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Hematological Findings in Children with Serum Vitamin B_{12} Deficiency

Serum Vitamin B_{12} Eksikliği Olan Çocuklarda Hematolojik Bulgular

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Abstract

Aim: Vitamin B_{12} is essential for DNA synthesis and hematopoiesis, and its severe deficiency not only causes anemia, but may also result in thrombocytopenia and leucopenia. In this study, we analysed the relationship between hematological status and vitamin B_{12} deficiency and draw attention to this issue.

Material and Method: The data gathered from our institute Gaziantep University Hospital outpatient clinics electronic database. Six hundred forty children were evaluated. The inclusion criteria were child patients with less than 200 ng/l vitamin B_{12} levels.

Results: Four hundred seventy-seven patients (74.5%) had no hematological abnormalities. Anemia was detected in 99 patients (15.5 %), two patients (0.3%) had a Hb-level<7 g/dl, anemia without macrocytosis in 91 (14.2 %), anemia with macrocytosis in 8 (1.3 %), leucopenia in 8 (1.3 %), neutropenia in 22 (3.4 %), lymphopenia in 9 (1.4 %), thrombocytopenia in 6 (0.9 %), and pancytopenia in 3 patients (0.4 %). An examination of the correlation, there were no correlations between vitamin B_{12} levels and hematological parameters.

Conclusion: Our results showed that hematological abnormalities were detected in only 25.5% of patients with Vitamin B_{12} deficiency, and anemia and neutropenia were the most common hematological abnormalities. In addition, serum vitamin B_{12} levels were not significantly correlated with the complete blood count parameters.

Keywords: Vitamin B_{12}, anemia, neutropenia

Öz

Amaç: Vitamin B_{12} DNA sentezi ve hematopoez için temeldir ve şiddetli eksikliği yalnızca anemiye neden olmaz aynı zamanda trombositopeni ve lökopeniye neden olabilir. Bu çalışmada, vitamin B_{12} eksikliği ve hematolojik durumlar arasındaki ilişkiye dikkat çekmek istedik.

Gereç ve Yöntem: Bu çalışmanın verileri Gaziantep Üniversitesi ayaktan hasta poliklinikleri elektronik veri sisteminden toplanmıştır. 640 çocuk değerlendirildi. Çalışmaya vitamin B_{12} seviyesi 200 ng/l'den düşük çocuklar dahil edildi.

Bulgular: 477 (%74,5) hastanın hemoglobin (Hb), lökosit ve trombosit değerleri normal sınırlardaydı. Hastalarda 99 (%15) anemi, 91 (%14,2) makrositozsuz anemi, 8 (%1,3) makrositozlu anemi, 8 (%1,3) lökopeni, 22 (%3,4) nötropeni, 9 (%1,4) lenfopeni, 6 (%0,9) trombositopeni ve 3 (%0,4) pansitopeni saptandı. Korelasyon değerlendirilmesinde vitamin B_{12} seviyesinin 200 ng/l'den düşük olmasıyla hematolojik parametreler arasında bir ilişki saptanmadı.

Sonuç: Bizim sonuçlarımız gösterdi ki vitamin B_{12} eksikliği saptanan olguların yalnızca %25,5'inde hematolojik anormallik saptandı, en yaygın hematolojik anormallik ise nötropeni ve anemi idi. Vitamin B_{12} eksikliği olan olgularda vitamin seviyesi ile tam kan sayımı parametreleri arasında ilişki gösterilemedi.

Anahtar Kelimeler: Vitamin B_{12}, anemi, nötropeni

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INTRODUCTION

Nutritional vitamin B_{12} deficiency is a worldwide problem, especially in developing and underdeveloped countries where the deficiency is common in all age groups. Vitamin B_{12} participates in DNA synthesis and plays a role in cell division and proliferation. Vitamin B_{12} deficiency is more frequent in populations with a low dietary intake of animal sources and in the gastrointestinal conditions leading to vitamin B_{12} malabsorption. Vitamin B_{12} deficiency is an important problem in Turkey.[1] In children, perhaps more than adults, functional or quantitative vitamin B_{12} deficiency are often clinically obscure. The symptoms differ according to age at onset and severity. Vitamin B_{12} deficiency can lead to serious pathology including certain neurological and hematologic abnormalities.[2,3]

