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Research Article

VITAMIN B12 DEFICIENCY: A CLINICAL REVIEW

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ABSTRACT

Vitamin B12 is an essential cofactor that is integral to methylation processes important in reactions related to DNA and cell metabolism. It is an essential nutrient needed for proper nervous system function and for the metabolism of carbohydrate, protein, and fat. Deficiencies in B12 can lead to inefficient erythropoiesis and megaloblastic anemia. Causes of B12 deficiency include malnutrition, lack of intrinsic factor (IF) and/or malabsorption of protein-bound vitamin B12, general malabsorption, and competition for vitamin B12. It's clinical manifestations are variable with neurological, psychiatric, oral and dermatological signs and symptoms. Treatment is replacement of B12 via parentral or oral administration. Biomarkers of B12 status include serum B12 levels, mean corpuscular volume (MCV), serum homocysteine and serum methylmalonic acid.

This review presents a concise summary of the most up to date evidence on how to diagnose and manage vitamin B12 deficiency.

Keywords: *Vitamin B12, Cobalamin, Deficiency, Review*

INTRODUCTION

Box 1: Common causes of vitamin B12 deficiency (Harmening, 2002; Provan *et al.*, 2010; Kaushansky *et al., 2010;* Grober *et al., 2013)*

Impaired intestinal absorption
- Ileal resection or disease—for example, Crohn's inflammatory bowel disease and tuberculous ileitis
- Blind loop syndrome
- Parasites: giardiasis, bacterial overgrowth, and fish tapeworm

Impaired gastric absorption
- Pernicious anaemia
- Gastrectomy—partial or total
- Zollinger-Ellison syndrome

Pancreatic insufficiency

Decreased intake
- Malnutrition
- Reduced intake of animal products
- Strict vegan diet

Congenital/inherited
- Intrinsic factor receptor deficiency/defect
- Congenital deficiency of intrinsic factor
- Transcobalamin deficiency

Increased requirements
- Haemolysis
- HIV

Drugs
- Alcohol
- Proton pump inhibitors
- H2 receptor antagonists
- Metformin

V itamin B12 (cobalamin) is a water-soluble vitamin that is crucial to normal neurologic function, red blood cell production, and DNA synthesis. Vitamin B12 is essential for three enzymatic processes: the conversion of homocysteine to methionine; the conversion of methylmalonic acid to succinyl coenzyme A; and the conversion of 5-methyltetrahydrofolate to tetrahydrofolate, a process necessary for DNA synthesis and red blood cell production. Deficiencies in B12 can lead to inefficient erythropoiesis and megaloblastic anemia. Furthermore, neurological disorders such as neuropathy, myelopathy, memory impairment, dementia, depression, and brain atrophy may occur in those with low B12 status. Neurological and psychiatric symptoms have been seen in patients without related anemia or macrocytosis, with B12 concentrations in the previously defined range of low-normal. This highlights the need for the prevention of B12 deficiency. As B12 cannot be manufactured by humans it must be regularly obtained from the ingestion of animal proteins or fortified cereal products.

Causes of Vitamin B12 Deficiency

Foods containing vitamin B12 are derived only from animals: meat, fish, and dairy. The recommended dietary allowance (RDA) of vitamin B-12 for adults in India is 1.0 µ g/day, whereas in USA it is 2.4 µ g/day. Vitamin B-12 intake by Indians derives from natural food products, i.e. dairy products viz. milk, yogurt, and cheese/butter, tea/coffee with milk or from vitamin B-12 supplements. The daily Western diet contains around 5-30 µg of vitamin B12 daily, of which 1-5 µg is absorbed. Body storage is relatively high, about 1-5 mg. Therefore deficiency from diminished intake or absorption may not manifest for several years after the depletion of stores (Harmening, 2002; Provan *et al.,* 2010)

Box 1 outlines the common causes of vitamin B12 deficiency.

At Risk Population

Deficiency can manifest in different groups as a result of periods when requirements are increased, such as during growth in children and adolescence or in pregnancy. Certain groups may have reduced intake, such as those with poor nutrition, older people, or people who adhere to a vegan or vegetarian diet. Deficiency within the elderly population is often the result of age-related gastric atrophy. This causes a decrease in acid and intrinsic factor production leading to B12 malabsorption. In the United Kingdom and United States the prevalence of vitamin B12 deficiency is around 6% in people aged less than 60 years, and closer to 20% in those aged more than 60 years. The prevalence of deficiency is much higher in African and Asian countries—for example, 70% in Kenyan schoolchildren, 80% in Indian preschool children, and 70% in Indian adults (Allen, 2009).

