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EDITORIAL

Beware of the Ailments of Vitamin B_{12} Deficiency

Antonis S. Manolis, MD, Theodora A. Manolis, RN, MS,
Emmanouil Poulidakis, MD, Helen Melita, MD

Evagelismos General Hospital, Athens, Onassis Hospital, Athens, Patras University Medical School, Patras, Greece

ABSTRACT

Vitamin B_{12} or cobalamin deficiency is a common problem in adult patients, which is however frequently missed. The most common cause of cobalamin deficiency is the food cobalamin malabsorption syndrome (>60% of all cases). Neuropsychiatric manifestations can be the presenting and only early sign of cobalamin deficiency even in the absence of hematologic abnormalities. The deficiency can occur despite serum cobalamin levels at low normal values; thus, normal vitamin B_{12} levels have been revised upward to >350 pg/ml. Early detection and treatment are important to prevent structural and irreversible damage. The causes and ailments of vitamin B_{12} deficiency are herein overviewed and a diagnostic and therapeutic strategy is outlined.

KEY WORDS: *vitamin B_{12}; cobalamin; methylmalonic acid; homocysteine; megaloblastic anemia; neuropsychiatric disorders; intrinsic factor; Helicobacter pylori; food-cobalamin malabsorption syndrome; atrophic gastritis; metformin*

Abbreviations
DNA = deoxyribonucleic acid
FDA = Food and Drug Administration
IF = intrinsic factor
MMA = methylmalonic acid
RNA = ribonucleic acid

Correspondence to:
Antonis Manolis, MD, Evagelismos Hospital, Athens, Greece;
e-mail: asm@otenet.gr

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INTRODUCTION

Vitamin B_{12} or cobalamin, a water-soluble vitamin, is vital for cell division, DNA synthesis, erythropoiesis, and normal function of the nervous system via the formation of myelin sheaths and the synthesis of neurotransmitters.[1-8] The human body does not manufacture this vitamin and gets it supplied from animal products or fortified cereals. It is produced by anaerobic bacteria and is found in foods of animal origin (e.g., meat, fish, dairy products, and eggs). On its way into the cell, vitamin B_{12} is absorbed by lysosomes and enters the cell with the aid of the transport protein CblF. It appears that a second transport protein, ABCD4, is also necessary for this step. Both proteins may be a cause of hereditary vitamin B_{12} deficiency.[9-11] In combination with folate, vitamin B_{12} is an essential cofactor in the metabolism of homocysteine and methylmalonic acid (MMA); when vitamin B_{12} is truly deficient, the levels of these two metabolites rise. Cobalamin is a cofactor and coenzyme in many biochemical reactions, including DNA synthesis, methionine synthesis from homocysteine and conversion of propionyl into succinyl coenzyme A from methylmalonate. Methionine is required for the formation of S-adenosylmethionine, a universal methyl donor for about 100 different substrates, including DNA, RNA, hormones, proteins, and lipids.

Daily requirements of vitamin B_{12}, recommended by the FDA and other medical societies, range between 2 and 5 μg (average 2.4 μg for recommended daily allowance).[2,4-6,8,12-16] A typical Western diet contributes 3–30 μg of cobalamin per day, while hepatic stores are multifold higher (>1.5 mg); cobalamin is also recycled via the enterohepatic cycle, whereby it is excreted in the bile and then reabsorbed in the small intestine. Thus, there is a significant 5-10-year delay between the onset of insufficient oral intake and the development of clinical ailments.

