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Original Article

Iron, Vitamin D and B12 Levels of Young Children with Autism Spectrum Disorder at Diagnosis

Otizmli Küçük Çocuklarda Tanı Anında Demir, Vitamin D ve B12 Seviyeleri

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ABSTRACT

Objective: The etiology of autism spectrum disorder (ASD) remains unclear. The study aims to evaluate the relationship between ASD and preventable nutritional risk factors as iron, vitamin D, and B12 deficiency.

Method: Medical records of ASD-diagnosed children, ages 1.5-4 years old, were retrospectively reviewed. Hemoglobin (Hb), hematocrit (Htc), mean corpuscular volume (MCV), red cell distribution width (RDW), ferritin, 25-hydroxyvitamin D, and vitamin B12 levels at diagnosis were compared with age and sex-matched typically developing control group.

Results: Eighty-five ASD-diagnosed and 63 typically developing children were included. Mean Hb, Htc, B12, and median MCV, RDW, ferritin and 25-hydroxyvitamin D levels were similar between the groups (p>0.05). Iron deficiency (ID) was in 39.5% and 35.5% of the children in the study and control groups, respectively (p>0.05). Iron deficiency anemia (IDA) prevalence in the study and control groups were 6.1% and 4.8% (p>0.05). Vitamin D insufficiency and deficiency were 15.1% and 16.4% in the study group, and 23.4% and 10.6% in the control group, respectively (p>0.05). B12 deficiency was detected in 20.2% and in 8.9% of the children in the study and control groups respectively, without significance (p>0.05). No relationship was observed between the severity of ASD symptoms and Hb, ferritin, B12, and 25-hydroxyvitamin D levels (p>0.05).

Conclusion: Young children with ASD did not at greater risk for ID, IDA, vitamin D and vitamin B12 deficiency than typically developing controls. But, our results support the necessity of evaluating children with ASD in terms of iron parameters, vitamin D and B12 levels.

Keywords: Autism spectrum disorder, iron, vitamin D, vitamin B12, children

ÖZ

Amaç: Otizm spektrum bozukluğunun (OSB) etiyolojisi halen belirsizliğini korumaktadır. Bu çalışmanın amacı OSB ile demir, D vitamini ve B12 eksikliği gibi beslenme ile ilişkili önlenebilir risk faktörleri arasındaki ilişkiyi değerlendirmektir.

Yöntem: Yaşları 1,5-4 arasında değişen OSB tanısı almış çocukların tıbbi kayıtları geriye dönük olarak incelendi. Tanı anındaki hemoglobin (Hb), hematokrit (Htc), ortalama eritrosit hacmi (MCV), eritrosit dağılım genişliği (RDW), ferritin, 25-hidroksivitamin D ve vitamin B12 düzeyleri benzer yaş ve cinsiyetteki sağlıklı gelişen kontrol grubu ile karşılaştırıldı.

Bulgular: OSB tanılı 85, sağlıklı gelişen 63 çocuk dahil edildi. Ortalama Hb, Htc, B12 ve ortanca MCV, RDW, ferritin ve 25-hidroksivitamin D düzeyleri gruplar arasında benzerdi (p>0,05). Demir eksikliği (DE) çalışma ve kontrol grubundaki çocukların sırasıyla %39,5'inde ve %35,5'inde saptandı (p>0,05). Çalışma ve kontrol gruplarında demir eksikliği anemisi (DEA) prevalansı %6,1 ve %4,8 idi (p>0,05). D vitamini yetersizliği ve eksikliği çalışma grubunda sırasıyla %15,1 ve %16,4, kontrol grubunda ise sırasıyla %23,4 ve %10,6 bulundu (p>0,05). B12 eksikliği, çalışma ve kontrol grubundaki çocukların sırasıyla %20,2'sinde ve %8,9'unda saptanırken, fark anlamlı değildi (p>0,05). OSB belirtilerinin şiddeti ile Hb, ferritin, B12 ve 25-hidroksivitamin D düzeyleri arasında ilişki gözlenmedi (p>0,05).

Sonuç: OSB'li küçük çocuklar, sağlıklı olarak gelişen kontrollere göre ID, DEA, vitamin D ve vitamin B12 eksikliği açısından artmış riske sahip değildir. Ancak sonuçlarımız OSB'li çocukların demir parametreleri, D vitamini ve B12 düzeyleri açısından değerlendirilmesi gerekliliğini desteklemektedir.

