ORIGINAL ARTICLE ÖZGÜN ARAŞTIRMA

Does Vitamin B12 Deficiency in Childhood Affect Hematological Parameters?

Çocukluk Çağında Görülen Vitamin B12 Eksikliğinin Hematolojik Parametrelere Etkisi Var mı?

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Abstract

Introduction: Vitamin B12 deficiency causes permanent neurological complications that can be resolved with early treatment. Studies have shown that changes in hematological parameters observed in the early period may contribute to early diagnosis.

Materials and Methods: A retrospective evaluation was made of the hematological parameters (hemoglobin, hematocrit, mean erythrocyte hemoglobin, mean erythrocyte hemoglobin concentration, erythrocyte count, mean erythrocyte volume, erythrocyte distribution width, leukocyte count, platelet count and mean platelet volume) of 74 patients with vitamin B12 deficiency and 74 healthy controls with normal vitamin B12 levels, who presented to the pediatric neurology clinic with various complaints. Patients aged 0-18 years with normal ferritin and folic acid levels, with no infections or chronic disease were included. The patients with low vitamin level were divided into subgroups: Group 1 (<150 pg/mL), group 2 (150-200 pg/mL) and group 3 (200-250 pg/mL). The control group was assigned as group 4 (>250 pg/mL).

Results: The vitamin B12 deficient group and the control group were similar in terms of sex and age. Vitamin levels were significantly different between the groups, while the hemogram parameters did not differ significantly. Group 1 included 16.21%, group 2 included 54.05% and group 3 included 29.72% of the patients. The subgroups were similar in terms of sex and age, and hemogram parameters did not differ significantly.

Conclusion: Changes in hemogram parameters may not be observed even at very low vitamin B12 levels, so vitamin levels should be checked in patients with clinical findings and a history suggestive of deficiency.

Keywords
Vitamin B12 deficiency, effect, pediatric, hematological parameters

Anahtar kelimeler
Vitamin B12 eksikliği, etki, pediatrik, hematolojik parametreler

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Öz

Giriş: Vitamin B12 eksikliğinde, erken tedavi ile önlenebilen, kalıcı nörolojik komplikasyonlar görülebilir. Çalışmalar, erken dönemde gözlenen hematolojik parametre değişikliklerinin, erken tanıya katkı sağlayabileceğini göstermiştir.

Gereç ve Yöntem: Vitamin B12 eksikliği saptanan 74 hasta ile çocuk nörolojisi polikliniğine farklı yakınma ile başvuran, vitamin B12 düzeyi normal olan, sağlıklı 74 kontrol hastasının hematolojik parametreleri (hemoglobin, hematokrit, ortalama eritrosit hemoglobini, ortalama eritrosit hemoglobin konsantrasyonu, eritrosit sayısı, ortalama eritrosit hacmi, eritrosit dağılım genişliği, lökosit sayısı, trombosit sayısı, ortalama trombosit hacmi) retrospektif olarak değerlendirildi. Ferritin ve folik asit düzeyi normal olan, enfeksiyon ve kronik bir hastalığı olmayan, 0-18 yaş aralığında olan hastalar çalışmaya alındı. Vitamin B12 düzeyi düşük olan hasta grubu üç sınıfa ayrıldı: Grup 1 (<150 pg/mL), grup 2 (150-200 pg/mL), grup 3 (200-250 pg/mL); grup 4 (>250 pg/mL) kontrol grubu olarak belirlendi.

Bulgular: B12 vitamini eksikliği olan grup ile kontrol grubu cinsiyet ve yaş özellikleri açısından benzerdi. Gruplar arasında vitamin düzeyleri anlamlı farklılık gösterirken, hemogram parametreleri anlamlı farklılık göstermedi. Hastaların %16,21'ini grup 1, %54,05'ini grup 2 ve %29,72'sini grup 3 oluşturdu. Alt gruplar cinsiyet ve yaş açısından benzerdi ve hemogram parametreleri anlamlı farklılık göstermedi.

