http://www.medscidiscovery.com

OPEN ACCESS JOURNAL ISSN: 2148-6832

MSD Medical Science and Discovery
2019; 6(9):186-91

Research Article Doi: 10.36472/msd.v6i9.290

Effects of vitamin B12 and folic acid deficiency on hemogram parameters in children

Kamil Şahin[1]*, Murat Elevli[1], Cihan Coşkun[2], Macit Koldaş[2]

Abstract

Objective: According to numerous studies, bicytopenia, pancytopenia, or isolated thrombocytopenia and anemia patients have folic acid (folate) and vitamin B12 (B12) deficiency. The purpose of this study is to analyze the effects of folate and B12 deficiency in childhood on several haemogram parameters such as platelet (PLT), mean platelet volume (MPV), hematocrit (HCT), mean corpuscular volume (MCV), and white blood cell (WBC) count.

Materials and Methods: The retrospective study included children who had applied to the pediatric outpatient clinic between 2015 and 2017. Patients were divided into 3 groups according to serum B12 and folate status. The results were evaluated by statistical methods.

Results: PLT and WBC levels of the folate and B12 deficiency group were found to be lower than the control group ($p=0.015$, $p<0.001$ respectively), and their MPV and HCT levels were higher (p: 0.015, $p<0.001$ respectively). MCV levels, however, were not different ($p>0.05$). No effect of PLT and MCV on folate levels was seen. Similarly, any effects of MPV, PLT and MCV independent variables on B12 levels were not observed. Although platelet and leukocyte count was decreased in folate and B12 deficiency, thrombocytopenia and leukopenia were observed only three patients.

Conclusion: Although peripheral blood cell lines are not always seen low during folate and B12 deficiency, and there is not obvious anemia and MCV highness at a patient with neurological and psychological symptoms, folate and B12 deficiency should be thought if there are leukocyte and thrombocyte levels lower than mean reference values.

Keywords: Children, Deficiency, Folate, Haemogram, Vitamin B12

Introduction

Vitamin B12 (B12) is necessary for the development of the fetus and the child. The exogenous intake of this vitamin is essential for human species who could not synthesize B12 (1). The vitamin is rarely found in food derived from plants; therefore, those following strict vegetarian diets are likely to have inadequate intakes of B12. Low B12 status may cause ineffective erythropoiesis because of the limited DNA synthesis due to inhibition of purine and thymidylate synthesis and this contributes to homocysteinemia and impairs the metabolic utilization of folate. As a result, B12 deficiency may produce various hematological, mucocutaneous, gastrointestinal or neurological signs and symptoms. The hematological sign is megaloblastic anemia. However, hematologic and neurologic signs may not emerge together all the time in B12-deficient subjects. In most deficient subjects, either anemia or neurologic signs predominate (2, 3). Folic acid (folate) is essential for normal embryonic growth and development. It is necessary to promote closure of the neural tube, defects of which result in malformations of the embryonic brain and/or spinal cord referred to as neural tube defects (NTDs).

Folate is widely distributed among food, particularly the ones with plant foliar origin, has an important role in the production of new cells through its functions in the synthesis of purine and thymidylate that are required for the de novo synthesis of DNA, and DNA replication and cell division. Disruption of these functions impairs cell division and results in macrocytic anemia of folate deficiency (3,4). Ineffective DNA synthesis depending on the deficiency of B12 and/or folic acid might also affect the development of megakaryocytes, as reflected by abnormal large polylobate megaloblastic megakaryocytes with a lack of cytoplasmic granules.

Received 07-08-2019 **Accepted** 11-09-2019 **Available Online** 13-09-2019 **Published** 30-09-2019
1 University of Health Sciences, Haseki Training and Research Hospital, Dept of Pediatrics, Fatih, İstanbul, TR
2 University of Health Sciences, Haseki Training and Research Hospital, Dept of Biochemistry, Fatih, İstanbul, TR
*** Corresponding Author:** Kamil Şahin **E-mail:** drkamil_sahin@hotmail.com **Phone:** +90 (212) 529 44 00