Pathophysiology

Vitamin B12 is a cofactor for only two enzymes: methionine synthase and l-methylmalonyl– coenzyme A mutase. The interaction between folate and B12 is responsible for the megaloblastic anemia seen in both vitamin deficiencies. Dyssynchrony between the maturation of cytoplasm and that of nuclei leads to macrocytosis, immature nuclei, and hypersegmentation in granulocytes in the peripheral blood (Figure 1A). The hypercellular and dysplastic bone marrow can be mistaken for signs of acute leukemia (Figure 1B) (Parmentier *et al.,* 2012). The ineffective erythropoiesis results in intramedullary hemolysis and release of lactate dehydrogenase, features that are similar to those of microangiopathic hemolytic anemia (Dalsania *et al.,* 2008). Vitamin B12 is necessary for the development and initial myelination of the central nervous system as well as for the maintenance of its normal function. Demyelination of the cervical and thoracic dorsal and lateral columns of the spinal cord, occasional demyelination of cranial and peripheral nerves, and demyelination of white matter in the brain (i.e., "combined-systems disease" or "subacute combined degeneration") can occur with vitamin B12 deficiency. Pathological analysis reveals a "spongy degeneration" due to the loss of and swelling of myelin sheaths; this degeneration is visible on magnetic resonance imaging. For unclear reasons, the severity of megaloblastic anemia is inversely correlated with the degree of neurologic dysfunction (Healton *et al.*, 1991) Less common conditions associated with vitamin B12 deficiency include glossitis, malabsorption, infertility, and thrombosis (including thrombosis at unusual sites such as cerebral venous sinus thrombosis) (Remacha *et al.*, 2011) Thrombosis has been attributed to the marked hyperhomocysteinemia seen in severe cases of vitamin B12 deficiency. Patients occasionally have hyperpigmentation, which clears with treatment.

Intrinsic factor is a protein, produced by the parietal cells of the cardiac and fundic mucosa of the stomach. It binds vitamin B12 to allow its absorption through the gastrointestinal tract, by way of a receptor on the intrinsic factor that is specific to cells at the terminal ileum (Figure 2). If there is resection or disease of the gastric mucosa or terminal ileum this leads to vitamin B12 deficiency as a result of malabsorption.

Pernicious anaemia is an autoimmune disease with atrophy of the gastric mucosa of the body and fundus of the stomach. This reduces the number of parietal cells that produce the intrinsic factor necessary for absorption of vitamin B12. Secretion of intrinsic factor parallels gastric acid; thus there will be reduced secretion in an alkaline environment created by the long term use of high dose proton pump inhibitors and similar drugs.

Box 2. Clinical manifestations of vitamin B12 deficiency:

Cutaneous
Hyperpigmentation
Vitiligo
Gastrointestinal
Glossitis
Jaundice
Hematologic
Anemia (macrocytic, megaloblastic)
Thrombocytopenia
Neuropsychiatric
Cognitive impairment
Gait abnormalities
Irritability
Peripheral neuropathy
Weakness

Clinical Features

The clinical manifestations of vitamin B12 deficiency (Box 2), (Quadros, 2010) represent the effects of depletion on multiple systems and vary greatly in severity depending on the degree and duration of deficiency. Mild deficiency manifests as fatigue and anaemia, with indices suggesting B12 deficiency but an absence of neurological features. Moderate deficiency may include an obvious macrocytic anaemia with, for example, glossitis and some mild or subtle neurological features, such as distal sensory impairment. Severe deficiency shows evidence of bone marrow suppression, clear evidence of neurological features, and risk of cardiomyopathy. However, it is important to recognise that clinical features of deficiency can manifest without anaemia and also without low serum vitamin B12 levels. In these cases treatment should still be given without delay.