Anahtar kelimeler: Otizm spektrum bozukluğu, demir, D vitamini, B12 vitamini, çocuklar

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INTRODUCTION

Autism spectrum disorder (ASD) is a neurodevelopmental disorder typically seen in early childhood, characterized by deficits in social interaction, restricted and repetitive behaviors [1]. It is estimated that one in 54 children has been identified with ASD with a global increase in the prevalence of ASD [2]. This increasing prevalence has encouraged researchers to question the relationship between ASD and preventable and changeable environmental risk factors for central nervous system dysfunction such as iron, vitamin D, and B12 deficiencies. Children with ASD are at risk for nutritional deficiencies because of frequently accompanying food selectivity and refusal of foods other than with certain colors and nutrient contents [3].

Vitamin D deficiency is the most frequently investigated environmental risk factor in infants and children with the diagnosis of ASD. Vitamin D plays an essential role in neuronal differentiation, proliferation, apoptosis, synaptic plasticity, immunomodulation, gene expression, and reduction of oxidative stress [4]. The findings of former studies on the relationship between serum vitamin D levels and ASD are controversial. Some studies suggested that vitamin D plays a role in the etiology of autism and low vitamin D levels are associated with the frequency and severity of autism, while others could not report any statistically significant interrelationship [5-9].

Another environmental risk factor investigated regarding the relationship with ASD is iron deficiency (ID). There is substantial evidence concerning the critical role of iron on learning, attention, memory, and psychomotor functions. Decreased iron concentration in the brain may affect serotonergic and dopaminergic systems, cortical networks, and myelin production [10-12]. Since children with ID often consume similar diets with their mothers, expectedly their mothers may also have ID [13]. Maternal ID can cause maternal depression, apathy, and low cognitive functioning. Therefore, ID can cause adverse developmental outcomes directly through its effect on brain functioning and indirectly through the non-responsive caregiver-child interaction [13]. Also, it has been shown that children with ID are less curious, less happy, and have less social interaction with the environment than healthy children [13]. Therefore, it can be considered that ID may increase the severity of ASD symptoms. Some studies have reported a high prevalence of ID and anemia in children with ASD [14-16], while others have reported that children with ASD are not at greater risk for ID than the general population [3,17].

Some studies have also investigated the relationship between ASD and vitamin B12, and reported that children with ASD had lower B12 and higher homocysteine levels compared with healthy controls [18-20], and indicated that treatment with B12 may alleviate ASD symptoms by reducing oxidative stress [21,22].

The studies exploring the relationship between vitamin D levels, iron parameters, and ASD have yielded conflicting results. There are limited data in the literature investigating the relationship between B12 and autism. Our research questions were: 1) Are the vitamin B12, vitamin D, and iron levels lower in children with newly diagnosed with ASD compared to their healthy peers? 2) Is there any relationship between severity of ASD symptoms and these micronutrients? Our study aimed to investigate the iron parameters, 25-hydroxyvitamin D, and vitamin B12 levels among children with ASD at the time of diagnosis and determine the relationship between severity of ASD symptoms and levels of these micronutrients.

MATERIALS and METHODS

This retrospective cohort study was conducted at the University of Health Sciences Turkey, Ankara Child Health and Diseases Hematology Oncology Training and Research Hospital, Developmental Behavioural Pediatrics (DBP) Outpatient Clinic. The study was approved by the Ethics Committee of the same hospital (decision no: 2019-144, date: 05/28/2019). Children aged 1.5-4 years and diagnosed with ASD between January 1, 2015, and May 1, 2019 with available hemogram, ferritin, 25-hydroxyvitamin D, and vitamin B12 test results at the time of diagnosis were included in the study.

The control group included children without any detected developmental delay admitted to the DBP Outpatient Clinic within the study period and underwent the tests required for the study group. Children in both groups used vitamin D regularly within the first year of life. Patients with a history of chronic neurologic, genetic and metabolic diseases, gestational age less than 32 weeks, birth weight less than 1500 grams, history of using iron and vitamin preparations within the last three months, and those using diuretics or antiepileptics were not included in the study. An additional exclusion criteria for the control group was malnutrition and eating disorders.

