cells and guarantee their appropriate development in culture, the conditioned medium is employed to capitalize on the chemicals the cells release, which may exert regenerative or therapeutic benefits[26]. In particular, it has been reported that the conditioned media from ADSC culture can be used as an antioxidant agent for skin to reduce the rate of collagen degradation and repair wounds in animal models[27].

Given its potential therapeutic implications, research on ADSC-M is essential when examining NB. ADSC-M has been shown to affect the behavior of NB cells by stimulating differentiation and preventing proliferation of these cells, which may result in the development of new therapeutic approaches. It has been demonstrated that ADSC-M, especially derived from dedifferentiated fat cells, induces neurite elongation and promotes the production of tubulin and neurofilament in NB cells, indicating its differentiation impact[15]. It has been also observed that the release of certain microRNAs, like miR-124, via exosomes produced from ADSCs might induce differentiation and depress the proliferation of NB cells[28]. Through controlling important biochemical pathways, such as the activity of GABBR1, ADSC-derived extracellular vesicles have been shown to suppress the proliferation of NB cells[29].

Phosphatidylinositol 3-kinase inhibitors in conjunction with ADSC-M greatly improve the inhibition of NB cell viability[15]. On the other hand, although ADSC-M exhibits promise, further research is necessary to completely comprehend its therapeutic efficiency in NB due to the intricacy of tumor microenvironments and possible diversity in responses. Tumor heterogeneity and resistance to therapy are greatly impacted by differences between NB cell lines in terms of stem cell markers. A poor prognosis and aggressive tumor behavior have been associated with linked to varied expression levels of stemness markers in NB[30]. Stem cell markers such as CD133 and CD15 exhibit variable expression levels in different NB cell lines. This variability affects the cancer stem cell (CSC)-like phenotype and identifies possible targets for the treatment of NB[31]. In another study, it was reported that stem cell markers such as CD133, KIT, HUMNF1ISO, GPRC5C and NOTCH1 were expressed at higher levels in malignant stem cells when compared to neuroblastic and substrate-adherent cells and potentially affected NB progression[32]. In a study by Vangipuram et al.[33], the authors demonstrated that in different NB cell lines, varying percentages of CD133+ stem-like cells affect resistance to chemotherapeutic agents. They reported that targeting CD133-expressing cells can improve the efficacy of NB treatment. In a research, they showed that NB cell lines expressed different versions of stem cell markers such CD133, NESTIN, and MSI1, suggesting the existence of a subpopulation that resembles CSCs that may have consequences in the clarification of tumor behavior[34]. In a flow cytometric study performed with MSCs, it has been demonstrated that ADSCs expressed CD90 [glycosylphosphatidylinositol-bound glycoprotein) and CD29 (integrin b1 chain), but not hematopoietic surface markers CD11b and CD45[35]. In this study, while CD90 expression was high in SH-SY5Y and KELLY NB cells, especially in cells treated with ADSC-M, CD45 was not expressed, which correlated with the literature data. In a study in gliomas, ADSC-M treatment led to a significant increase in migration in the wound model compared to the control (DMEM SF)[36]. CD44 is an adhesion protein that plays a role in tumor progression, metastasis and stemness in different cancers. In a study, high CD44 expression was shown to be associated with reduced survival in high-grade human NB, independent of MYCN amplification. It has also been reported that CD44 positive cells may lead to the development of more tumourigenic, metastatic and aggressive neuroblastic tumours with a high frequency after transplantation[37]. In this study, while CD44 expression was abolished by ADSC-M application in KELLY cell line, which can be considered as an indication of poor prognosis, it led to a decrease in its expression in SH-SY5Y cells, suggesting that ADSC-M application should be evaluated in terms of prolongation of survival in N-MYC-expressing NBs with poor prognosis. Stigliani et al. reported that high CD14 expression in primary tumors of high-risk NB patients was predictive of better survival.

They suggested that increased CD14 expression may affect the immune status of the tumor and the natural history of this pediatric cancer[38]. In another study, expression levels of genes representing tumour-associated macrophage infiltrates (CD33, CD16, etc.) were found to be significantly higher in metastatic tumours of young patients (<18 months) compared to tumors of patients aged ≥18 months. This suggests that the inflammatory response and tumor microenvironment may have important effects on the natural history and outcome of NB in certain patient groups[39]. In the present study, the disappearance of CD14 and CD33 expression in KELLY cells with ADSC-M treatment, while it remained at a similar level in SH-SY5Y cells, was consistent with the results of a study performed by Stiglani et al. in patients with NB. These changes in expression levels of CD14 and CD33 may be especially important in terms of their relationship with poor prognosis in NB. Again, the change in CD14 expression indicates that the potential effect of ADSC-M administration should be evaluated in terms of modulation of the immune microenvironment and contribution to prognosis in terms of NB cells with and without N-MYC expression. In a different study, the association of CD34 surface expression (in 92 patients) with NB stage/clinical outcomes was investigated and it

was shown that CD34 positivity in NB may be associated with advanced disease stage and poor prognosis in high-risk patients with N-MYC amplification[40]. In the present study, ADSC-M application eliminated CD34 expression in N-MYC positive cells and decreased it by half in N-MYC negative SH-SY5Y cells, indicating that ADSC-M application should be considered especially in terms of contribution to survival in poor prognostic NB. In a study by Jain et al. in a cohort of 87 NB patients, loss of protein expression of CD73 was associated with poor overall survival and relapse-free survival in high-risk, MYCN-amplified and high-risk non-MYCN-amplified subgroups. Furthermore, overexpression or silencing of CD73 was found to regulate classical cadherins (E-cadherin, N-cadherin, vimentin) during epithelial-mesenchymal transition (EMT), stemness maintenance (Sox2, Nanog, Oct3/4), self-renewal capacity (Notch) and inhibition of differentiation by leukemia inhibitory factor proteins. It has been suggested that loss of CD73 in NB may contribute to the maintenance of activated EMT and stemness and subsequently promote disease progression[41]. In this study, while CD73 expression was abolished in N-MYC positive KELLY cells with ADSC-M application, it decreased by half in SH-SY5Y cells in the absence of N-MYC, indicating that the mechanism of ADSC-M in NB in terms of contribution to the reduction in disease progression should be evaluated. AADSC-M is a good alternative source for stem cells compared to other media. ADSCs can firstly transform into neurospheres, and then into stem cell-like structures[42]. It was determined that these stem cell-like structures can effectively induce the differentiation of SH-SY5Y human NB cells and can also form myelin structure with neuronal neurites[42]. In another study, it was found that MSCs express brain natriuretic factor and β-neuronal growth factor specific to their subpopulations[43]. It was determined that BDNF expression levels of SH-SY5Y NB cell line and co-culture systems formed with MSCs supported development of neuritis by increasing NB cell viability[43]. It was found that human ADSCs can transform into neuronal phenotype and can be positive for GFAP, NeuN[44]. Recently, it was found that olfactory ensheathing cells and B104 NB cells showed neuronal cell, and both progenitor and mature neuronal properties such as nestin, PGP 9.5 and MAP2 after treatment with ADSCs[14].

ADSC environment around NB cells may stimulate the transformation of NB cells into neuronal mature cells by creating a special microenvironment and regulating the immune microenvironment[15,45].

Many signalling pathways are involved in especially embryonic development of NB. Among these, especially the Hippo signalling pathway plays a role in stem cells, CSCs and tumourigenesis[46]. It is suggested that ADSC-M may activate the Hippo pathway at certain stages, leading to inhibition of cell proliferation and promotion of apoptosis; however, the exact mechanisms underlying the interaction between ADSC-M and the Hippo pathway are still under investigation and may vary depending on the specific experimental conditions and cell types involved[47]. We believe that stem cells and associated media have the potential to be used in the treatment of some types of cancer, especially NB. The goal of conventional treatment strategies has been to lower the total tumor load. These techniques, however, frequently ignore the CSCs, a vital group of cells that are in charge of high recurrence rates and drug resistance. Further studies are needed to elucidate this issue.

Research on precision medicine that selectively targets CSCs has been sparked by the shortcomings of conventional therapy. Its scope covers the recognition and targeting of certain surface markers, signaling pathways, and the particular microenvironments in NB that sustain the development of CSCs[48]. High-risk NB patients who undergo stem cell transplantation now have better survival rates thanks to a variety of factors, including more effective treatment modalities, enhanced stem cell sources, tailored approaches, reduced toxicity, integration with targeted therapies, and better long-term care[48]. CSCs which are resistant to treatment with conventional medications, are important targets for novel therapeutic approaches[49]. In the field of regenerative medicine, there is growing awareness regarding capability of stem cell media to act as therapeutic agent solvents. According to the results of a research study by Ackermann et al.[1] on stem cell materials, their compositions, and methods of use a wide range of bioactive components found in stem cell-CM can improve the effectiveness of treatment According to our research, stem cells and similar bioactive material may one day be used for the treatment of various cancer types, particularly NB. Stem cell media may have the potential to be used as a solvent of therapeutic agents so that blastic cells can differentiate into mature cells and contribute to the recovery of the disease.

CONCLUSION

In this study, it has been shown for the first time that exposure to adipocyte-derived cell media leads to changes in stem cell markers that may results in the

improvement in prognosis and prolongation of survival, especially in N-MYC-expressing NB cells. In the light of the results of the study, it is necessary to evaluate more comprehensively whether ADSC environment can be a treatment option in terms of modulation of the immune microenvironment by revealing its favorable effects on molecular and functional changes.

Ethics

Ethics Committee Approval: Not applicable.

Informed Consent: Not applicable.

Footnotes

Author Contributions

Surgical and Medical Practices: S.K.Ö., Z.A., E.S., S.A., P.E., N.O., Concept: S.K.Ö., Z.A., E.S., S.A., P.E., N.O., Design: S.K.Ö., Z.A., E.S., S.A., P.E., N.O., Data Collection or Processing: S.K.Ö., Z.A., E.S., S.A., P.E., N.O., Analysis or Interpretation: S.K.Ö., Z.A., E.S., S.A., P.E., N.O., Literature Search: S.K.Ö., Z.A., E.S., S.A., P.E., N.O., Writing: S.K.Ö., Z.A., E.S., S.A., P.E., N.O.