Dietary cobalamin is bound to animal proteins and is absorbed via a complex process.[2,4-8] First, it is released in the stomach via the effect of hydrochloric acid and pepsin. Synthetic vitamin B_{12}, added to fortified foods and dietary supplements, is already in free form and, thus, there is no need for a release process. Free cobalamin in the stomach is bound by glycoproteins (R-proteins) secreted from salivary glands and parietal cells. Intrinsic factor (IF), a weak binder of cobalamin in the presence of R proteins, is also released by parietal cells in the stomach. In the duodenum, dietary- and bile-secreted cobalamin-R complexes are cleaved by pancreatic enzymes, and free cobalamin is then bound to IF with more affinity. Cobalamin–IF complexes are taken up by endocytosis in the distal ileal mucosa. Once inside the cell, cobalamin dissociates from IF. Free cobalamin is then bound to transporter proteins (transcobalamins) and transported to the liver. The biologically-active form of the vitamin can be taken up by cells via endocytosis for metabolic purposes. Between 1-5% of free cobalamin is absorbed along the entire intestine by passive diffusion, which renders feasible the absorption of high doses (>1000 μg daily) of oral supplemental cobalamin, despite malabsorption disease processes.

Disruptions in the metabolic pathways of cobalamin produce elevated levels of homocysteine and methylmalonic acid (MMA).[17] Homocysteine is neurotoxic, through overstimulation of the N-methyl-D-aspartate receptors, and toxic to the vasculature because of activation of the coagulation system and adverse effects on the vascular endothelium. MMA, a product of methylmalonyl-CoA, can cause abnormal fatty-acid synthesis, affecting the neuronal membrane. MMA and homocysteine levels are elevated before any clinical manifestations of vitamin B_{12} deficiency and often precede low serum vitamin B_{12} levels. Neuropsychiatric symptoms usually precede hematologic signs and are often the presenting manifestation of cobalamin deficiency.[17] Diagnosing and treating vitamin B_{12} deficiency is crucial since it is a reversible cause of anemia and demyelinating nervous system disease.

CAUSES

The most common cause of cobalamin deficiency is the food-cobalamin malabsorption syndrome (>60% of all cases) (Table 1).[1-8,12-18] Other causes comprise pernicious anemia (15%–20% of all cases), insufficient dietary intake, malabsorption, and rare hereditary syndromes (e.g., Imerslund-Grasbeck syndrome), which though appear in newborns and therefore do not involve adult patients. Food-cobalamin malabsorption, which has only recently been identified as a significant cause of cobalamin deficiency, particularly among the elderly, is characterized by the inability to release cobalamin from food or a deficiency of intestinal cobalamin transport proteins or both. Food-cobalamin malabsorption is caused primarily by gastric atrophy. Gastric atrophy may or may not be related to *Helicobacter pylori* infection. Other factors that may contribute to this syndrome include microbial proliferation (blind or stagnant loop syndrome), long-term ingestion of metformin, H2-receptor antagonists and proton pump inhibitors, chronic alcoholism, gastric surgery, partial pancreatic exocrine failure and Sjogren's syndrome.[5,19] A major risk factor contributing to vitamin B_{12} deficiency in developing countries is indeed a smoldering infection with *Helicobacter pylori* (Table 1).[18] It is estimated that a 50-90% of the population is infected by *H. pylori* and this has been shown to have a direct implication in decreasing vitamin B_{12} absorption because of gastric atrophy observed in around 30% of individuals with *H. pylori* infection, leading to inadequate binding of vitamin B_{12} and intrinsic factor.[7] *H. pylori* has been detected in up to 78% of those with severe vitamin B_{12} deficiency compared with 44% of those with normal levels of vitamin B_{12}. Following treatment of *H. pylori*, 40% of patients have their vitamin B_{12} levels restored to normal within 2 years. *Giardia lamblia* and other intestinal