http://dx.doi.org/10.36472/msd.v6i9.290

Corresponding changes in the blood smear include anemia with oval macrocytes, anisocytosis, and poikilocytosis; leukopenia with hyper segmented polymorphonuclear cells; and thrombocytopenia (5). On the other hand, different results were reported on this study. In a retrospective design study involving 120 subjects suffering from anemia resulting from B12 deficiency, it was reported that only 28% of the subjects had thrombocytopenia, 29% had leukopenia and 17.3% had pancytopenia (6). In another study, it was declared that deficiency of B12 and/or folate might be a possible cause of isolated thrombocytopenia, an, increased platelet volume may also accompany with thrombocytopenia (7). The mean platelet volume (MPV), index of platelet reactivity, is frequently used in outpatient and inpatient healthcare facilities because of being a relatively low cost hemogram parameter and a useful one to determine platelet volume (8).

In this study, how the platelet count and the mean platelet volume (MPV) were affected from different levels of B12 and folate in children were analyzed. The relationship between B12 and folate with other hematological parameters such as red blood cell (RBC), mean corpuscular volume (MCV), and white blood cell (WBC) were also examined. In addition, the effects of independent variables such as age and gender on these parameters have been also evaluated.

Materials and Methods

This retrospective study was performed with an approval from local ethical committee (University of Health Sciences, Haseki Training and Research Hospital Ethical commission). The study included 371 children (204 females and 167 males) between the ages of 9 months and 18 years who had applied to the pediatric outpatient clinic with simple complaints between the dates 1.01.2015 and 31.12.2017. During this period, 806 patients received simultaneous vitamin B12, folate and hemogram. The study excluded 388 patients with acute and chronic infection and hematologic disease, and 47 patients whose results could not be reached due to a technical reason. The study groups were divided into 3 groups according to their B12 and folate levels. Cut-off points established by the kit manufacturer which used in this study. 180 pg/ml was accepted as cut-off point for B12 while 5.9 ng/ml was considered as cut-off point for folate. Accordingly, while Group 1 included 79 people (43 females, 36 males) whose folate (>5.9 ng/ml) and B12 levels (>180 pg/ml) were normal, Group 2 comprised of 191 people (103 females, 88 males) who suffered from B12 deficiency (<180 pg/ml). On the other hand, Group 3 consisted of 101 people (43 females, 58 males) who had folate deficiency. The hematological condition in both vitamin B12 and folate deficiency is as in vitamin B12 deficiency, even if folate is normal. Therefore, both low patients were not enrolled in the study. The necessary information and relevant test results of the patients were obtained from the hospital information management system. Those recorded in the system as having chronic disease, acute-chronic infection and hematologic problems were excluded from the study.

Serum folate and B12 levels were analyzed by using UniCel DxI 600 autoanalyser (Beckman Coulter, Inc. USA) with chemiluminescence method. The platelet count was determined by hydrodynamic centering factor whereas MPV values were obtained by calculating through the formula: MPV (fl)= [(PCT (%)/platelet count (×109/L)]. Platelet related tests were studied with Sysmex XE-2100 hematology analyzer (TOA Medical Electronics, Kobe, Japan).

It was confirmed that the internal quality control results of the tests were at ±2 standard deviation on the day of the analysis. The lowest detectable level of folate distinguishable from zero with 95% confidence was 0.5 ng/mL (1.1 nmol/L). Total imprecision of folate (CV%) was 4.34. The lowest detectable level of B12 distinguishable from zero with 95% confidence was 50 pg/mL (37 pmol/L). Total imprecision of B12 (CV%) was 8.4.

Statistical Analysis

Statistical evaluations were performed using the Statistical Package for Social Sciences (SPSS) 21 software (IBM, New York, USA). The Kolmogorov-Smirnov test was used to determine whether the numerical data showed normal distribution and Levene test was used to evaluate the homogeneity of the variances of each group. Categorical variables were assessed by Chi-square test whereas comparisons among study groups in terms of the numerical variables were performed with One-way ANOVA test.

Two-way ANOVA test and covariance analysis (ANCOVA) were used to evaluate the common effect of multiple independent variables on a given dependent variable. The gender distributions of the groups were evaluated by Student's T test. The correlation between hemogram parameters and folate/ B12 levels was evaluated by Pearson correlation test. The significance level of P value was accepted as <0.05. The results were expressed as mean ± standard deviation.