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Original Article

Challenges in Interpreting Cerebrospinal Fluid Viral Polymerase Chain Reaction Results: Understanding the Results Related to HHV-6, HHV-7, and Enterovirus

Beyin Omurilik Sıvısında Viral Polimeraz Zincir Reaksiyonu Sonuçlarının Yorumlanmasındaki Zorluklar: HHV-6, HHV-7 ve Enterovirüs ile İlgili Sonuçların Anlaşılması

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ABSTRACT

Objective: We have aimed to evaluate our experience in interpreting polymerase chain reaction (PCR) test results of cerebrospinal fluid (CSF) samples for human herpesvirus (HHV)-6, HHV-7, and enterovirus in children with suspected viral meningoencephalitis.

Method: Children aged 1 month to 5 years underwent PCR analyses. Samples were collected via lumbar puncture and assessed using real-time PCR for the identification of enterovirus, HHV-6, and HHV-7.

Results: Most (79.8%) of 109 CSF samples analyzed did not show the presence of any viral particles. Among the positive samples, 8.3% were positive only for HHV-6, 6.4% for HHV-7, and 1.9% for enterovirus. Two samples showed positivity for both HHV-6 and HHV-7; one sample for HHV-7 and enterovirus; and another sample for HHV-6, HHV-7, and enterovirus. Among the PCR-positive patients, fever (77%) and seizures (59%) were the most prevalent presenting symptoms. A statistically significantly higher incidence of seizures was observed in patients with HHV-7 positivity compared to those in whom no virus was detected (p=0.003). At discharge, three patients received alternative diagnoses.

Conclusion: The most frequently detected virus was HHV-6, followed by HHV-7. Enterovirus was detected at a lower frequency than expected, most probably due to the rapid clearance of enterovirus from the CSF and coronavirus disease 2019 mitigation. Considering the possible latency or chromosomal integration (for HHV-6), clinical presentations, CSF findings, and patient-specific additional diagnostic work-up were influential on the decision-making process for diagnosis. In the absence of advanced molecular techniques, it is crucial to recognize that HHV-6 and HHV-7 may be bystanders, and other potential pathogens and diagnoses should be considered.

Keywords: HHV-6, HHV-7, enterovirus, meningoencephalitis

ÖZ

Amaç: Viral meningoensefalit şüphesi olan çocuklarda insan herpes virüsü (HHV)-6, HHV-7 ve enterovirüs açısından beyin omurilik sıvısı polimeraz zincir reaksiyonu (PCR) sonuçlarını yorumlama deneyimimizi değerlendirmeyi amaçladık.

Yöntem: Bir ay ile beş yaş arasındaki çocuklar analiz edildi. Numuneler lomber ponksiyon yoluyla elde edildi ve enterovirüs, HHV-6 ve HHV-7 tespiti için gerçek zamanlı PCR kullanılarak değerlendirildi.

Bulgular: Analiz edilen 109 beyin omurilik sıvısı numunesinin %79,8'inde herhangi bir viral partikül bulunmadı. Pozitif numuneler arasında %8,3'ü sadece HHV-6, %6,4'ü HHV-7 ve %1,9'u enterovirüs pozitifti. İki numunede hem HHV-6 hem HHV-7, bir numunede HHV-7 ve enterovirüs, bir numunede ise HHV-6, HHV-7 ve enterovirüs pozitifliği

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saptandı. PCR pozitif hastalar arasında en sık görülen semptomlar ateş (%77) ve nöbet (%59) idi. HHV-7 pozitif olan hastalarda, hiçbir virüs saptanmayanlara göre istatistiksel olarak daha yüksek nöbet insidansı gözlendi (p=0,003). Üç hastaya meningoensefalit dışında tanılar konuldu.

Sonuç: En sık tespit edilen virüs HHV-6, ardından HHV-7 oldu. Enterovirüs, muhtemelen beyin omurilik sıvısından hızlı bir şekilde temizlenmesi ve koronavirüs hastalığı 2019 önlemleri nedeniyle beklenenden daha düşük sıklıkta tespit edildi. HHV-6 için olası latens veya kromozomal entegrasyon göz önünde bulundurulduğunda, klinik bulgular, beyin omurilik sıvısı bulguları ve hastaya özgü ek tanısal incelemeler tanı sürecinde etkili olmuştur. İleri moleküler tekniklerin bulunmadığı durumlarda, HHV-6 ve HHV-7'nin yalnızca "bystander" virüsler olabileceği ve diğer potansiyel patojenler ve tanıların göz önünde bulundurulması gerektiği unutulmamalıdır.

Anahtar kelimeler: HHV-6, HHV-7, enterovirüs, meningoensefalit

INTRODUCTION

In recent years, the landscape of central nervous system (CNS) infections in children has undergone a significant shift, primarily attributed to the widespread administration of conjugated vaccines. As a result, viruses have emerged as the leading cause of CNS infections in this population[1]. Among viral pathogens, enteroviruses have been identified as responsible for over 75% of the cases with viral meningitis caused by a specific pathogen[2,3]. However, other T-lymphotropic viruses, such as human herpesvirus (HHV)-6 and HHV-7, which exhibit high seroprevalence rates in childhood, are believed to contribute to CNS infections less frequently. Seroprevalence studies have revealed that HHV-6 exhibits a seroprevalence rate of 80% in children older than 2 years, while HHV-7 antibody prevalence rate reaches 75% in children aged between 3 and 6 years[4-7]. Infections caused by enteroviruses, HHV-6, and HHV-7 can present with a range of clinical signs, including fever, rash, and seizures[8-10]. When these viruses infect the CNS, the clinical manifestations can vary widely, spanning from asymptomatic infections to severe cases of encephalitis[10,11]. The analysis of cerebrospinal fluid (CSF) samples using polymerase chain reaction (PCR) tests has indeed facilitated the rapid diagnosis of viral nucleic acids, including HHV-6. However, interpreting the results of these tests to determine whether the infection is acute, latent, or due to chromosomal integration can be challenging[12,13]. The bystander effect refers to the presence of HHV-6 and/or HHV-7 in CSF that does not directly contribute to the emergence of infection. Clinically, the bystander effect complicates diagnosis and treatment, as it becomes challenging to determine whether the detected pathogen is causing an active infection or is merely a result of latency or chromosomal integration. Misinterpreted results may lead to incorrect diagnoses. Therefore, clinical symptoms, laboratory findings, and the patient's immune status must be carefully evaluated to ensure accurate interpretation of test results. Studies have suggested that up to 80% of HHV-6 DNA isolated on multiplex panels of CSF may be clinically irrelevant.[14-16] Similarly, HHV-7 has been considered to cause CNS infections in immunocompromised patients or co-infections with other viruses. However, CNS infections due to HHV-7 in immunocompetent children is considered as rarely seen entities.[17,18] As HHV-7 establishes lifelong latency; it is often difficult to interpret the clinical relevance of HHV-7 detection in the CNS.[17] As a result, the actual frequency of HHV-6 and HHV-7-related diseases remains uncertain.

The use of molecular tests has facilitated the rapid diagnosis of viral meningitis. Nevertheless, challenges have arisen in interpreting molecular test results about both prevalent and rarely seen viral CNS pathogens. In this study, we aimed to review our experience with in-house PCR to detect HHV-6, HHV-7, and enteroviruses in children younger than 5 years old with suspected viral meningoencephalitis.

MATERIALS and METHODS

Study Design and Population

This prospective study was conducted at a tertiary care children's hospital, a referral center for pediatric patients, between December 28, 2019, and March 31, 2022.

A total of 110 children aged 1 month to 5 years with suspected CNS infection were included in the study. The diagnostic inclusion criteria for viral meningoencephalitis were: nausea, vomiting, neck stiffness or bulging fontanelle, seizures, irritability, and altered mental status, which are commonly observed in the clinical course of such infections.[19] The patients with bacterial growth in their conventional CSF cultures were excluded from the study.

CSF samples obtained through lumbar puncture were analyzed using real-time PCR test to detect enteroviruses, HHV-6, and HHV-7. The following variables were recorded: age, gender, presenting symptoms, CSF white blood cell counts, protein, and glucose levels, PCR test results, and discharge diagnosis of the patients. CSF samples with >5 white blood cell (WBC)/μL were

considered pleocytic. Protein values under 45 mg/dL and glucose above 50 mg/dL were considered normal[19].

Detection of the Viral Nucleic Acid

Viral nucleic acid extraction from 200 µL of each CSF sample was performed using the spin column extraction method, resulting in an average yield of approximately 4-6 µg nucleic acid per sample.

The detection of enterovirus, HHV-6, and HHV-7 was performed using kits (Procomcure Biotech PhoenixDX®, Thalgua, Austria) containing primers targeting the DNA and RNA sequences of these viruses. Real-time PCR tests were performed, and virus detection method was verified using signals from the Fluorescein amidite (FAM) channels to detect HHV-6 and HHV-7 DNA, while Cy5, and Rhodamine X (ROX) probes were used to detect the PCR positive control, and passive reference (if required) for HHV-6 and HHV-7, respectively. The three-step process included 1 cycle at 94 °C for 5 minutes, followed by 45 cycles at 94 °C for 15 seconds and at 55 °C for 70 seconds. A comparison of the signals obtained with positive controls allowed for the identification of PCR-positive and negative samples. The analytical sensitivity of the kit is 95%, and 40 copies can be detected at each reaction.

Enterovirus RNA was detected with the signals from the FAM channel (for enterovirus enterovirus RNA), from the Hexachlorofluorescein VIC channel (for human extraction control), and from the ROX channel (for passive reference, if requested). The four-step process included 2 cycles at 50 °C for 5 and at 95 °C for 5 minutes, followed by 45 cycles at 95 °C for 15 and at 62 °C for 1 minute.

Statistical Analysis

Statistical analysis for this study was performed with IBM SPSS Statistics 22 (Chicago, IL, USA). Continuous variables are presented as medians, and ranges varying between minimum and maximum values. Categorical variables are presented as frequencies and percentages, and compared using Pearson's χ^2 and Fisher's exact tests (Table 1). A p-value of <0.05 was considered to be statistically significant.

Ethics approval for the study was obtained from the Institutional Review Board of University of Health Sciences Turkey, Dr. Behçet Uz Children's Hospital (approval number: 2019/364, dated: 19.12.2019).

Informed consent for this study was obtained from the parents of the patients.

RESULTS

A total of 110 CSF samples were obtained from 110 patients. Unfortunately one of these samples that did not contain sufficient volume of CSF sample for testing was excluded from the statistical analysis. As a result, a total of 109 patients were included in the study, with a median age of 8.5 months (range: 1-60 months). Study population consisted of 44 (40%) female, and 66 male (60%) patients. With the exception of Patient 7 with DiGeorge syndrome, none of the patients had a previously known immunodeficiency.

