International Journal for Multidisciplinary Research (IJFMR)
E-ISSN: 2582-2160 ● Website: www.ijfmr.com ● Email: editor@ijfmr.com

Study on Clinical Data Related to High Serum Vitamin B12 Levels

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Abstract

Back ground

Serum vitamin B12 measurements are routinely done to rule out vitamin B12 deficiency. Surprisingly, the number of samples for vitamin b12 measurement display high serum vitamin B12 levels. The present study investigated to identify the types of diseases that are showing high serum vitamin B12 levels and to find out the significant association between kidney diseases and high serum vitamin B12 levels. Based on earlier literature, elevated vitamin B12 levels can be found in patients with a variety of diseases, including diseases of the liver, kidney, and immune system as well as several different malignancies. In clinical practice, biomarkers are used as tests for diagnosis, prognosis, screening, and monitoring diseases. In these context, measurement of serum Vitamin B12 is requested to diagnose (or rule out) cobalamin deficiency in patients with the symptoms of haematological, gastrointestinal, and neurological kind.

Aims and Objectives

The present study aims to assess the significant association between high serum vitamin B12 levels and kidney diseases and to identify the types of diseases that are showing high serum vitamin B12 levels.

Methods

The present is a retrospective and observational study, in which the inpatient serum sample values that were obtained from January-June 2018 as part of routine analysis in the department of biochemistry, St. john's hospital, Bengaluru, Karnataka, India after approval by ethics committee. Inpatient serum vitamin B12 values are obtained from laboratory information system. Software data for the period of JANUARY 2018 to JUNE 2018. Exclusion criteria are; patients below 18 years of age, incomplete medical files. In our study, the reported data of vitamin B12 tests are segregated as follows;

1) Low serum vitamin B12 levels- ≤ 211 pg/ml.
2) high serum vitamin B12 levels- ≥2001 pg/ml.

The medical record of patients who had high serum vitamin B12 levels are seen and the clinical diagnoses is recorded.

STATISTICAL ANALYSIS

Data entered in a Microsoft Excel spread sheet and the association of kidney diseases with high serum vitamin B12 levels are statistically analysed by odds ratio.

RESULT

Amongst 486 inpatient samples, 68 samples show high serum vitamin B12(>2001pg/ml) and 72 samples show low serum vitamin B12 levels (<211 pg/ml).

We have observed high serum levels are significantly associated with kidney diseases with an odds ratio of 4.2. This could be due to lack of clearance from kidney damage. High serum vitamin B12 levels are also observed in leukaemia, hepatic diseases and autoimmune diseases.

We included 486 inpatients, of whom 68 patients showed ≥2001 pg./ml. Among those 68 patients, 15 patients had Neurological disorders and they were administered with vitamin B12 injections. 22 patients who were having anaemia showed high serum vitamin B12 status due to administration of vitamin B12 supplements and 2 patients with autoimmune diseases showed high serum vitaminB12 levels. 1 patient with chronic pancreatitis showed high serum vitamin levels.2 patients with rickettsia fever showed high serum vitamin B12 levels without any administration of VitaminB12 supplements and injections. 12 patients who had kidney diseases showed vitamin B12 levels ≥2001 pg./ml, who were not administered with vitamin B12 injections and supplements.

CONCLUSION

High serum vitamin B12 are as frequent as low serum vitaminB12 levels in clinical practice. Kidney diseases have shown significant association with high serum vitamin

B12 levels with an odds ratio of 4.2. Hypercobalaminemia is not specific for any one particular disease. Hence the present results do not advocate the use of serum vitamin B12 as a biomarker for any specific disease. High serum vitamin B12 are as frequent as low serum vitaminB12 levels in clinical practice.

INTRODUCTION

It is common practice to measure serum levels of vitamin B12 in persons suspected of vitamin B12 deficiency based on prevalent symptoms like anaemia, neuropsychological complaints, such as fatigue, cognitive dysfunction and paraesthesia, and diarrhoea and glossitis. Persons at risk of developing B12 deficiency include vegetarians and those with malabsorption due to pernicious anaemia, inflammatory bowel disease, or atrophic gastritis.

Vitamin B12 is an essential micronutrient involved in mitosis and one-carbon and odd chain fatty acid metabolism. In a clinical context, risk factors for vitamin B12 deficiency include a vegetarian diet, existing autoimmune or gastrointestinal or previous gastrointestinal surgery. Vitamin B12 deficiency can cause anaemia and neurological symptoms. Therefore, these factors and symptoms are the indications for measuring vitamin B12 levels.[4]

However, Recent studies suggest that these indications are often not present in patients referred for plasma vitamin B12 measurement. Based on earlier literature, elevated vitamin B12 levels can be found in patients with a variety of diseases, including diseases of the liver, kidney, and immune system as well as several different malignancies.

The pathogenesis underlying these associations is only partly, understood, as are the clinical implications of elevated vitamin B12 levels. There are 3 types of pathophysiological mechanisms that leads to high serum vitamin B12 levels in the body.