The best-known morbid effects of vitamin B_{12} deficiency are hematological. There is a large body of information, derived from studies of various populations, on the prevalence of vitamin B_{12} deficiency as determined by biochemical assays, but it is uncertain how much of this apparent deficiency is translated into morbid hematological change.[4] Vitamin B_{12} deficiency is manifest by macrocytic anemia resulting from megaloblastic change in the bone marrow. This form of abnormal hematopoiesis may lead to anemia, leukopenia, thrombocytopenia, and pancytopenia however, they occur in later periods of the disease.[4,5] An increase in mean corpuscular volume, and macroovalocytosis and hypersegmentation in neutrophils in peripheral blood smear may suggest the diagnosis. There is not much data in literature about overlooked hemogram parameters (ie. red cell distribution width, mean platelet volume, platelet distribution width) in vitamin B_{12} deficiency.

In present retrospective study evaluates the varying hematological manifestations in 640 children diagnosed as vitamin B_{12} deficiency.

MATERIAL AND METHOD

The data gathered from our institute Gaziantep University Hospital biochemistry laboratory electronic database. The period of this cross-sectional retrospective study was from 2019 to 2020. Six hundred forty children were evaluated: 278 females (43.4%) and 362 (56.6%) males. Ages of the patients were from 1 to 16 years old. The inclusion criteria were child patients with less than 200 ng/l vitamin B_{12} levels. The hematological parameters were measured using a Sysmex XN1000 analyzer. Serum vitamin B_{12} levels were done by Beckman Coulter, UniCel DXI 800 Access immunoassay system. The study protocol was approved by the Medical Ethics Committee of Gaziantep University.

We used vitamin B_{12} levels cutoff of<200 ng/l for children to estimate vitamin B_{12} deficiency since this was a widely used criterion in previous studies.[1,6,7] Macrocytosis was defined as MCV of >85 fL.[8] Anemia was defined as hemoglobin levels less than 11 g/dL, thrombocytopenia platelet counts less than 150000 /mm^3 and leucopenia white blood cell (WBC) counts less than 4000 /mm^3 and lymphopenia levels as lymphocyte counts less than 1500/ mm^3 with neutropenia levels as absolute neutrophil counts (ANC) less than 1500 / mm^3 for children in our study.[8] Pancytopenia was defined as hemoglobin levels <11 g/dL, WBC counts<4000 /mm^3, and platelet counts <150000 /mm^3. Their red cell indices (MCV, mean corpuscular hemoglobin [MCH], mean corpuscular hemoglobin concentration [MCHC], and RDW), were noted.

Statistical Analysis

Data were analyzed using SPSS 23.0 software (SPSS, Inc., Chicago, IL, USA). Demographic data were shown as means and SD or percentages. Categorical variables were compared by using chi-square test. Spearman's correlation analysis was performed to examine the correlations between age and vitamin B_{12}, and hematological measures. Two-tailed significance values are reported throughout. A probability level of $p<.05$ was used to indicate statistical significance.

RESULTS

The sample consisted of 640 children with vitamin B_{12} deficiency (362 males, 278 females) between 1 and 16 (mean±SD=8±4.5) years. The male-female ratio was 1.3. This study group was divided into two groups as group 1, cases<150 ng/l (n=279), and group 2, cases 150-200 ng/l (n= 361) according to vitamin B_{12} levels.

The mean ± SD of laboratory measures, including vitamin B_{12} levels, and hematological values, are summarized in **Table 1.** Four hundred seventy-seven patients (74.5%) had no hematological abnormalities. Anemia was detected in 99 patients (15.5%), two patients (0.3%) had a Hb-level<7 g/dl, anemia without macrocytosis in 91 (14.2%), anemia with macrocytosis in 8 (1.3%), leucopenia in 8 (1.3%), neutropenia in 22 (3.4%), lymphopenia in 9 (1.4%), thrombocytopenia in 6 (0.9%), and pancytopenia in 3 patients (0.4%). Leucopenia coexisted with anemia in 3 (0.4%) patients, leucopenia coexisted with thrombocytopenia in 1 patient, and anemia coexisted with thrombocytopenia in 2 (0.3%) patients.

Vitamin B_{12} levels was significantly lower, and platelet levels was significantly higher in group 1 (vitamin B_{12} levels<150 ng/l). There was no statistical significant difference between vitamin B_{12} levels and RBC, hemoglobin, hematocrit, MCV, MCH, MCHC, RDW, WBC, lymphocyte, neutrophil and MPV levels in all measures.