CSF samples of 109 patients were analyzed, and 79.8% (n=87) did not show the presence of any viral particles. CSF samples were reverse transcription-PCR positive only for HHV-7 in 9 (8.3%) for HHV-6 in 9 (8.3%), for HHV-7 in 7 (6.4%), enterovirus in 2 (1.9%), for both HHV-6 and HHV-7 in 2, HHV-7 and enterovirus in 1, and HHV-6, HHV-7, and enterovirus in 1 patient. No bacterial growth was observed in the conventional CSF cultures.

Table 1 illustrates the demographic data, clinical features, laboratory findings, and discharge diagnoses of 22 patients with at least one positive viral particle in their CSF samples. Among the PCR-positive patients, fever (77%, 17/22) and seizures (59%, 13/22) were the most prevalent presenting symptoms. Notably, 27% (6/22) of patients exhibited significantly higher WBC counts in their CSF samples indicative of meningoencephalitis, with the exception of 2 patients with bloody CSF samples. Furthermore, 41% (9/22) of patients demonstrated elevated protein levels, and 14% (3/22) displayed decreased glucose levels. At discharge, three patients received alternative diagnoses. No antiviral treatment was used in any of the patients for the treatment of HHV-6, HHV-7, and enterovirus detected in the CSF.

Our study could not reveal any statistically significant difference in terms of the incidence rates of altered mental status, fever, and seizure between patients with and without at least one positive HHV-6 or HHV-7 identified in their CSF samples. Similar symptom frequencies were noted in patients with only HHV-6 positivity and those without detectable viral particles. Nonetheless, a statistically higher incidence of seizures was observed in patients with HHV-7 positivity compared to those without (p=0.003).

No antiviral treatment was used in any of the patients for the treatment of infections caused by HHV-6, HHV-7, and enterovirus detected in the CSF samples. However, all patients who tested positive for influenza A/B virus via the respiratory viral panel received oseltamivir treatment.

Table 1. Demographics, clinical characteristics, laboratory results and discharge diagnoses of the patients in whom at least one viral particle was identified in CSF samples

Patient number	Age (month)	Gender (M/F)	Presenting symptoms	WBC/ mm^3	Protein (mg/dL)	Glucose (mg/dL)	Positive for HHV-6	Positive for HHV-7	Positive for enterovirus	Discharge diagnoses
1	60	M	Seizure	0	23.6	83	Yes	No	No	Influenza A + *Bordetella pertussis*
2	19	F	Fever, seizure	0	25.1	60	No	Yes	No	Influenza B
3	2	M	Fever	0	48	47	Yes	No	No	Influenza A
4	11	F	Fever, seizure	0	27.5	63	Yes	Yes	No	Meningitis
5	2	M	Fever	0	76.4	52	No	No	Yes	Meningitis + myocarditis
6	24	F	Seizure	50	20.9	46	No	Yes	No	Meningitis
7	5	M	Fever, seizure	0	37.5	61	Yes	Yes	No	Meningitis,
8	3	M	Fever	30	46.6	60	No	Yes	Yes	Meningitis + Kawasaki syndrome
9	60	F	Fever, altered mental status	BloodyLP	31.6	79	No	Yes	No	Encephalitis
10	19	F	Fever, seizure	0	43.8	70	No	Yes	No	Meningitis
11	1	F	Fever, seizure	0	132.5	50	Yes	Yes	Yes	Meningitis
12	7	F	Fever, seizure	10	23.6	62	No	Yes	No	Meningitis
13	13	M	Fever, seizure	>500	77.9	46	No	Yes	No	Meningitis
14	60	M	Seizure, altered mental status	0	317	74	Yes	No	No	Encephalitis
15	21	M	Fever, seizure	0	4	93	No	Yes	No	Meningitis
16	1	F	Seizure	0	26.9	57	Yes	No	No	Meningitis
17	2	M	Fever	0	41.3	51	Yes	No	No	Meningitis
18	10	F	Fever	>500	55.3	53	Yes	No	No	Meningitis
19	4	M	Fever	10	14.6	68	Yes	No	No	Meningitis
20	10	M	Fever, petechial rash	0	45.4	52	Yes	No	No	Meningitis
21	18	M	Seizure	0	28.5	55	No	No	Yes	Meningitis
22	2	M	Fever	Bloody LP	153.8	54	Yes	No	No	Meningitis

CSF: Cerebrospinal fluid, HHV-6: Human herpes virus-6, HHV-7: Human herpes virus-7, WBC: White blood cell, LP: Lumbar puncture

DISCUSSION

In this prospective study conducted over 15 months in children under 5 years of age with suspected CNS infection, most frequently HHV-6 followed by HHV-7 were identified in CSF samples. However, enterovirus was detected at a lower frequency than expected. Concomitantly, three viruses were also identified. HHV-6 and enterovirus were not specifically associated with any particular symptoms, but in patients with HHV-7 infection, seizures were significantly more frequent. Clinical presentations, CSF findings, and patient-specific additional diagnostic work-up were influential in the diagnostic decision-making process.

Identifying HHV-6 DNA in CSF samples could indicate primary infection, latency, reactivation, or chromosomally integrated HHV-6 (ciHHV-6) complicates interpretation of the results obtained[14]. Immunohistochemical analysis of nasopharyngeal swabs of our solely HHV-6-positive patients revealed concomitant influenza A virus and *Bordetella pertussis* positivities in Patient 1, and influenza A positivity in Patient 3 possibly associated with symptoms which made us to consider the plausibility of latency or chromosomal integration. Moreover, Sugaya et al.[20] reported that certain instances of influenza-related encephalopathy might result from a co-infection with influenza virus and HHV-6, HHV-7, or both. Additionally, an alternate scenario was the reactivation of latent HHV-6 or HHV-7 virus in the brain by influenza virus, leading to encephalopathy or febrile convulsions[20]. Although based on their symptoms, and results of immunohistochemical examination of their CSF samples, Patients 14 and 18 likely had HHV-6 as the causative agent, normal CSF biochemistry and the absence of cells in direct CSF examination in Patients 16, 17, and 20 led us to consider the presence of a causative culpritt microorganism as a lower possibility. However, CSF abnormalities are rarely reported in patients with HHV-6 infection[21,22]. Pleocytosis may be absent or minimal during primary HHV-6 infection of the central nervous system[23]. Since HHV-6 meningitis was observed at postnatal 1 and 2 months as in Patients 16, and 17, the possibility of congenital infection was entertained. Finally, if lumbar puncture specimen is contaminated with blood as in Patient 22, the source of HHV-6 positivity could not be definitively assessed.

Limited data exists on HHV-7 infections involving CNS. A systematic review indicated that the prevailing symptoms commonly include headache and fever, with documented cases of rash and seizures[24]. Furthermore, analysis of spinal tap specimens typically reveals an elevated cell count with lymphocytic predominance and normal to slightly elevated protein levels, aligning with patterns observed in other viral CNS infections[24]. In our study, several cases (Patients 6, 12, and 13) were suspected of having an acute HHV-7 infection based on presenting symptoms and/or analysis of CSF specimens. Despite the presence of symptomatic meningitis, analysis of CSF samples may not reveal evidence of an alternative causative agent as in Patients 10 and 15. As in Patient 9, despite the potential contamination of spinal tap specimen with peripheral blood the altered mental status was considered indicative of HHV-7 encephalitis.

The simultaneous detection of both HHV-7 and influenza B virus in Patient 2 implies the possibility of coinfection or reactivation of HHV-7 induced by influenza B.

Co-detection of multiple viruses in CSF samples sometimes makes the determination of the causative agent more challenging. HHV-6 and HHV-7 might represent bystanders; however, it is established that enterovirus induces acute infection, does not persist latently, and undergoes rapid elimination by the immune system akin to other RNA viruses.[25,26] Hence, as in our study, in instances where HHV-6 and/or HHV-7 are concurrently detected with enterovirus, the causative agent is deemed to be the enterovirus, considering the patient's clinical characteristics. Nevertheless, the possibility of coinfection could not be ruled out. Notably, Patient 8, testing positive for HHV-7 and enterovirus, received the diagnosis of Kawasaki disease during the hospitalization. It has been reported in the literature that both HHV-6 and HHV-7 may reactivate in Kawasaki patients and aggravate symptoms of Kawasaki disease[27]. In addition, Kawasaki disease triggered by enterovirus or Kawasaki disease concurrent with enteroviral infection can also be seen[28].

In our study, the detection rate of enterovirus, recognized as the most prevalent cause of aseptic meningitis in childhood, was lower than anticipated. It has been reported that enterovirus is swiftly eliminated from the CSF, resulting in decreased viral load and lower rates of positive detection in the CSF samples among patients with symptoms lasting over 2 days[29]. We did not consider duration of symptoms as one of the study parameters. Consequently, it is plausible that enterovirus may not have been detected at the expected frequency. On the other hand, since the study was carried out during the coronavirus disease 2019 (COVID-19) pandemic

period, it was hypothesized that there may have been a decline in the frequency of enterovirus, like some other viruses, due to the effect of the mitigation strategies. Sun et al.[30] investigated the impact of non-pharmaceutical interventions on enterovirus infections in children in Hangzhou, China, and found a significant decrease in enterovirus-positive cases during the pre-COVID-19 and COVID-19 pandemic, with a gradual increase observed after the relaxation of nonpharmaceutical interventions in 2023. Their findings support our hypothesis.

Study Limitations

Our study has certain limitations. Firstly, no serologic confirmation was conducted on peripheral blood samples. Although detection of maternal antibody in young infants was observed, immunoglobulin detection in older children could provide insight into acute infection. Additionally, quantitative real-time PCR tests in serum or plasma samples were not performed. It has been noted that individuals with ciHHV-6 exhibit a persistent presence of HHV-6 DNA in their serum or plasma, indicating that identification of viral DNA alone is inadequate for diagnosing active infection. Moreover, it has been proposed that whole blood HHV-6 DNA viral loads exceeding >5.5 log 10 copies of HHV-6 DNA per mL strongly indicate the presence of ciHHV-6[31]. Due to concerns of cost-effectiveness, access to these tests is limited in many centers across Turkey. The second limitation of our study was its small patient sample size.

CONCLUSION

Our study holds importance in assessing CSF HHV-6, HHV-7, and enterovirus positivity without utilizing advanced molecular or serological tests. When evaluating the clinical significance of HHV-6 and HHV-7 positivity, it is necessary to assess the symptoms, immune status, and CSF characteristics of the patients in combination. In the absence of advanced molecular techniques, it is crucial to recognize that HHV-6 and HHV-7 may be bystanders, and other potential pathogens and diagnoses should not be discounted.