Sonuç: Hemogram parametrelerindeki değişiklikler, çok düşük vitamin B12 düzeylerinde dahi görülmeyebilir, bu nedenle klinik bulgusu olan ve öyküsü vitamin B12 eksikliğini düşündüren hastalarda mutlaka vitamin B12 düzeyi bakılmalıdır.

Introduction

Vitamin B12 deficiency is a global problem that is common in all age groups. Vitamin B12 participates in DNA synthesis and plays a role in cell division and proliferation, and deficiencies can lead to serious neurological and hematological signs and symptoms (1,2). Hematological abnormalities accompanying vitamin B12 deficiency vary, and may not correlate strongly with vitamin B12 levels until late periods of the disease. Hemogram parameters are not always sufficient to predict vitamin B12 deficiency (2,3).

There is a scarcity of literature on hemogram parameters in vitamin B12 deficiency, although some studies have shown that changes in hematological parameters may be observed in the early period and may contribute to early diagnosis (3-6). In the present study, we evaluated the relationship between the severity of vitamin B12 deficiency and hematological parameters and assessed the value of hemogram parameters for detecting vitamin B12 deficiency.

Materials and Methods

Following the granting of approval by the Süleyman Demirel University Faculty of Medicine Clinical Research Ethics Committee (approval number: 20/317, date: 05.11.2021), this retrospective study was launched with 74 patients with vitamin B12 deficiency and 74 age- and sex-matched healthy controls with normal vitamin B12 levels who presented to the Pediatric Neurology Outpatient Clinic of the Süleyman Demirel University Faculty of Medicine between January 2019 and January 2021, with various complaints. Included in the study were patients who were 0-18 years of age with normal ferritin and folic acid levels, and with no infections, chronic disease, or history of chronic drug use. Patients with ferritin and folic acid levels outside the normal range, or with any infections or chronic diseases that may affect hematological parameters, and those with a history of chronic drug use were excluded from the study. The patients' age, sex, symptoms on admission, vitamin B12 levels and the following hematological parameters were retrieved from the patient's records: hemoglobin (Hb), hematocrit (HTC), mean erythrocyte hemoglobin (MCH), mean erythrocyte hemoglobin concentration (MCHC), erythrocyte count (RBC), mean erythrocyte volume (MCV), erythrocyte distribution width (RDW), leukocyte count (WBC), platelet count (PLT) and mean platelet volume (MPV). A cut-off point of <250 pg/L was used to identify vitamin B12 deficiency. Anemia was defined as an Hb level of <11.5 g/dL, thrombocytopenia as a PLT of <150,000/mm^3 and leucopenia as a white blood cell count of <1500/mm^3. Patients with low vitamin B12 levels were divided into three subgroups as: Group 1 (<150 pg/mL), group 2 (150-200 pg/mL) and group 3 (200-250 pg/mL), while the control group was assigned to group 4 (>250 pg/mL).

Statistical Analysis

All statistical analyses for this study were performed using IBM SPSS Statistics (version 20.0. Armonk, NY: IBM Corp.). Descriptive measurements were presented as mean ± standard deviation (median; minimum-maximum) and frequency (percentage). The normality of continuous numerical data was analyzed with a Kolmogorov-Smirnov test, the results of which revealed a non-normal distribution ($p<0.05$), and thus, non-parametric tests were preferred for group comparisons. A One-Way Analysis of Variance was used for the comparison of the vitamin B12 groups, and a chi-square analysis was used to compare categorical data. For a type 1 error rate of 5%, a p-value of <0.05 was considered statistically significant.

Results

The study sample included 74 patients and 74 healthy controls, totaling 148 participants, in the 0-18 years of age group. The vitamin B12 deficient group and the control group had the same sex ratio

and a similar mean age. In both groups, 73% of the participants were female and the remainder were male. The mean age was 10.79±6.11 years in the vitamin B12 deficiency group and 10.93±5.92 years in the control group.

The mean vitamin B12 level was 181.95±36.90 pg/mL in the patient group and 275.13±21.46 pg/mL in the control group, indicating a significant difference (p<0.001), while hemogram parameters (Hb, HTC, RBC, MCH, MCHC, MCV, RDW, WBC, PLT and MPV) did not differ significantly (Table 1).