Results

There was no statistically significant difference between the groups in terms of mean age and gender. While MPV, PLT, WBC, and HCT showed significant differences among groups, there was not any significant difference between the groups in terms of MCV. Although platelet and leukocyte count was decreased in folate and B12 deficiency, thrombocytopenia and leukopenia were observed only three patients. (Table 1).

There was difference between normal group and groups with folate and B12 deficiency in terms of MPV level while there were not any differences between these two groups with deficiency. In terms of PLT levels, on the other hand, statistically significant difference was found only between the normal group and group with B12 deficiency. Although the mean PLT level in normal group was higher than the mean PLT level in the group with folate deficiency, this difference was not statistically significant. (Figure 1,2).

http://dx.doi.org/10.36472/msd.v6i9.290

Although the MCV level of normal group showed a remarkable highness than the deficiency groups, no significant difference was determined between the MCV averages of the three groups. When the HCT averages were compared, the difference between the Hct level averages of the normal group and the averages of both deficiency groups was significant. Similarly, there is a significant difference between the normal group and deficiency groups in terms of mean WBC.

While the HCT levels of deficiency groups were higher than the normal group, their WBC levels were lower. There were not any differences between deficiency groups in terms of the averages of these two parameters (Figure 3,4).

As a result of the multiple linear regression analysis where the effect of haemogram parameters as independent variables on the folate and B12concentrations were evaluated, it was determined that MPV, HCT and WBC had effects on folate concentrations.

A correlation between the increase in MPV and HCT levels and the decrease in folate levels was found whereas it was determined that there was a correlation between the decrease of WBC and folate below the normal range. However, PLT and MCV had no effect on folate concentration (R=0.401, p<0.001). Likewise, no effect of MPV, PLT and MCV independent variables on B12 level was monitored. Similar to the relations with folate, the increase of MPV and Hct levels were found to be related to the decrease in B12 level while a correlation between the decrease in WBC and lowness of B12 was determined (R=0.354, p<0.001). Furthermore, there was a highly strong negative correlation between age and especially folate level (r=-0.677, p<0.001), and a reasonable negative correlation was determined between B12 and age (r=-0.426, p<0.001).

No difference was found between folate and B12 levels in terms of gender. However, when the haemogram parameters were analyzed, differences between females and males were found in terms of HCT and MPV levels (Table2).

Table 1. Demographic characteristics and laboratory test results of study groups

Variables	Group 1	Group 2	Group 3	P value
Gender (F/M)	43/36	103/88	58/43	0.84
Age (years)	11.3±0.5	12.6±0.3	12.7±0.4	0,78
Folate (ng/ml)	16.2±4.4	8.4±5.0	4.7±0.8	<0.001
Vitamin B_{12} (pg/ml)	480±110	144±20	288±76	<0.001
MPV (fL)	9.6±0.9	10.0±0.9	10.0±1.0	0.002
PLT (10^3/µL)	318±87	276±79	290±80	0.015
MCV (fL)	93.1±9.6	80.1±5.6	79.4±5.2	0.07
HCT (%)	36.5±4.8	38.8±4.0	38.7±3.6	<0.001
WBC/ mm^3	8.1±2.6	6.9±2.3	6.7±2.0	<0.001

F: female, M: male, MPV: mean platelet volume, PLT: platelet, MCV: mid corpuscular volume, HCT: hematocrit, WBC: white blood cell.

