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Retrospective evaluation of patients with vitamin B12 deficiency in the pediatrics outpatient clinic

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Ethics Committee Approval
The study was approved by the Ethics Committee of Bagcilar Training and Research Hospital with the registry number 352 on December 1, 2010.
All procedures in this study involving human participants were performed in accordance with the 1964 Helsinki Declaration and its later amendments.

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

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Abstract

Background/Aim: This study examines patients diagnosed with vitamin B12 deficiency in our department. Although rare, vitamin B12 deficiency is one of the causes of megaloblastic anemia, which can lead to negative outcomes in patients. We aim to promote earlier diagnosis to protect patients from these negative effects. Therefore, this study will contribute to raising awareness in the literature.

Methods: Retrospectively, we included a total of 127 outpatient children, aged 0–18 years (0–215 months), who were diagnosed and treated for vitamin B12 deficiency at the Bagcilar Training and Research Hospital Child Health and Disease Clinic between October 2014 and February 2015.

Results: Among the patients, 67 (53%) with vitamin B12 deficiency were female, while 60 (47%) were male, indicating a higher occurrence in girls. Vitamin B12 deficiency was most commonly observed in the age groups of 0-2 years and 12-17 years (adolescents). The mean vitamin B12 level was 168.1 (34.1) pg/mL, the mean hemoglobin level was 12 (1.9) g/dL, and the mean MCV (mean corpuscular volume) was 78.4 (8.1) fl. Anemia was observed in 38% of the patients, bicytopenia in 4%, neutropenia in 6%, thrombocytopenia in 9%, pancytopenia in 3%, and macrocytosis in 2%. Non-iron-deficient patients showed no difference in mean hemoglobin and RDW (red cell distribution width) when compared to iron-deficient patients. However, their B12 levels were lower. The prevalence of iron deficiency did not differ between girls and boys. Comparing patients with vitamin B12 levels lower than 150 pg/mL to those with higher levels, there were no significant differences in average Hb, MCV, and RDW.

Conclusion: It should be noted that macrocytic anemia is not exclusive to vitamin B12 deficiency. Vitamin B12 deficiency can manifest as normocytic anemia and should not be overlooked in biochemical assessments. It is recommended to conduct nationwide and regional prevalence studies to evaluate vitamin B12 deficiency as a public health issue and to develop new solutions accordingly.

Keywords: pediatrics, vitamin B12, deficiency

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Introduction

The main source of water-soluble vitamin B12 is animal foods, predominantly found in beef liver and kidney. While humans can fulfill their cobalamin requirements through animal food intake, deficiency symptoms arise when intake is insufficient [1]. Vitamin B12 plays a crucial role in cell development and division by participating in DNA (Deoxyribonucleic Acid) synthesis. Its deficiency particularly affects rapidly growing tissues with high cell turnover [2]. The consequences of deficiency include somatic and motor neuron developmental delays and megaloblastic anemia [3]. Severe deficiency can lead to serious conditions, ranging from the inability to perform basic functions, such as holding the head and sitting up, to coma [4]. Vitamin B12 deficiency represents a significant public health issue, particularly in developing countries. This study aims to retrospectively investigate the frequency, demographic, clinical, and biochemical characteristics of patients with vitamin B12 deficiency. By examining the medical records and computer data of patients treated for vitamin B12 deficiency at the Bagcilar Training and Research Hospital Pediatrics Clinic between October 2014 and February 2015, we seek to compare our findings with existing literature.