TABLE 1. Causes of Vitamin B_{12} Deficiency

• Food-cobalamin malabsorption syndrome (atrophic gastritis, chronic gastritis, drug interactions, small intestinal bacterial overgrowth)
• Type B atrophic gastritis (associated with helicobacter pylori infection)
• Lack of intrinsic factor (IF) due to immune mediated destruction of gastric parietal cells (pernicious anemia)
• Nutritional deficiency (vegans, vegetarians, elderly, alcoholics, HIV positive, breast fed infants of vegetarian mothers)
• Pathology of distal ileum (distal ileal resection, inflammatory bowel disease like Crohn's disease, ileocaecal tuberculosis, Whipple's disease, tropical sprue)
• Surgical gastrectomy / ileal resection
• Colonization of small bowel with bacteria or intestinal parasites (tapeworm)
• Nitrous oxide inhalation (functional deficiency)
• Drugs (metformin, antacids, proton pump inhibitors, H2 blockers, colchicine, phenytoin, zidovudine)
• Increased demands (pregnancy / lactation)
• Congenital syndrome (Imerslund-Grasbeck syndrome)

HIV = human immunodeficiency virus.

parasites may also be responsible for chronic diarrhea and malabsorption, with approximately one-third of the infected population having decreased vitamin B_{12} levels.[7]

Pernicious anemia, or Biermer's disease, an autoimmune disease, is a classical cause of cobalamin deficiency and accounts for 15-20% of all cases.[20] It is characterized by a cell-mediated destruction of the fundal gastric mucosa that leads to neutral or slightly acidic gastric secretions despite the presence of gastrin (which normally increases acidity), which contain little or no intrinsic factor. The disease is also characterized by the presence of anti-intrinsic factor or antigastric parietal cell antibodies. Due to gastric atrophy with hypochlorhydria, patients have a reactive hypergastrinemia. A positive Schilling test conducted with use of radiolabeled vitamin B_{12} and combined with the addition of a test for anti-intrinsic factor antibodies, virtually confirms the diagnosis (specificity >99%); however, this test has dropped out of favor and is not currently available.[4,8] Pernicious anemia is associated with many other autoimmune disorders and with an increased frequency of gastric neoplasms, requiring periodic endoscopic surveillance. In contrast with the malabsorption syndrome, there is near absence of mucosal *H. pylori* in patients with pernicious anemia.

Low serum vitamin B_{12} levels compromise the immune response to pneumococcal vaccine because of impaired humoral immunity.[21] Similarly, low serum B_{12} levels may affect the results of tests like the Quantiferon Gold test for tuberculosis.

CLINICAL MANIFESTATIONS

Vitamin B_{12} deficiency occurs in 4 stages, starting with declining blood levels of the vitamin (stage I), progressing to low cellular concentrations of the vitamin and metabolic abnormalities (stage II), an increased blood level of homocysteine and methylmalonic acid and a decrease in DNA synthesis with emergence of neuropsychiatric symptoms (stage III), and ultimately, macrocytic anemia (stage IV).

The clinical manifestations of vitamin B_{12} deficiency include (Table 2):[1-8,12-17,20,22-26]

- hematologic: megaloblastic anemia and pancytopenia
- neurologic: dementia; parasthesias, peripheral neuropathy and subacute combined degeneration of the spinal cord
- psychiatric: irritability, personality change, memory impairment, depression and psychosis
- gastrointestinal and other constitutional symptoms: stomatitis, diarrhea, constipation, loss of appetite, fatigue, weakness, weight loss and premature birth, and possibly
- cardiovascular, possibly related to increased homocysteine levels conferring increased risk of myocardial infarction and stroke.

Vitamin B_{12} crosses the placenta and is present in breast milk. Pregnant women with cobalamin deficiency may give birth to children with neural tube defects.[27] Breast-fed children of mothers with vitamin B_{12} deficiency are at increased risk of failure to thrive, hypotonia, ataxia, and anemia.[28]