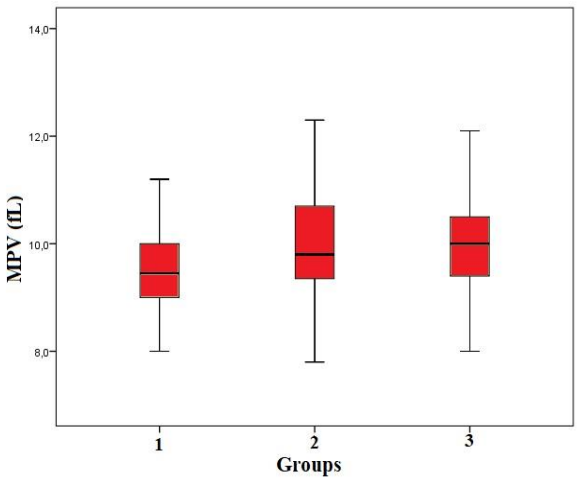

Figure 1. Box-plot graph showing mean platelet volume (MPV) of the groups

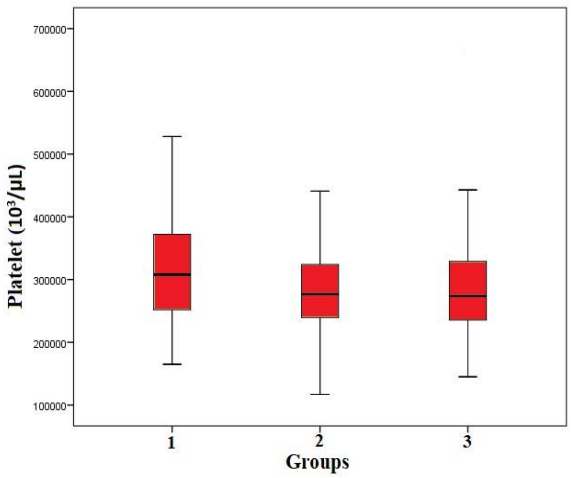

Figure 2. Box-plot graph showing the platelet count of the groups

http://dx.doi.org/10.36472/msd.v6i9.290

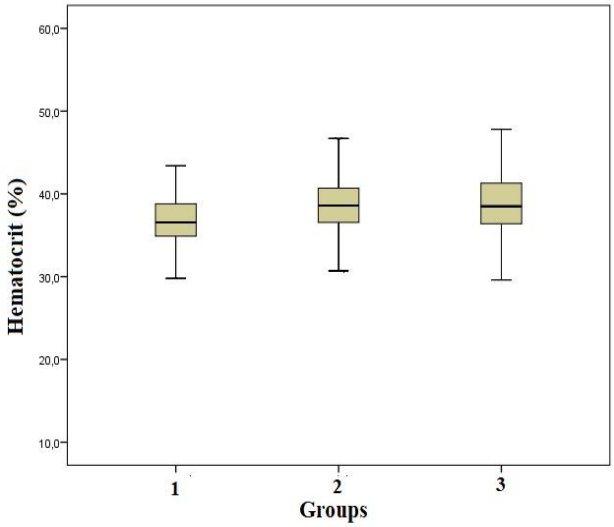

Figure 3. Box-plot graph showing hematocrit of the groups

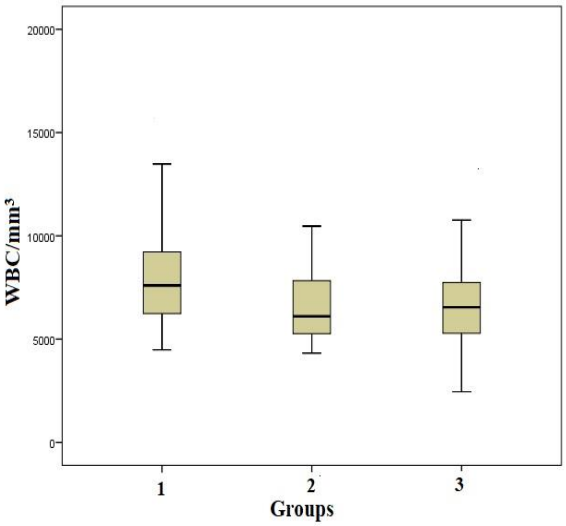

Figure 4. Box-plot graph showing white blood cell (WBC) of the groups

Table 2. Comparison of the values of the laboratory tests used in the study in females and males (Men±SD)

Variables	Female	Male	P value
Folate (ng/ml)	8.8±5.4	9.3±6.2	0.326
VitamineB_{12} (pg/ml)	280±196	261±166	0.307
MPV (fL)	10.0±1.0	9.7±0.9	0.005
Platelet (10^3/µL)	298±88	293±83	0.565
MCV (fL)	83.4±6.6	81.7±6.1	0.727
HCT (%)	37.6±2.9	39.0±5.0	<0.001
WBC/ mm^3	7.4±2.5	7.2±2.3	0.680

Discussion

Body needs B12 for tetrafolate production and methylation reactions that are required for DNA synthesis. In addition, folic acid is required together with B12 for the formation from homocysteine to methionine and the formation of shaped elements in the bone marrow. Therefore, it was reported that thrombocytopenia might occur as well as anemia and leukopenia as a result of the damage of DNA synthesis due to B12 and/or folate deficiency (9). In this study, it was observed that folic acid and B12 deficiency affected the platelets numerically and dimensionally. It was observed that platelet volume increased whereas platelet count decreased in the groups with deficiencies.