The developmental evaluation was conducted by developmental and behavioral pediatricians based on Bronfenbrenner and Ceci's [23] bioecological theory, International Classification of Functioning, Disability, and Health framework [24], family-centered care [25], and Guide for Monitoring Child Development [26]. Bronfenbrenner and Ceci's [23] bioecological theory postulates that early childhood development holds through dynamic interactions between the child's biological, psychological, and social functioning and his/her immediate environment consisting of parents and other family members, and the distant environment including social opportunities that support this basic structure. This theory offers a helpful perspective for understanding and supporting child development. The child's health status was evaluated as an integral part of the body functions and structures, the participation and limitations in his/her activities, and the environmental and personal factors [24]. Health and education services were planned, delivered, and evaluated by a mutual collaboration with families who had a unique role in the child's life [25]. The Guide for Monitoring Child Development [26] is a validated method based on an open-ended interview with the primary caregiver for the developmental monitoring and early detection of developmental difficulties in low and middle-income countries [26]. Children whose development was compatible with their age in terms of expressive, and receptive language, relationship, fine and gross motor domain according to developmental evaluation were included in the control group. The ASD was diagnosed based on the Diagnostic and Statistical Manual of Mental Disorders-Fifth Edition criteria for ASD [1]. The severity of ASD symptoms was evaluated by the Childhood Autism Rating Scale (CARS) [27-29]. The CARS is a behavioral rating scale used to diagnose and evaluate the severity of ASD [28]. The Turkish validity and reliability of the scale was performed [27]. Sum scores can range from 15 to 60, and scores more than 30 indicate that the child is in the autistic range. Scores between 30-36 and 37-60 are categorized as mild, and severe autism, respectively [28].

Serum ferritin, 25-hydroxyvitamin D, and vitamin B12 levels were measured by the radioimmunoassay method on Beckman Coulter DXI800 autoanalyzer. Hemogram was studied on Beckman Coulter HmX hematology analyzer.

Hemoglobin (Hb), hematocrit (Htc), mean corpuscular volume (MCV), red cell distribution width (RDW), and ferritin levels were used to evaluate serum iron parameters. ID was defined as ferritin <12 ng/mL; and iron deficiency anemia (IDA) as Hb <11 gr/dL, Htc <34%, MCV <73 fL, and ferritin <12 ng/mL [30,31]. American Academy of Pediatrics considers serum 25-hydroxyvitamin D levels ≤15 ng/mL as vitamin D deficiency and 25-hydroxyvitamin D levels between >15-20 ng/mL as vitamin D insufficiency [32]. We categorized vitamin D levels measured between 21 March, and 23 September as summer, and between 23 September-21 March as winter 25-hydroxyvitamin D levels. A serum vitamin B12 level below 200 pg/mL was considered as vitamin B12 deficiency [33].

Statistical Analysis

The data obtained were analyzed using the SPSS 20 package program for MAC. Chi-square test were used for comparing the percentages between groups. Statistical differences of the mean values between groups were analyzed using the t-test for the variables with normal and Mann-Whitney U test without normal distribution. Paired t-test was used for the time comparison in the group for the normally distributed data and the Wilcoxon test for not normally distributed data. Descriptive statistics were expressed as numbers and percentages for categorical variables and as mean ± standard deviation or median (minimum-maximum) for the continuous variables. Spearman tests were used for evaluating the correlation between different variables. For all tests, statistical significance was defined as $p<0.05$.

RESULTS

We followed 192 children with ASD between January 2015 and May 2019. The laboratory results were available at the time of diagnosis in 121 of them. We excluded 36 patients with chronic conditions such as neurologic (n=23), genetic (n=7), surgical (n=4), allergic (n=7) diseases and history of very low birth weight (n=5). We finally assessed a total of 148 children including 85 with an ASD diagnosis, and 63 controls. Demographic characteristics of the cases are shown in Table 1. The mean age of the children was 30.3±8.6 months. The age, gender, birth weight, gestational age, and fathers' educational level were similar between the groups ($p>0.05$). The percentage of mothers who were educated more than 8 years was higher in the control group ($p=0.007$).

Hemogram, ferritin, vitamin B12, and vitamin D levels were available in 144, 143, 140, and 120 children, respectively. In the study group, 60 of 85 children's CARS scores were available. The comparison of Hb, Htc, MCV, RDW, ferritin, vitamin D and vitamin B12 levels between both groups is shown in Table 2.