Materials and methods

The files of patients aged 0–18 years (0–215 months) who were diagnosed with vitamin B12 deficiency and received follow-up and treatment at the Bagcilar Training and Research Hospital Pediatrics Outpatient Clinic between November 2014 and March 2015 were retrospectively analyzed. Information about the selected patients was obtained by reviewing their medical records and using the hospital automation system records. A total of 127 patients were identified and included in the study. The lower limit for diagnosing vitamin B12 deficiency was set at 200 pg/ml, and patients with values above this threshold were excluded from the research. The study received ethical approval from our hospital's ethics committee, dated 31.12.2010, and numbered 352. The patient follow-up form recorded age, sex, complete blood count, serum iron, total iron-binding capacity, serum ferritin levels, folic acid, and vitamin B12 levels. All patients had vitamin B12 levels below 200 pg/ml, which was considered deficient. The normal range for serum vitamin B12 was defined as 200–800 pg/ml. Iron deficiency was defined as ferritin levels below 20 ng/ml. Hemoglobin (Hb) and hematocrit (Hct) levels below 2 standard deviations from the normal values for age were considered indicative of anemia, while a white blood cell count (WBC) below 4000/mm^3 indicated leukopenia. Neutropenia was defined as a neutrophil count below 1500/mm^3, and a platelet count below 150,000/mm^3 was classified as thrombocytopenia [5]. Bicytopenia was defined as the presence of two impairments in hemoglobin, WBC, or platelet values, while pancytopenia referred to the presence of three impairments. The upper limit for mean corpuscular volume (MCV) in each patient was calculated using the formula [84fl + (age (year) × 0.6)] until it reached the adult value of 96 fl after 6 months. Macrocytosis, defined as an elevated MCV level according to age, was calculated separately for each patient [6]. Macrocytosis was not evaluated in infants younger than 6 months. Vitamin B12, folic acid, ferritin, serum iron, and total iron-binding capacity (TIBC) were measured using the Cobas-Roche (E170, Japan) hormone analyzer with electrochemiluminescence immunoassay (ECLIA) method and Roche brand mass. TIBC was calculated as the sum of total iron and partial iron-binding capacity (PIBC). PIBC were measured using the spectrophotometric method on the Cobas-Roche E170 hormone analyzer with Roche brand mass. Complete blood counts were performed using an automatic blood count device, the Beckman LH750 device, and the Coulter brand kit. Homocysteine levels were measured using the High-performance liquid chromatography (HPLC) fluorescence detector method on an Agilent brand device. The laboratory's normal serum level ranges was as follows: vitamin B12 200–800 pg/ml, folic acid 4.6–18.7 ng/ml, iron 60–158 µg/dl, ferritin 20–275 ng/ml, homocysteine 5–14 µmol/L, and LDH 95–500 U/L.

Statistical analysis

Descriptive statistics were employed to analyze the data, including mean, standard deviation, median, minimum, maximum, frequency, and ratio values. The distribution of variables was assessed using the Kolmogorov-Smirnov test. The Mann-Whitney U test was utilized for analyzing quantitative data, while Spearman correlation analysis was employed for correlation analysis. The analysis was performed using the SPSS 22.0 program.

Results

The patients were categorized by sex, revealing that 67 (53%) were female and 60 (47%) were male (Table 1). There was no significant difference observed in the occurrence of vitamin B12 deficiency between girls and boys (P=0.744). Likewise, no significant correlation was found between age and vitamin B12 levels (P=0.834) (Table 2). Except for anemia, most of our patients were diagnosed with upper respiratory tract infections, and their follow-up and treatments were conducted through outpatient clinic visits (Table 3).

Table 1: Anemia morphology of patients

		Mean (SD)	Median (Min-Max)
Age, year		7.6 (5.9)	8.0 (1.0-17.0)
		n	**%**
Gender	Female	67	53%
	Male	60	47%
Value by age	Hyperchromic	3	2%
	Hypochromic	41	32%
	Normochromic	83	65%

Table 2: Vitamin B12 characteristics by sex and age

		Vitamin B12 Level			*P*-value
		Mean (SD)	Median	Min-Max	
Gender	Female	170.4 (26.4)	175	102-200	0.834
	Male	173.4 (23.3)	180	107-200	

Mann-Whitney U test / Spearmen Correlation

The mean vitamin B12 level for the evaluated 127 patients was 168.1 (34.1) pg/ml, with a median value of 175.7. The mean hemoglobin (Hb) level was 12.0 (1.9) g/dl, with a median value of 12.1. The mean MCV was 78.4 (8.1) fl, with a median value of 80.0. The mean RDW was 13.4 (3.5), with a median value of 12.4. The mean folic acid level was 13.5 (5.3) ng/ml, with a median value of 13. No significant correlations were found between vitamin B12 levels and Hb (P=0.481) or MCV (P=0.448). While macrocytic anemia is typically associated with vitamin B12 deficiency, 65% of our patients had normocytic anemia, 32% had microcytic anemia, and only 2%

had macrocytic anemia. Anemia was observed in 38% of our patients, and there was no significant correlation between vitamin B12 levels and hemoglobin. Thrombocytopenia was detected in 9% of cases, neutropenia in 6%, bicytopenia in 4%, and pancytopenia in 3%. The mean platelet count was 333.0 (102.6) thousand/mm^3, with a median value of 311 ×10^3/mm^3, while the mean leukocyte count was 8.8 (2.7) ×10^3/mm^3, with a median value of 8.5 ×10^3/mm^3. The mean vitamin B12 levels in patients with neutropenia, bicytopenia, thrombocytopenia, and pancytopenia were not significantly lower than those without these conditions (P=0.095). Additionally, the mean vitamin B12 levels in patients with thrombocytopenia and pancytopenia did not significantly differ from those with anemia, neutropenia, and bicytopenia. No significant difference was observed in mean vitamin B12 levels between patients with and without anemia (P=0.073) (Table 4).