Bone Marrow

The classic hematologic expression of vitamin B12 deficiency is a megaloblastic macrocytic anemia characterized by an elevated mean corpuscular volume and mean corpuscular hemoglobin, and a peripheral smear containing macroovalocytes and hypersegmented neutrophils. About 28 percent of affected patients may have a normal hemoglobin level, and up to 17 percent may have a normal mean corpuscular volume (Savage *et al.,* 1994). Anaemia may range from mild to severe, with symptoms of fatigue on exertion, dyspnoea, palpitations, and pallor. All cell lines can be affected, with macrocytic anaemia, low white cell count or neutropenia, and thrombocytopenia.

Tissues and Organ Dysfunction

Epithelial changes with vitamin B12 deficiency include skin hyperpigmentation and glossitis. Reproductive tissue can be affected, manifesting as infertility. Deficiency can also result in osteoporosis,

with reduced bone derived alkaline phosphatase and plasma osteocalcin. Rarely, cardiomyopathy can occur (Stabler, 2013).

Neurological Features

Neurological impairment includes motor disturbances, sensory loss, abnormal balance and reflexes, cognitive impairment, and memory loss.

Extreme cases may present with stupor or psychosis. An estimated 20% of patients with neurological signs do not manifest anaemia. Clinical features of anaemia may be minimal and the blood indices may not reflect important anaemia. Subacute combined degeneration of the spinal cord involves demyelination of the posterior and lateral tracts. Initial bilateral peripheral neuropathy can progress to axonal degeneration and neuronal death if left untreated. This is followed by disturbances of proprioception, vibratory sense, and areflexia. Patients may mention clumsiness, poor coordination, and difficulty walking. Without treatment, weakness and stiffness may develop, manifesting as spastic ataxia. Damage to peripheral nerves results in sleepiness, altered taste and smell, and optic atrophy. In severe deficiency or advanced stages, a dementia-like illness may be seen, and frank psychosis with hallucinations, paranoia, and severe depression (Grober *et al.*, 2013; Quadros, 2010).

Investigations

Several investigations reflecting physiological, static, and functional B12 status are available. Box3 outlines when testing for vitamin B12 deficiency should be considered.

Box 3: When to consider testing for vitamin B12 deficiency

- Anaemia
- Macrocytosis mean cell volume >100 fl
- Clinical symptoms of vitamin B12 deficiency
- Known gastrointestinal disorder associated with vitamin B12 deficiency vegan diet

Vitamin B12 Level

Vitamin B12 level is actually a measurement of serum cobalamin. Measurement of vitamin B12 in serum is the most common assay used to evaluate vitamin B12 levels. The test, however, also measures both serum holohaptocorrin and serum holotranscobalamin, and as such may mask true deficiency or falsely imply a deficient state.

The test is widely available at low cost and uses an automated method and competitive-binding immune chemiluminescence (Devalia *et al.*, 2014; Lindenbaum *et al.*, 1988; Solomon, 2007; Clarke *et al.*, 2007; Valente *et al.*, 2011). It has been suggested that serum cobalamin <148 pmol/L (200 ng/L) would be sensitive enough to diagnose patients with vitamin B12 deficiency.

Holotranscobalamin

Holotranscobalamin, the metabolically active form of vitamin B12, can be measured by immunoassay. Emerging evidence indicates that a low level of holotranscobalamin is a more reliable marker of impaired vitamin B12 status than is a low level of serum vitamin B12 (Valente *et al.*, 2011). Holotranscobalamin may be the earliest marker for vitamin B12 depletion. A second confirmatory test, such as for methylmalonic acid levels, is recommended if the result is in the intermediate range (Sobczynska-Malefora *et al.*, 2014).

Methylmalonic Acid

Accumulation of methylmalonic acid occurs in B12 deficiency as conversion of methylmalonic acid to succinyl-CoA requires B12 as a cofactor. An increase in methylmalonic acid persists for several days even after replacement is started.2 Measurement of methylmalonic acid may be the most representative marker of metabolic vitamin B12 insufficiency. False positive results can occur in older patients (>65 years) and those with impaired renal function. Methylmalonic acid is measured using gas chromatography mass spectrometry, a high cost test.

Total Homocysteine

Plasma total homocysteine can increase early in the course of deficiency. It is a sensitive but non-specific marker and it is also high in folate deficiency, B6 deficiency, renal failure, and hypothyroidism. Most laboratories regard levels >15 μmol/L as high.