TABLE 2. Clinical Manifestations of Vitamin B_{12} Deficiency

Hematological Manifestations
Anemia
Pancytopenia (rare)
Neurological Manifestations
CNS manifestations
1. Dementia
2. Depression
3. Parkinson's disease
4. Acute psychosis, reversible manic and schizophreniform states (Megaloblastic madness)
5. Cerebrovascular disease (homocystenemia is an independent risk factor for stroke)
Spinal cord manifestations
1. Myelopathy (Subacute combined degeneration of spinal cord), ataxia, spasticity and abnormal gait
PNS manifestations
1. Neuropathy
• motor-sensory polyneuropathy (parasthesias, numbness and weakness)
• mononeuropathy (optic or olfactory)
• autonomic neuropathy (impotence, urinary or fecal incontinence
2. Myeloneuropathy (combined myelopathy and neuropathy)
Cardiovascular Manifestations
Effects from anemia
Increased cardiovascular risk / angina (hyperhomocysteinemia)
Venous thromboembolic disease
GI Manifestations
Glossitis
Jaundice
Mucocutaneous ulcers (rare)
Dyspepsia (?)
Other
Vaginal mucosal atrophy
Vaginal & urinary chronic (especially fungal) infections
Hypofertility / miscarriages
Vitiligo / Hyperpigmentation

CNS = central nervous system; GI = gastrointestinal; PNS = peripheral nervous system

Although the true prevalence of vitamin B_{12} deficiency is difficult to estimate due to varying laboratory values, methods and criteria, the Framingham Heart Study reported in

1994 the prevalence of vitamin B_{12} deficiency, as defined by a serum vitamin B_{12} level <200 pg /ml and elevated levels of serum homocysteine, methylmalonic acid, or both, to be 12% among 548 elderly patients.[29] According to unpublished data from the National Health and Nutrition Examination Survey, 3.2% of U.S. adults older than 50 years are estimated to have a serum vitamin B_{12} level less than 200 pg/ml.[8] Other estimates indicate that >20% of the elderly individuals suffer from vitamin B_{12} deficiency,[3] but the condition may often go unrecognized because the clinical manifestations are subtle. However, they are also potentially serious, since they may lead to severe, albeit reversible at early stages, neuropsychiatric symptomatology.[17,22-26] Indeed, the non-hematologic clinical features of vitamin B_{12} deficiency can be manifest despite the absence of anemia.

DIAGNOSIS

Although many clinical laboratories define vitamin B_{12} deficiency at a level of <150 pg / ml (<110 pmol/L; pmol/L = pg/ml X 0.738), or in some cases <200 pg/ml (<148 pmol/L), patients with values above these levels may be symptomatic and benefit from treatment.[5,8,30] Vitamin B_{12} levels >350 pg/ml (>258 pmol/L) seem to be protective against symptoms of vitamin B_{12} deficiency. Nevertheless, there is evidence that serum vitamin B_{12} concentrations might not accurately reflect intracellular concentrations. Furthermore, large amounts of folic acid can conceal the detrimental effects of vitamin B_{12} deficiency by correcting the megaloblastic anemia caused by vitamin B_{12} deficiency without remedying the neurological damage that also develops.[12] Also, high serum folate levels might not only mask vitamin B_{12} deficiency, but could also worsen the anemia and the cognitive symptoms produced by cobalamin deficiency. The ensuing delay in treatment might produce permanent nerve damage. Thus, in healthy individuals folic acid intake generally should not exceed 1 mg daily.

In patients with clinical symptoms of vitamin B_{12} deficiency and low levels of serum vitamin B_{12}, no further confirmatory testing is generally needed before treatment is initiated.[6] Verification with serum methylmalonic acid (MMA) and/or serum homocysteine level[6,31,32] may be necessary in asymptomatic patients with high-risk conditions, symptomatic patients with low-normal levels of vitamin B_{12} (200 to 350 pg/ml), or symptomatic patients in whom vitamin B_{12} deficiency is unlikely but must be excluded.[8] Elevated levels of serum homocysteine and methylmalonic acid have been shown to be highly sensitive markers for vitamin B_{12} deficiency. Testing is widely available, but expensive, and some conditions (e.g. renal insufficiency) may falsely elevate serum homocysteine and methylmalonic acid levels.[6] Because serum methylmalonic acid level is as sensitive as, but more specific than serum homocysteine level for vitamin B_{12} deficiency, it is the confirmatory test of choice. Measurement of cobalamin bound to transcobalamin would be a more physiologic measure of cobalamin status, but this assay is not yet routinely available and further research is needed about its clinical utility.[4,6] Vitamin B_{12} levels may be found falsely lower in some coexisting conditions, such as multiple myeloma, pregnancy, folate deficiency and intake of oral contraceptives, while patients with liver or renal disease, or myeloproliferative disorders may have falsely normal levels.[6]

