

ORIGINAL ARTICLE

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Retrospective investigation of the effect of Vitamin B12 deficiency on hemogram parameters

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Abstract

Vitamin B12 deficiency is a common health problem in the population. In this study, any hemogram parameter that can detect Vitamin B12 deficiency was investigated. A total of 121 patients, including 67 patients with vitamin B12 deficiency and 54 patients without vitamin B12 deficiency, were retrospectively investigated for vitamin B12 deficiency in Abant İzzet Baysal University, Faculty of Medicine, Department of Internal Medicine. The findings were analyzed statistically. All non-hemogram laboratory parameters were found to have a similar distribution. Among the hemogram parameters, the following parameters were significantly different between the groups; platelet-crit (PCT) value from normally distributed values ($p=0.017$), platelet distribution width (PDW) value from non-normally distributed values ($p=0.030$), and PDW/platelet (PLT) ratio ($p=0.037$) from values calculated from hemogram parameters. In addition, haemoglobin (HGB) and mean corpuscular volume (MCV) values were found to be significantly correlated ($p=0.0001$). There was a significant difference in the presence of anaemia between the groups according to gender ($p=0.0001$). When evaluated separately, this significant difference persisted only in women ($p=0.0001$). ROC analysis results of PDW, PCT and PDW/PLT ratio values, which were significant in the comparisons, revealed that PDW predicted Vitamin B12 deficiency with the highest specificity (63.7%) and sensitivity (64.2%). Our results showed that not only HGB and MCV but also PCT, PDW and PDW/PLT parameters can be useful in detecting the Vitamin B12 deficiency-related processes.

Keywords: Anaemia, hemogram parameters, vitamin B12

Introduction

Vitamin B12 deficiency is a common health problem. Generally, there are enough vitamin B12 in foods to be necessary for the human body [1]. Vitamin B12 has a coenzyme role in the reactions that take place during the DNA synthesis phase [1,2]. Vitamin B12 is necessary for the nervous system and hematopoietic cells to maintain their functions [2]. The causes of Vitamin B12 deficiency can be listed as follows; Vitamin B12 deficiency in the diet, decreased intrinsic factor production, pernicious anaemia, gastrectomy, *Helicobacter pylori* (HP) infection, blind-loop syndrome, intestinal parasites, pancreatic insufficiency, decreased ileal absorption of Vitamin B12 (due to surgical

resection or Crohn's disease), and transcobalamin deficiency [3].

Hematologically, macrocytic anaemia, thrombocytopenia, and neutropenia are observed in Vitamin B12 deficiency, and pancytopenia is observed in severe Vitamin B12 deficiencies [3,4]. Neurologically, paresthesia, peripheral neuropathy, optic neuropathy, incontinence problems (faecal or urinary), and problems with memory can be seen [4,5]. Regarding the gastrointestinal system, loss of appetite, dyspepsia, nausea-vomiting, abdominal pain, aphthous ulcers, atrophic glossitis (Hunter's tongue), jaundice, diarrhoea and a decrease in the regeneration rate of the gastrointestinal epithelium may be observed [4,5]. In the cardiovascular system, the atherosclerotic process can be seen due to hyperhomocysteinemia [5,6].

In our study, the effect of Vitamin B12 deficiency on hemogram parameters was examined and a reliable parameter to detect it in the early period was investigated.

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Materials and Methods

Ethical Approval

Our study has approved the provisions of the World Medical Association's Declaration of Helsinki, which was developed as a statement of ethical principles to guide physicians and other participants in medical research involving human subjects. Our study was approved by the local Ethics Committee (no:293). All patients were given detailed information about the content of the study and written informed consent was obtained.

Patients

A total of 121 patients, including 67 patients with vitamin B12 deficiency and 54 patients without vitamin B12 deficiency, were retrospectively investigated for vitamin B12 deficiency in Abant İzzet Baysal University, Faculty of Medicine, Department of Internal Medicine for 4 months. Values above 187 pg/ml, which is the cut-off value of our laboratory, were considered normal for serum Vitamin B12 levels. According to WHO criteria the presence of anaemia was accepted as values below 13 g/dl in men and below 12 g/dl in women. Other laboratory tests include urea, creatinine, estimated glomerular filtration rate (eGFR), sodium, potassium, alanine aminotransferase (ALT), aspartate aminotransferase (AST), thyroid-stimulating hormone (TSH), ferritin, iron, iron-binding capacity (IBC), total iron-binding capacity (TIBC), Vitamin B12, folate, sedimentation, c-reactive protein (CRP), white blood cell (WBC), red blood cell (RBC), haemoglobin (HGB), hematocrit (HCT), mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC), neutrophil (NEU), eosinophil (EOS), basophil (BASO), monocyte (MONO), lymphocyte (LYM), platelet (PLT), mean platelet volume (MPV), plateletcrit (PCT), platelet distribution width (PDW) values were recorded. The normal ranges of our hospital's laboratory were

taken as a reference for all these parameters.