Ethics

Ethics Committee Approval: Ethics approval for the study was obtained from the Institutional Review Board of University of Health Sciences Turkey, Dr. Behçet Uz Children's Hospital (approval number: 2019/364, dated: 19.12.2019).

Informed Consent: Informed consent for this study was obtained from the parents of the patients.

Footnotes

Author Contributions

Concept: İ.D., N.B., Design: E.B., İ.D., N.B., Data Collection or Processing: E.B., E.K., Ş.Ş., A.A.K., K.Ö.O., H.A., F.Y.A., D.Z., S.K., S.T., Analysis or Interpretation: E.B., İ.D., E.K., F.Y.A., D.Z., S.K., Literature Search: E.B., İ.D., E.K., Ş.Ş., A.A.K., K.Ö.O., H.A., S.T., Writing: E.B., İ.D., N.B.

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Original Article

Root Cause Analysis of Patient Safety Incidents in Pediatric Anesthesia and Consequences of the Second Victim Phenomenon

Pediatrik Anestezide Hasta Güvenliği Olaylarının Kök Neden Analizi ve İkinci Mağdur Fenomeninin Sonuçları

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ABSTRACT

Objective: This study investigates the root causes of patient safety incidents (PSIs), and the ensuing second victim phenomenon (SVP) among healthcare professionals involved in pediatric anesthesia. It also aims to analyze the impact of adverse events on healthcare professionals providing pediatric anesthesia and identify underlying factors contributing to these incidents.

Method: Using qualitative analysis, focus group discussions were conducted with twelve pediatric anesthesiologists. Thematic analysis was applied to the discussions using MAXQDA22 software, focusing on identifying root causes and consequences of SVP specific to pediatric anesthesia.

Results: The analysis revealed six primary root causes of PSI leading to SVP in pediatric anesthesia settings: individual, systemic, medical, administrative factors, legal responsibilities, and violence. The adverse consequences of SVP for healthcare professionals included psychological distress, self-doubt, and burnout, affecting both personal well-being and national healthcare outcomes because of unnecessary treatments and tests.

Conclusion: The study highlights the complex interplay of factors leading to PSI and SVP, underscoring the need for comprehensive strategies to address these root causes. Improving patient safety culture and supporting affected healthcare professionals are crucial for enhancing healthcare quality and safety on a global scale.

Keywords: Health and safety, quality in healthcare, qualitative research, quality improvement, second victim phenomenon

ÖZ

Amaç: Bu çalışma, pediatrik anestezi bağlamında hasta güvenliği olgularının (HGV) kök nedenlerini ve buna bağlı olarak sağlık çalışanları arasında ortaya çıkan ikinci mağdur fenomenini (İMF) araştırmaktadır. Çalışmanın amacı, pediatrik hastalarda advers olayların sağlık çalışanları üzerindeki etkisini analiz etmek ve bu olaylara katkıda bulunan temel faktörleri belirlemektir.

Yöntem: Çalışmada nitel araştırma yöntemi kullanılmış olup, pediatrik anestezi alanında çalışan on iki uzman doktor ile odak grup görüşmeleri gerçekleştirilmiştir. Görüşmeler, MAXQDA22 yazılımı kullanılarak tematik analiz yöntemi ile incelenmiş ve pediatrik anesteziye özgü HGV'nin kök nedenleri ile İMF'nin sonuçları belirlenmiştir.

Bulgular: Analiz sonucunda, pediatrik anestezi ortamında HGV'ye neden olan altı ana kök neden belirlenmiştir: Bireysel faktörler, sistemsel faktörler, tıbbi faktörler, idari faktörler, hukuki sorumluluklar ve şiddet olayları. Sağlık çalışanları için sonuçlar ise psikolojik stres, özgüven kaybı ve tükenmişlik olup, bu durum bireysel refahı etkilediği gibi, gereksiz tetkik ve tedaviler yoluyla ulusal sağlık sistemine de olumsuz yansımaktadır.

Sonuç: Çalışma, HGO ve İMF'nin ortaya çıkışındaki karmaşık etkileşimleri ortaya koyarak, bu kök nedenleri ele almak için kapsamlı stratejilere duyulan ihtiyacı vurgulamaktadır. Hasta güvenliği kültürünün geliştirilmesi ve etkilenen sağlık çalışanlarının desteklenmesi, küresel ölçekte sağlık hizmetlerinin kalitesini ve güvenliğini artırmak için kritik öneme sahiptir.

Anahtar kelimeler: Hasta güvenliği, sağlıkta kalite, nitel araştırma, kalite iyileştirme, ikinci mağdur fenomeni

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INTRODUCTION

Patient safety (PS) is a comprehensive concept involving the assessment, prevention, and management of potential risks that could negatively impact patients' health during the delivery of healthcare services. PS incidents (PSIs) refer to adverse events such as medical errors, side effects of treatments, and communication gaps that may occur among healthcare institutions and professionals. These incidents typically encompass various components of healthcare services, including medical interventions, medication administrations, surgical procedures, and patient communication[1].

PSIs may increase the likelihood of patients encountering unexpected and undesirable outcomes, exerting serious adverse effects on both healthcare professionals and patients. Recognizing, and effectively managing PSIs is critically important for improving the quality of healthcare systems and ensuring patients' trust. PSIs and near-miss events frequently occur in intensive care units (ICUs). PS holds particular importance in these units, where mortality rates are already high[2].

Second victim phenomenon (SVP) is used to describe the experiences of emotionally distressed healthcare professionals as a result of being involved in an event adversely affecting PS[3]. Professionals who experience SVP often fear legal proceedings arising from harm to the patient and damage to their professional reputation. They feel primarily responsible in such situations, doubting their clinical skills and knowledge[4]. Professionals exhibit not only emotional responses such as guilt, shame, anger, and fear but also display physical reactions such as insomnia and extreme fatigue[5].

Root cause analysis is a methodology used to understand the fundamental issues affecting a problem or an event and the relationships among these causes. This analysis aims not only to identify the apparent causes of a specific event but also to uncover the underlying root causes. Root cause analysis for PSI can contribute to understanding errors, deficiencies, and risks in healthcare systems, thereby helping to develop strategies to prevent similar PSIs in the future[6].

The primary aim of this study is to systematically analyze the root causes of SVP among pediatric anesthesiologists following occurrence of a PSI and to understand the factors that contribute to these adverse events. Root cause analysis reveals not only the surface causes of events but also the underlying systemic, organizational, or individual factors that lead to emergence of these events which make efforts to improve PS more effective by assisting healthcare providers and managers in identifying and resolving real underlying problems[7].

Root cause analysis can be used as a focal point for quality improvement efforts in the field of PS, guiding strategies to prevent the recurrence of errors in pediatric anesthesia and improve overall PS. This research is conducted to learn about the root cause analysis of unwanted incidents happening in pediatric anesthesiology and to understand the consequences of these incidents, leading to SVP.

MATERIALS and METHODS

In this study, a qualitative research approach was employed to understand the root causes of SVP following PSIs. Focus group discussions with twelve pediatric anesthesiologists were conducted to gain in-depth insights and gather various perspectives. The interviews were organized in three separate sessions, with each session consisting of a group of four participants. This grouping was designed to increase the rate of volunteer participation and ensure that participants felt comfortable during the interviews. Doctors actively serving in the field of pediatric anesthesia who volunteered to participate in the study were interviewed. Anesthesiologists who did not volunteer to participate and those not actively practicing their profession for the time being (being retired or on sick leave, etc.) were not included in the survey. Participants were selected using purposive sampling with the snowball method. Purposive sampling allows for in-depth research based on the objectives of the study[8]. The participants were convened under the moderation of a facilitator, and the data were transcribed by a rapporteur. The sessions of focus group discussions took 3.5 hours and data saturation was achieved. Transcripts were returned to participants for their comments.

The analysis of the focus group discussions was conducted using MAXQDA22 qualitative software (Verbi Gmbh, Berlin, Germany). This software provides researchers with the opportunity to systematically and effectively analyze qualitative data. The opinions of the pediatric anesthesiologists were analyzed using thematic analysis, and the emerging themes were systematically categorized using the MAXQDA22 qualitative software. The names of all participants were anonymized. Through the use of computer-assisted qualitative data analysis software, detailed observations and in-depth

descriptions were performed while adhering to the criteria for ethical conduct, validity, and reliability of the research, also ensuring transparency. The report of this research study was prepared in accordance with the consolidated criteria for reporting qualitative research criteria[9].

The focus group discussions used in the study were designed to explore the observations, experiences, and opinions of healthcare professionals in depth. This method allowed for a more comprehensive understanding of pediatric anesthesiologists' perceptions and experiences regarding PSI. As a result, this research aims to understand the root causes of PSI from the perspective of pediatric anesthesiologists using qualitative data collection and analysis methods. This approach can be considered as an important step in identifying and improving safety gaps in healthcare systems.

The study was conducted in accordance with the World Medical Association Declaration of Helsinki Ethical Principles for Medical Research Involving Human Participants and approved by the Ethics Committee of İzmir Democracy University (approval number: 2024/01-9, dated: 31.01.2024).

RESULTS

The study was conducted with seven female and five male volunteer pediatric anesthesiologists, all serving in the department of pediatric anesthesia. Only six participant anesthesiologists had experienced SVP. The average duration of clinical experience of all participants in anesthesia was 19.9 (±8.7) years. However, there was a statistically significant difference in the duration of clinical experience as anesthesiologist between those who had and had not experienced SVP (25.8±6.3 vs. 14.0±6.5 years; p=0.01). The demographic characteristics of the participants are presented in Table 1.

When examining the data obtained from focus group discussions conducted with healthcare professionals regarding their experiences of SVP following PSI, six themes emerged as root causes which can be classified as individual, systemic, medical and administrative factors, legal responsibilities, and incidents of violence.

Upon examining the consequences of SVP arising from PSI, we have observed that PSIs may be categorised into two main headings. The first one concerns with the effects of SVP on pediatric anesthesiologists, while the second one pertains to the broader i.e. nationwide implications of SVP (Table 2).

Main Theme I. Root Causes of the SVP Following PSI

The data obtained regarding the root causes of PSI leading to SVP were examined within the scope of healthcare workers' experiences as second victims. In this context, issues stemming from both individual and systemic factors, the impact of errors made by other staff members, the effects of patients' medical conditions, the turnover of personnel, and incidents of violence have emerged prominently, as illustrated in Figure 1.