Table 3: Diagnoses of the patients with the exception of anemia

Diagnoses	n	%
Acute bronchiolitis	1	1%
Allergic rhinitis	2	2%
Anemia	48	38%
Asthma	4	3%
Enuresis	2	2%
Growth retardation	18	14%
Urinary infection	3	2%
Conjunctivitis	2	2%
Lymphadenitis	3	2%
Myalgia	4	3%
Obesity	4	3%
Oral aphtha	1	1%
Rhinitis	1	1%
Syncope	1	1%
Synovitis	1	1%
Upper respiratory infection	31	24%
Vitamin D deficiency	1	1%

Table 4: Hemogram and other anemia parameters

	Median	Min- Max	Mean (SD)
Vitamin B12	175.7	14.4-200.0	168.1 (34.1)
Ferritin	24.0	1.9-158.0	32.6 (28.5)
TIBC	381.0	44.0-636.0	375.7 (69.9)
Iron	56.5	16.8-167.7	59.5 (32.6)
Folic Acid	13.0	4.6-20.0	13.5(5.3)
Hemoglobin	12.1	6.2-16.6	12.0 (1.9)
Hematocrit	37.0	4.9-51.7	37.0 (6.1)
MCV	80.0	52.0-94.2	78.4 (8.1)
RDW	12.4	1.0-37.7	13.4 (3.5)
WBC	8.5	3.0-20.3	8.8 (2.7)
Platelet	311.0	105.0-729.0	333.0 (102.6)
Vitamin D	17.8	3.0-63.0	22.5 (16.6)

TIBC: Total iron-binding capacity, MCV: Mean corpuscular volume, RDW: Red blood cell distribution width, WBC: White blood cell

Discussion

Vitamin B12 is a water-soluble, red complex coenzyme that contains cobalt ions and cannot be synthesized by humans. It has a molecular weight of 1355.42 Daltons [3]. Structurally, vitamin B12 belongs to the class of compounds known as corrins. The corrin ring refers to the cobalamin tetrapyrrole ring without cobalt and other side chains [7]. Vitamin B12 is composed of three parts:

- The core consists of a cobalt atom surrounded by four reduced pyrrole rings.
- A nucleotide group is attached to both the cobalt atom and one of the pyrrole rings via a phosphate chain. This group differs from the typical nucleotide as it contains 5,6-dimethylbenzimidazole as a ribose-linked basic substance.
- The cobalt atom is further bonded to a small group (CN, OH^-, H_2O, SO3, methyl, 5'-deoxy-adenosyl) through coordination-type bonds [2].

The compound formed by the first two large groups is known as cobalamin (Cbl). Adding one of the molecules from the third group to cobalamin results in the formation of different vitamin B12 derivatives that exhibit enhanced effectiveness [3]. Cobalamin derivatives important for humans include cyanocobalamin (CNCbl), hydroxycobalamin (OHCbl), deoxy-adenosylcobalamin (AdoCbl), methylcobalamin (MeCbl)

Cyanocobalamin and hydroxycobalamin are both stable compounds that can be utilized as drugs. Hydroxycobalamin is eliminated from the body at a slower rate compared to cyanocobalamin. However, its use as a drug is generally not recommended due to the development of antibodies against the complex it forms with transcobalamin, leading to tolerance to its effects. Consequently, cyanocobalamin is more commonly employed as a medicinal treatment. Deoxy-adenosylcobalamin and methylcobalamin, conversely, serve as active coenzymes in tissues [8].

Humans are unable to synthesize vitamin B12 on their own. It is exclusively produced by certain bacteria and mold fungi [9]. While the bacteria in the large bowel do produce vitamin B12, the amount generated is insufficient to meet the body's requirements, both due to its occurrence in the distal part of the absorption area and the inadequate synthesis [9]. Consequently, individuals need to obtain vitamin B12 precursors from animal-based foods. The highest concentrations of vitamin B12 can be found in beef liver and kidney (40–50 µg/100 g). Plant-based foods do not serve as sources of vitamin B12, although they may

Although humans fulfill their cobalamin requirements by consuming animal-based foods, signs of vitamin B12 deficiency can occur due to insufficient intake. The World Health Organization (WHO) recommends the following dietary intake levels to prevent deficiency: 1 µg for adults, 1.3 µg for lactating mothers, 1.4 µg for pregnant women, and 0.1 µg for infants [11]. In children, the daily requirement starts at 0.4 µg/day during the early months and gradually increases with age, reaching 2.4 µg/day during puberty [12].