The mean Hb, Htc, vitamin B12, and median MCV, RDW, and ferritin levels were similar between the groups (p>0.05). The median vitamin D levels in summer and winter were similar between the study and the control groups (p=0.535 and p=0.569). In the study group 39.5% and in the control group 35.5% of the children had ID (p=0.623). We determined that 6.1% of the study, and 4.8% of the control group had IDA without astatistically significant intergroup difference (p=0.744). Vitamin D insufficiency and deficiency were observed in 15.1% and 16.4% of the study (p=0.249), and 23.4% and 10.6% of the control group, (p=0.374) respectively. We found vitamin B12 deficiency in 20.7% and 8.9% of the study and the control groups without a statistically significant difference (p=0.072) (Table 3).

Table 1. Demographic characteristics of the groups

	Study group n=85	Control group n=63	Total n=148	p
Gender*				
Male	72 (84.7)	48 (76.2)	120 (81.1)	0.191
Female	13 (15.3)	15 (23.8)	28 (18.9)	
Age, month†	31.12±8.32	29.27±8.91	30.33±8.60	0.197
Mothers' age‡	28 (18-42)	27 (19-42)	28 (18-42)	0.197
Fathers' age‡	31 (20-55)	31 (22-48)	31 (20-55)	0.704
Education levels of mothers*				
≤8 years	36 (42.9)	11 (20.4)	47 (34.1)	0.007
>8 years	48 (57.1)	43 (79.6)	91 (65.9)	
Education levels of fathers*				
≤8 years	26 (31)	15 (27.8)	41 (29.7)	0.690
>8 years	58 (69)	39 (72.2)	97 (70.3)	
Birth weight, gram†	3236±518	3263±413	3246±480	0.757
Gestational age, week‡	40 (35-42)	40 (37-42)	40 (35-42)	0.903

*n (%), †mean ± Standard deviation, ‡median (minimum-maximum). Missing data of ten parents educational information are excluded

Table 2. Comparison of Hb, Htc, MCV, RDW, ferritin, vitamin D, and vitamin B12 levels in the groups

	Study group n=85	Control group n=63	Total n=148	p
Hb*	12.36±1.04	12.21±0.84	12.30±0.96	0.357
Htc*	36.53±2.84	36.06±2.48	36.33±2.69	0.302
MCV†	75.8 (16.5-88.3)	76 (60.1-83.3)	75.9 (16.5-88.3)	0.482
RDW†	14.8 (12.2-26.4)	14.6 (12.6-19.9)	14.7 (12.2-26.4)	0.368
Ferritin†	15.9 (1.9-63.3)	14.7 (2-53)	14.8 (1.9-63.3)	0.717
Vitamin D†	24.3 (10-70.3)	24 (7.5-52.9)	24 (7.5-70.3)	0.851
Vitamin D, winter†	24.1 (10-61)	24.8 (12.3-52.9)	24.3 (10-61)	0.569
Vitamin D, summer†	25.2 (10.4-70.3)	23.8 (7.5-43.6)	23.9 (7.5-70.3)	0.535
Vitamin B12*	375.0±186.7	364.1±179.4	370.6±183.2	0.729

*mean ± Standard deviation, , †median (minimum-maximum). Hb: Hemoglobin, Htc: Hematocrit, MCV: Mean corpuscular volume, RDW: Red cell distribution width

Table 3. Comparison of iron, vitamin D, vitamin B12 deficiency, and iron deficiency anemia between the groups

	Study group n=85	Control group n=63	Total n=148	p
Iron deficiency	32 (39.5)	22 (35.5)	54 (37.8)	0.623
Iron deficiency anemia	5 (6.1)	3 (4.8)	8 (5.6)	0.744
Vitamin D insufficiency	11 (15.1)	11 (23.4)	22 (18.3)	0.249
Vitamin D deficiency	12 (16.4)	5 (10.6)	17 (14.2)	0.374
B12 deficiency	17 (20.2)	5 (8.9)	22 (15.7)	0.072

Available micronutrient results (study/control group): Ferritin (81/62); hemogram (82/62); vitamin D (73/47); vitamin B12 (84/56)

Mild and severe autism symptoms were found in 8 and 52 children according to CARS scores, respectively. There was no correlation between CARS scores and Hb, serum ferritin, 25-hydroxyvitamin D, and vitamin B12 levels (Table 4). There was no significant relationship between the severity of autism symptoms and 25-hydroxyvitamin D, Hb, ferritin, and vitamin B12 levels (p>0.05).

