Progress in Nutrition 2020; Vol. 22, N. 3: e2020016 DOI: 10.23751/pn.v22i3.7788

Original article

Comparison of hemogram parameters according to the severity of Vitamin B12 deficiency in children aged three months-16 years

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Summary. *Introduction* Vitamin B12 is essential for neurological development in childhood. Anemia, leukopenia, and thrombocytopenia can be listed among hematologic findings in vitamin B12 deficiency, however, these changes occur in the advanced stages. The objective of this study was to investigate the relationship between the severity of vitamin B12 deficiency and hemogram parameters and to evaluate the value of hemogram parameters in early detection of vitamin B12 deficiency. *Methods:* The patients were divided into two groups. A vitamin B12 level ≤200 pg/mL was considered severe deficiency (Group 1) and a vitamin B12 level >200 pg/mL was considered as a moderate deficiency (Group 2). Vitamin B12 level, hemoglobin, hematocrit, red cell distribution width, platelet count, mean erythrocyte volume, mean platelet volume, lymphocyte and neutrophil counts were compared between the two groups. *Results*The mean red blood cell count (RBC) in Group 1 was significantly lower than in Group 2 (p=0.047) and the mean red cell distribution width (RDW) in Group 1 was significantly higher than in Group 2 (p=0.003). The mean hemoglobin and hematocrit levels in Group 1 was found to be significantly lower than in Group 2 (p=0.026 and p=0.025). As a result of the logistic regression analysis performed with these parameters, it was observed that the only factor affecting vitamin B12 deficiency in children in our region was elevated RDW (p=0.01). *Conclusion*In this study, it was observed that only elevated RDW can be used as an early indicator of vitamin B12 deficiency in children.

Key words: Vitamin B12 deficiency anemia, child, RDW, RBC

Introduction

Vitamin B12 is a complex water-soluble organic compound. Nowadays, nutritional vitamin B12 deficiency is a considerably important health problem, especialy in developing countries. In studies conducted in regions with a low socioeconomic level, it has been reported that The prevalence of vitamin B12 deficiency has been reported to range between 22% and 65% in regions of low socioeconomic levels (1,2).

The most important function of vitamin B12 is to support the synthesis of deoxyribonucleic acid (DNA) with folic acid. Therefore, the system that is most sensitive to its deficiency is the hematopoietic system, which has a high rate of cell proliferation, especially the erythropoietic series. Furthermore, it is also necessary for regeneration and proliferation of rapidly proliferating cells, such as intestinal epithelial cells. Another important function of vitamin B12 is the maintenance of some normal structures and functions of the central and peripheral nervous system (3). Its deficiency in children causes physical and neuromotor growth retardation, as well as megaloblastic anemia. In addition to being able to cause delay in acquiring motor and mental functions such as holding the head, smiling, making eye contact, swallowing, sitting, walking, and speaking, and loss of these acquired functions in its severe deficiency, it can lead to serious clinical

pictures such as coma in more severe cases (4). For this reason, first of all, the prevention and identification, then the treatment of the deficiency are crucial for the healthy development of children.

Vitamin B12 deficiency leads to an increase in homocysteine levels, and homocysteine has been previously shown to be associated with conditions involving inflammation (1,5-7). Besides, mean platelet volume (MPV) and red blood cell distribution width (RDW) have been reported to be associated with inflammation (8,9). There are studies suggesting that MPV and red cell distribution volume (RDW) deterioration starts from the early period of vitamin B12 deficiency and questioning the usability of these parameters in the early diagnosis (10-12).

However, it is observed that the number of these studies are limited number in the literature; therefore, in this study, the correlation of the severity of vitamin B12 deficiency with hemoglobin (Hb), hematocrit (Htc), reticulocyte distribution width, platelet count, mean red cell volume, MPV , lymphocyte (LYM), and neutrophil (NEUT) count was investigated in children who were followed up for vitamin B12 deficiency in Istanbul Bağcılar Region, and it was aimed to determine the usability of these parameters in the early diagnosis.

Patients and method

Selection of Patients

Records of 237 patients aged 3 months-16 years, who were followed up for and treated due to vitamin B12 deficiency between January 2016 and November 2016 in the pediatrics clinic at Bağcılar Training and Research Hospital, were retrospectively examined. The patients were divided into two groups as those with a vitamin B12 level below 200 pg/mL(Group 1) and those with a vitamin B12 value between 200-300 pg/mL (Group 2). Children with a known chronic disease were not included in the study. For the study, approval dated 08.02.2017 and numbered 541 was received from the ethics committee of our hospital.