The incidence of vitamin B12 deficiency is influenced by factors such as race, environment, gender, age, socioeconomic level, and dietary habits. A study conducted by Allen et al. in Mexico between 1982–1986 revealed a 43% frequency of vitamin B12 deficiency among 219 patients aged 18–36 months [13]. Another study reported by Garcia et al. in Venezuela in 2005 documented an incidence of 11.4% among 5658 cases aged 0–18 years [14].

In our country, there is no prevalence study available that demonstrates the frequency of vitamin B12 deficiency. However, there are a few limited case reports from certain regions. Cetinkaya et al. [15] found a frequency of 0.64% among 3117 children (4–24 months old) hospitalized in Istanbul in 2007. Similarly, Baytan et al. [16] reported an incidence of 0.3% among 3980 cases aged between 3 months and 13 years in Bursa in 2007.

Vitamin B12 deficiency is prevalent among individuals with low socioeconomic status and those under 2 years of age [17]. In a study conducted by Taskesen et al. [18] in Diyarbakir, it was found that 82% of the patients were under the age of 2 years. In our study, we observed that 35% of the patients were under 2 years old, 8% were between 2-5 years old, 16% were between 6–11 years old, and 39.2% were between 12–17 years old. There were no significant differences in the frequency of deficiency between boys and girls. Additionally, no distinction was found in the mean vitamin B12 levels between girls and boys.

The primary hematological manifestation of vitamin B12 deficiency is megaloblastic anemia, characterized by an increased MCV, red cell distribution width (RDW), and hypersegmentation and macrocytosis in neutrophils observed in peripheral smears [3]. In our study, 38% of patients with vitamin B12 deficiency exhibited anemia. Durmus et al. [19] reported that 25% of patients with megaloblastic anemia did not display elevated MCV levels. In the study conducted by Bay et al., MCV values were below 90 fl in one-third of the cases [20]. In contrast to the literature, our study did not identify a significant negative moderate correlation between vitamin B12 levels and MCV. The mean MCV in our study was 78.4 (8.1) fl.

Additionally, the mean MCV of individuals with iron deficiency was not significantly lower than those without iron deficiency. We observed that 14% of patients with vitamin B12 deficiency also had iron deficiency. When comparing children with iron deficiency to those without deficiency in our study, there was no significant difference in the means of RDW; however, the mean RDW was 13.4% (3.5), indicating a slight increase. This suggests an elevation in RDW not only in cases of iron deficiency but also in cases of vitamin B12 deficiency. In contrast, Gupta et al. reported a significantly greater increase in RDW values in megaloblastic anemia compared to aplastic anemia, suggesting its potential use in differential diagnosis [21].

Additional hematological findings associated with vitamin B12 deficiency include leukopenia, neutropenia, bicytopenia, and pancytopenia. In the study conducted by Taskesen et al. [18], neutropenia was observed in 11% of 131 patients with megaloblastic anemia (mean vitamin B12 level of 69 pg/ml), while thrombocytopenia was present in 40% and pancytopenia in 6% of the cases. Cetinkaya et al. [15] reported anemia in 61.6%, thrombocytopenia in 30%, pancytopenia in 5%, and neutropenia in 15% of 20 cases with vitamin B12 deficiency (mean vitamin B12 level of 66.9 pg/ml). In our study, anemia was detected in 38% of our patients, but no significant correlation was found between vitamin B12 levels and hemoglobin. We observed thrombocytopenia in 9%, leukopenia in 6%, bicytopenia in 4%, and pancytopenia in 3% of the cases. The mean platelet count was 333.0 (102.6) thousand/mm^3, with a median of 311 $\times10^3$/mm^3. The mean leukocyte count was 8.8 (2.7) $\times10^3$/mm^3, with a median of 8.5 $\times10^3$/mm^3. In our study, the mean vitamin B12 levels in children with leukopenia, bicytopenia, thrombocytopenia, and pancytopenia were not significantly lower than those without. Furthermore, there was no significant difference in the mean vitamin B12 levels between patients with thrombocytopenia and pancytopenia compared to those with anemia, leukopenia, and bicytopenia.

Limitations

As our study relied on retrospective analysis of patient records, the available information was limited to what had been documented in the files. The records did not consistently provide clear documentation of the improvement in clinical findings for each patient, thereby preventing a comprehensive evaluation of clinical improvement.

Conclusion

This study confirms the high prevalence of vitamin B12 deficiency in children. The concentration of patients within the first 2 years and 12–17 years age groups indicates that these specific age ranges are particularly at risk. Contrary to common knowledge, it was observed that vitamin B12 deficiency could be associated with normocytic anemia in addition to macrocytic anemia. Therefore, the overall assessment should consider the laboratory results. In patients with iron deficiency and inadequate consumption of animal-based foods, vitamin B12 levels should be checked. Furthermore, it is important to recommend animal-based foods to adolescents, considering their increased nutritional needs.

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