Table 1. Demographic data of the study participants

Participants	Focus group number	Gender	Hospital units	Age (years)	Clinical experience (years)	SVP experience
1	1	Male	Operating room	42	12	No
2	1	Male	Critical care	36	6	No
3	1	Female	Critical care	48	15	Yes
4	1	Female	Operating room	48	19	No
5	2	Female	Critical care	55	24	Yes
6	2	Female	Critical care	58	27	Yes
7	2	Female	Critical care	61	31	Yes
8	2	Male	Critical care	56	25	Yes
9	3	Male	Operating room	45	16	No
10	3	Female	Critical care	62	33	Yes
11	3	Female	Operating room	53	23	No
12	3	Male	Operating room	39	8	No

SVP: Second victim phenomenon

Subtheme I. Individual Factors

Individual factors include; public ignorance and decreasing respect for the medical profession, burnout syndrome among healthcare personnel, and the consequences of preventive medicine. Most participants indicated that the primary underlying factors of the second victim incident (SVI) were public ignorance, doctors engaging in non-medical pursuits, and lack of time for reading, and emphasized their efforts to enhance their knowledge on preventive medicine.

• "Sometimes, due to the ignorance of the public." (Participant 1)

• "Physicians now engage in pursuits outside the medical profession and personal development, leading to the stagnation and failure to renew their medical knowledge. The burnout syndrome we are experiencing can sometimes push us into emptiness at the patient's bedside." (Participant 6)

• "There are opportunities where we can improve our knowledge of preventive medicine." (Participant 9)

• "Not being able to find time to read." (Participant 10)

Table 2. Main themes and subthemes of the analysis

Main themes	Subthemes
I. Root causes of the SVP following patient safety incidents	Individual factors Systemic factors Medical factors Administrative factors Legal responsibilities Incidents of violence
II. Consequences of the SVP following patient safety incidents	Consequences of the SVP on health care professionals Nationwide consequences of the SVP

SVP: Second Victim Phenomenon

Subtheme II. Systemic Factors

Systemic factors are underscored by a disrupted educational continuum. Participants detailed encountering complications rooted in these systemic issues, highlighting a shift where doctors increasingly divert their focus to activities beyond their medical practice, leading to a stagnation or decline in their basic professional knowledge.

• "But indirectly, complications occur because of the system, meaning that sometimes due to the problems in nursing care, the patient is not properly monitored, and some situations are overlooked. Generally, I don't blame myself, I blame the system." (Participant 5)

• "I think the broken education chain of doctors (starting with congresses) has been effective here for a long time. Physicians now engage in pursuits outside the medical profession and personal development, leading to the stagnation and failure to renew their medical knowledge." (Participant 8)

Subtheme III. Medical Factors

The impact of patients' medical conditions emerged prominently. One participant emphasized that acute medical conditions experienced by patients have a significant negative impact.

• "What affects me the most are the acute medical conditions experienced by patients; which make me feel bad. I have a lot of trouble when it comes to the patient. For example, we, the anesthesiologist, surgeon, nurse, and the whole team, were sued for cautery burns of a patient. This incident affected us so deeply that when a pediatric orthopedic patient with a slightly blurred consciousness transported to the operating room for anesthesia the other day, we wrapped all the iron parts of the arm splints, fearing that the child might involuntarily touch iron parts and get burned. So, that lawsuit affected us so much that we felt a strong obligation to take

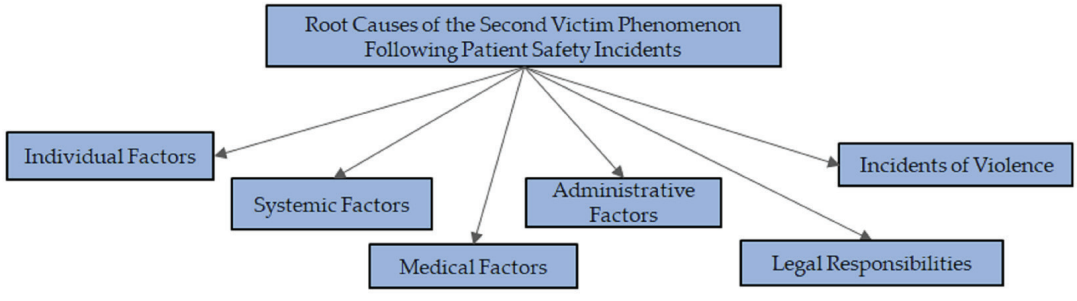

Figure 1. Root causes of the second victim incidents (MAXQDA 22-Hierarchical Code-Subcode Model)

very strict precautions in case a patient with impaired consciousness touches somewhere and gets burned. But these bad experiences do not prevent us from doing what needs to be done, of course, we reperformed the necessary procedure." (Participant 7)

Subtheme IV. Administrative Factors

Personnel turnover significantly affected the situation. A male participant stated that he rarely questioned himself much about patient's cause of death amidst high patient mortality rates, feeling unaccountable for outcomes like cardiac arrest due to medication errors made by the staff.

• "Most of the patients admitted to us have very high mortality rates. I don't question myself much about why the patient died or what more could I have done better. Few patients could benefit. Even in these fatalities, thankfully, we can't say that we did this major malpractice and lost the patient. What I've seen, of course, I can't blame myself for that either. For example, if I prescribe this medication to the patient, and the nurse mixes up the medications and, in the meantime, the patient who received the wrong medication goes into cardiac arrest, then I can't really blame myself as the primary culprit in that case. I don't think about whether I should have administered the medication myself. I don't want to think about the medication errors made by the auxiliary healthcare personnel either." (Participant 2)

• "Especially during the pandemic period, there was a high turnover of auxiliary staff. They were working without adapting themselves to the unit they were working in. Apart from the incidents that occurred during that period, I also think that the training of auxiliary healthcare personnel needs to be improved." (Participant 11)

Subtheme V. Legal Responsibilities

Errors made by colleagues notably influenced participants. One participant shared the idea of "how others' mistakes also affected them", prompting a sense of responsibility and efforts to understand the underlying cause.

• "The mistake made by someone else also affects you. A mistake made by a nurse, a technician, or a staff member can cause harm to the patient. In such situations, we try to solve it because we feel responsible, and at the same time, we try to understand why it happened. We experience intense distress in the meantime."(Participant 4)

Subtheme VI. Incidents of Violence

Incidents of violence stood out significantly. Within this context, participants articulated their feelings that these events were out of their control, acknowledging that mistakes could lead to violence, which profoundly affected them even to the extent of undermining their willingness to go to the hospital.

• "When I feel verbal or physical violence, the feeling I experience weighs heavier, it sticks with you, even if I have not been directly subjected to verbal or physical violence. But the thought of inducing adverse incidents during the procedure due to an intervention performed by myself is of course a bad feeling." (Participant 7)

• "Verbal abuse and physical assaults affect me more deeply." (Participant 8)

• "Verbal abuse affects me more profoundly. We do not engage in interventions that deliberately have a negative impact on patients. Medical errors happen. Of course, we are extremely careful to avoid adverse events, but drug interactions are not within our control. Of course, we feel sad, we wish it hadn't happened, but when I experience verbal or physical abuse after putting in so much effort, I immediately become disillusioned with the profession. I don't even want to come to the hospital, to be honest." (Participant 10)

Main Theme II. Consequences of SVP Following PSI

The consequences of SVP emerged prominently, both in terms of its nationwide effects and its effects on healthcare personnel, as depicted in Figure 2.

Subtheme VII. Consequences of SVP on Healthcare Professionals

When examining the data on the consequences of SVP on specialist doctors, most participants expressed experiencing psychological issues and self-doubt.

• "I'm currently trying to monitor patients non-invasively. Indeed, there were times when I questioned myself during invasive interventions." (Participant 1)

• "Of course, sometimes we question what we're doing. I'm a very questioning person. While I do this questioning every 10 patients, someone with a very relaxed character may not even feel that concern, even if they make a mistake. I think it's somewhat of a personal matter." (Participant 5)

• "You know, maybe we don't leave much room for error for ourselves here, but if we were to expose the patient to the same thing the second time, maybe I could see myself as experiencing SVP." (Participant 2)

• "I blamed myself a bit on that." (Participant 6)

• "From the first patient onwards, your morale is down. Then you examine 50 more patients working without a secretary during these processes. You start questioning why you became a doctor. I think doctors are the ones who suffer the most in these kinds of discussions." (Participant 7)

However, participants also expressed feelings of anxiety, unrest, and burnout as a result of experiencing SVP:

• "I feel such anxiety when I see a patient with a circulatory disorder due to a bad experience I had during my residency. It's not easy to shake it off." (Participant 8)

• "You feel restless because you've done too much." (Participant 10)

• "The burnout syndrome we're in can sometimes push us into a void at the bedside." (Participant 11)

Ultimately, pediatric anesthesiologists highlighted that the SVP leads to the formation of a discontented cohort, underscoring the systemic roots of this distressing condition:

• "Doctors who were equipped priorly with essential medical knowledge, and also renewed themselves and thought only about the patient and medical issues are extinct. There's a completely different group now, dealing with various issues like medical secretarial work, performance anxiety, burnout syndrome, and pondering over different cases." (Participant 2)

• Of course, this unhappy group expresses its feelings towards the problem of victimization in two ways as exemplified below:

• Yes, I admit that I have shortcomings concerning this issue." (Participant 7) or

• "There is a faulty system that drives me to experience these things, so I don't criticize myself at all, in fact, I am justified, I am not at fault at all. There is a system that hinders my development, that demands irrelevant things from me." (Participant 8)

Subtheme VIII. Nationwide Consequences of the SVP

A study participant noted that to prevent SVP, extra treatments and unnecessary tests are sometimes conducted to preclude negative responses from patients' families:

• "There are situations where excessive treatment is administered, which also burdens the country's economy." (Participant 9)

• "Sometimes, unnecessary tests are ordered, and antibiotics are prescribed to avoid reactions from patients' families." (Participant 11)

World Cloud Analysis

A word cloud was generated to visually represent the frequency and distribution of key terms identified during the focus group analysis (Figure 3). This visualization highlights the most frequently mentioned terms, as detailed in Table 3, and their impact on professional experiences and systemic issues in healthcare. The top 15 words, listed in descending order of frequency, emphasize the primary stressors and challenges faced by healthcare providers, with keywords "patient," "care," and "safety" leading the list.