Tests to Determine the Cause of Vitamin B12 Deficiency

If the patient consumes sufficient amounts of vitamin B12 and has clinically confirmed B12 deficiency, then malabsorption must be present. A positive test for anti–intrinsic factor or anti–parietal-cell antibodies is indicative of pernicious anemia; surveillance for autoimmune thyroid disease is reasonable in patients with positive antibody tests. Chronic atrophic gastritis can be diagnosed on the basis of an elevated fasting serum gastrin level and a low level of serum pepsinogen I (Quadros, 2010). Some experts recommend endoscopy to confirm gastritis and rule out gastric carcinoid and other gastric cancers, since patients with pernicious anemia are at increased risk for such cancers. The Schilling test of radioactive absorption is no longer available.

Treatment

Treatment should be started within a few days of a confirmed diagnosis. If there are neurological disturbances then treatment should be expedited and started without delay. Neurological presentation may occur in the absence of haematological changes, with early treatment essential to avoid permanent neurological disability. Emergency treatment with packed red cell transfusion may be required for major anaemia in the presence of cardiovascular compromise (Carnel, 2008).

Parenteral Treatment

Standard practice is to begin parenteral treatment with intramuscular hydroxocobalamin. This bypasses the possibility of the debate about whether the treatment will be adequately taken, absorbed, and metabolised. Treatment for patients without neurological involvement is 1000 μg intramuscularly three times a week for two weeks. If there are neurological symptoms then 1000 μg intramuscularly on alternate days should be continued for up to three weeks or until there is no further improvement (Stabler, 2013). In irreversible cases, for example, pernicious anaemia, the treatment should be continued for life. For temporary causes, such as pregnancy, the treatment can be reviewed when the patient is fully replete and the causative agent removed.

Oral Treatment

Cyanocobalamin is an oral preparation that can be given at a dose of 50-150 μg daily. The duration is determined by the cause of the deficiency. This is a drug preparation requiring conversion to metabolically active cobalamins. A Cochrane review of two randomised controlled trials in 108 people with vitamin B12 deficiency found that high oral doses of B12 (1000 μg and 2000 μg daily) were as effective as intramuscular treatment in achieving haematological and neurological responses (Vidal-Alaball, 2005). High dose oral cobalamin may be a suitable alternative in selective cases, where intramuscular injections are not tolerated and compliance is not a problem (Quadros, 2010). Oral treatment may be considered in certain situations—for example, in mild or subclinical deficiency with no clinical features and when absorption and compliance are definitely not a problem (Stabler, 2013). Treatment with vitamin B12 leads to the production of new erythrocytes, which results in an intracellular influx of potassium. This may produce severe hypokalemia, which requires monitoring and appropriate treatment.

Treating Concomitant Deficiencies

If there is concomitant vitamin B12 and folic acid deficiency then vitamin B12 must be started first to avoid precipitating subacute combined degeneration of the spinal cord. In patients with isolated vitamin B12 deficiency and anaemia, additional folic acid supplementation is recommended until vitamin B12 is replete; to prevent subsequent folate deficiency after replenishment of B12 stores (Cranel, 2008). Iron deficiency can be treated with oral ferrous sulphate (or suitable alternative) 200 mg three times daily with vitamin C supplementation. If this is not tolerated or effective then referral to a specialist may be required.

Assessment of Response to Treatment

Patients often have a sense of improvement within the first 24 hours of treatment; however, the haematological response can take several days before the effects are first noticed and up to two months to complete response (Quadros, 2010). Initially, a full blood count and reticulocyte count after 7-10 days of treatment is useful to document the response, and a further check should be done after eight weeks to confirm a normal blood count. When there is inadequate reticulocytosis, an incorrect diagnosis may be responsible. Within eight weeks of treatment the mean cell volume should have normalised (77-95 fL). Iron and folate status should be checked because coexisting deficiency is often obscured in vitamin B12 deficiency (Galloway, 2007; Finch *et al.*, 1956). Homocysteine or methylmalonic acid should normalise during the first week of treatment. Failure to do so suggests an incorrect diagnosis, unless renal failure or other causes of increases in the metabolites coexist. Cobalamin and holotranscobalamin levels are not helpful because they increase with vitamin B12 influx regardless of the effectiveness of treatment, and retesting is not usually required. They can be tested 1-2 months after starting treatment or if there is no response to treatment. Neurological recovery may take some time; improvement begins within one week and complete resolution usually occurs between six weeks and three months. Progression should prompt reassessment of the diagnosis. Patients with delayed improvement should be referred for rehabilitation, including physiotherapy. Residual disability is seen in up to 6% of patients. Damage is likely to be irreversible if diagnosis and treatment are delayed by six months (Quadros, 2010; Carnel, 2008).