Statistical Evaluation

The collected data were recorded in the SPSS 20 (SPSS 20.0 for Windows, IBM, Chicago, USA) program. The Kolmogorov-Smirnov test was used to evaluate whether the variables were normally distributed. The comparison of normally distributed parameters was done with the Student-T test. Parameters that were not normally distributed were compared with the Mann-Whitney U test. Categorical data were compared with the Chi-square test. The Chi-square test (Monte Carlo) was used to compare multiple categorical data. The correlation between Vitamin B12 level, MCV and HGB levels were determined by the Pearson test. Sensitivity and specificity ratios for significant hemogram parameters were determined by ROC curve analysis. The statistical significance level of $p<0.05$ was accepted.

Results

Demographic findings

In our study, the gender distribution of the groups with and without Vitamin B12 deficiency was found to be similar ($p=0.076$) (according to the Chi-square test with Yate's correction). It was observed that the mean age of the groups was evenly distributed ($p=0.157$). The mean age of women with Vitamin B12 deficiency was 46.83 ± 20.13 years, and the mean age of women without Vitamin B12 deficiency was 44.19 ± 16.34 years ($p=0.513$). The mean age of men with Vitamin B12 deficiency was 46.81 ± 20.19 years and the mean age was 41.83 ± 14.08 years ($p=0.388$). When the mean ages of the groups with and without Vitamin B12 deficiency were compared, no significant difference was found ($p=0.334$). There was no significant difference in age between the groups according to Vitamin B12 deficiency (female $p=0.513$, male $p=0.388$, overall $p=0.334$) [Table-1].

Table 1. Demographic characteristics (age and gender) of the study group

	Vitamin B12 Deficient	Vitamin B12 normal	p-value
Number (n)	67	54	-
Gender	Female n(%)	41 (49.4)	0.076
	Male n(%)	26 (68.4)	
The mean age	Female (mean age $\pm$ SD)	46.83 $\pm$ 20.13	0.513
	Male (mean age $\pm$ SD)	46.81 $\pm$ 20.19	0.388
	Overall (mean age $\pm$ SD)	46.82 $\pm$ 19.99	0.334

Abbreviations: SD: Standard deviation, the comparison of the genders was made with the Chi-Square X² test, the comparison of the means was made with Student's T-test. $p<0.05$

Comparison of hemogram and non-hemogram laboratory parameters

As a result of the comparison of non-hemogram laboratory parameters, it was determined that all parameters had a similar distribution [Table-2]. Of the hemogram parameters, uniformly distributed parameters were RBC, HGB, PLT, LYM, BASO, MPV,

and PCT. Non-uniformly distributed parameters were WBC, HCT, MCV, MCH, MCHC, MONO, NEU, EOS, and PDW. When the uniformly distributed hemogram parameters were compared, only the PCT ($p=0.017$) value was found to be significantly lower [Table-3]. Only PDW value was found to be significantly higher in non-uniformly distributed hemogram parameters ($p=0.030$) [Table-3]. Neutrophil/lymphocyte ratio (NLR), platelet/

lymphocyte ratio (PLR), PDW/PLT ratio, MPV/PLT ratio were calculated from hemogram parameters. It was determined that these values were not homogeneously distributed. When these parameters were compared between the groups, only the PDW/PLT ratio was found to be significantly higher ($p=0.037$) [Table-3].

Correlation between Vitamin B12, haemoglobin and MCV values

Of the Vitamin B12, haemoglobin and MCV values, only haemoglobin was uniformly distributed. Therefore, the Spearman Correlation test was preferred and haemoglobin and MCV were found to be significantly correlated ($p=0.0001$).