DISCUSSION

The root cause analysis employed in this survey elucidates the multifaceted experiences pediatric anesthesiologists experiencing PSI face. It categorizes

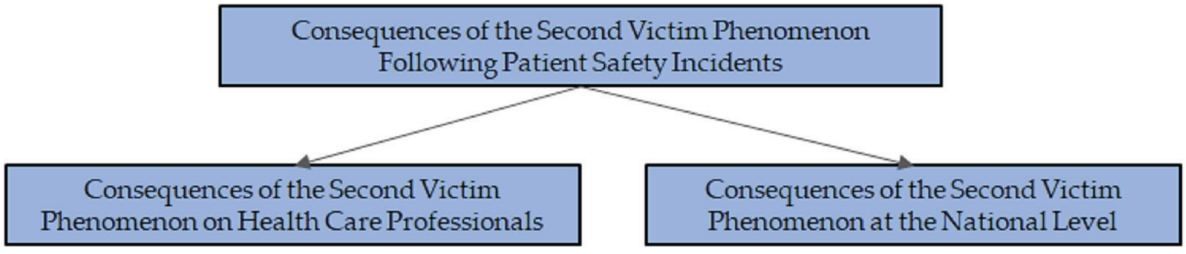

Figure 2. Consequences of the SVP (MAXQDA 22-Hierarchical Code-Subcode Model)

SVP: Second Victim Phenomenon

Figure 3. Word cloud depicting the most frequently used terms in focus group discussion

Table 3. The top 15 words, frequency counts, and percentages within the top words subsets

Order of frequency	Words	Frequency counts	Top 15 words (%)
1	Patient	20	13.2
2	Care	18	11.8
3	Safety	12	7.9
4	Affect	11	7.2
5	Lack	11	7.2
6	Mistake	10	6.6
7	Adverse	9	5.9
8	Victim	9	5.9
9	Feel	8	5.3
10	System	8	5.3
11	Error	8	5.3
12	Issue	7	4.6
13	Violence	7	4.6
14	Impact	7	4.6
15	Legal	7	4.6

the underlying factors into six distinct themes for comprehensive understanding. Individual factors encapsulate professionals' competencies, experiences, and communication skills, highlighting the personal dimension of PSI. Systemic factors reveal organizational and structural flaws within healthcare systems, pointing to broader institutional issues. Medical reasons include clinical factors such as medical errors, malpractices, diagnostic shortcomings, and treatment planning intricacies. Administrative factors are related to management policies and procedures. Legal responsibility underlines the significant influence of regulatory practices on PS, whereas incidents of violence draw attention to threats endangering workers' physical and emotional well-being.

The most frequently used terms according to the word cloud analysis emphasize the central role of patient care and safety in discussions about SVP and the influence of systemic factors such as "lack" and "support" on healthcare outcomes. The distribution of these words reflects that healthcare professionals see patient-centered care and safety as paramount issues, but systemic deficiencies, personal stress, and legal pressures create barriers to fulfilling these duties effectively. This interpretation suggests a need for focused interventions that address both external (systemic support) and internal (psychological well-being) dimensions to foster a safer and more supportive work environment for healthcare providers.

The results have shown that more experienced anesthesiologists were significantly more likely to have encountered SVP in their professional careers. With increased years in practice, they more frequently experience PSIs, making seasoned professionals more vulnerable to SVP. Anesthesiologists assuming

leadership roles often bear greater responsibility for patient outcomes, experiencing their feelings of self-blame and emotional distress related to adverse events more intensely. Additionally, cumulative exposure to medical errors over time may amplify psychological burdens, making experienced anesthesiologists more susceptible to guilt, anxiety, and professional self-doubt. Unlike early-career clinicians who may attribute complications to systemic factors, senior anesthesiologists often perceive greater personal accountability, exacerbating their distress. These findings highlight the need for continuous psychological support tailored to experienced professionals. While younger novice anesthesiologists receive mentoring, senior practitioners often face greater professional isolation and may hesitate to seek help due to workplace norms discouraging emotional vulnerability.

Analyzing the aftermath of PSI and SVP, it's evident that the primary impact is on pediatric anesthesiologists, manifesting as psychological trauma, professional discontent, and diminished job satisfaction. On a broader scale, these incidents erode national confidence in the healthcare system, deteriorate public trust, and provoke legal ramifications.

Research has shown that medical errors are more common in ICUs and that PS needs to be more closely monitored in such settings[10]. According to a study, a life-threatening event more frequently (29%) occurs during a patient's stay in the ICU. Another study found that the rate of medical errors in medical-surgical ICUs was 1.7 per patient per day. These high rates of medical error may contribute to the more frequent occurrence of SVP among healthcare professionals working in ICUs[11].

Limited research has delved into the foundational causes of medical errors. A study segmented the origins of PSI that led to medical harm into three principal categories. Human factors were foremost, with elements like fatigue, insufficient training, communication gaps, time constraints, decision-making mistakes, logical errors, and abrasive personalities all contributing to this category. The second category, institutional factors, includes issues related to workplace design, policy implementation, administrative and financial frameworks, leadership dynamics, shortcomings in resource allocation, and mismanagement of staff. The third category encompasses technical factors, such as the lack of adequate technology, malfunctioning or subpar equipment, insufficient decision-making support, and integration deficits[12]. Our investigation corroborates that these individual, organizational, and technical dimensions significantly influence the incidence rates of medical errors and the emergence of SVP.

A study on PS in ICU indicated that medical errors arise not only from personal but also from technical and administrative factors. Due to these factors beyond the control and intervention of healthcare personnel, greater stress and pressure may arise. Therefore, medical errors should not be perceived as personal mistakes and healthcare providers should not be blamed, and punished accordingly. Instead, efforts should focus on reducing the sense of pressure, increasing frequency of reporting errors, and improving the system to prevent occurrence of these medical errors[13].

Root cause analysis plays a crucial role in devising effective strategies to combat PSI and foster improvements in the healthcare sector. This analytical method focuses on pinpointing the underlying causes of errors and developing interventions to prevent their recurrences. A study presented two illustrative scenarios of medical errors, thoroughly dissecting their root causes. In the first scenario, a miscommunication led to the erroneous administration of a blood thinner (warfarin sodium) to the wrong patient due to a last-minute room switch and a failure in identity verification of the patient which necessitated the cancellation of the scheduled surgery of the patient. The subsequent root cause analysis unveiled several underlying issues: inadequate training and orientation for healthcare staff, lack of experience, pervasive communication gaps among medical personnel, teamwork discrepancies, neglect in confirmation of the patient's identity, and general oversight. The second scenario depicted an accident where a janitor was wounded with a needle protruding from a medical waste bag. This incident, occurring three months previously during the janitor's shift, was promptly reported to the unit supervisor and then to the employee safety committee. Analysis of this scenario pinpointed critical safety concerns: insufficient training of new employees, the oversight of mandatory vaccinations upon employment commencement, and inadequate use of personal protective equipment. Identification of these root causes facilitated the development of specific prevention strategies targeted at each identified issue which underscores instrumental role of root cause analysis in mitigating risks associated with PSI and improving safety protocols for both patients and healthcare workers, thereby reinforcing the overall integrity and efficacy of the healthcare system[14].

A descriptive study was conducted at a university hospital to determine the frequency and root causes of commonly encountered patient falls. It was reported that 32.8% of falls occurred within the first three days of hospitalization, and 36.1% of them between 04:01 a.m. and 08:00 a.m. The hospital fall rate was determined to be 0.33%, with the highest rate observed in the neurology clinic. The identified causes for falls were associated with patient distraction and inattention (32.8%), his/her physical condition (32.8%), and lack of an attendant (22%). Using tree diagrams, a total of 241 root causes were identified and classified for each fall incident, with 3-4 root causes were identified for each event. The majority of falls occurred due to patient-related factors (45%), non-compliance with rules (23%), technical errors (15.8%), and organizational failures (8%). In light of these results, it was recommended to use a fall risk assessment scale to identify causes of falls in high-risk or fallen patients and implement appropriate, and individualized preventive measures[15]. In this context, establishing fall and risk assessment committees, monitoring falls at the institutional level, evaluating outcomes, and developing measures are critically important issues. These root cause analyses can improve the quality of healthcare delivery and reduce the incidence of SVP.

Another study aimed at decreasing PS events examined fall incidents in the hospital. In this study, root cause analysis was conducted using the fishbone diagram method. This method visually presents the causes of the problem, using statistical methods and analyzes results to identify the causes of the event and demonstrate the cross-relationships between the results and the underlying causes. The head of the fish represents the main problem. The fishbone diagram typically progresses from right to left, with more detail shown in smaller bones, allowing each major bone to branch out when further details are examined. The detailed analysis of the problem is carried out in four steps: clarifying the main problem, developing a fishbone diagram by defining sub-dimensions, while incorporating stakeholder analysis, and creating an unbiased view based on problem analysis[16]. A fall incident that occurred during the patient's transfer to the operating room was examined using the fishbone diagram method. An action was developed for all identified root causes (such as the three-level locking system of the patient bed in use, the unexpected failure of these locks, lack of competence in using the patient bed by staff, inadequacy of the assigned female staff for lifting and transport of the patient), and a person responsible for implementing the action was appointed which aimed to prevent future unwanted (sentinel) events and SVP[17].

Another factor that influences the PS Culture has been identified as the prevalence of illegitimate tasks performed in a hospital. The findings of the study underscore the association between the frequency of perceived illegitimate tasks, and duties regarded as unnecessary or outside one's professional responsibilities- with their relevant negative outcomes. Specifically, the perception of a higher frequency of illegitimate tasks was linked to a higher risk of reporting a low safety rating within hospital units and a higher likelihood of completing safety event reports which suggests that addressing the prevalence of illegitimate tasks could be a crucial step toward enhancing PS and improving the overall working conditions for healthcare professionals[18].

Regardless of the cause, experiencing an adverse event is stressful for healthcare professionals and reduces their work efficiency, and productivity. A cross-sectional study conducted in the Republic of Colombia covering the period from 2017 to 2021 highlights the prevalence and consequences of acute stress among healthcare workers following adverse events[19]. This study, which surveyed 838 healthcare professionals across various Colombian regions, found that 33.8% of respondents experienced adverse events, leading to significant stress reactions. Specifically, 21.91% of these professionals reported experiencing medium-high emotional overload, and 3.53% faced extreme acute stress. The findings underscore the substantial psychosocial risks healthcare workers face, underscoring the imperative for health institutions to proactively address these issues within the framework of PS and occupational health programs.