If patients are symptomatic there should be no question of treating the deficiency and a low vitamin B_{12} level should never be ignored. The medical profession has often relied on the presence of megaloblastic anemia as an indication to check vitamin B_{12} status. Changes in the blood film with macrocytosis and hypersegmented neutrophils are late manifestations of folate or vitamin B_{12} deficiency and should not be relied upon as an indication for testing of vitamin B_{12} levels. In addition, those with a very low vitamin B_{12} and a normal folate may not have a megaloblastic picture but will still be at risk of developing neuropsychiatric and cardiovascular sequelae. Serum concentrations of vitamin B_{12} may be low in the presence of normal tissue levels if there is concomitant folate deficiency, pregnancy, iron deficiency or in certain rare inherited disorders of vitamin B_{12} metabolism. Vitamin B_{12} deficiency should be suspected in all patients with unexplained anemia, unexplained neuropsychiatric symptoms, and/or gastrointestinal manifestations with glossitis, anorexia, and diarrhea.[4] Patients at risk of developing cobalamin deficiency include the elderly due to increased incidence of atrophic gastritis in this group, the vegetarians and the vegans, and patients with intestinal diseases. Other groups at risk include patients with autoimmune disorders (Graves' disease, thyroiditis, vitiligo), as well as patients receiving metformin, proton pump inhibitors, or histamine receptor antagonists for prolonged periods of time.[12,17,19]

Additional testing in order to determine the cause of vitamin B_{12} deficiency comprises antibodies to intrinsic factor which establish the diagnosis for pernicious anemia; however, only about 70% of patients with pernicious anemia have these antibodies. Other tests that are considered useful for detecting the cause of vitamin B_{12} deficiency include levels of pepsinogen and/or levels of plasma gastrin which are suggestive but not specific. For years, Shilling's test, in which labeled vitamin B_{12} is administered orally alone (Shilling's test I) or together with intrinsic factor (Shilling's test II), has been deemed the gold standard test to investigate whether lack of the vitamin is caused by lack of intrinsic factor. Shilling's test is, however, no longer available due to increasing difficulties in obtaining labeled vitamin B_{12} and intrinsic factor.[4,8]

TREATMENT

There is no universal agreement on the recommendations for the treatment of vitamin B_{12} deficiency in those who do not have pernicious anemia. Nevertheless, most physicians proceed with supplemental therapy in symptomatic patients.[33] However, apart from the symptomatic patient, a far more prevalent presentation is ***subclinical vitamin B_{12} deficiency*** in an asymptomatic individual with borderline serum B_{12} levels and elevated homocysteine or methylmalonic acid levels, or both.[8,33] Such patients pose a therapeutic dilemma because there are no guidelines for their treatment. Some physicians elect to treat these patients aiming at having the metabolite markers normalized, while others prefer to withhold therapy and follow patients closely.

All patients with vitamin B_{12} <300-350 pg/ml should have their *H. pylori* status ascertained with serum antibodies (if they have not had previous treatment), breath test or stool antigen, and a discussion about the importance of a diet high in animal source foods and the usefulness of fortified cereals. Certain medications adversely affect vitamin B_{12} levels, including proton pump inhibitors, H2 antagonists, and metformin (Table 1). Therefore patients using these medications will be unable to build up and maintain their stores of vitamin B_{12} and will remain deficient, even if diet improves and *H. pylori* is treated.