The effect of Vitamin B12 deficiency on anaemia and MCV

When the anaemia status of those with and without Vitamin B12 deficiency was compared in general and according to gender, there was no significant difference between the groups ($p=0.089$). MCV values had a similar distribution between those with Vitamin B12 deficiency and those with normal ($p=0.911$). When compared according to gender, there was no significant difference between the groups in terms of MCV values ($p=0.798$ for women, $p=0.678$ for men). When the presence of anaemia and MCV values were compared, a significant difference was observed between the groups ($p=0.0001$). When evaluated according to gender, this significant difference persisted only in women ($p=0.0001$). When the groups

were compared in pairs, there was only a significant difference between microcytic anaemia and normocytic anaemia ($p=0.0001$). This significant difference was also found in women ($p=0.0001$).

ROC analysis of significant hemogram parameters

ROC analysis of the PDW, PCT, PDW/PLT ratio parameters, which were found to be significant in the comparison between the groups, was performed, and it was seen that PDW predicted Vitamin B12 deficiency with the highest sensitivity and specificity (64.2% sensitivity and 63.7% specificity) [Table-4, Figure-1].

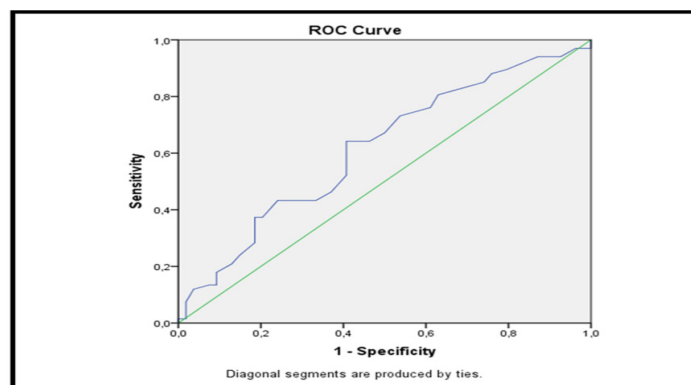


Figure-1. ROC curve of PDW

Table 2. Comparison of non-hemogram laboratory parameters

	Vitamin B12 Normal $\pm$ SD	Vitamin B12 Deficient $\pm$ SD	p-Value
Folate (ng/ml)	8.09 $\pm$ 3.20	7.65 $\pm$ 8.54	0.718
TSH (μ u/l)	1.82 $\pm$ 1.48	2.29 $\pm$ 5.92	0.574
Ferritin (ng/ml)	50.44 $\pm$ 87.57	50.66 $\pm$ 66.77	0.987
Iron (μ g/dl)	78.65 $\pm$ 30.54	77.84 $\pm$ 43.48	0.908
IBC (μ g/dl)	255.76 $\pm$ 78.57	256.57 $\pm$ 82.47	0.957
TIBC	334.41 $\pm$ 67.91	334.21 $\pm$ 69.85	0.987
ALT (u/l)	16.15 $\pm$ 7.41	16.16 $\pm$ 9.67	0.992
AST (u/l)	22.28 $\pm$ 32.54	17.63 $\pm$ 5.63	0.253
Urea (mg/dl)	28.71 $\pm$ 16.78	29.16 $\pm$ 12.16	0.865
Creatinine (mg/dl)	0.75 $\pm$ 0.24	0.76 $\pm$ 0.13	0.623
eGFR	102.94 $\pm$ 22.36	101.75 $\pm$ 19.70	0.755
Sodium (mmol/l)	138.0 $\pm$ 2.33	138.96 $\pm$ 2.99	0.133
Potassium (mmol/l)	4.46 $\pm$ 0.39	4.39 $\pm$ 0.38	0.289
Sedimentation	23.11 $\pm$ 16.22	24.98 $\pm$ 20.28	0.647
CRP (mg/l)	7.68 $\pm$ 23.66	7.52 $\pm$ 15.97	0.965

Abbreviations: ALT: Alanine aminotransferase, AST: Aspartate aminotransferase, CRP: C-reactive protein, eGFR: Estimated glomerular filtration rate, IBC: Iron-binding capacity, SD: Standard deviation, TIBC: Total iron-binding capacity, TSH: Thyroid-stimulating hormone. The comparison of the means was made with Student's T-test. $p<0.05$

Table 3. Comparison of hemogram parameters

	Vitamin B12 Normal	Vitamin B12 Deficient	p-Value
PCT (%)	0.19 $\pm$ 0.05	0.17 $\pm$ 0.04	0.017
PDW	17.55 (16.40-20.20)	18.00 (15.60-20.40)	0.030
PDW/PLT	0.069 (0.03-0.11)	0.075 (0.04-0.12)	0.037