To improve the PS culture in institutions, it is necessary to first openly declare incidents of medical error and conduct root cause analyses. In cases where discussing medical errors is not encouraged, these errors are often covered up, and solutions cannot be developed. This study addresses the root causes of SVI experienced by expert physicians and the nationwide consequences of these incidents, and their impact on healthcare professionals. The study findings contribute to understanding the challenges and areas for improvement in the healthcare sector.

Improving PS culture in healthcare institutions requires an initial step of transparently acknowledging medical errors and thoroughly undertaking detailed root

cause analyses. In environments where open discussions about mistakes are discouraged, medical errors tend to be concealed, obstructing the development of effective solutions. This study examines the underlying reasons for SVI as encountered by pediatric anesthesiologists, while exploring the impact of these incidents on healthcare workers and the broader national healthcare landscape. The insights garnered shed light on the existing challenges and pinpoint crucial opportunities for advancement in the healthcare domain, aiming to foster a culture of safety and continuous improvement.

CONCLUSION

In conclusion, this article provides an important foundation for making improvements in healthcare systems by examining the complexity and various causes of SVP. The findings from this study can shed light on future research focusing on issues that affect the daily practices of healthcare professionals, while offering solutions to these problems.

Ethics

Ethics Committee Approval: The study was conducted in accordance with the World Medical Association Declaration of Helsinki Ethical Principles for Medical Research Involving Human Participants and approved by the Ethics Committee of İzmir Democracy University (approval number: 2024/01-9, dated: 31.01.2024).

Informed Consent: Informed consent was obtained from all subjects involved in the study.

Footnotes

Author Contributions

Surgical and Medical Practices: A.G.A., P.A., Concept: A.G.A., P.A., Design: A.G.A., P.A., Data Collection or Processing: A.G.A., P.A., Analysis or Interpretation: A.G.A., P.A., Literature Search: A.G.A., Writing: A.G.A., P.A.

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Case Report

Early Surgical Repair in a Patient with Post-Traumatic Complete Posterior Urethral Rupture Associated with both Vaginal and Rectal Injury

Hem Vajinal Hem de Rektal Laserasyon ile İlişkili Posttravmatik Komplet Posterior Üretral Rüptür Olgusunda Erken Cerrahi Onarım

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ABSTRACT

Posterior urethral rupture in females are uncommon. However, when it occurs it is usually a complete rupture often associated with vaginal laceration in 75%, and rectal injury in 33% of the cases. There are controversial approaches to the management of posterior urethral injuries, including primary repair of the urethral ends, primary cystostomy with delayed repair and endoscopic realignment of the urethral ends. Herein, we present a female child who developed complete urethral rupture associated with vaginal and rectal injury due to traffic accident which was urgently repaired after the traumatic event.

A-5-year-old-female patient was admitted to our emergency department with a blunt injury as a result of a traffic accident. Computed abdominal tomography revealed a hematoma around bladder neck, fracture of the superior pubic ramus, and a high-situated urinary bladder completely filled wirh extravased contrast material. Urethroplasty was performed immediately through transpubic approach. The serosal defect of the rectum and the anterior aspect of lacerated proximal vagina were also repaired.

Early surgical repair of a complete post-traumatic urethral rupture associated with vaginal and rectal injuries yields satisfactory outcomes, as in our case.

Keywords: Urethral rupture, vaginal injury, urethral trauma

ÖZ

Kız çocuklarında posterior üretral rüptür nadirdir. Ancak kızlarda meydana geldiğinde genellikle tam rüptürdür ve vakaların %75'inde vajinal yırtılma ve %33'ünde rektal yaralanma eşlik eder. Üretral uçların primer onarımı, gecikmiş onarım ile primer sistostomi ve üretral uçların endoskopik olarak yeniden hizalanması dahil olmak üzere posterior üretral yaralanmaların tedavisinde tartışmalı yaklaşımlar vardır. Bu çalışmada trafik kazası sonucu vajinal ve rektum yaralanmasına bağlı olarak tam üretra rüptürü gelişen ve yaralanma sonrası erken dönemde ameliyat edilen bir kız çocuğu sunulmuştur.

Trafik kazası sonucu künt yaralanma ile 5 yaşındaki bir kız çocuğu acil servisimize başvurdu. Abdominal tomografide, mesane boynu çevresinde hematom, superior pubik ramus kırığı ve kontrast madde ekstravazasyonu ile birlikte yüksek yerleşimli ve kontrast dolu bir mesane saptandı. Transpubik yaklaşımla acil üretroplasti yapıldı. Rektumun serozal defekti ve anteriordan lasere vajenin proksimali onarıldı.

Kızlarda travma sonrası komplet üretral rüptürün hemen onarımı, eşlik eden vajinal ve rektal yaralanmaların gösterilmesinde ve tedavisinde yararlıdır ve bizim olgumuzda da tatmin edici sonuçlar elde edilmiştir.

Anahtar kelimeler: Üretral rüptür, vajinal yaralanma, üretral travma

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INTRODUCTION

Urethral trauma in pediatric patients is most commonly caused by straddle injuries, pelvic fracture or iatrogenic urethral interventions. The estimated incidence of pediatric urethral rupture ranges between 1 and 5%[1,2]. Although the pathogenesis of urethral rupture in children is similar to that observed in adults, some differences need to be considered a) traumatic injury of urethra in children is less predictable due to the intra-abdominal location of the bladder, b) distraction defects of the urethra tend to be longer due to the bladder prominently protruding upward into the abdomen, c) simultaneous injuries of the membranous urethra and bladder neck are more common, d) prepubertal perineal size may make it hard to reach the highly

located proximal urethral end, e) smaller diameter of the urethra[3]. The injuries most commonly associated with posterior urethral ruptures are pelvic fractures and their effects on urinary and sexual functions[2,3]. Posterior urethral rupture in girls has a lower incidence due to the higher mobility and shorter length of the female urethra. Disruption at the level of the proximal urethra is usually complete, with vaginal laceration seen in 75%, and rectal injury in 33% of the cases[3,4].

There are several approaches to the management of posterior urethral injuries, including endoscopic realignment of the urethral ends and primary repair of the urethra. However, the widely accepted management is primary cystostomy and delayed urethroplasty with end-to-end urethral anastomosis using the transpubic approach[1,3,5,6].

Herein, we present a female child who had complete urethral rupture associated with vaginal and rectal injury due to traffic accident and was operated immediately after injury.

CASE REPORT

A-5-year-old-girl was admitted to our emergency department with a blunt injury as a result of a traffic accident. On physical examination, abdominal distention and tenderness with bleeding from the urogenital region were detected. Contrast-enhanced computed abdominal tomography and subsequently taken lateral abdominal radiography showed a hematoma around bladder neck, a high-situated and contrast filled bladder associated with superior pubic ramus fracture and extravasation of contrast material (Figure 1). Retrograde urethrography demonstrated extravasation of the contrast material with empty bladder. The patient underwent emergency surgery at the 6th hour after the traumatic event to determine the site of injury and repair the injurious site once and for all. Complete distraction of the urethra at the level of the bladder neck and injury of anterior vaginal wall were detected by retrograde urethroscopy and vaginoscopy performed before surgical treatment. Through Pfannenstiel incision, completely transected posterior urethra and extremely elevated bladder were identified during exploration (Figure 2A). After evacuation and excision of massive hematoma, partially transected vagina was noted at the anterior cervix junction (Figure 2B). Then, the posterior vagina was explored and serosal laceration was detected in the anterior part of the rectum (Figure 2C). The serosal defect of rectum was sutured. Thereafter, the anterior side of lacerated proximal vagina was sutured to the uterus following the insertion of a Fogarty balloon catheter. The proximal end of the urethra was detected just below the highly elevated bladder.

The distal part of the urethra was barely visible under the pubis. After a reverse Foley catheter was inserted into the distal urethra, the balloon of the catheter was inflated at the level of the external meatus of the urethra. The catheter was pulled up from the suprapubic region to elevate the urogenital diaphragm. Thus, the distal urethra could be exposed and a tension-free, end-to-end anastomosis was performed. While the last sutures were being placed, the reverse Foley catheter was removed and a Foley catheter was inserted into the bladder. There was no need for blood transfusion. This urethral catheter was left *in situ* for 3 weeks. While changing the Foley catheter, cystoscopic examination was performed which revealed lack of any urinary extravasation. The presence of a watertight anastomosis was confirmed with cystoscopy and vaginoscopy performed one month later and then the catheter was removed. Cystoscopic and vaginoscopic examinations performed during the

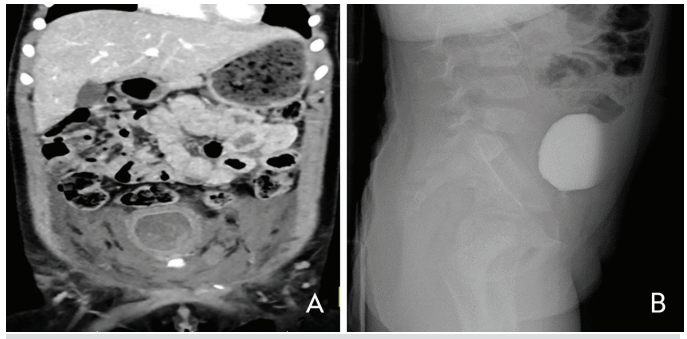

Figure 1. A) Computed abdominal tomography showing a hematoma around bladder neck, associated with fracture of superior pubic ramus and extravasation of contrast material. B) Subsequently performed lateral abdominal radiography demonstrating a high-situated bladder filled with contrast material

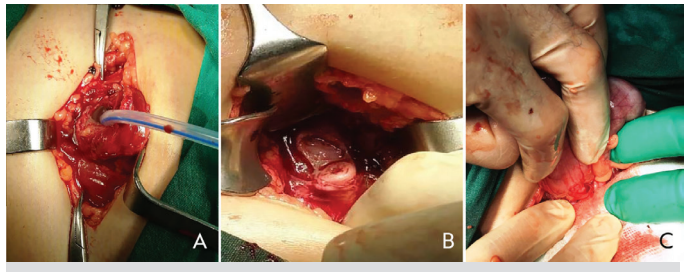

Figure 2. A) Completely transected posterior urethra and very highly-situated bladder. B) Partially transected vagina at the junction of anterior servix. C) Serosal laceration at the anterior side of rectum

follow- up period lasting for 2 years could not reveal any evidence of stenotic urethral and vaginal anastomosis. Any sign of urinary incontinence was not observed. Written informed consent was obtained from the parents of the child.