An examination of the correlation, there were no correlations between vitamin B_{12} levels and, hemoglobin, ($r=0.26$), $p>0.05$, hematocrit, ($r=0.19$), $p>0.05$, RBC, ($r=0.05$), $p>0.05$, RDW levels ($r=-0.56$), $p>0.05$, age ($r=0.09$), $p>0.05$, MCH ($r=0.02$), $p>0.05$, MCHC ($r=0.32$), $p>0.05$, MCV ($r=-0.17$), WBC ($r=-0.45$), $p>0.05$, neutrophil ($r=0.28$), $p>0.05$, lymphocyte counts, ($r=-0.58$), $p>0.05$, and platelet counts ($r=-0.69$), $p>0.05$, and $p>0.05$, MPV ($r=0.36$), $p>0.05$.

Table 1. Vitamin B_{12}, and hematological values

	Total n=640	Group 1, vitamin B_{12} levels <150 ng/l (n=279)	Group 2, vitamin B_{12} levels 150-200 ng/l (n= 361)	p Value
Vitamin B_{12} (ng/L)	150.7±33.8	120±25	175±14	<0.05
Hb(g/dL)	16±2.2	12.3±1.7	12.4±1.4	>0.05
RBC (/mm^3)	4.80 ±0.49	4.79 ±0.51	4.82±0.48	>0.05
Htc (%)	37.5±4	37.6±4.5	37.6±3.7	>0.05
MCV (fL)	78.5±6.3	78.9±5.8	78.3±6.7	>0.05
MCH (pg)	25.8±2.6	25.9±2.5	25.8±2.8	>0.05
MCHC(g/dL)	32.8±1.3	32.8±1.3	32.8±1.4	>0.05
RDW (%)	13.9±1.9	14.1±2.1	13.9±1.8	>0.05
WBC (/mm^3)	8245±2737	8460±3030	8080±2480	>0.05
Lymphocyte (/mm^3)	3691±1820	3820±2110	3590±1560	>0.05
Neutrophile (/mm^3)	3625±2061	3730±2380	3540±1780	>0.05
Platelet (/mm^3)	339.350±103.610	350.000±180.000	331.000±100.000	>0.05
MPV (/fl)	9.7±0.8	9.7±0.9	9.8±0.9	>0.05

Hemoglobin indicates; Hb, hemoglobin; RBC, red blood cell; Hct, hematocrit; MCV, mean corpuscular volume; MCH, mean corpuscular hemoglobin; MCHC, mean corpuscular hemoglobin concentration, RDW, red cell distribution width
P<0.05 was accepted to be statistically significant.

DISCUSSION

As Turkey is a country dependent on agriculture, plant-derived foods are cheaper than meat and dairy products. The most frequent cause of B_{12} deficiency in children is inadequate consumption due to dietary restriction or lack of access to food.[7] Studies in patients with anemia due to vitamin B_{12} deficiency have shown that impaired DNA synthesis and its sequelae are key elements in the increased hematopoietic cell death that characterizes these anemias. More severe deficiency of vitamin B_{12} is associated with hematological manifestations, such as anemia, leucopenia, thrombocytopenia and pancytopenia.[9] This study, based on real data from a single institution with a large number of consecutive cases with well documented vitamin B_{12} deficiency, confirms several other findings that have been previously reported.[10-14]

There is little consensus on appropriate cut-off point for determining normal and abnormal vitamin B_{12} deficiency.[1,6] The World Health Organisation (WHO) has recommended that a level of less than 203 ng/l be used as the threshold for defining Vitamin B_{12} deficiency.[7] Our laboratory used <200 pg/ml as the cut off point for vitamin B_{12} deficiency during our investigation.

