Detection of vitamin B12 levels with the aid of some hematological and biochemical parameters that are more sensitive

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ABSTRACT: Vitamin B12 insufficiency causes neurological and hemostatic problems; it mostly affects the elderly. We set out to determine if, after accounting for a number of hematological and biochemical factors, vitamin B12 levels in the blood may be a good proxy for tissue levels. The age range of the 128 patients who were included in the study was 16–90, and all of them had low vitamin B12 levels. There were 53 women among them. We looked at things like homocysteine levels, complete blood counts, APA, peripheral blood smears, and gastroduodenoscopy results. The average number of red blood cells in the first group of patients (group 1) with a vitamin B12 level below 60 pg/mL was 107 fl. As a result, 85% of patients had elevated homocysteine levels, 82% had neutrophil hyper-segmentation, 53% had atrophic gastritis, and 47% tested positive for APA. It has been determined that the average age in this group is 65. Group 2 patients with vitamin B12 levels between 61 and 100 pg/mL had a high homocysteine level and an MCV of 91 fl. In group 3, whose vitamin levels range from 101 to 140 pg/mL, 27% of subjects had increased homocysteine levels and 89% had MCV. Vitamin levels in group 4 range from 141 to 178 pg/mL, with 16% of cases showing an increase in homocysteine and MCV as

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INTRODUCTION

Megaloblastic anemia, which may be caused by a lack of vitamin B12 or folic acid, requires prompt diagnosis and appropriate treatment. The diagnosis of vitamin B12 and folic acid insufficiency is straightforward, and the treatment for both conditions is quite inexpensive. Serious consequences, including anemia, permanent brain damage, neural tube abnormalities, and thromboembolism, might result from treatment delays (1-3). More people than previously thought suffer from vitamin B12 insufficiency (4, 5). In the elderly, the incidence is close to 10% to 20%.

Nevertheless, asymptomatic vitamin B12 insufficiency affects around 5-10% of people. Neuropsychiatric symptoms, glossitis, macrocytic anemia, and various cytopenias are the hallmarks of traditional clinical presentation. Vitamin B12 levels in the blood, together with those of homocysteine and methylmalonic acid, must be determined in order to make a proper diagnosis (6).

One of the current diagnostic tools, vitamin B12 levels in the blood, is known to not be indicative of the actual tissue levels (7). Vitamin B12 deficient individuals often have anti-thyroid antibodies (T4), anti-intrinsic factor antibodies (AIs) in 60% of cases, and anti-parietal cell antibodies (APAs) in 90% of cases (8). Detecting vitamin B12 insufficiency is easier when the levels of homocysteine and methyl malonic acid, which are increased in the early stages of vitamin B12 deficiency, are measured. Considering the sensitivity (94%) and specificity (99%) of elevating at least one metabolite. Serological detection of antibodies to intrinsic factor and parietal cells has often supplanted the Schilling test, which was formerly used to identify peripheral anemia (9).

Clinicians may perform needless treatments and interventions on individuals whose blood vitamin B12 levels are determined to be reduced, even while they show no clinical indicators of abnormalities. We set out to determine if hematological and biochemical tests might detect vitamin B12 deficits more effectively in situations where blood vitamin B12 levels were found to be lower than reference values.

MATERIALS AND METHODS

Selection of the cases and design of the study

Of the patients that have been applied to our center for anemia between April 2005 and November 2007 or are referred to he- matology consultation for having lower vitamin B12 levels, atotal of one hundred fifty eight cases whose ages are between16 and 90 and fifty three of those are female; have been re- trieved retrospectively for the information regarding headings of diagnosis/referring diagnosis, cause of application, physi- cal examination findings, assessment/plan laboratory data, anti

parietal antibodies result, radiology/endoscopy/ pathol- ogy, consultation, etiology, treatments and advises heading from computer software (Avicenna HBSY, Ankara, Turkey) for the nutritional anemias. The patients with the combined iron deficiency, folic acid deficiencies or thalessemia carriers have been excluded from the study.

Patients have been categorized into four groups based on their vitamin B12 levels. The 34 patients whose vitamin B12 levels are lower than < 60 pg/mL, their ages ranged from 32 to 90and 13 of them are females constituted the group 1 one; the 32 patients, whose vitamin B12 levels are between 61 and 100 pg
/mL, their ages ranged from 19 to 87 and 14 of them are fe- males constituted the group 2, 29 patients one, whose vitamin B12 levels are between 101 and 140 pg/mL, their ages rangedfrom 21 to 88 and 12 of them are females constituted the group 3, 28 patients, whose vitamin B12 levels are higher than 141 to 178 pg/ mL, their ages ranged from 16 to 62 and 14 ofthem are females constituted the group 4. A total of 128 pa-tients have been included into study. Demographic informa- tion and clinical findings of the patients have been recorded.