DISCUSSION

The aim in the management of urethral rupture is to maintain urethral continuity and to minimize the risk of complications such as recurrent urethral stricture, impotence and urinary incontinence[2,5,6].

If urethral injury is suspected in any child with pelvic fracture, appropriate evaluation has a critical importance when the patient-s clinical condition is stable[2,7]. Urethral catheterization should be avoided to prevent worsening of the urethral injury. Computed tomography (CT) helps to examine the genitourinary system in detail. A CT scan also searches for major accompanying abdominal traumatic injuries and identifies the types of pelvic fracture. Plain abdominal X-ray after contrast-enhanced CT scan may disclose shape, position of the bladder, and extravasation of urine (if any). On radiologic imaging, a hematoma around the bladder neck or a high positioned bladder may be a sign of posterior urethral injury. On cystography, bladder neck closure is indirect evidence of the robustness of the proximal sphincter mechanism. An impaired bladder neck and extravasation of contrast medium may also be an evidence of a damaged proximal sphincter[3]. However, retrograde urethrography performed with the patient in the lateral oblique position is the most effective diagnostic examination to detect urethral injury. Extravasation of contrast material or complete loss of urethral continuity can be determined by retrograde urethrography[8,9]. Although radiographic and endoscopic findings provide information about the location and severity of the injury, the most accurate assessment is made during surgical exploration[2,7]. Since the distal urethral sphincter mechanism in the membranous urethra is damaged during trauma, preservation of the bladder neck sphincter mechanism in these cases is important to prevent development of incontinence after urethroplasty[6,7].

Management of posterior urethral injuries can be performed urgently or may be delayed. Endoscopic urethral realignment or open surgery can be preferred for the repair of posterior uretral defects. The widely accepted management is primary cystostomy and delayed urethroplasty[6-8]. Before delayed urethroplasty, however, some authors recommend early endoscopic urethral realignment to shorten the distraction defect, reduce the likelihood of developing strictures, and facilitate open repair. Furtermore, some authors suggest that early endoscopic urethral realignment which is easy to perform and requires only minimal manipulationmay may be successful as a definitive treatment and alleviate the need for open urethroplasty in a reasonable number of patients[10,11].

The advantages of primary cystostomy and delayed urethroplasty or delayed primary endoscopic urethral realignment include elimination of the necessity of performing major operative procedure in acutely traumatized patients, minimal blood loss, shorter hospital stay, reduced likelihood of encountering infected pelvic hematoma, and prevention of injury to the penile nerve supply[2,3,10,11]. The disadvantages of primary cystostomy and delayed urethroplasty include the need for prolonged suprapubic drainage (3-6 months) and inevitable development of urethral stricture requiring one or more urethroplasties. Local complications such as urethral fistula, pseudo diverticulum, or stone may also occur secondary to delayed repair[3,10]. The disadvantages of primary endoscopic urethral realignment are that it cannot be performed in all patients and additional interventions are required in about half of the patients. Scarberry et al.[5] suggested urethral reconstruction within 3-6 weeks when the lesions are stabilized after acute phase of trauma has passed and the perineum felt softened on physical examination.

Emergency urethroplasty for the repair of acute urethral rupture is a difficult procedure due to coexisting pelvic hematoma and edema. Also, anatomic reconstruction via suprapubic approach irequires taking on a great challenge in finding the distal part of the urethra that is hidden below the pubis[2,7,8]. In our case, we planned to perform primary endoscopic urethral realignment, but we failed due to high-situated bladder. Therefore, we priorly decided to perform urethroplasty through a suprapubic approach and, if not possible we would proceed with primary urethral realignment.

Additionally, if possible, emergency urethroplasty during acute urethral rupture leads to a faster recovery process for the patient. However, if it is not feasible due to presence of hematoma and edema, other methods should also be considered.

Study Limitations

This study has several limitations. First of all, the results of this case report should be supported by data coming from further relevant studies. Besides, studies

with longer follow-up should be perdormed to assess long-term continence and late-term complications of surgical interventions performed to manage urethral ruptures.

CONCLUSION

In conclusion, immediate repair of a post-traumatic complete urethral rupture in girls is useful to demonstrate associated vaginal and rectal injuries and it also gave satisfactory results in our case such as shorter hospital stay, less psychological impact and treatment of associated injuries at the same time. If emergency urethroplasty is not possible, primary realignment of urethral cut ends can be done during open surgery.

Ethics

Informed Consent: Written informed consent was obtained from the parents of the child.

Footnotes

Author Contributions

Surgical and Medical Practices: A.S., S.C.K., Concept: A.S., Design: A.S., S.C.K., Data Collection or Processing: A.S., Analysis or Interpretation: A.S., S.C.K., Literature Search: A.S., Writing: A.S., S.C.K.

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Letter to the Editor

Should Pertussis Vaccine be Administered During Pregnancy?

Gebelerde Boğmaca Aşısı Yapılmalı mı?

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Pertussis, caused by *Bordetella pertussis*, is an acute respiratory infection. While this infection can affect individuals of all ages, severe infections and mortality predominantly occur in the neonatal period. Despite the inclusion of pertussis vaccination in routine childhood immunization programs globally, pertussis remains a significant cause of death among infants under one year of age. The infection is primarily transmitted through respiratory droplets produced by coughing or sneezing. Initial symptoms typically appear after an incubation period of 7 to 10 days post-exposure, manifesting as mild fever and rhinorrhea. As the infection progresses, paroxysmal coughing episodes begin, intensifying as the body attempts to expel mucus from the lungs.

Following expulsion of mucus, rapid inhalation of air produces the characteristic "whooping" sound. Post-tussive vomiting may also occur. In infants, paroxysmal cough may be absent, with respiratory distress being more prominent. In severe cases, apnea and cyanosis may develop. The period of highest pertussis-related mortality and morbidity in infants occurs within the first two months of life[1-3]. This period also precedes the administration of the first dose of the pertussis vaccine, typically given at two months of age as part of the national immunization schedule.

Between October 2023 and July 2024, respiratory polymerase chain reaction (PCR) tests were requested from 634 patients hospitalized at our institution due to upper respiratory tract infections. It was observed that 295 of these patients were under 1 year of age, and 280 patients were between 1 and 18 years old. *B. pertussis* was detected in 12 (1.89%) of the throat swab/sputum samples sent for PCR testing. Most of these 12 patients (n. 10; 83.3%) were infants younger than 2 months (33-59 days) at the time the test. One infant was 71 days old at the time of the test, and considering the incubation period of pertussis, it was thought that the infection developed before the vaccine could elicit an effective immune response. Six of these infants were treated and monitored in the pediatric ward, and five infants were managed in the intensive care unit due to clinical indications before being discharged. The remaining 15-year-old patient was treated in the pediatric ward and subsequently discharged.

A study conducted in the United States similarly demonstrated that over 80% of pertussis cases requiring hospitalization involved infants younger than 2 months of age[4]. In a multicenter study conducted in Turkey in 2023, involving 6601 children monitored in 11 different pediatric intensive care units, *B. pertussis* infection was detected in 50 cases (0.76%) through PCR testing. The median age of the patients diagnosed with pertussis was 9.14 weeks (range, 7.29-15.3 weeks)[5].

Data from the Centers for Disease Control and Prevention (CDC) also indicate that the risk of pertussis-related morbidity and mortality is highest among infants who have not yet reached two months of age. Since the pertussis vaccine, administered to infants aged two months as part of the routine immunization program, does not provide protection to these very young infants, the CDC recommends that pregnant women should receive a single dose of the tetanus-diphtheria-acellular pertussis (Tdap) vaccine between the 27th and 36th weeks of gestation. This strategy has been shown to prevent 78% of pertussis cases and reduce hospitalizations related to pertussis by 91% in infants younger than two months. Furthermore, vaccinating during pregnancy helps reduce the risk of the mother contracting pertussis postpartum and subsequently transmitting the infection to her infant. Adverse effects following Tdap vaccination in pregnant women are generally mild to moderate and self-resolving, with reports of serious side effects being extremely rare. Additionally, no teratogenic effects have been reported. Another strategy to protect infants younger than two months against pertussis is the "cocooning" approach, which involves vaccinating everyone in close contact with the infant. However, this method is noted to be both costly and challenging. Besides, it is difficult to ensure that all individuals in close contact with the baby are vaccinated. Moreover, vaccination must be done at least two weeks before contact with the infant to ensure immunity is established, presenting another logistical challenge[6].

Similarly, data reported to the European Centre for Disease Prevention and Control in 2019 revealed that pertussis mostly affects infants under 1 year of age. The three reported fatalities that year were all occurred in infants too young to have received the vaccine. Following an increase in hospitalization and mortality rates due to pertussis in 2012, the United Kingdom recommended administering an acellular pertussis vaccine to pregnant women between the 28th and 32nd weeks of gestation. Thanks to the transfer of antibodies from vaccinated mothers via the placenta to the fetus, a reduction in pertussis-related mortality was observed within the first year of the program. Additionally, the pertussis vaccination during pregnancy was found to reduce hospitalizations in infants younger than two months by 75-88%. After 2012, other European countries, including Italy, Spain, Belgium, and Portugal, also began implementing pertussis vaccination during pregnancy. To investigate the potential limiting effect of maternal pertussis vaccination on the immune response to the pertussis vaccine administered as part of routine childhood immunization, a study was conducted comparing infants born to mothers who received Tdap vaccine during pregnancy with those born to unvaccinated mothers during the 2-11 month period. It was observed that administering at least one dose of the pertussis vaccine as part of the routine immunization program reduced the need for hospitalization to similar levels in both groups. However, the study noted that the sample size was not large enough to conclusively determine whether maternal Tdap vaccination has any immunological dampening effect on the subsequent doses of the pertussis vaccine[7].

In our hospital, 83.3% of the patients treated for pertussis were infants who had not yet reached the second month of their lives and, thus, they were vulnerable to whooping cough due to the lack of protection against its deleterious effects. Our findings align with previous studies highlighting the high risk of hospitalization for infants under two months of age due to pertussis. The positive outcomes associated with Tdap vaccination during pregnancy, as demonstrated by studies from the CDC and other health authorities, have increased confidence in this preventive approach among health authorities. On the other hand, the alternative "cocooning" strategy presents disadvantages such as high costs and logistical challenges. Overall, these data strongly support the vaccination of pregnant women to protect infants younger than two months from pertussis.

Ethics

Ithics Committee Approval: The Scientific Research Ethics Committee of Trabzon Faculty of Medicine granted ethical approval for this study (approval number: 10496660-115, dated: 10.09.2024)

Footnotes

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