1) A direct increase in serum vitamin B12 levels by excess oral intake of vitamin B12 supplements or administration.

2) An increase in serum vitamin B12 levels through lack of clearance by kidney.

3) A direct increase in serum vitamin B12 by liberation from an internal reservoir mainly liver.[2]

Since the late 1940s, Measurement of plasma cobalamin levels has been a cornerstone in diagnosing cobalamin deficiency, although this biomarker has a high rate of both false-negative and false-positive results for diagnosing cobalamin deficiency. Other biomarkers have been established, namely measurement of methyl malonic acid(MMA), homocysteine, and holo-TC. However, the costs, limitations, and availability of these markers as well as clinical tradition hinder their full integration into routine clinical practice. Therefore, the measurement of serum cobalamin remains the first-choice biomarker in the routine diagnosis of cobalamin deficiency.[4]

Vegetarians have a lower intake of vitamin B12 than omnivores, and although clinical signs of deficiency are uncommon, biochemical markers of status indicate functional vitamin B12 deficiency.

The total concentration of cobalamin is measured when measuring serum cobalamin levels, so it is the combined levels of cobalamin-saturated HC and the holo-TC that are measured. In the human circulation, 90% of TC is unsaturated, and TC binds approximately 20% of circulating cobalamin while HC binds the remaining 80%. Inactive cobalamin forms, the so-called cobalamin analogues. Total HC, total TC, and holo-TC can be measures separately, but they are not measured in routine clinical practice.[6]

In clinical practice, biomarkers are used as tests for diagnosis, prognosis, screening, and monitoring diseases. In this context, measurement of serum Vitamin B12 is requested to diagnose (or rule out) cobalamin deficiency in patients with the symptoms of haematological, gastrointestinal, and neurological kind.

However, recent studies suggest that elevated cobalamin levels have most consistently been associated with diseases of the chronic renal failure, acute renal failure. The pathogenesis causing high cobalamin levels in renal disease patients is not well characterised. Areekul et al. showed elevated Tc levels in patients with acute renal disease due to malaria and typhus, and Carmel et al. reported high HC levels in renal disease patients.[4] In autoimmune diseases, elevated plasma cobalamin levels and levels of cobalamin binding proteins have been studied in detail. For rheumatoid arthritis and systemic lupus erythematosus, elevated unsaturated TC levels have been identified.[2]

AIM AND OBJECTIVES

1) The present study aims to assess the significant association between high serum vitamin B 12 levels and kidney diseases
2) Identify the diseases that are showing high serum vitamin B12 levels.

NEED FOR THE STUDY

1) A systematic search is needed to know what types of diseases are associated with high serum vitaminB12 levels.
2) E. Andres in his study concluded that more studies are needed to better understand the clinical data that are associated with high serum vitamin B12 levels.[1]

REVIEW OF LITERATURE

Vitamin B12, also known as cyanocobalamin, is a water-soluble hematopoietic vitamin that is required for the maturation of erythrocyte. The generic term vitamin B12 refers to a group of physiologically active substances chemically classified as cobalamins or corrinoids. They are composed of tetrapyrrole rings surrounding central cobalt atoms and nucleotide side chains attached to the cobalt.[6]

The cobalamin tetrapyrrole ring, exclusive of cobalt and other sidechains, is called a corrin. All compounds containing this corrin nucleus are corrinoids. The cobalt-corrin complex is termed cobamide. In cobalamins, 5,6-dimethylbenzimidazole riboside is bound to the cobalt atom by one of its imidazole nitrogen's, and its 2′-ribose carbon is linked with an ester of amino isopropanol and propionic acid to the corrin ring.

Cobalamins differ in the nature of additional side groups bound to cobalt. Examples include
- Methyl (methylcarbylamine)
- 5′- deoxyadenosine [deoxy adenosyl (short form, adenosyl), cobalamin, or coenzyme B12],
- Hydroxyl(hydroxocobalamin),
- H2O (aquocobalamin, or vitamin B12b), and
- Cyanide (cyanocobalamin)

Cyanocobalamin is a stable compound that forms dark red, needle-like crystals; it is the reference compound for measuring serum cobalamin concentration. Less stable serum cobalamins may be converted to this compound for quantitation.

The predominant physiologic form of cobalamin in serum is methyl cobalamin, whereas that in cytosols is adenosyl cobalamin. It is recommended that the term vitamin B12 be used as the generic descriptor for all corrinoids exhibiting qualitatively the biological activity of cyanocobalamin. Cyanocobalamin has a molecular weight of 1355 Da and a solubility of 12 g/L in water at 20 °C. It is soluble in lower alcohols and aliphatic acids, but is insoluble in acetone, ether, and chloroform. It is gradually destroyed on exposure to light. Aqueous solutions of cyanocobalamin exhibit a distinctive absorption spectrum with maxima at 278, 361, and 550 nm. The spectrum isindependent of pH but changes when cyanocobalamin binds to intrinsic factor (IF). Because of its stability in aqueous solutions and its distinct absorption spectrum, accurate concentrations of cyanocobalamin are prepared and used as calibrators for the measurement of serum cobalamin concentrations.[6]

DIETARY SOURCES

All vitamin B12 is ultimately the product of microbial synthesis. Because plants do not use the vitamin, the main dietary sources are meat and meat products, dairy products, fish and shellfish, and fortified ready-to-eat cereals [6].