However, only a correlation between platelet volume increase and folate level decrease was determined. It was thought that the reason why there was no correlation between platelet count and the deficiency of these two vitamins could be the fact that the platelet decrease in the groups with folate and B12 deficiency was not at thrombocytopenia level, and that platelet levels of all the three groups showed changes within the reference ranges. Nevertheless, in a retrospective study by Jaggia A and Northern A, they argued that deficiency of B12 and/or folate might be a possible cause of isolated thrombocytopenia and platelet formation which is greater than normal levels (7).

The inverse correlation between platelet count and volume, which is a finding of this study, is similar to this study. In another study on a pediatric group, it was suggested that B12 deficiency may cause isolated thrombocytopenia without megaloblastic anemia and/or leukopenia (10).

In another retrospective designed study by Nafil H et.al., involving 120 subjects suffering from anemia resulting from B12 deficiency, it was reported that only 28% of the subjects had thrombocytopenia, 29% had leukopenia and 17.3% had pancytopenia (6). In this sense, different results have been obtained so far in the studies on this subject.

No correlation between corpuscular volume and folate and B12 levels was observed in the study. However, it was determined that HCT levels were higher in the groups where these vitamins were deficient, and that there was an inverse correlation between the deficiency of these two vitamins and HTC. Moreover, it was found out that leukocyte level was significantly lower in the groups with folate and B12 deficiency, and that there was a correlation. In some of the previous studies, only anemia due to B12 deficiency was observed, whereas other studies reported bicytopenia or pancytopenia (11,12).

In another study conducted by Refsum et. al., it was observed that the study group with B12 deficiency unexpectedly rarely had anemia and the reason for this was interpreted as that the adequate intake of folate suppressed

anemia (13). It is suggested to exclude B12 and folate deficiency primarily at the etiology of the patients applied with pancytopenia and bicytopenia in the literature (14).

However, it is needed to mention that 10% of the children in the study groups were suffering from anemia (HCT< 35) and there was no child with leukopenia in the study groups.

The differences among the haemogram parameters in the study represented the changes within reference ranges. Furthermore, it is necessary to consider that the folate and B12 deficiencies in the study groups were not at advanced levels. Nonetheless, in the literature, the lower limit level for serum B12 vitamin is stated to be as the 257 pg/mL in terms of homocysteine, and the 219 pg/mL in terms of urinary methyl malonic acids pillage. It is suggested to accept serum B12 lower limit level as the 250 pg/mL at routine practices for safety (15).

As the B12 averages of the deficiency group of the study were lower than these values, neurologic and psychiatric symptoms were started, but lower levels and low levels for longer period of time might be needed for the appearance of hematologic anomalies.

On the other hand, the retrospective design of the study prevented the clinical assessment of some tests and subjects. Measurement of plasma total homocysteine and serum methyl malonic acid levels as well as B12 levels for the diagnosis of B12 deficiency is also recommended by various guidelines (7).

In addition, the reliability of the study shall be enhanced by conducting a prospective design study of bone marrow examinations of patients. Another important factor is the duration of the symptoms of patients suffering from folate and/or B12deficiency. In our study, similar to some previous studies, there are limitations such as not knowing the duration of deficiency, not having bone marrow and methyl malonic acid and homocysteine levels done. The age average of the group without folate and B12 deficiency in the study was lower than that of the deficiency groups. However, no age based deficiency classification could be seen in the previous studies. We could only find a classification in accordance with suggested age for measurements of folate and B12 to be realized in plasma (16).

But we could not get use of this classification since we conducted the folate and B12 measurements in the study on serum samples. For this reason, cut-off values obtained with reference range studies by kit manufacturer were taken into consideration.

Conclusion

As a result, it was observed that folate and/or B12 deficiency decreased platelet count and WBC while increasing platelet volume and HCT. On the other hand, it was observed that corpuscular volume was not affected by the deficiency of these two vitamins.

Moreover, it was determined that these changes were not at a level to cause thrombocytopenia, leukopenia, or anemia.