Laboratory tests

The complete blood count, thyroid function tests, folic acid and homocysteine levels and the peripheral blood smear eval-uations have been assessed in the venous samples of the pe- ripheral blood that have been drawn from the fasting patients. The preparations have been used to detect the auto-anti bodies against to parietal cells commercially (Euroimmun, AG, Ger- many). They have been incubated with 1/10 patient serum forinitial dilution. Then they have been incubated with IgG – FITC and the parietal antibodies have been detected with im- mune fluorescent microscope (Euro star, Euroimmune, AG, Germany). The reference range has been detected as the 179 – 883 pg/mL, with the chemiluminescent microparticle immune assay (Abbott Laboratories, Abbott Park, IL, USA) in the Arci-tect device. The homocysteine levels have been measured in the AXYM device (Abbott park, IL, USA) with the fluorescencepolarization immune assay method and reference range has been taken as 5.9 – 16 µmol/L in males, 3.36–20.44 µmol/ L in females. The blood counts have been analyzed with the Cell Dyn 37000 automatic blood count analyzer (Abbott park, IL, USA) . Thyroid function tests have been measured with Chemiluminescent Microparticle Immunoassay in the Arcitectdevice (Abbott Park, IL, USA). The patients with combined folic acid and Vitamin B12 deficiencies have been excluded from the study.

The helicobacter pylori positivity, has been shown either with the fast urease test (Pronto Dry, Medical Instr, Solothurn, Swit-zerland) or the histopathological assessment in the patients who underwent endoscopy. The quality control measure- ments have been performed with Biorad External Quality As-say System 3517 in the laboratory.

The data that were gathered have been transferred to SPSS (15th November 2001) statistical software package and analyzed with one way ANOVA and Pearson Chi square. P values that are less than 0 .05 were considered as statistically significant.

RESULTS

The correlations between the vitamin B12 levels and the age,hemoglobuline, homocysteine, APA, hyper segmentation finding in peripheral blood smear, MCV, thyroid function tests and patients with clinically diagnosed neurological prob-lems have been depicted in table 1. The age, MCV, hemoglob- ine, homocysteine, hypersegmentation in neutrophils, atroph- ic gastritis, positivity of helicobacter pylori, APA positivity and neurological problems are obviously higher in the group 1, in which the vitamin B12 level has been detected lower thanthe 60/pg/mL. This group has been found as the eldest groupwith the mean age of 65.1±14.8. When The group 1 has been compared individually to other groups, the age, hemoglobin value, MCV, level of homocysteine, APA positivity, hyperseg- mentation, gastric atrophy and neurological problems have been significantly different in statistical analysis ($p<0.05$). There have been no statistically significant differences of thy-roid function tests and helicobacter pylori positivity status between group 1 and other study groups ($p >0.05$).

DISCUSSION

Traditionally, measures of blood vitamin B12 levels have been used to diagnose vitamin B12 insufficiency. Some studies have shown that measuring homocysteine and methylmalonic acid levels, which rise in the early stages of vitamin B12 insufficiency, is the most accurate way to screen for the condition.

Serological detections of antibodies against parietal cells and intrinsic factor have mostly superseded the Schilling test, which was once used to identify pernicious anemia (10). Compared to tests that are less specific for vitamin B12 levels between 100 and 400 pg/mL, it has been shown that measurements are more specific for levels below 100 pg/mL (10,11). A total of 406 individuals diagnosed with vitamin B12 insufficiency had elevated levels of homocysteine (found in 95.9% of patients) and methylmalonic acid (in 98.4% of patients). It has been suggested that individuals with normal levels of homocysteine and methylcobalamin should clinically rule out the obvious shortage of vitamin B12.

TABLE 1. The correlation of some hematological and biochemical parameters with the groups that have been formed according to the vitamin B12 levels.