ABSORPTION, TRANSPORT, METABOLISM AND EXCRETION

The uptake of vitamin B12 from the intestine into the circulation is a complex mechanism, involving five separate vitamin B12–binding molecules, receptors, and transporters. Vitamin B12 released from food in the stomach is bound to haptocorrin (R protein, a salivary protein) and travels with it into the intestine,

where the haptocorrin is digested by pancreatic enzymes. Liberated vitamin B12 then binds to IF, a glycoprotein with a molecular weight of approximately 50 kDa that is produced by the gastric mucosa. When the vitamin B12–IF complex reaches the distal ileum, it is bound by receptors on the surface of mucosal epithelial cells and then enters the cells. The vitamin B12–IF complex is dissociated within the mucosal epithelial cells, with vitamin B12 then binding with transcobalamin II (TcII). The B12-TcII complex is then transported across the cell membrane while bound to a TcII receptor and is released into the plasma of the mucosal capillaries and subsequently to the blood in the portal vein. Almost all vitamin B12 is taken up by hepatocytes as the blood in the portal vein passes through the liver. It is the blood in the portal vein passes through the liver. It is stored in the liver and is released to plasma to meet physiologic demands. If the quantity of vitamin B12 exceeds the demands. If the quantity of vitamin B12 exceeds the capacity of hepatocyte receptors, most of the excess is excreted by the kidneys. Normally, approximately 1 mg of vitamin B12 is stored in the liver—a quantity equivalent to the daily metabolic requirement for 2000 days. Thus, when the dietary supply of vitamin B12 is interrupted or mechanisms of absorption are impaired, vitaminB12 deficiency does not become evident for 5 years or longer. IF, a glycoprotein with a molecular weight of approximately 50 kDa, is secreted by the parietal cells of the stomach. Many other substances bind vitamin B12, but no other known substance has the property of transporting it across the intestinal wall. One molecule of IF binds one molecule of vitamin B12. Gastric secretion of IF is stimulated by food, histamine, and gastrin; it is inhibited by vagal blockade.[6]

The ileal receptor for the IF–vitamin B12 complex has an association constant of approximately 5 × 109 mol/L between pH 6.4 and 8.4. Binding does not appear to be specific for the configuration of the vitamin B12 molecule, because complexes of IF with various analogues of vitamin B12 bind equally well to the ileal receptors.[6]

The most important vitamin B12 transport protein in plasma is TcII, a β-globulin. It is synthesized mainly in the liver but also in other tissue. TcII is a polypeptide with a molecular weight of approximately 43 kDa; it has a single vitamin B12–binding site per molecule. TcII is less specific for vitamin B12 than is IF; it also binds cobalamins that are physiologically inactive. TcII transports vitamin B12 to receptors on cell membranes throughout the body. Binding is very rapid: if TcII–vitamin B12 is injected intravenously, it is almost completely cleared in one passage through tissue, mostly by the liver. The TcII–vitamin B12 complex enters the cell by pinocytosis. Lysosomal proteolysis degrades TcII and releases the vitamin B12. Unbound vitamin B12 can enter the tissue cells, but the process is much less efficient.

Two types of vitamin B12 binders are found in human gastric juice—
- One with slow (S) and
- One with rapid (R) mobility in zone electrophoresis.

The slow component is IF, and the rapid component is R protein. Immunologically identical R proteins are found in plasma, amniotic fluid, milk, saliva, ascitic fluid, and granulocytes. However, this granulocyte-derived protein is differentiated from the other R proteins electrophoretically. It is called transcobalamin III, whereas the R protein from other sources is designated transcobalamin I. Collectively, these two binders are called cobalophilins. They are glycoproteins with molecular weights between 60 and 150 kDa. Heterogeneity of R proteins may be due to variations in the carbohydrate moieties (sialic acid

residues) rather than in the apoproteins. They have one binding siteper molecule and bind due to variations in the carbohydrate moieties (sialic acid residues) rather than in the apoproteins. They have one binding site per molecule and bind to vitamin B12 analogues to some extent. In gastric juice at pH 2, the cobalophilins have much greater affinity than IF and bind almost all vitamin B12. It has been postulated that cobalophilins aid in host defense against bacteria by depriving them of access to vitamin B12. However, the physiologic function of these proteins is unknown.[4]

Vitamin B12 is continually secreted in the bile, but most is reabsorbed and is available for metabolic functions. If circulating vitamin B12 concentrations exceed the binding capacity of the blood, the excess will be excreted in the urine, but in most circumstances, the highest losses of vitamin B12 occur through the feces.[6]