The group of patients with low vitamin B12 levels was divided into three subgroups, as group 1 with 12 patients (16.21%), group 2 with 40 patients (54.05%) and group 3 with 22 patients (29.72%), with corresponding vitamin B12 levels of <150 pg/mL, 150-200 pg/mL and 200-250 pg/mL, respectively. The control group was assigned to group 4 (>250 pg/mL). A comparison of the groups revealed no significant difference in the sex ratio (p=0.958), and there was also no significant difference in the mean ages of the groups (p=0.487). A comparison of the vitamin B12 levels of the groups revealed a significant difference (p<0.001). The hemogram parameters of the groups did not differ significantly according to vitamin B12 levels, and there was no significant correlation between the hemogram parameters and vitamin B12 levels (Table 2).

The evaluation of the vitamin B12 subgroups also revealed no significant difference in the Hb values, or leukocyte and PLTs (Table 3).

Among the most common symptoms of vitamin B12 deficient patients who applied to the pediatric neurology outpatient clinic were fatigue (14.9%), paresthesia (13.5%), headache (13.5%), dizziness (12.2%), breath-holding spells (8.1%) and personality changes (6.8%) (Table 4).

A statistically significant difference was determined in clinical findings according to the age. The age of the patients with hypotonia, breath-holding spells, and developmental delay was significantly lower than the age of the patients with other neurological symptoms (p<0.001) (Table 4).

We compared the neurological findings in terms of hematological parameters. The hemogram parameters did not vary significantly among the patients with different clinical findings (p=0.540), and there was no significant difference in the sex distribution of the patients (p=0.257).

Table 1. Characteristics and hematologic parameters of the study groups

	B12 deficient group (B12 <250) (n=74)	Control group (B12 >250) (n=74)	p
Female (n, %)	54 (73.0)	54 (73.0)	
Male (n, %)	20 (27.0)	20 (27.0)	
Age	10.79±6.11 (12; 1-18)	10.93±5.92 (13; 1-18)	0.892
Vit B12 (pg/mL)	181.95±36.90	275.13±21.46	<0.001*
Hb (g/dL)	13.48±1.42	13.52±1.64	0.869
HTC (%)	39.58±3.81	39.95±6.17	0.665
RBC/(mm^3)	4.88±0.36	4.91±0.39	0.627
MCV (fl)	79.83±11.40	80.96±6.39	0.572
MCH (pg)	27.64±2.61	27.55±2.67	0.842
MCHC (g/dL)	33.80±2.39	38.29±6.05	0.287
RDW (%)	13.93±1.43	14.04±1.60	0.662
WBC/(mm^3)	7787±2580	7954±1939	0.658
PLT/(mm^3)	307,283±107,105	297,635±71,798	0.521
MPV (fl)	8.22±0.95	8.24±0.83	0.920

*: Significant at the 0.05 level according to the Mann-Whitney U test. Hb: Hemoglobin, WBC: White blood cell, PLT: Platelet, HTC: Hematocrit, RBC: Erythrocyte count, MCV: Mean erythrocyte volume, MCH: Mean erythrocyte hemoglobin, MCHC: Mean erythrocyte hemoglobin concentration, RDW: Erythrocyte distribution width, MPV: Mean platelet volume