Although one or several of the peripheral blood cell lines are not always low during folate and B12 deficiency, and there is not obvious anemia and MCV highness at a patient with neurological and psychological symptoms, the probability of folate and B12 deficiency should be considered if there are leukocyte and thrombocyte levels lower than mean reference values. In parallel with the decrease in platelet count, MPV values are also higher. However, further studies are also suggested considering the limitations mentioned above.

Acknowledgements: None

Conflict of Interest: The authors declare no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

Author's Contributions: **KŞ, ME,:** Patient examinations, **CC, MK:** Biochemical Analysis**, KŞ:** interpretation of the data, preparation of the manuscript, application of the statistical analyses

References

1. Akcaboy M, Malbora B, Zorlu P, Altınel E, Oguz MM, Senel S. Vitamin B12 deficiency in infants. The Indian Journal of Pediatrics. 2015 Jul 1;82(7):619-24.

2. Koury MJ, Ponka P. New insights into erythropoiesis: the roles of folate, vitamin B12, and iron. Annu. Rev. Nutr.. 2004 Jul 14;24:105-31.

3. Combs Jr GF, McClung JP. The vitamins: fundamental aspects in nutrition and health. Academic press; 2016 Dec 15.

4. Mahmood L. The metabolic processes of folic acid and Vitamin B12 deficiency. Journal of Health Research and Reviews. 2014 Jan 1;1(1):5.

5. Green R, Mitra AD. Megaloblastic anemias: nutritional and other causes. Medical Clinics. 2017 Mar 1;101(2):297-317.

6. Nafil H, Tazi I, Sifessalam M, Bouchtia M, Mahmal L. Clinical, biological and therapeutic profile of anemia by vitamin B12 deficiency in the department of hematology of Marrakech (Morocco). Bulletin de la Societe de pathologie exotique (1990). 2013 May;106(2):83-8.

7. Jaggia A, Northern A. Vitamin B12 and/or Folate Deficiency is a Cause of Macro Thrombocytopenia. Global Journal of Hematology and Blood Transfusion. 2015;2:20-4.

8. Chu SG, Becker RC, Berger PB, Bhatt DL, Eikelboom JW, Konkle B, Mohler ER, Reilly MP, Berger JS. Mean platelet volume as a predictor of cardiovascular risk: a systematic review and meta-analysis. Journal of Thrombosis and Haemostasis. 2010 Jan;8(1):148-56.

9. Guilland JC, Aimone-Gastin I. Vitamin B9. La Revue du praticien. 2013 Oct;63(8):1079-81.

10. Okur M, Ozkan A, Gunes C, Kaya M, Kocabay K. A case of isolated thrombocytopenia due to cobalamin deficiency. International journal of hematology. 2011 Nov 1;94(5):488-90.

11. Stabler SP, Allen RH, Savage DG, Lindenbaum J. Clinical spectrum and diagnosis of cobalamin deficiency [see comments]. Blood. 1990 Sep 1;76(5):871-81.

12. Sarode R, Garewal G, Marwaha N, Marwaha RK, Varma S, Ghosh K, Mohanty D, Das KC. Pancytopenia in nutritional megaloblastic anaemia. A study from north-west India. Tropical and geographical medicine. 1989 Oct;41(4):331-6.

13. Refsum H, Yajnik CS, Gadkari M, Schneede J, Vollset SE, Örning L, Guttormsen AB, Joglekar A, Sayyad MG, Ulvik A, Ueland PM. Hyperhomocysteinemia and elevated methylmalonic acid indicate a high prevalence of cobalamin deficiency in Asian Indians. The American journal of clinical nutrition. 2001 Aug 1;74(2):233-41.

14. Davies JK, Guinan EC. An update on the management of severe idiopathic aplastic anaemia in children. British journal of haematology. 2007 Feb;136(4):549-64.

15. Erdol S. An Important Public Health Problem: Vitamin B12 Deficiency. GUNCEL PEDIATRI-JOURNAL OF CURRENT PEDIATRICS. 2017 Aug 1;15(2):30-6.

16. Hicks JM, Cook J, Godwin ID, Soldin SJ. Vitamin B12 and folate. Pediatric reference ranges. Archives of pathology & laboratory medicine. 1993 Jul;117(7):704-6.