FUNCTIONS OF VITAMIN B12

Vitamin B12 is required in coenzyme form for more than 12 different enzyme systems.
In humans it is required in
(1) adenosyl cobalamin, coenzyme to l-methyl malonyl-CoA mutase in the conversion of l-methyl malonyl CoA to succinyl-CoA; and
(2) methyl cobalamin, coenzyme to methionine synthase in the conversion of homocysteine to methionine.

mutase is a mitochondrial matrix enzyme that binds 2 moles of adenosyl-cobalamin (Cbl)/dimer376 and participates in a complex reaction using radical chemistry. The conversion of l-methyl malonyl-CoA to succinyl-CoA links propionyl- CoA, which is formed from amino acids such as valine, isoleucine, and methionine with odd-chain fatty acids with the tricarboxylic acid (TCA) cycle. Congenital defects of mutase synthesis or inability to synthesize adenosyl-Cbl results in life-threatening methylmalonic aciduria and metabolic ketoacidosis.[6]

In the latter reaction, methylcarbylamine serves as an intermediate in the transfer of a methyl group from 5-methyltetrahydrofolate to homocysteine for the formation of methionine. Methionine is required for protein synthesis and as the methyl donor, S-adenosylmethionine. Congenital defects in methionine synthase or the synthesis of methyl-Cbl results in severe hyperhomocysteinemia. [6]

Requirements and Reference Nutrient Intakes
Total body stores of vitamin B12 are estimated to be between 2 and 5 mg in the adult man, of which about 1 mg is in the liver and a smaller amount in the kidney. A daily obligatory loss of vitamin B12 of about 0.1% of body pool is believed to occur, irrespective of size, suggesting that a daily requirement to maintain stores would be 2 to 5 µg.

The daily diet of Western countries contains between 5 and 30 µg of vitamin B12, with average ingestion of 7 to 8 µg/d by adult men and 4 to 5 µg/d by adult women. Additional small amounts may be available from vitamin B12 synthesis by intestinal microorganisms. Of the amount ingested, between 1 and 5 µg is absorbed. The RDA for vitamin B12 is based on the amount necessary for maintenance of hematologic status and normal serum vitamin B12 concentrations; it assumes 50% absorbance of ingested vitamin B12.

The RDA for adults (19 to 50 years) has been set at 2.4 µg/d, with an increase to 2.6 µg/d in pregnancy and to 2.8 µg/d in lactation. RDAs for children are 0.9 µg/d at 1 to 3 years, 1.2 µg/d at 4 to 8 years, 1.8 µg/d at 9 to 13 years, and 2.4 µg/d at 14 to 18 years. Because 10% to 30% of older persons may be unable to absorb naturally occurring vitamin B12, it is recommended that those older than 50 years meet their RDA mainly by consuming foods fortified with vitamin B12 or with a vitamin B12–containing supplement.[6]

Deficiency of vitamin B12

Deficiency of vitamin B12 in humans is associated with megaloblastic anaemia and neuropathy. The most common cause of vitamin B12 deficiency is pernicious anaemia, an autoimmune disease in which chronic atrophic gastritis results from antibodies to gastric parietal cells and IF, directed against gastric parietal cell H+/K+-ATPase.

One population study showed that 1.9% of persons older than 60 years have undiagnosed pernicious anaemia, although the diagnosis is made most commonly inyoung to middle-aged black women (mean age, 53 years) and in middle-aged to elderly whites. Pernicious anaemia may also occur in children because of failure of IF secretion or secretion of biologically inactive IF.

Other groups at risk for vitamin B12 deficiency include those

(1) older than 65 years of age;

(2) with malabsorption;

(3) who are vegetarians;

(4) with autoimmune disorders; and

(5) taking prescribed medication known to interfere with vitamin absorption or metabolism, including nitrous oxide, phenytoin, dihydrofolate reductase inhibitors, metformin, and proton pump inhibitors; as well as

(6) infants with suspected metabolic disorders Intestinal malabsorption of vitamin B12 may be caused by gastrectomy or ileal resection, with an inverse relationship noted between the length of ileum resected and absorption of vitamin B12. Other causes of malabsorption include tropical sprue, inflammatory disease of the small intestine, intestinal stasis with overgrowth of colonic bacteria, which consume vitamin B12 ingested by the host, and human immunodeficiency virus (HIV) infection. Another cause of vitamin B12 malabsorption is failure to extract cobalamin from food. Some patients fail to absorb cobalamin bound to food, whereas absorption of non–food-bound cobalamin in the Schilling test is unimpaired. This is particularly a problem in patients with compromised gastric status or early in the course of development of pernicious anaemia.[6]

Vegetarians have a lower intake of vitamin B12 than omnivores, and although clinical signs of deficiency are uncommon, biochemical markers of status indicate functional vitamin B12 deficiency. In a study of lactovegetarians or lacto-ovo-vegetarians, 29 vegans, and 79 omnivores, the incidence of low holotranscobalamin II was 77%, 92%, and 11%, respectively, in the three groups; of elevated methylmalonic acid (MMA), 68%, 83%, and 5%; and of elevated total homocysteine, 38%, 67%, and 16%.[4]