Table 2. Distribution of patients according to the vitamin B12 levels and hematological values					
	Vitamin B12 subgroups				
	B12 <150 (n=12)	B12:150-200 (n=40)	B12:200-250 (n=22)	B12 >250 (control) (n=74)	p
Female (n,%)	8 (66.7)	31 (77.5)	15 (68.2)	54 (73.0)	0.958
Male (n,%)	4 (33.3)	9 (22.5)	7 (31.8)	20 (27.0)	
Age (year)	12.16±6.08 (15; 1-18)	9.80±6.21 (13; 1-18)	11.86±5.89 (14; 1-18)	10.93±5.92 (13; 1-18)	0.487
Vit B12 (pg/mL)	122.63±27.11	177.86±14.85	221.75±15.38	275.13±21.46	<0.001*
Hb (g/dL)	13.47±1.46	13.20±1.40	13.99±1.36	13.52±1.64	0.308
HTC (%)	39.15±3.72	38.96±3.70	40.96±3.86	39.95±6.17	0.295
RBC/(mm³)	4.78±0.43	4.87±0.35	4.94±0.35	4.91±0.39	0.626
MCV (fl)	82.32±8.68	78.77±11.62	80.14±13.08	80.96±6.39	0.171
MCH (pg)	28.27±3.34	27.06±2.65	28.35±1.87	27.55±2.67	0.074
MCHC (g/dL)	34.38±0.80	33.86±0.95	33.40±4.17	38.29±6.05	0.244
RDW (%)	14.19±1.84	14.00±1.48	13.67±1.07	14.04±1.60	0.877
WBC/(mm³)	7,908±2,303	7,865±2,835	7,581±2,315	7,954±1,939	0.674
PLT/(mm³)	317,833±78,775	324,225±124,525	270,727±76,281	297,635±71,798	0.137
MPV (fl)	8.05±0.94	8.14±0.87	8.47±1.08	8.24±0.83	0.714

*: Significant at the 0.05 level according to the Kruskal-Wallis test. Hb: Hemoglobin, WBC: White blood cell, PLT: Platelet, HTC: Hematocrit, RBC: Erythrocyte count, MCV: Mean erythrocyte volume, MCH: Mean erythrocyte hemoglobin, MCHC: Mean erythrocyte hemoglobin concentration, RDW: Erythrocyte distribution width, MPV: Mean platelet volume

Table 3. Distribution of hematological parameters according to the vitamin B12 level subgroups						
		Vitamin B12 subgroups				
		<150 (n=12)	150-200 (n=40)	200-250 (n=22)	>250 (n=74)	p
Sex, n (%)	Female	8 (66.7)	31 (77.5)	15 (68.2)	54 (73.0)	0.958
	Male	4 (33.3)	9 (22.5)	7 (31.8)	20 (27.0)	
Hb (g/dL)	<11.5	1 (11.1%)	6 (66.7%)	2 (22.2%)	11 (14.9%)	0.705
	>11.5	11 (16.9%)	34 (52.3%)	20 (30.8%)	63 (85.1%)	
WBC/(mm³)	<4,500	0	1 (33.3%)	2 (66.7%)	1 (1.4%)	0.896
	>4,500	12 (16.9%)	39 (54.9%)	20 (28.2%)	73 (98.6%)	
PLT/(mm³)	<150,000	0	1 (50.0%)	1 (50.0%)	1 (1.4%)	0.910
	>150,000	12 (16.7%)	39 (54.2%)	21 (29.2%)	73 (98.6%)	

Hb: Hemoglobin, WBC: White blood cell, PLT: Platelet

Discussion

Patients with vitamin B12 deficiency present with hematological findings that vary from anemia to pancytopenia. In cases of vitamin B12 deficiency, pancytopenia is caused by ineffective erythropoiesis, leukopoiesis and thrombopoiesis associated with programmed cell death in the absence of vitamin B12, and the reduced survival of precursors in peripheral blood that may occur in the later phases of the disease (7). Literature contains a few studies examining the frequency of hematological findings associated with vitamin B12 deficiency in children. A previous study reported 22.7% of patients with vitamin B12 deficiency had anemia, while none of the children had leukopenia or thrombocytopenia (8). Another study reported no significant difference in anemia or complete blood count parameters between cases with and without vitamin B12 deficiency (9). Emen et al. (10), on

Table 4. Clinical features of patients with vitamin B12 deficiency			
Clinical findings	n (%)	Age (mean ± SD)	p
Fatigue	11 (14.9)	10.27±5.69	
Headache	10 (13.5)	12.7±4.37	
Paresthesia	10 (13.5)	14.9±1.2	
Dizziness	9 (12.2)	14.78±2.44	
Breath-holding spells	6 (8.1)	1	
Personality changes	5 (6.8)	5.4±6.11	
Developmental delay	4 (5.4)	2.5±3	<0.001
Memory impairment	4 (5.4)	16.5±2.38	
Tremor	4 (5.4)	16.25±1.5	
Hypotonia	3 (4.1)	1	
Syncope	3 (4.1)	14.67±1.15	
Vision blurring	2 (2.7)	12	
Difficulty in concentration	1 (1.4)	6	
Muscle weakness	1 (1.4)	15	
Poor school performance	1 (1.4)	11	
SD: Standard deviation			

