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ORIGINAL ARTICLE

Frequency of Hematological Findings Associated with Severe Plasma Vitamin B12 deficiency in Infants and Adolescents

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SUMMARY

Background: **The aim of this study was to determine the hematological status of severe vitamin B12 deficiency in infants and adolescents.**
Methods: **This study involved 95 infants and 117 adolescents with severe plasma vitamin B12 deficiency (< 120 pg/mL) and normal plasma folate and ferritin. Infants were aged between one and 24 months. Adolescents were aged between 11 and 17 years .**
Results: **Macrocytic anemia was associated with nine (9.5%) out of 95 infants with severe vitamin B12 deficiency. Neutropenia was found in 16 (16.8%) out of 95 infants with severe vitamin B12 deficiency. Thrombocytopenia was not found in 95 infants with severe vitamin B12 deficiency. Macrocytic anemia was found in two (1.7%) out of 117 adolescents with a severe vitamin B12 deficiency. Neutropenia was associated in one (0.8%) out of 117 adolescents with severe vitamin B12 deficiency. Thrombocytopenia was not found in 117 adolescents with severe vitamin B12 deficiency.**
Conclusions: **Low clinical or hematological findings for B12 deficiency in infants and adolescents living in regions at risk, such as those with low consumption of meat and other animal products warrant the measurement of vitamin B12 level.**
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KEY WORDS

cobalamin deficiency, hematological values, infants, adolescents

INTRODUCTION

Nutritional cobalamin deficiency is a worldwide problem, especially in developing and underdeveloped countries where the deficiency is common in all age groups [1,2]. In infants, inadequate cobalamin intake is mainly due to maternal depletion of the vitamin, and in older children and adults, a low consumption of animal-source foods (particularly meat) is the main cause in poor populations worldwide. In most studies, serum vitamin B-12 concentration is correlated with intake of this vitamin [3,4].

The incidence of vitamin B12 deficiency in pregnant Turkish women ranges from 48.8% to 80.9%. Based on such data, vitamin B12 deficiency is an important problem in Turkey, and infants born to deficient mothers are at high risk for developing manifestations of vitamin B12 deficiency [5,6]. In children, perhaps more than adults, functional or quantitative cobalamin deficiency is often clinically obscure. The symptoms differ according to age at onset and severity. Detection of cobalamin deficiency in infants can be challenging for clinicians, but the stakes are high because early treatment can prevent irreversible neurologic damage [7]. Neurologic symptoms of cobalamin deficiency in adolescents may predominate such as dementia, headache, vertigo, and

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syncope [8]. Hematological manifestations completely resolve following vitamin B12 supplementation, but neurologic findings may persist; therefore, early diagnosis and treatment are important [9,10].

The aim of this study was to determine the hematologic status of severe vitamin B12 deficiency in infants and adolescents.

MATERIALS AND METHODS

This study involved 95 infants and 117 adolescents with severe plasma vitamin B12 deficiency and normal plasma folate and ferritin. Infants were aged between one and 24 months. Adolescents were aged between 11 and 17 years.

One hundred infants and 100 adolescents, all healthy children with normal plasma ferritin (above 20 ng/mL), folat (above 5ng/mL), vitamin B12 levels (above 200 pg/mL), and normal hemoglobin electrophoresis were chosen as the control group.

Anemia was defined as hemoglobin (Hb) value in the complete blood count less than 11 g/dL and 12 g/dL for infants and adolecents, respectively. Peripheral blood smear screening for macrocytosis and hypersegmentation was performed. Hemoglobin electrophoresis was used to rule out thalassemia trait in children.

Most infants with vitamin B12 deficiency were admitted to the hospital with non-specific manifestations, such as apathy, pallor, failure to thrive, developmental delay, weakness, refusal of solid foods, or any combination of these problems.

Most adolescents with vitamin B12 deficiency were admitted to the hospital with non-specific manifestations, such as headaches, amnesia, weakness, dizziness, lack of attention, or any combination of these problems.

A low consumption of animal-source foods (particularly meat approximately 100 - 200 gram/person/weekly) is the most common cause of the cobalamin deficiency in adolescents and infants in our region.

In two hundred and twelve children blood counts and plasma vitamin B 12 levels were evaluated. The diagnosis is based on a high index of suspicion leading to demonstration of a low plasma cobalamin level and confirmed by response to parenteral cobalamin therapy. The reference value for severe plasma vitamin B12 deficiency was < 120 pg/mL [6,11].