DISCUSSION

Our data have shown that children with ASD aged between 1.5 and 4 years were not at a greater risk for ID, IDA, vitamin D, and vitamin B12 deficiency compared with age and gender-matched healthy controls.

There are many studies investigating the relationship between vitamin D levels and ASD. A variety of case-control studies from different countries and races reported that children and adolescents with ASD had lower vitamin D levels [20,34-47], however, some studies in the literature have indicated the opposite of these results [48-54]. Recently, in a meta-analysis of 24 case-control studies, Wang et al. [55] showed that children and adolescents with ASD had significantly lower vitamin D levels than the control group. Quantitative integration of 10 case-control studies which reported odds ratio among these studies revealed that low vitamin D levels were associated with increased risk of ASD (odds ratio: 5.23, 95% confidence interval: 3.13; 8.73, p<0.0001, I2=78.2%). Surprisingly we did not observe any statistically significant difference in vitamin D levels between children with ASD diagnosis and healthy controls. Only 6 of the 24 case-control studies in the meta-analyses by Wang et al. [55] included children below five years of age. Our results may be associated with some relevant factors. Firstly, newly diagnosed children below four years of age were included in our study. Secondly, parents had not yet restricted dietary milk protein or casein. In addition, children's daily routines and exposure to sunlight were probably similar to the control group, as they were newly diagnosed and have not started to receive early intervention services yet.

Geographic location and latitude are also factors affecting vitamin D levels [55]. Studies from African and Asian countries showed that ASD diagnosed children had lower vitamin D levels than the control group. However, studies from Europe reported higher but non-significant vitamin D levels in the ASD diagnosed children than the control group. Studies from America reported no difference in vitamin D levels between the children with and without ASD. The mean vitamin D level of children living in the low-latitude areas was higher than children living in high and middle latitudes. Studies from Turkey also showed different results. While some studies reported that vitamin D concentration in children with ASD was lower than the controls, others reported no difference in vitamin D concentration [20,42,50,56]. Guler et al. [50] reported that mean vitamin D concentrations in children with ASD were 25.58±10.31, and 25.35±9.92 in the control group (p>0.05). We observed similar vitamin D levels in our study with children of younger age group. Also, approximately one-third of children in both the study and the control groups had vitamin D insufficiency or deficiency (≤20 ng/mL) in our study. This high rate may be related to the characteristics of the city where this study was conducted. Ankara has high latitude and receives short-term sunlight exposure. Unexpectedly we could not find a seasonal variation in vitamin D levels. These results may be related to the small sample size and the study was not designed to detect the difference in seasonal variations.

Increasing data from preclinical studies and case-control studies about the relationship between vitamin D levels and ASD have prompted randomized controlled trials (RCTs) investigating the effect of vitamin D supplementation on symptoms of ASD. However, the results of RCTs have been inconsistent, and a recent meta-analysis of RCTs showed that vitamin D supplementation in children with ASD provides a small improvement in hyperactivity scores but there was no statistically significant effect on the severity of core symptoms of ASD such as disrupted social interaction,

Table 4. Correlations between the Childhood Autism Rating Scale scores and ferritin, vitamin D, vitamin B12, and hemoglobin levels

	CARS	Ferritin	Vitamin D	Vitamin B12	Hb
CARS	1.000	-	-	-	-
Ferritin	0.013	1.000	-	-	-
Vitamin D	0.193	0.103	1.000	-	-
Vitamin B12	-0.068	0.107	0.133	1.000	-
Hb	-0.082	0.272**	0.125	0.044	1.000

**Correlation is significant at the 0.01 level (2-tailed). Hb: Hemoglobin, CARS: Childhood autism rating scale

communication, repetitive and restricted behaviors and interests [57]. Contrary to studies that found a significant negative correlation between serum 25-hydroxyvitamin D levels and CARS, in our study no relationship was found between severity of ASD and serum vitamin D levels [45,47]. This result may be related to our sampling method which included mostly the children with severe ASD symptoms, and we could not reach the CARS score of the entire sample because of the retrospective design of the study.

Although there is some biological evidence showing the possible potential relationship between vitamin D levels and ASD, the fundamental mechanism of this relationship is not well understood. While interpreting the vitamin D levels of children diagnosed as ASD, it is important to consider some variables such as methodologic differences in the studies (e.g diagnostic methods/criteria, method of measuring vitamin D levels), duration of the sunlight exposure, diet, geography, age, ethnic and genetic factors.