Hematologic findings in patients with vitamin B_{12} deficiency vary from anemia to pancytopenia. Ineffective erythropoiesis, leucopoiesis and thrombopoiesis resulting from programmed cell death in the absence of vitamin B_{12}, and decreased survival of precursors in peripheral blood are causes of pancytopenia in vitamin B_{12} deficiency. In the English literature, there were a few studies with respect to frequency of hematological findings associated with vitamin B_{12} deficiency in children.[15] Previous studies showed that megaloblastic anemia, thrombocytopenia, neutropenia, or pancytopenia was case-based in children with vitamin B_{12} deficiency.[10-13] In the present study, anemia was detected in 99 patients (15.5%), two patients (0.3%) had a Hb-level<7 g/dl, anemia without macrocytosis in 91 (14.2%), anemia with macrocytosis in 8 (1.3%), leucopenia in 8 (1.3%), neutropenia in 22 (3.4%), lymphopenia in 9 (1.4%), thrombocytopenia in 6 (0.9%), and pancytopenia in 3 patients (0.4%). In a study including 201 adults with documented vitamin B_{12} deficiency, approximately 10% of the patients were found to have life-threatening hematological manifestations.[14] Among these were pancytopenia (5%), severe anaemia (defined as a haemoglobin level <6.0 g/dL) 2.5% and haemolytic anaemia (1.5%). Also, they found that 14% of patients (n =28) had a leucopenia, with severe neutropenia (neutrophil count <1 x 109/l) in six patients (3%), and 10% (n =20) had a thrombocytopenia, with two cases of symptomatic thrombocytopenia (platelet count <50 x 109/l). Sezer et al.[16] a total of 79 patients were included in the study, anemia was present in 18 (22.7%) children. None of the children had leukopenia, thrombocytopenia. In a study from Turkey, Atay et al.[15] in children found that had macrocytic anemia (10%), neutropenia (16.8%), bicytopenia (6%), and macrocytosis (3%) but none had pancytopenia and thrombocytopenia. Another study by Colak et al.[17] a total of 404 children (mean age: 3.2±1.1 years, median age: 4 years, age range: 1-5 years; 183 females/221 males were included. Ninety-four (23.3%) children had vitamin B_{12} deficiency. Patient's age, gender, and anemia and the levels of complete blood count parameters were not significantly different in cases with and without B_{12} deficiency (p>0.05). Emen et al.[18] in their study reported a cross-sectional study in 367 vitamin B_{12} deficiency subjects, assessed laboratory features and serum levels of vitamin B_{12}, no statistical significance was found between the B_{12} levels; age, gender, and peripheral blood parameters. In this study, there was no statistical significant difference between vitamin B_{12} levels and complete blood count parameters excluding platelet counts. The most common hematologic manifestation in our study was anemia and it was present in 15.5% of the patients. Also, anemia is just one possible outcome of vitamin B_{12} deficiency and should not therefore be used as a sole marker.

In our study vitamin B_{12} levels were not significantly correlated with the complete blood count parameters ($p>0.05$). Serum vitamin B_{12} levels were not significantly correlated with the complete blood count parameters ($p>0.05$) in Colak et al.[17] study Jain et al.[19] reported 89% patients were having normal or low MCV even they are vitamin B_{12} deficient. Vidja PJ.[20] in study there is no correlation between serum vitamin B_{12} level and MCV in the majority of cases. Hematologic findings may not always correlate with vitamin B_{12} levels. Therefore, vitamin B_{12} deficiency should not be excluded on the basis of a normal blood count. The findings of our study are in consistence with other studies.

Isolated thrombocytopenia is a common indication for hematologic consultation. Testing for vitamin B_{12} deficiency is commonly performed during the evaluation of cytopenias. In the series by Erkurt et al.[21] 5% of the patients admitting with thrombocytopenia had megaloblastic anaemia. Another study, Gupta et al.[22] analyzed the varying clinico-hematological manifestations in 50 children diagnosed as megaloblastic anemia over a four year period. Thrombocytopenia was reported in 30% patients in the study. In our study isolated thrombocytopenia was detected in 0.9%.

Limitations of the retrospective study are the lack of analysis of other variables that reflect the tissue deficiency of vitamin B_{12} such as serum methylmalonic acid and homocysteine. Further, this study did not collect any data about the medical history, diet, iron and folic acid status of the population. Also, we did not evaluate the dietary intake of vitamin B_{12} to better assess the vitamin B_{12} status of participants. Our results reflect an outpatient population, and it is not representative of general population.

CONCLUSION

Hematological abnormalities accompanying vitamin B_{12} deficiency are not common, hematological abnormalities were detected in approximately 25.5% of our cases, anemia and neutropenia were the most common hematological abnormalities, and also hematological parameters may not correlate with vitamin B_{12} levels.

ETHICAL DECLARATIONS

Ethics Committee Approval: This study was approved by institutional Review Board of Gaziantep University (Aproved number, 2020, 81).

Informed Consent: Due to the retrospective design of the study, informed consent of the patients was not necessary.

Referee Evaluation Process: Externally peer-reviewed.

Conflict of Interest Statement: The authors have no conflict of interest to declare.

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Author Contributions: H.T.A., and M.O. performed the research; H.T.A. designed the research study; H.T.A., and M.O. contributed essential reagents or tools; H.T.A. analyzed the data; and H.T.A. wrote the paper.

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