The patients were treated with vitamin B12 as follows: 1.0 mg/day IM for one week, followed by 1.0 mg IM for two weeks, and then monthly 1.0 mg injections and vitamin B12 levels were checked after three months of treatment.

An automatic hemocytometer (LH-780, Beckman Coulter, USA), which is calibrated on a daily basis, was used for complete blood counts. Hb, hematocrit, mean corpuscular volume (MCV), mean platelet volume (MPV), white blood cell, neutrophil and platelet count were measured from 2.0 ml of venous blood.

Vitamin B12 levels were measured in venous blood obtained in the morning after eight hours of fasting using the chemiluminescent technique by Immulite 2000 (Diagnostic Products Corporation, USA).

Written informed consent was obtained for blood from all patients and healthy children. The study had the approval of the local ethics committee.

Reference ranges were calculated as ± 2 standard deviation (SD) to generate 95% confidence intervals for MCV in adolescents and infants, using a 2-tailed distribution with 2.5% of the population expected to be in the highest tail. Student's *t*-test was performed for the evaluation of MPV in vitamin B12 deficient and control group.

RESULTS

The mean age of infants with severe vitamin B12 was 12 months (one to 24 months). Among the infants, 36 (42%) were males and 49 (58%) were females. The mean age of adolescents with severe vitamin B12 deficiency was 13.3 years (11 to 17 years). In adolescents, 57 (48%) were males and 60 (52%) were females. 10% of infants had macrocytosis or macroovalocytosis and hypersegmented neutrophils. 12% of adolescents had macrocytosis or macroovalocytosis and hypersegmented neutrophils.

The hematologic parameters in children with severe vitamin B12 deficiency are shown in Table 1. The mean (min-max) hemoglobin (Hb) and MCV of 100 healthy control infants were 12.4 (11.3 to 14.4 g/dL) and 79 (73 to 88 fL), respectively.

The mean plasma vitamin B12 level, Hb, and MCV of 70 infants without anemia, despite a severe vitamin B12 deficiency, were 92 (45 to 119 pg/mL), 12 (11 to 14.6 g/dL), and 77 (67 to 88 fL), respectively. Vitamin B12 level after treatment was 576 (236 to 1364 pg/mL).

The mean plasma vitamin B12 level, Hb, and MCV of 25 infants with anemia and a severe vitamin B12 deficiency were 69 (10 to 107 pg/mL), 9 (5.6 to 10.9 g/dL), and 86 (70 to 110 fL), respectively. Vitamin B12 level rose to 552 (245 to 1186 pg/mL) after the treatment. There was no correlation between vitamin B12 levels and anemia in 95 infants with a severe vitamin B12 deficiency ($p = 0.61$).

The 97% percentile for MCV in healthy control infants was 87 fL.

Macrocytic anemia was found in nine (10%) out of 95 infants with severe vitamin B12 deficiency. The MCVs of the remaining anemic patients were within normal range.

Neutropenia (< 1500/μL) was found in 16 (16%) out of 95 infants with severe vitamin B12 deficiency. None of the infants out of 95 with severe vitamin B12 deficiency had thrombocytopenia (< 150.000/μL).

The mean Hb and MCV of 100 healthy control adolescents were 13.4 (12 to 16 g/dL) and 82 (74 to 94 fL), respectively.

The mean plasma vitamin B12 levels, Hb, and MCV of

Table 1. Hematological values in infants and adolescents with severe vitamin B-12 deficiency below 120 pg/mL.

	Infant (n = 95)		Adolescents (n = 117)	
	n * = 70	n ** = 25	n * = 101	n ** = 16
Vit B12 < 120 pg/mL ***	92 (45 - 119)	69 (10 - 107)	95 (10 - 120)	96 (28 - 119)
Hb (g/dL) ***	12 (11 - 14.6)	9 (5.6 - 10.9)	13.5 (12 - 16.4)	10 (6.3 - 11.8)
MCV (fL) ***	77 (67 - 88)	86 (70 - 110)	84 (74 - 95)	82 (77 - 106)
Macrocytosis (fL)	n = 3 88 (88 - 88)	n = 0	n = 5 95 (98 - 101)	n = 0
Macrocytic anemia Hb (g/dL) *** MCV (fL) ***	n = 0	n = 10 8.8 (5.6 - 10.8) 95 (88 - 110)		n = 2 7.6 (6.3 - 9) 98 (96 - 101)
Neutropenia (cell/µL) ***	n = 10 1173 (434 - 1450)	n = 6 911 (21 - 1200)	n = 1 427	n = 0

*** No anemia, ** anemia, *** (mean; range)**

101 adolescents without anemia despite severe vitamin B12 deficiency were 95 (10 to 120 pg/mL), 13.5 (12 to 16.4 g/dL), and 84 (74 to 95 fL), respectively. Vitamin B12 level increased to 807 (280 to 1132 pg/mL) after the treatment.