A large number of disorders are associated with cobalamin deficiency in infancy or childhood. Of these, the most commonly encountered is the Imerslund-Graesbecksyndrome, a condition that is characterized by inability to absorb vitamin B12, with or without IF, and proteinuria. It appears to be due to an inability of intestinal mucosa to absorb the vitamin B12–IF complex. The second most common of these is congenital deficiency of gastric secretion of IF. Very rarely, congenital deficiency of vitamin B12 in a breast-fed infant is due to deficiency of vitamin B12 in maternal breast milk resulting from unrecognized pernicious anaemia in the mother. This is rare because most women with undiagnosed and untreated pernicious anaemia are infertile. Additionally, some rare methylmalonic acidemias (acidurias) caused by inborn errors in homocysteine and methionine metabolism are responsible for disorders of vitamin B12 status.[6]

The hematologic effects of vitamin B12 deficiency are indistinguishable from those of folate deficiency. Classical morphologic changes in the blood, in approximate order of appearance, are as follows: hyper segmentation of neutrophils, macrocytosis, anaemia, leukopenia, and thrombocytopenia, with megaloblastic changes in bone marrow accompanying peripheral blood changes. The cause of the hematologic abnormalities is thought to be an imbalance of decreased deoxyribonucleic acid (DNA) synthesis and adequate ribonucleic acid (RNA) synthesis caused by the secondary block in folate metabolism caused by vitamin B12 deficiency. Many immature cells die in the bone marrow, possibly by apoptosis, leading to the release of bilirubin and lactate dehydrogenase (LD) into the blood. This is termed ineffective erythropoiesis. All bone marrow lesions can be reversed by vitamin B12.[6]

In addition to hematologic changes, vitamin B12 deficiency can lead to a demyelinating disorder of the central nervous system in man. Serious and often irreversible neurologic disorders can occur, such as burning pain or loss of sensation in the extremities, weakness, spasticity and paralysis, confusion, disorientation, and dementia. This condition has been given the name subacute combined degeneration of the spinal cord.

Neurologic symptoms may occur without any discernible hematologic changes in the blood; indeed, an intriguing inverse relationship between the hematologic and the neurologic has been observed.

The incidence of neurologic complications is between 75% and 90% of all individuals with clinically observable vitamin B12 deficiency; in about 25% of cases, these may be the only clinical manifestation of deficiency. The mechanism of the disorder is uncertain, although indirect evidence suggests that disorders of both enzyme systems requiring vitamin B12 coenzymes are necessary before neurologic symptoms occur. The response of neurologic symptoms to vitamin B12 replacement is often dependent on the duration of the symptoms.

Vitamin B12 deficiency may be associated with other mainly gastrointestinal complications, such as glossitis of the tongue, appetite and weight loss, flatulence and constipation, mental changes, and infertility. Interest in a possible link between vitamin B12 status and cognitive decline is increasing, but data remain inconclusive even though supplements of vitamin B12 and folate may normalize homocysteine concentrations.[6]

Toxicity

No adverse effects have been associated with excess vitamin B12 intake from food or supplements in healthy people. Daily oral doses of up to 2 mg of cyanocobalamin have been used for treatment of deficiency in those who tolerate oral supplementation. Data in the literature are insufficient to propose a tolerable upper intake amount for vitamin B12.[6]

Laboratory Assessment of vitamin B12 Status

Both direct and indirect (functional) methods are available for assessment of vitamin B12 status. Indirect tests include assays for urinary and serum concentrations of MMA, plasma homocysteine, the deoxy uridine suppression test, and the vitamin B12 absorption test.

Cytochemical staining of red blood cell (RBC) precursors and the test for IF blocking antibodies are other ancillary methods of assessing vitamin B12 status. A comprehensive review of methods for measuring vitamin B12 in various biological samples has been published.[6]

Microbiological, competitive protein binding (CPB), and immunometric assays have been used for quantitation of serum vitamin B12. Microbiological assays have largely been replaced by the other, more convenient and precise methods, although they remain reference methods for the determination of biologically active vitamin B12. The most widely used procedures use Euglena gracilis, Lactobacillus leishmannii, or a mutant of Escherichia coli, although each of these organisms is susceptible to growth inhibitionby antibiotics or other drugs, such as methotrexate, that may be present in a patient's serum. Furthermore, these assays require at least 24 hours to establish adequate growth of the microorganism. However, use of microtiter enzyme-linked immunosorbent assay (ELISA) plate technology has enhanced the utility of some microbiological assays.[6]

Commercial kits are available for CPB assays of vitamin B12. The vitamin B12 binder used is often nonhuman IF, usually obtained from hog stomach. If the IF is not highly purified, it may contain R proteins, which bind not only vitamin B12 but also related metabolically inactive compounds, yielding higher values. IF therefore must be highly purified or must have cobamamide (a vitamin B12 analogue) added to the IF to saturate all binding sites on the R proteins. Cobinamide is not bound by IF.