Abbreviations: PCT: Plateletcrit, PDW: Platelet distribution width, PDW/PLT: Platelet distribution width/Platelet, PLT: Platelet, The comparison of means was done with Student's T-test, and the comparison of medians was done with the Mann-Whitney-U test. $p<0.05$

Table 4. ROC analysis of significant hemogram parameters

	Cut-off	Sensitivity	Specificity
PDW/PLT (k/ul)	0.0716	%58.2	%55.6
PCT (%)	0.1795	%44.8	%44.4
PDW	17.650	%64.2	%63.7

Abbreviations: PCT: Plateletcrit, PDW: Platelet distribution width, PDW/PLT: Platelet distribution width/Platelet

Discussion

Vitamin B12 deficiency, which is encountered at rates ranging from 3% to 40% from country to country in the world, constitutes an important health problem [7,8]. In many studies in the literature, no relationship was found between Vitamin B12 deficiency and gender [9,10]. In our study, no significant difference was found between patients with Vitamin B12 deficiency and gender. The high rate of the female population in our study was attributed to the high female population applying to the polyclinic during working hours.

In our study, we did not find a significant relationship between age and Vitamin B12 levels. In the study of Framingham, although there was a tendency to decrease in direct proportion between Vitamin B12 level and increasing age, no significant difference was found in age groups [11]. In a study involving the 35-64 age group, no relationship was found between age and Vitamin B12 level [12]. In a study conducted in Ankara with 310 patients, it was evaluated that age may be an independent risk factor that may cause Vitamin B12 deficiency [13]. In a prospective study conducted in Izmir in which people over the age of 65 were included in the study, a direct ratio was found between age and Vitamin B12 deficiency [14]. This situation was thought to be related to the fact that we received quite a few patients with isolated vitamin B12 deficiency from the other outpatient clinics; patients with absorption problems that increase with age often apply to the gastroenterology outpatient clinic and the different nutritional and socioeconomic status of the population in our region.

The reasons for the decrease in Vitamin B12 levels in elderly patients are the decrease in vitamin absorption compared to young people and the more frequent occurrence of atrophic gastritis due to chronic HP infection [15,16]. In a study conducted with patients with atrophic gastritis, no relationship was found between Vitamin B12 level and age [17]. And this was associated with the young age average we determined in our study and the very small number of patients in the gastroenterology outpatient clinic.

In our study, we did not find any significant difference with non-hemogram laboratory parameters (folate, TSH, ferritin, iron, IBC, TIBC, ALT, AST, urea, creatinine, eGFR, sodium, potassium, sedimentation, CRP). Among these parameters, the average of the parameters of iron deficiency and the average of TSH were determined close to the optimal ranges, showing that there were no situations that could be misleading by affecting MCV except for Vitamin B12. In a study, anaemia was not found in 44% of 86 patients whose Vitamin B12 levels were found to be low, MCV was <100 fl in 36%, and LDH value was found within normal ranges in 43% [18]. In another study, anaemia or macrocytosis was not found in 28% of 141 patients diagnosed with neuroanatomic

syndrome due to Vitamin B12 deficiency [19]. The absence of significant anaemia or MCV elevation in patients with Vitamin B12 deficiency in our study was attributed to the low number of patients with severe Vitamin B12 deficiency.

Although Vitamin B12 values above 180 pg/ml are generally accepted as normal, there are also studies suggesting that the lower limit value should be set above 250 pg/ml and even 300 pg/ml [20]. Bernard et al., in their study with elderly patients with Vitamin B12 levels less than 150 pmol/L, concluded that 90% of the patients had high homocysteine levels [21]. Although we considered values below 187 pg/ml to be a deficiency in our study, we concluded that it would have been more accurate to take at least 300 pg/ml as a reference in Vitamin B12 treatment. The biggest problem encountered in the diagnosis of Vitamin B12 deficiency is encountered in Vitamin B12 deficiency, which occurs before the symptoms of anaemia appear. This has led to the search for early diagnostic criteria and methods based on the measurement of MMA and homocysteine in Vitamin B12 deficiency have been developed [22]. The classical technique for detecting Vitamin B12 deficiency is measuring the level in the serum. However, Vitamin B12 levels are found to be normal in approximately 50% of the patient group evaluated as subclinical [23]. Holo-Tc, the active form of Vitamin B12, plays a role in the uptake of Vitamin B12 into the cell. In some studies, holo-Tc was found to be more sensitive in the early diagnosis of Vitamin B12 deficiency [23]. In the study conducted by Obeid and Hermann with a group of 1018 patients, they concluded that the measurement of holo-Tc is more sensitive and specific in detecting Vitamin B12 deficiency compared to the total cobalamin level [24]. In the study conducted by Woo et al. with 184 patients, they found that the holo-Tc level was more sensitive in patients with Vitamin B12 levels of 151-300 pmol/l [25]. In the study of 244 elderly patients, Robinson et al. found a strong correlation between holo-Tc and depressive syndromes [26]. In addition, this related condition was not found in total Vitamin B12 levels and homocysteine. Thus, they concluded that holo-Tc is more sensitive and specific [27]. The fact that we did not detect the relationship between MCV and Vitamin B12 expected in Vitamin B12 deficiency in our study suggests that more specific parameters such as holo-Tc are needed.