A **B**

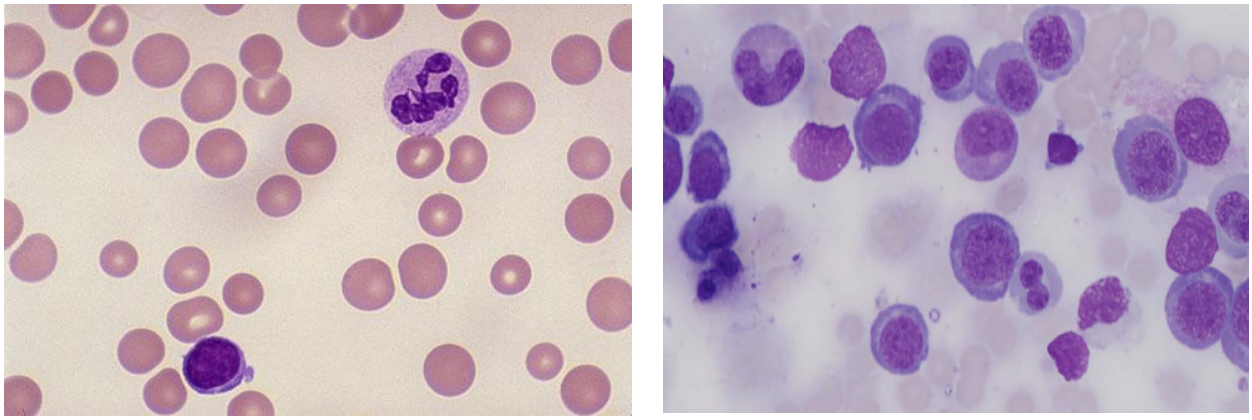

Figure 1: Blood film (A) and bone marrowaspirate (B) indicating megaloblastic features

Prevention

The Institute of Medicine estimates that adults younger than 50 years absorb approximately 50 percent of dietary vitamin B12, and that between 10 and 30 percent of older patients may not be able to absorb adequate amounts from normal dietary sources. The Institute of Medicine recommends daily consumption of 2.4 mcg of vitamin B12 in adults older than 18 years to prevent vitamin B12 deficiency. Because crystalline formulations are better absorbed than naturally occurring vitamin B12, patients older than 50 years should consume foods fortified with vitamin B12 and vitamin B12 supplements, rather than attempting to get vitamin B12 strictly from dietary sources. Strict vegetarians must obtain their vitamin B12 from supplements or consumption of fortified cereal products to prevent deficiency (Institute of medicine, Washington, 1998). Because of the high incidence of vitamin B12 deficiency in patients undergoing gastric bypass surgery, daily prophylactic supplementation with 1 mg is recommended.

Special Considerations

Vitamin B12 deficiency is the major cause of hyperhomocysteinemia in countries with folate fortified food, such as the United States. Epidemiologic studies show significant associations between elevated homocysteine levels and vascular disease and thrombosis. However, large randomized trials of combined highdose vitamin B therapy in patients with vascular disease have shown no reduction in vascular events

(Yang *et al.*, 2012). Vitamin B12 status should be evaluated in patients with hyperhomocysteinemia before folic acid treatment is initiated.

The potential role of mild vitamin B12 deficiency in cognitive decline with aging remains uncertain. Epidemiologic studies indicate an inverse association between vitamin B12 supplementation and neurodegenerative disease, but results of randomized trials have been largely negative (Nachum-Biala, 2012). Besides oral tablets, vitamin B is available in sublingual preparations, oral sprays, nasal gels or sprays, and transdermal patches. Data on the absorption and efficacy of these alternative preparations are lacking.

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