In a widely used CPB assay, vitamin B12 (cobalamin) competes with Co-labelled cobalamin for a limited number of binding sites on IF. Some assays require a preliminary step in which the specimen is boiled in a buffered solution containing dithiothreitol, KCN, and Co-labelled tracers to release vitamin B12 from endogenous binding proteins. Alternatively, other procedures irreversibly denature endogenous binding proteins by increasing the pH from 12 to 13 and then readjusting the pH to 9.3 before the binding reagent is added. Subsequent separation of bound and free folate and vitamin B12 is achieved by contact with dextran-coated charcoal, which absorbs the free (unbound) molecules, leaving protein bound vitamin B12 in the solution.[6]

Most immunometric methods use solid-phase separation by immobilizing the IF binder on beads or magnetic particles. The free vitamin B12 then remains in the supernatant, and the bound analytes become part of the solid-phase suspension. For simultaneous folate/vitamin B12 measurement, a gamma

scintillation counter that discriminates between the energy levels of 57Co (for vitamin B12) and 125I (for folate) must be used Multiple automated and semiautomated systems are available for measuring vitamin B12 and folate, using, for example, chemiluminescence as a signal. The assays are standardized with 7.5-minute incubation, magnetic particle separation, and an acridium ester signal. The precision of automated systems allows specimens to be analysed in singlet while CVs less than those found for the mean of duplicates of radio immunoassays are maintained.

Indirect tests assess the functional adequacy of vitamin B12. Serum methylmalonic acid concentration is increased when lack of adenyl-Cbl causes a block in the conversion ofmethyl malonyl-CoA to succinyl-CoA. It is a sensitive test of status, being often the first analyte to be raised in subclinical vitamin B12 deficiency. It has a further advantage in that it is unaffected by folate deficiency. Early methods for methylmalonic acid lacked sensitivity and specificity; this situation has been resolved by the adoption of gas chromatographic– mass spectrometric methods, although these methods require specialized handling.[6]

Plasma total homocysteine concentration is a sensitive indicator of vitamin B12 status because methyl-Cbl is required for the remethylation of homocysteine to methionine, but it is not specific, being elevated in deficiencies of folate and vitamin B6 and vitamin B12. Plasma concentrations of total homocysteine can be reliably measured by HPLC with fluorescent or electrochemical detection, and with enzymatic and capillary gas chromatography–mass spectroscopy methods. Plasma samples for homocysteine analysis must be obtained soon after venipuncture to reduce preanalytical increases that may occur on standing, although these can be minimized by the use of a fluoride– ethylenediaminetetraacetic acid (EDTA) tube. Increased screening of plasma total homocysteine concentrations as an independent risk factor for cardiovascular disease may lead to identification of additional cases of subclinical vitamin B12 deficiency. The measurement of holotranscobalamin II is potentially useful as a specific marker of biologically available vitamin B12, because only cobalamin bound to TcII is specifically available for uptake by all cells. Other methods have been described for the measurement of holotranscobalamin in serum, using an immobilized monoclonal antibody to human transcobalamin, followed by measurement of released cobalamin by CPB645 or an automated assay by enzyme immunoassay. Another method uses magnetic beads coated with cobalamin to precipitate apotranscobalamin, followed ELISA. Although these methods are claimed to be precise and simple to perform, there remains doubt over interpretation of the measured concentrations and their sensitivity and specificity in the diagnosis of vitamin B12 deficiency.

The deoxyuridine suppression test measures the effects of prior addition of deoxyuridine on uptake of radiolabelled thymidine into the DNA of cultured bone marrow cells, peripheral blood lymphocytes, or whole blood. Normal samples that contain vitamin B12 can convert deoxy uridine to thymidine and therefore do not take up as much thymidine. Samples from patients who are deficient in vitamin B12 showless suppression than those from normal patients. Because it is relatively time-consuming, the deoxy uridine suppression test is not widely available for use as a diagnostic test.

The Schilling test is primarily a test of vitamin B12 absorption and not of status, but it permits differentiation of causes of vitamin B12 deficiency (pernicious anaemia or intestinal malabsorption). The proportion absorbed from orally administered57Co- or 58Co-labeled vitamin B12 is measured by

determining radioactivity in feces, urine, or serum or by externally scanning the liver. The usual procedure is to externally scanning the liver. The usual procedure is to measure radioactivity in a 24-hour urine sample, which is collected after oral administration of 0.5 µg of radioactive Co-labelled vitamin B12 after an overnight fast. In normal individuals, 8% or more of the dose administered is excreted in the urine, whereas in people with pernicious anaemia, less than 7% (often 0 to 3%) is excreted. A confirmatory test for lack of IF requires ingestion of vitamin B12 and IF.[6]

Reference Intervals

Depending on the laboratory and the procedure used, reference intervals vary widely. The WHO, in its report in 1968, defined a serum vitamin B12 concentration less than 150 ng/L (110 pmol/L) as deficient, and a concentration of 201 ng/L (147 pmol/L) or higher as acceptable. A dietary and nutritional survey of British adults in 1990 published a reference interval of 206 to 678 ng/L (151 to 497 pmol/L). Changes in serum vitamin B12 concentration as a function of age in healthy adults have been the subject of contradictory reports. [6]

Data from a study population in the United States (Framingham Study) showed an increased prevalence (40.5% of 222 subjects) of low serum vitamin B12 concentration (<258 pmol/L) in elderly subjects in comparison with a control group of younger subjects (17.9% incidence).