The mean plasma vitamin B12 level, Hb, and MCV of 16 adolescents with anemia and severe vitamin B12 deficiency were 96 (28 to 119 pg/mL), 10 (6.3 to 11.8 g/dL), and 82 (77 to 106 fL), respectively. The vitamin B12 level was 456 (270 to 1086 pg/mL) after the treatment.

There was no correlation between vitamin B12 levels and anemia in 117 adolescents with severe vitamin B12 deficiency ($p = 0.57$).

The 97th percentile for MCV in healthy control adolescents was 92 fL.

Macrocytosis was found in five (5%) out of 101 adolescents without anemia despite a severe vitamin B12 deficiency. Macrocytic anemia was found in two (12%) out of 16 adolescents with anemia and severe vitamin B12 deficiency. The MCVs of the remaining anemic patients were within normal range.

MPV of infants with vitamin B12 deficiency was 7.7 ± 1.3 and that of the control group was 7.5 ± 1.3. The difference was statistically insignificant ($p = 0.4$). In the adolescent group with vitamin B12 deficiency, MPV was 8.2 ± 5.7. In the control group of adolescents, MPV was similar (8.0 ± 5.9). There was no statistical difference ($p = 0.08$)

Neutropenia was found in one out of 117 adolescents with severe vitamin B12 deficiency. Thrombocytopenia was not found in 117 adolescents with severe vitamin B12 deficiency.

DISCUSSION

Our study is the first to examine the relationship between frequency of hematological findings and severe plasma vitamin B12 deficiency in infants and adolescents.

The hematologic values of severe vitamin B12 deficiency observed in the present study were neutropenia, macrocytosis, and macrocytic anemia. Thrombocytopenia was not found. Koc et al. and Balci et al. [6,12] reported that the incidence of vitamin B12 deficiency in Turkish infants and adolescents was 41%.

The diagnosis is particularly challenging in infants, where symptoms may be nonspecific and difficult to detect, partly due to the large variation in normal development in this age group.

Due to the considerable physiological changes in hematological indices during the first year of life, the diagnosis in this age group might be complicated.

Hematologic findings in patients with vitamin B12 deficiency vary from anemia to pancytopenia. In the literature, there were no studies with respect to frequency of hematological findings associated with severe plasma vitamin B12 deficiency in children. Previous studies showed that megaloblastic anemia, thrombocytopenia, or neutropenia was case-based in infants with vitamin B12 deficiency [13-15].

In the present study, the infants had macrocytic anemia (10%), neutropenia (16.8%), bicytopenia (6%), and macrocytosis (3%) but none had pancytopenia and thrombocytopenia.

Although significant megaloblastosis could be seen in bone marrow, hematological findings in the peripheral blood is not so common, as we found red blood cells with a normal MCV, a finding that initially made it more difficult to reach a correct diagnosis.

Adolescence is considered a particularly nutritionally vulnerable period, characterized by rapid growth and

often great changes in dietary habits. Previous studies found that adolescents with vitamin B12 deficiency had megaloblastic anemia, thrombocytopenia, or neutropenia [16]. In the present study, the adolescents had macrocytic anemia (1.7%), macrocytosis (4%), and neutropenia (1%), but none had pancytopenia and thrombocytopenia.

Andres E et al. reported that elderly patients with vitamin B12 deficiency had anemia (37%), leukopenia (13.9%), thrombopenia (9.9%), and macrocytosis (54%) [17].

Hematologic findings were seen more frequently in infants than in adolescents. Hematologic findings were seen less frequently in children than in older patients.

In conclusion, clinical and/or hematological findings of vitamin B12 deficiency are relatively low in infants and adolescents especially in those living in regions at risk for deficiency. Thus, the measurement of vitamin B12 levels of children in those regions with low consumption of meat and other animal products should not await for the occurence of hematological or clinical findings. Indeed, studies which will focus on vitamin B12 supplementation in areas at risk are needed. They might help us to develop programs like vitamin D supplementation programs, and find a relatively cheap and effective way to overcome this problem.

Declaration of Interest:
None.

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