In a case-control study, Bener et al. [40] showed that in a case group with a mean of 5.39±1.66 years, mean serum iron, Hb, ferritin, Htc were significantly lower than the control group. Gunes et al. [58] similarly found significantly lower mean serum iron level, Hb, ferritin, Htc in children diagnosed as ASD with a mean age of 9.73±4.20 years than the healthy control group, with a significant correlation between ID parameters and autism severity. In contrast to these case-control studies, a recent meta-analysis reported that the available evidence was inconsistent about whether children with ASD had lower iron levels or not [17]. In our study, we did not detect any significant differences between the groups in terms of Hb, Htc, MCV, RDW, and ferritin levels. We observed that frequency rates of IDA and ID were strikingly high in the whole sample (5.6%, and 37.8%, respectively). Compared to the control group, the frequency of IDA and ID were higher in the study group, without any statistically significant difference. The role of iron in brain development is noteworthy, and as in the study of Pivina et al., [59] our study also emphasizes that we should follow iron parameters of the children both with an ASD diagnosis and healthy individuals. In contrast to the study of Gunes et al., [58] we did not find any relationship between Hb, Htc, ferritin levels and severity of ASD.

Methyl B12 is an essential cofactor in the antioxidant system and has a role in the transmethylation pathway by providing methyl groups for the methionine-homocysteine cycle. Methyl B12 administration was reported to improve cellular methylation capacity, decrease oxidative stress, and alleviate ASD symptoms in a subgroup of children with the diagnosis of ASD [21,22]. Additionally, this responder subgroup exhibited significant improvement in blood plasma levels of glutathione, a potential biomarker of response, and a mechanism of improvement, which may include increased antioxidant capacity and reduced oxidative stress. These preliminary results indicated a strong trend toward improvement following methyl B12 administration in a subgroup of children with autism, warranting further research into the efficacy of methyl B12 and potential biomarkers in the evaluation of response to this treatment. Lower serum vitamin B12 levels, and significantly higher homocysteine levels were detected in children diagnosed with ASD [19,20,60-62]. In contrast to the literature, our study revealed lack of any significant difference between children diagnosed as ASD and healthy controls in terms of mean vitamin B12 levels which may be related to the younger age of the study sample compared with the studies in the literature. Although vitamin B12 deficiency was more frequent in the study group compared to the control group (20% and 8,9%, respectively), this intergroup difference was not statistically significant. However, our findings support the need for monitoring vitamin B12 levels of children diagnosed as ASD.

Study Limitations

Our findings can not be generalized due to the results of a single center. One of the limitations of our study is the variables affecting the level of the micronutrients, such as nutrition-related factors, longevity of sunlight exposure, selective nutrition, sensory hypersensitivity were not evaluated because of the retrospective design of the study. Another limitation is that serum iron and iron-binding capacity were not evaluated. However, the inclusion of a gender-, and age-matched control group and the evaluation of the data of youngest aged ASD children at the time of diagnosis are the strengths of our study.

CONCLUSION

In conclusion, although children diagnosed with ASD are not at a greater risk for ID, IDA, vitamin D, and vitamin B12 deficiency, our preliminary findings support the necessity of monitoring these preventable risk factors. Parents of children with ASD should be guided about providing a balanced diet for their children,

appropriate exposure to sunlight, iron and vitamin D supplementation during infancy, and not following non-evidence-based diets. Further studies are necessary to determine the potential relationship between ASD and iron, vitamin D and B12 deficiency, and their underlying mechanisms.

Ethics

Ethics Committee Approval: The study was approved by the Ethics Committee of the University of Health Sciences Turkey, Ankara Child Health and Diseases Hematology Oncology Training and Research Hospital (decision no: 2019-144, date: 05/28/2019).

Informed Consent: Retrospective study.

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Author Contributions

Surgical and Medical Practices: P.Ç., İ.A.S., Concept: P.Ç., H.İ.Y., Design: P.Ç., İ.A.S., H.İ.Y., Data Collection and/or Processing: P.Ç., İ.A.S., Analysis and/or Interpretation: P.Ç., Literature Search: P.Ç., Writing: P.Ç., İ.A.S., H.İ.Y.

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