Vitamin B12 concentrations within the reference interval may not necessarily reflect adequate vitamin B12 status, because serum concentrations may be maintained at the expense of tissue stores. Conversely, low serum vitamin B12 concentrations may not be indicative of vitamin B12 deficiency. Most of the vitamin B12 in serum is bound to TcI, which is released by granulocytes and has no functional role in the transport ofvitamin B12 to cells. Low serum vitamin B12 concentration may be due to a reduction in TcI as a consequence of low total granulocyte mass. This has been observed in benign neutropenia, multiple myeloma, and leukemic reticuloendotheliosis and may be expected in other conditions in which the bone marrow is hypoplastic, aplastic, or replaced by malignant cells.[6]

MATERIALS AND METHODS

SOURCE OF DATA

This is a retrospective study Done in the department of clinical biochemistry, St. John's medical college and hospital Bangalore. In which the inpatient serum vitamin B12 values were obtained from laboratory information system. The study was approved by ethics committee of the institution.

EXCLUTION CRITERIA

Patients below 18 years of age, incomplete medical files.

METHOD OF DATA COLLECTION:

Inpatient serum vitamin B12 values are obtained from laboratory information system. Software data for the period of JANUARY 2018 to JUNE 2018.

In our study, the reported data of vitamin B12 tests are segregated as follows;

1)Low serum vitamin B12 levels- ≤ 211 pg/ml.

2)high serum vitamin B12 levels- ≥2001 pg/ml.
The medical record of patients who had high serum vitamin B12 levels are seen and the clinical diagnoses is recorded.

STATISTICAL ANALYSIS
Data entered in a Microsoft Excel spread sheet and the association of kidney diseases with high serum vitamin B12 levels are statistically analysed by odds ratio.

METHOD OF ESTIMATION
VITAMIN B12 ESTIMATION

PURPOSE OF EXAMINATION;
a) To measure Vitamin B 12 quantitatively in the specimen by Automated Method.
b. To use Vit B 12 measurements in the diagnosis of anaemia and conditions marked by high turnover of myeloid cells, as in leukaemia's.

SPECIMEN TYPE REQUIRED- Serum

TYPE OF CONTAINER AND ADDITIVES FOR- Golden yellow capped serum separator tube (SST)/ Red capped vacutainer with clot activator.

PRINCIPLE OF TEST METHOD - CHEMILUMINISCENT MICROPARTICLE IMMUNO ASSAY(CMIA).

Vitamin B12 is measured using Fully automated analyser Architect I system (ARCHITECT ci8200). Determination of vitamin B12 in human serum is done by using CMIA technology (Chemiluminescent microparticle immunoassay).

PRINCIPLE- A sample is added to a reaction vessel along with potassium cyanide and alpha monothioglycerol. This treatment denatures vitamin B12 binding proteins and converts all forms of vitamin B12 to the cyanocobalamin form, the pre-treated sample and intrinsic factor coated paramagnetic microparticles are combined. The vitamin microparticles are held in a magnetic field while unbound materials are washed away.

After washing vitamin B12 acridinium-labelled conjugate is added to create a reaction mixture. Then the chemiluminescent substrate, hydrogen peroxide and sodium hydroxide are added to the vessel, the resulting chemiluminescent reaction is measured as relative units (RLUs). there is an inverse relation between the amount of vitamin B12 in the sample and the RLUs detected by the Architect I system optics.[7]

PROCEDURE
1.Sample and Pre-treatment reagent 1, Pre-treatment reagent 2, and Pre-treatment reagent 3 are combined.

2.An aliquot of the pre-treated sample is aspired and transferred into a new reaction vessel (RV). The pre-treated sample, assay diluent, and intrinsic factor coated paramagnetic microparticles are combined. The B12 present in the sample binds to the intrinsic factor coated microparticles.
3.After washing, B12 acridinium-labelled conjugate is added to create a reaction mixture.
4.Following another wash cycle, pre-trigger and trigger solutions are added to the reaction mixture.
5.The resulting chemiluminescent reaction is measured as relative light units (RLUs). There is an inverse relation between the amount of B12 in the sample and the RLUs detected by the ARCHITECT I system optics [7].