Variables of the Study

The age at admission, gender, full blood count variables of Hb, Htc, reticulocyte distribution width, platelet count, mean red cell volume, MPV, LYM and NEUT counts, and vitamin B12 levels were recorded in patient follow-up forms.

The normal range of serum vitamin B12 was considered to be 300-800 pg/mL, and a vitamin B12 level below 200 pg/mL was considered severe deficiency (13). The values below 2 standard deviations of the normal value of hemoglobin (Hb) and hematocrit (Htc) levels according to age were considered as anemia (14).

The upper limit of MCV in children was calculated using the formula [84 fl + (age (year) x 0.6)] until the adult level of 96 fl was reached following the age of 6 months, and the macrocytosis definition was made by calculating the macrocytosis limit of MCV separately for each patient by age (14). Macrocytosis was not assessed in infants younger than six months of age.

Laboratory methods

Vitamin B12 was studied in a Cobas-Roche brand (E170, Japan) hormone analyzer with a Rocher brand kit by the electrochemiluminescence immunoassay (ECLIA) method.

Complete blood counts were performed in a Beckman LH750 brand automatic blood count device with a Coulter brand kit.

Statistical Analysis

Statistical analyses were performed using the NCSS (Number Cruncher Statistical System) 2007 Statistical Software (Utah, USA) package program.

In the evaluation of the data, in addition to descriptive statistical methods (mean, standard deviation), the independent t-test was used for the comparison of paired groups, and the chi-square test was used for the comparison of qualitative data. Logistic regression analysis was performed to determine the factors affecting the low level of vitamin B12. A p value of less than 0.05 was considered statistically significant.

Results

146 (61.6%) patients were female, and 91 (38.3%) were male. No statistically significant correlation was observed between vitamin B12 values and gender groups (p=0.635).

Of 118 patients with a vitamin B12 level ≤200 pg/mL, 72 (61%) were female and 46 (39%) were male (Group 1), and of 119 patients with a vitamin B12 level >200 pg/mL, 74 (62.1%) were female and 45 (37.9%) were male (Group 2). No statistically significant difference was observed in terms of gender distributions between Group 1 and Group 2 (Table 1; p=0.853).

Although macrocytic anemia is often expected in vitamin B12 deficiency, the mean MCV in Group 1 and Group 2 was found to be 80.12±7.25 and 80.57±5.33, respectively and there was no statistically significant difference between the groups (Table 1; p=0.585).

There was no significant difference between the two groups in terms of mean white blood cell count (WBC) (p=0.903), platelet (p=0.227), mean corpuscular volume (MCV), MPV (p=0.327) and platelet distribution width (PDW) (p=0.869) (Table 2). Moreover, no statistically significant difference was observed in mean LYM, monocyte (MON), NEUT, eosinophil (EOS), and basophil (BASO) (p>0.05) between the groups (Table 1).

While the mean red blood cell (RBC) count in Group 1 was 4.61±0.57, it was found to be 4.74±0.39 in Group 2 which was statistically significantly lower than in Group 1 (Table 1; p=0.047).

The mean Hb level in Group 1 was 12.35±1.74 g/dL, and the mean Hb level in Group 2 was 12.80±1.31 g/dL, which was statistically significantly lower than in Group 1 (Table 1; p=0.026).

The mean Htc level in Group 2 was statistically significantly higher than in Group 1 (38.43±3.7 and 37.11±5.17, respectively) (Table 1; p=0.025).

The mean RDW in Group 1 was statistically significantly higher than in Group 2 (12.64±1.78, and 12.05±1.2, respectively) (Table 1; p=0.003).

The logistic regression analysis was performed with RBC, Hb, Hct, and RDW, which were found to be statistically significant parameters, to determine the factors affecting the low level of vitamin B12. It was observed that the factor relevant to severity of vitamin B12 deficiency in children in our region was elevated RDW alone (p=0.01) (Table 2).