	Group1 (n:34)	Group2 (n:32)	Group3 (n:29)	Group4 (n=28)	p
age, year savg±SD	65.1±14.8	61.5±18.1	48.4±15.2	40±13.1	<0.001
Hb, gr/dl, avg±SD	8.9±2.9	13.0±1.6	13.3±1.4	12.9±2.5	<0.001
MCV, fL, avg±SD	107.8±12.39	91.4±9.7	89.1±5.9	86.8±3.5	<0.001

Hom.>16,umol/L	85(29)	44(14)	27(8)	18(5)	<0.001
APA (+), %(n)%(n)	47(16)	34(11)	24(7)	3(1)	0.002
HS, %(n)	82(28)	41(13)	24(7)	3(1)	<0.001
Ferritin, avg±SD	143±153	55±32	65±28.21	48±6.8	<0.001
Abnormal TFT,%(n)	18(6)	12(4)	7(2)	7(2)	0.121
G. atrophy, %(n)	53(18)	25(8)	24(7)	3(1)	<0.001
H. pylori(+), %(n)	41(14)	40(13)	45(13)	43(12)	0.834
NP% (n)	56(19)	25(8)	10(3)	7(2)	<0.001

Avg: average, SD: standart deviation, Hb: hemoglobin, MCV: mean erythrocyte volume, Hom: Homocysteine, APA: anti parietal antibody, HS: hypers egmentation, TFT: thyroid function tests, G. Atrophy : gastric atrophy, NP: neurological problems Group 1; B12 60 – 100 pg/mL, Group 2; B12 101–140 pg/mL, Group 3; B12 101-140 pg/mL, Group 4; B12 141-178

malonic acid, since the presence of normal levels has only been seen in a single instance. As for hematocrit and MCV, the same research indicated that 28% of patients had normal values, while 17% had normal results (12). Hence, it has been suggested that individuals with neurological problems or low or below normal vitamin B12 levels or those with methyl malonic acid and homocysteine levels should be tested for vitamin B12 deficiency.A 94% sensitive and a 99.99 percent specific increase in metabolite levels is required for diagnosis (13).

The results of this research demonstrate that patients with vitamin B12 levels below 60 pg/mL are clinically more specifically diagnosed with vitamin B12 insufficiency. In situations with greater levels, the diagnosis of a true vitamin B12 insufficiency should be thoroughly assessed. Researchers found no therapeutic benefit from treating individuals with low vitamin B12 levels who otherwise had normal metabolites in a 1992 trial (14). This research found that low vitamin B12 levels were not always indicative of a true deficiency in this nutrient. It may be possible to differentiate between vitamin B12 and folate deficits by measuring metabolites. In cases of folate deficiency, homocysteine levels rise while methyl malonic acid levels remain stable. There is a new, vitamin B12-independent method for treating folate-related hemolytic diseases in people with vitamin B12 deficiency. Damage from vitamin B12 deficits, which may worsen if left untreated or undetected, can occur in these instances (15).

In excess of 90% of cases, antibodies directed against parietal cells are detected. Since it may provide a favorable result in otherwise healthy people, the specificity is undesirable. Furthermore, antibodies against intrinsic factors were positive in 70% of patients and antibodies against thyroid glands were positive in about 40% of patients (16).

Vitamin B12 deficiency is often caused by one of three main things: malabsorption, eating disorders, or problems with the stomach or intestines (17). Atrophic gastritis, the leading cause of hypochlorhydria, is the leading cause of vitamin B12 insufficiency in the elderly (18). Vitamin B12 deficiency in the elderly can have several causes; one study linked it to malabsorption in 53% of cases, pernicious anemia in 33%, and poor diet in 2%. In 11% of cases, the exact cause of the deficiency remained unknown (19). Atrophic gastritis, caused by Helicobacter pylori, is more common in the elderly. Vitamin B12 malabsorption and reduced acid secretion are the end outcomes (4).

As many as 56% of individuals with vitamin B12 deficiency tested positive for helicobacter pylori in one research (20). Serum vitamin B12 levels were found to be raised in 40% of patients after helicobacter pylori eradication. Apart from the increased vitamin B12 levels in the blood (146-271 pg/ml), it has been noted that homocysteine levels have dropped from an average of 41 µmol/L to an average of 13 µmol/L after treatments for helicobacter pylori (21).

Our research found that out of all the patients with vitamin B12 levels below 60 pg/ml, 47% had pernicious anemia, 52% had atrophic gastritis, and 4% tested positive for helicobacter pylori infection. Seventeen percent of patients in the first group tested positive for anti-thyroid antibodies. Instead of basing the diagnosis of vitamin B12 deficits just on blood vitamin B12 levels, it has been suggested in previous research that more sensitive criteria such homocysteine and methyl malonic acid levels should be used (11, 12, 13). Neuropsychiatric diseases without hematological changes may be treated in older people with vitamin B12 levels below 200 pg/ml (22, 23). Vitamin B12 levels below 60 pg/ml were associated with an increased risk of neurological complications in our research.