Macrocytosis (MCV>96 fl) due to Vitamin B12 deficiency has an important place in the diagnosis of Vitamin B12 deficiency, and the MCV value tends to increase even before a decrease in haemoglobin parameters [28,29]. However, when Vitamin B12 deficiency is accompanied by iron deficiency or thalassemia, it can be detected as normal or lower than normal. In a study, less than half of the patients with an MCV value higher than 115 fl had a normal level of Vitamin B12 and a low level of Vitamin B12 was found in one patient with an MCV value higher than 130 fl

[28]. Since most of the Vitamin B12 in the serum is transported by haptocorrin, the measurement of the serum Vitamin B12 level is of low diagnostic value [29]. In addition, it is difficult for Vitamin B12, which is in complex with haptocorrin, to be taken into the cell [29].

Another factor that causes a change in serum Vitamin B12 level is the level of binding proteins [30]. While false Vitamin B12 elevation can be seen in myeloproliferative diseases, false Vitamin B12 deficiency may be observed in cases such as folate deficiency, transcobalamin deficiency and pregnancy. On the other hand, Bacterial overgrowth can cause false Vitamin B12 measurements by causing inactive Vitamin B12 forms [29,30]. The reasons why we could not detect MCV elevation in our study were as follows; inactive forms of Vitamin B12 due to bacterial overgrowth, the presence of proteins to which Vitamin B12 binds in plasma, and transcobalamin deficiency. In addition, we did not detect a significant increase in MCV values in patients with Vitamin B12 deficiency in our study. In our study, a significant difference was found between MCV and HGB in the general population and the female population, but no significant relationship was found in the male population. This is due to the small male population and the fact that our sample could not reflect the total male gender.

Bozkurt et al. concluded in their study that MPV values increased after treatment in patients with active infection and MPV was useful in demonstrating active infection [31]. In our study, no significant difference was found in the mean CRP and sedimentation values of patients with Vitamin B12 deficiency, so secondary factors that might affect MPV were excluded. However, in our study, we did not find a significant difference in terms of MPV value in patients with Vitamin B12 deficiency. We think that the relationship between MPV and Vitamin B12 deficiency should be examined in more details with more comprehensive studies to be done with more patients.

Platelets are of great importance in vascular pathologies. The MPV value indicates the activated platelets, and the PDW value indicates the heterogeneity of the platelets [32,33]. It is known that PDW is more important in platelet activation and that MPV and PDW values should be evaluated together in the coagulation process [33]. In our study, the PDW value was found to be significantly higher in patients with Vitamin B12 deficiency. Our results suggest that PDW, hence heterogeneity in platelets, increases in those with Vitamin B12.

In a study conducted with a group of patients who were found to be positive for HBsAg, it was found that the PCT level was significantly lower and the PDW value was significantly higher, and it was concluded that platelet activation was increased in these patients [33]. We also evaluated the similar results we found in our study. However, we think that this idea should be investigated by supporting it with larger patient groups.

Conclusion

Our findings showed that not only HGB and MCV but also other hemogram parameters such as PCT, PDW and PDW/PLT may be affected. These parameters can be useful in detecting the Vitamin B12 deficiency-related processes. Therefore, more comprehensive studies are needed to reveal the effects of Vitamin B12 deficiency on hemogram parameters.

Conflict of interests

The authors declare that there is no conflict of interest in the study.

Financial Disclosure

The authors declare that they have received no financial support for the study.

Ethical approval

Our study was approved the provisions of the World Medical Association's Declaration of Helsinki, which was developed as a statement of ethical principles to provide guidance to physicians and other participants in medical research involving human subjects. Our study was approved by the local Ethics Committee (no:293).

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