Table 1. The relationship of the severity of vitamin B12 deficiency with gender and hemogram parameters

		Group 1 n:118		Group 2 n:119		p
Age		7.99±6.24		9.23±5.27		0.101
Gender	**Female**	72	61.02%	74	62.18%	
	Male	46	38.98%	45	37.82%	0.853
WBC		8.8±2.93		8.85±2.83		0.903
RBC		4.61±0.57		4.74±0.39		**0.047**
Hemoglobin		12.35±1.74		12.80±1.31		**0.026**
Hematocrit		37.11±5.17		38.43±3.7		**0.025**
Platelet		311.96±99.13		297.35±86.35		0.227
MCV		80.12±7.25		80.57±5.33		0.585
MPV		7.91±5.64		7.39±1.46		0.327
PDW		18.28±1.93		18.32±2.01		0.869
LYM		4175.25±2207.86		3813.7±1885.83		0.176
MON		667.29±240.73		662.52±240.94		0.879
NEUT		3682.35±2238.67		4060.26±2036.94		0.175
EOS		241.43±262.54		252.29±199.01		0.721
BASO		86.96±73.44		83.28±52.34		0.657
RDW		12.64±1.78		12.05±1.2		**0.003**

Group 1: Patients with a vitamin B12 level of ≤ 200 pg/ml
Group 2: Patients with a vitamin B12 level of > 200 pg/ml

Discussion

Vitamin B12 deficiency and associated megaloblastic anemia are usually the problem of children, pregnant, and the elderly in developing countries, and its cause has been reported to be insufficient intake of vitamin B12 through the diet (1). The most important function of vitamin B12 is to provide the DNA synthesis necessary for cell division and proliferation. Its deficiency causes psychiatric and hematological disorders and physical and neuromotor growth retardation, as well as megaloblastic anemia. Especially due to its demyelination effect, brain and nervous system development is significantly affected. In children with vitamin B12 deficiency, all motor functions, such as head

Table 2. Logistic regression analysis with RBC, Hemoglobin, Hematocrit, and RDW

					OR %95 GA	
	B	**S.E.**	**P**	**OR**	**Lower Limit**	**Upper Limit**
RBC	-0.69	0.48	0.156	0.50	0.20	1.30
Hemoglobin	-0.22	0.34	0.523	1.24	0.64	2.40
Hematocrit	-0.03	0.12	0.825	0.97	0.77	1.24
RDW	0.37	0.14	**0.01**	1.45	1.09	1.92

control, sitting, walking, and speaking can be delayed (15,16).

Early diagnosis and treatment of vitamin B12 deficiency in childhood is important. Although the cost of treatment is quite low, delayed treatment can lead to serious complications such as deep anemia, irreversible neurological damage, etc. (17). Variances in hematologic parameters (anemia, alone or with leukopenia or thrombocytopenia) and neurological findings may occur in the late periods of the condition (18). It is easy to make a diagnosis at these stages. However, early diagnosis necessary to prevent irreversible neurological damage is considerably difficult due to inadequate laboratory methods and lack of symptoms. Macroovalocytes in erythrocytes and hypersegmentation in NEUT nuclei are major findings in the peripheral distribution of vitamin B12 deficiency. On the other hand, Htc and MCV may be normal even in cases with neurological findings (15,16).

In the literature, no correlation has been reported between vitamin B12 deficiency and gender (19). In this study also, no difference was found in vitamin B12 deficiency between male and female genders.

Some authors have reported that the sensitivity of the value of 200 pg/mL, which is considered to be the threshold value for the serum vitamin B12 level, was low and that symptoms were present even in individuals with a serum concentration up to 350 pg/mL (20,21). In this present study, the threshold value for vitamin B12 was accepted as 200 pg/mL.

In our study, there was no significant difference in MCV, white blood cell, and platelet counts between the two groups. It has been stated that MCV value alone was not always a reliable parameter in the diagnosis of megaloblastic anemia (22). Iron deficiency may mask macrocytosis, and also, normal or low MCV due to vitamin B12 deficiency with iron deficiency may lead to a false diagnosis (23). In this present study, it was observed that the MCV was below 90 fL in both groups, The fact that the iron parameters were not studied is a limitation in this study. However, it is a well-known fact that iron deficiency is common in our country. In this case, vitamin B12 deficiency becomes more important for the clinician and this major deficiency becomes more insidious. Bor et al. (24) determined that only 2 of 98 patients with an MCV value above 100 fL had vitamin B12 deficiency. Kwok et al. (25) reported that there was no significant difference between patients with vitamin B12 deficiency and healthy controls in terms of WBC, MCV, and platelet count. The results of our study are consistent with the literatures.