Significant morphological alterations in blood and bone marrow are among the most noteworthy manifestations of vitamin B12 insufficiency. Megaloblastic anemia develops when the DNA synthesis process is interrupted. Macrocytic erythrocytosis (MCV>110 fl) and neutrophil hyper-segmentation are the morphological abnormalities that are seen. Iron deficiencies or thalassemia carrier status may mask the increased erythrocyte volume. So, it's possible that one-third of people with vitamin B12 insufficiency have normal mean cell hemoglobulin (MCV) values. A research that looked at individuals who responded clinically to vitamin B12 therapy found that hematocrit levels dropped below 25% of their value in only 20% of instances (15). Compared to other groups, the one with vitamin B12 insufficiency levels below 60 pg/ml had an average MCV of 108 fl in our research. MCH levels were found to be within normal range in the other groups. Vitamin B12 deficiency is associated with neutrophil hyper-segmentation at an 82% incidence rate, however it is less common in situations where vitamin B12 levels are greater. Furthermore, it has been shown that patients with low tissue vitamin B12 levels had much lower hemoglobulin levels.

Finally, our research found that vitamin B12 levels below reference values may not be indicative of actual tissue levels, and that values measured at or below 60 pg/dl may be more indicative of signaling clinical deficiencies than values evaluated higher.

REFERENCES

1. Karmel, Green, Rosenblatt, and Watkins (1). A Refresher on Homocysteine, Folate, and Cobalamin... Am Soc Hematol Educ Program, Hematology, vol. 1, 2003, pp. 62–81.

2. 2. Mu-tated methylene tetrahydrofolate reductase as a risk factor for spina bifida (Van Der Put NM, Steegers-Theunissen RP, Frosst P.). The Lancet 346:1070-1071, 1995.
3. 3. A meta-analysis of the effects of the MTHFR 677C→T polymorphism on the risk of coronary heart disease. Klerk, Verhoef, and Clarke. Journal of the American Medical Association, 288(2023–2031), 2002.
4. Phokonen P, Laxen F, Huotari K, and Harkonen M. were the authors of the fourth article. Atrophic gastritis and Helicobacter pylori infection are associated with elevated homocysteine levels and low vitamin B12 levels in blood samples taken from an older male population. Scandinavian Journal The gastroenterologist published an article in 2003 with the reference number 38: 1209-16.
5. The authors of the study include Clarke, Grimley, Schneede, and others. Inadequate levels of vitamin B12 and folate in old age. Ageing Research 33: 34–41, 2004.
6. 6. Grisold W, Lechner K, Födinger M, and others. Insufficiency of vitamin B12. Latest information about a previously covered topic. Published in 2005 in Wien Klin Wochenschr., 117:579–591.
7. 13. Wiesersinga WJ, de Rooij SE, Huijmans JG, et al. Vitamin B12 insufficiency diagnosis updated. Volume 149, Issues 2789–294, Ned Tijdschr Ge-neeskd., 2005.
8. Megaloblastic anemias, an article by Anthony AC. Schwartz R, Shattil SJ, Furie B., Benz EJ. Churchill Livingstone Inc., New York, 2000, pp. 446-484. Hematology: A Comprehensive Guide to Basic Principles and Practice, Third Edition.
9. Hvas AM, Ellegaard J, and Nexo E made a 9. There is no correlation between an elevated plasmamethylmalonic acid level and the development of vitamin B12 deficient symptoms. Published in 2001 in the Annals of Internal Medicine, 161: 1534–1541.
10. Oh R, DL Brown. Insufficiency of vitamin B12. March 2003, American Family Physician 67: 993-994.
11. The eleventh is snow CF. Folate and vitamin B12 insufficiency confirmed in the lab. May 1999, Arch Intern Med 159: 1289-1298.
12. Authors: Savage DG, Lindenbaum J, Stabler SP, and Allen RH. Diagnostic sensitivity of total homocysteine and serum methylmalonic acid for the diagnosis of folate and cobalamin deficiency. United States Journal of Medicine 96: 239-246, 1994.
13. Hvas AM, Ellegaard J, and Nexo E.: 13. There is no correlation between an elevated plasmamethylmalonic acid level and the development of vitamin B12 deficient symptoms. Published in 2001 in the Annals of Internal Medicine, 161: 1534–1541.
14. fourteen. Stabler SP, Allen RH, Savage DG, Lindenbaum J. Cobalamin insufficiency: a clinical spectrum and diagnostic guide. Journal of the American Medical Association 76: 871-881, 1990.
15. 15. Primary Care Case Reviews 5(2): 53-60, 2002. Craven, Elizabeth MD. Vitamin B12 deficiency: recognition and treatment.