While oral treatment can be effective, its limitation is that with higher doses the ileal receptors for vitamin B_{12} intrinsic factor complex become saturated.[33-35] The recommended dietary intake for adults is 2.4 μg/day (higher for pregnant or breastfeeding women) but only about 56% of a 1 μg oral dose will be absorbed. Absorption rates fall dramatically as dosage increases; in a >25 μg dose only 1% is absorbed. Even in people with normal absorption only 10 μg of 1000 μg will be absorbed; in those with *H. pylori* or *G. lamblia,* or those on proton pump inhibitors, metformin or H2 antagonists, the absorption rate will be even less.[12,19]

Oral doses of 1000–2000 μg/day, then weekly, then monthly have been proposed as effective as intramuscular injection in achieving a clinical response.[4,8,33-35] However, the standard recommendation for patients with vitamin B_{12} deficiency due to pernicious anemia is 1 mg (1000 IU) of vitamin B_{12} given intramuscularly daily for 1 week, weekly for 1 month, then monthly indefinitely.[8,36] Other protocols in pernicious anemia use 1 mg weekly for 1 month, and monthly thereafter. All patients should have their levels checked at 3-4 months and regularly afterwards.[33]

There appears to be evidence supporting the existence of an alternate system for the absorption of vitamin B_{12} that is independent of intrinsic factor or even an intact terminal ileum. Approximately 1% of a large dose of vitamin B_{12} (e.g. 1000 μg) is absorbed by this second mechanism. This pathway is important in relation to oral replacement therapy. Once absorbed, vitamin B_{12} binds to transcobalamin II and is transported throughout the body. A clinical trial (project OB_{12}) has been designed to compare the effectiveness of orally and intramuscularly administered vitamin B_{12} in the treatment of patients ≥65 years of age with vitamin B_{12} deficiency.[37]

Treatment with vitamin B_{12} is considered safe, even when very high vitamin serum levels are reached with doses 1000 times the recommended daily allowance.[1] Cobalamin has not been shown to be toxic or cause cancer, birth defects, or mutations.[1,8] However, one should be cognizant of the potential risk of hypokalemia and fluid overload early during treatment of patients with megaloblastic anemia due to increased erythropoiesis, cellular uptake of potassium, and increased blood volume. An entirely different situation exists when in the absence of cobalamin therapy, high plasma cobalamin levels (>800 pg/ml) are unexpectedly detected, whereby high plasma cobalamin denotes an alteration in cobalamin metabolism with either increased synthesis or decreased clearance of cobalamin-binding proteins (transcobalamin and/or haptocorin) or release of cobalamin from body stores. An association of unexpected high cobalamin levels has been reported with hematologic malignancies, liver disease and cancer, autoimmune disease, renal disease and infections.[38]

There has been a great deal of interest in the link between elevated levels of homocysteine, a direct consequence of vitamin B_{12} deficiency, and cardiovascular disease. No studies have directly evaluated the cardiovascular effects of correcting vitamin B_{12} deficiency in patients with known cardiovascular disease, although numerous studies have failed to demonstrate that correction of hyperhomocysteinemia itself reduces cardiovascular mortality or cardiovascular complications.[12,39-45] The routine use of vitamin B_{12} to lower levels of serum homocysteine in patients at high risk of cardiovascular events is not recommended.[12]

CONCLUSION

Vitamin B_{12} deficiency is a common, albeit frequently missed, problem in adult patients, particularly in the elderly. Neuropsychiatric manifestations can be the presenting and only sign of cobalamin deficiency even in the absence of hematologic abnormalities. The deficiency can occur despite "normal" serum cobalamin levels; thus, measuring homocysteine and MMA in patients having vitamin B_{12} levels <350 pg/ml can decrease false-negative findings. Early detection and treatment are important to prevent structural and irreversible damage. Oral high-dose treatment has been proposed as efficacious as parenteral treatment but further confirmatory data are needed. Devising a strategy to select patients with subtle or subclinical vitamin B_{12} deficiency who would benefit from supplemental therapy remains an important issue for future research.

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