In this study, it was observed that Hb and Htc values were significantly lower in the patient group with a vitamin B12 level below 200 pg/mL, which is consistent with anemia. Moreover, in their study involving adults, Aktaş et al. (10) reported that there was no significant difference in terms of Hb and Htc values between subjects with a vitamin B12 level below and above 250 pg/mL. In this study, vitamin B12 deficiency was diagnosed according to Hb and vitamin B12 levels. Hb and Htc levels were found to be significantly lower in patients with a vitamin B12 level below 200 pg/mL. However, demonstration of vitamin B12 deficiency at the metabolic level is possible with the homocysteine and methylmalonyl acid measurements (25). The fact that homocysteine and methylmalonyl acid levels were not studied in this study is one of the limitations of the present study.

It was found out in this study that there was no significant difference in MPV value between those with a vitamin B12 level below and above 200 pg/mlL. MPV shows the platelet size and bone marrow response. The platelet volume is a parameter that determines the platelet function. Platelets with a larger volume are more active (26). It has been shown that chemokines released from the membranes of activated platelets play a role in the immune response and act as an acute phase reactant and work as NEUTs, granulocytes, and MONs, and even have a direct antimicrobial effect (27,28). Activated platelets release inflammatory factors such as chemokines and cytokines and lead to an increase in platelet size. There are studies revealing the association of MPV with inflammation (28-30). These findings support the presence of large and active platelets in the peripheral blood in megaloblastic anemia. The relationship of vitamin B12 deficiency and MPV is not well-known. Thrombocytopenia is observed in vitamin B12 deficiency due to ineffective megakaryopoiesis (31). It has been reported that MPV was increased following vitamin B12 treatment (32). In a study by Aktaş et al. (10), it was found out that

MPV in patients with vitamin B12 deficiency was significantly lower compared those without vitamin B12 deficiency. Elevated serum homocysteine levels and MPV have been identified as a risk factor for atherosclerosis and arterial and venous thromboembolism. Therefore, it can be said that homocysteine and MPV together may be associated with the course of inflammation (33,34). In their study, Mohan et al. (35) reported that homocysteine levels increased the platelet activation and supported this hypothesis. In addition, alterations in MPV have been identified as a risk factor for myocardial infarction and atherosclerosis (33). One of the possible explanations for MPV changes in vitamin B12 deficiency associated with inflammation is that affected megakaryopoiesis results in forming smaller platelets in the bone marrow. Another explanation is that active platelets play a role in inflammation due to their wider and larger diameters and inactive smaller platelets may cause a reduction in MPV (32).

Vitamin B12 deficiency may be associated with inflammation through the pathway that increases homocysteine. It is known that homocysteine levels increase in vitamin B12 deficiency (1) and homocysteine is thought to be associated with inflammation. Since homocysteine plays an important role in inflammation, it may be thought that vitamin B12 deficiency is also associated with inflammatory conditions.

The relationship between MPV and vitamin B12 deficiency is not clearly explained in the literature. No statistically significant relationship was determined between the severity of vitamin B12 deficiency and MPV in this study.

In the present study, it was observed that the mean RDW was significantly higher in patients with a vitamin B12 level below 200 pg/mL. It was revealed that elevated RDW was associated with inflammatory diseases (9, 36). Vitamin B12 deficiency causes defects in DNA synthesis, which leads to unbalanced growth of hematopoietic cells and impaired division. Therefore, both normal size and enlarged erythrocytes leading to an increase in RDW are produced in bone marrow. Another explanation may be the relationship between vitamin B12 deficiency and inflammation. Since it has been revealed that RDW is associated with inflammation and vitamin B12 deficiency is thought to be associated with inflammation due to elevated homocysteine, elevated RDW in vitamin B12 deficiency reflects the inflammatory burden of the disease (10). In their study, Aktaş et al. (10), found out that RDW was significantly higher in patients with low vitamin B12 levels. There are studies on elevated RDW levels in vitamin B12 deficiency that were published by Ponstaporn et al. (37) and Bhatia et al. (22). The result of the present study was found to be consistent with the literature.

Conclusion

It was observed in this study that elevated RDW alone can be used as an inflammation-related early indicator in children with vitamin B12 deficiency. It has been discussed in the literature that vitamin B12 deficiency may be associated with inflammation as a reason for elevated homocysteine. As seen in the literature, vitamin B12 deficiency affects both RDW and MPV. In this study, it was observed that only RDW was affected. Studies on this subject were performed mostly in the adult age group. The number of studies conducted on children is very limited. This retrospective study has some limitations. One of them is that the homocysteine level was not studied. According to the results of the present study, it can be said that elevated RDW can be used as an indicator in the early diagnosis of vitamin B12 deficiency, however, further comprehensive prospective studies are needed.

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