the other hand, reported no statistically significant association between vitamin B12 levels and complete blood count parameters, excluding PLTs. In the present study, anemia was detected in nine (12.1%), leucopenia in three (4.05%) and thrombocytopenia in two (2.7%) of the patients with vitamin B12 deficiency, while in the control group, eleven (14.9%) had anemia, one (1.4%) had leukopenia and one (1.4%) had thrombocytopenia. There was no statistically significant difference between the two groups in this regard. Although macrocytic anemia is expected in cases of vitamin B12 deficiency, the mean MCVs were 79.83 fl and 80.96 fl in the deficiency and control groups, respectively, with no statistically significant difference between them. A study of 1,100 individuals evaluating the correlation between vitamin B12 and MCV could establish no correlation, identifying a high MCV value in only 14.59% of patients, and concluded that MCV should not be the only criterion for diagnosis of vitamin B12 deficiency (11). An MCV value within the reference range may be misleading if used as a screening parameter.

There have been studies suggesting that MPV and RDW deterioration starts in the early period of vitamin B12 deficiency, prior to the development of cytopenia, leading the authors to question the usability of these parameters in early diagnosis. Aktas et al. (3) reported no significant difference in the Hb and HTC levels of cases with vitamin B12 below and above 250 pg/mL, but identified a significantly higher RDW in patients with vitamin B12 deficiency, concurring with the studies by Pongstaporn and Bhatia, which also reported an elevated RDW in cases of vitamin B12 deficiency (5,6). A study involving an adult age group reported higher MPV and RDW values in patients with vitamin B12 deficiency than in the control group, which suggested these parameters could be used as indicators during the early periods (4).

That finding, however, contrasts with the findings of the present study, in which vitamin B12 levels were significantly different between the vitamin B12 deficient and control groups, while the hemogram parameters did not differ significantly. In another study, involving 640 pediatric patients, no relationship could be identified between vitamin levels and complete blood count parameters in vitamin B12-deficient cases (12). In a study by Colak et al. (9). comparing the hemogram parameters of patients with normal vitamin B12 levels and those of patients with deficiency, it was established that hemogram parameters were not predictive, and vitamin B12 deficiency may not always be reflected in hemogram parameters.

Hematological findings may not always correlate with vitamin B12 levels, and thus vitamin B12 deficiency should not be dismissed purely based on a normal blood count. The findings of the present study are consistent with those of most studies.

Neurological symptoms can be observed in vitamin B12 deficiency such as hypotonia, dizziness, ataxia, tremor, paresthesia, fatigue, developmental delay/ regretion; neuropsychiatric abnormalities including mood changes, attention deficit, memory impairment, and behavioral abnormalities (13,14).

In a study, where 38 pediatric patients with vitamin B12 deficiency were evaluated, mean serum B12 level was found to be 137.18 (40-196) mg/dL and most common neurological symptoms included syncope, dizziness, hypotonia, convulsion, paresthesia, fatigue and concentration difficulty; only 23.6% of the patients were anemic (2). Similar results were obtained in our

vitamin B12- deficient group; there was no clinical correlation with hematological parameters.

These results showed that clinical findings in vitamin B12 deficiency are variable and non-specific and may not be associated with hematological abnormalities. Clinicians must consider vitamin B12 deficiency in children with unexplained neurological manifestations.

Conclusion

In vitamin B12 deficiency, changes in hemogram parameters may not be observed even at very low vitamin levels, and hematological parameters may not be associated with vitamin B12 levels and clinical findings. Since clinical manifestations are so variable, a high level of suspicion is required for early diagnosis of vitamin B12 deficiency to prevent irreversible neurological complications by means of early diagnosis and treatment.

Ethics

Ethics Committee Approval: This was approval by the Süleyman Demirel University Faculty of Medicine Clinical Research Ethics Committee (approval number: 20/317, date: 05.11.2021).

Conflict of Interest: No conflict of interest was declared by the authors.

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