Calibration procedures
a. Calibrator: 7K61--01 ARCHITECT Bl2 Calibrators.
b. Calibrator traceability: The ARCHITECT B 12 assay is traceable to B12 World Health Organization International Standard 03/178.
c. Calibration Scheme: Test Calibrators A-Fin duplicate.
d. Calibration Frequency: As manufacturer's recommendation.
i. Every new lot of reagent cartridge.
ii. As indicated in laboratory quality control procedures.
iii. After major maintenance or service, if indicated by IQC results.[7]
SPECIFIC PERFORMANCE CHARACTERISTICS:
a. Assay Range /Analytical Measurement Range: 150 - 2000 pg/mL
b. LoQ : 150 pg/ Ml

QUALITY CONTROL PROCEDURES:
Internal Quality Control (IQC): IQC material with known Vitamin B 12 concentrations are analyzed on the days of use and data is reviewed.
a. QC product material: Serum: LYPHOCHECK Immunoassay Plus Control (IPC). Level I, II and Ill from Bio-Rad Laboratories.
b. Frequency: Any two Levels IPC once a day.
c. Lab mean, SD and CV% is established for the IQC. If the value lies within the cut off limits as specified by the lab or pack insert for Quality Control (before establishing the lab n1ean), the test results are released. Levey-Jennings control charts are maintained.
RESULT

In order to identify significant association between high serum vitamin B12 levels and kidney diseases the sample size required was 68 patients with high serum vitamin B12 levels.
Amongst 486 inpatient samples, 68 samples showed high serum vitamin B12 levels (>2001) and 72 samples showed low serum vitamin B12 levels. (<211).
Analysing the data by using statistical method odds ratio, Kidney diseases are significantly associated with high serum vitamin B12 levels with an odds ratio of 4.2.

Amongst the 68 patients with high serum vitamin B12 levels

NO. OF PATIENTS	CLINICAL DIAGNOSIS
12 patients	Kidney diseases
5 patients	Liver diseases
5 patients	leukemia
15 patients injection	Neurological disorders and patients were administered withvitamin B12
22 patients	Anemia and patients were administered with vitamin B12 injections.
2 patients	Autoimmune diseases
2 patients	Rickettsia fever
1 patient	Chronic pancreatitis

In the study period, the distribution of serum vitamin B12 levels in the hospital patients was as follows:

SERUM VITAMIN B12 LEVELS	NO. OF PATIENTS
≤211 pg/ml	72(14.8%)
≥2001 pg/ml	68(13.9%

We included 486 inpatients, of whom 68 patients showed ≥2001 pg/ml. Among those 68 patients, 15 patients had Neurological disorders and they were administered with vitamin B12 injections. 22 patients who were having anaemia showed high serum vitamin B12 status due to administration of vitamin B12 supplements and 2 patients with autoimmune diseases showed high serum vitaminB12 levels. 1 patient with chronic pancreatitis showed high serum vitamin levels.2 patients with rickettsia fever showed high serum vitamin B12 levels without any administration of VitaminB12 supplements and injections. 12 patients who had kidney diseases showed vitamin B12 levels ≥2001 pg./ml, who were not administered with vitamin B12 injections and supplements.

DISCUSSION

The study was undertaken to identify the significant association between kidney diseases and high serum vitamin B12 levels and to identify the types of diseases that are showing high serum vitamin B12 levels. Analysing the data by using statistical method odds ratio, we have observed that Kidney diseases are significantly associated with high serum vitamin B12 levels with an odds ratio of 4.2. This could be due to lack of clearance from kidney damage.

Results were adjusted with 95% confidence intervals, p = 0.001.

The suggested mechanisms of hypercobalaminemia are –

a) excess vitamin B12 intake/ administration.

b) Damage to internal reservoir (liver disease) and direct release of vitamin B12 into the plasma,

c)Excess production or lack of clearance of cobalamin.

In our studies, Renal disease patients showed significant association with high serum vitamin B12 levels. This could be due to lack of clearance of cobalamin from kidney damage.15 In patients who were having neurological disorders showed high serum vitamin B12 status because of administration of vitamin B12 injection. 22 patients who were having anaemia showed high serum vitamin B12 status due to administration of vitamin B12 supplements and 2 patients with autoimmune diseases showed high serum

vitaminB12 levels. 1 patient with chronic pancreatitis showed high serum vitamin levels.2 patients with rickettsia fever showed high serum vitamin B12 levels without any administration of VitaminB12 supplements and injections.

Six studies assessed the prevalence of certain diagnoses in patients with high plasma cbl levels. All populations consisted of patients referred for plasma cobalamin measurement, were cross-sectional, and had sample sizes ranging from 135-16,497 patients; however, three studies reported associations between elevated cbl levels and renal, liver, and autoimmune diseases and cancer, both haematological and solid tumour cancers.

Carmel et al. were the only authors to describe the underlying alterations in cbl-binding proteins, reporting that both saturated and unsaturated HC and TC were elevated. Jeffery et al. and Remecha et al. found that between 18% and 25% of patients with elevated Cbl levels had an immune complex with Cbl or Cbl-binding proteins, although the majority of the patients in the study were treated with high-dose Cbl. There was not full consensus across the studies on Cbl level cut-off points, prevalence of elevated Cbl levels, and which diseases were highly prevalent in patients with elevated Cbl levels. Only Brah et al. conducted a multicentre study, and none of the studies used direct measurement of Cbl-binding proteins.[4]

The 58 studies assessing the prevalence of elevated Cbl levels in certain diagnoses are presented below. Elevated Cbl levels have most consistently been associated with diseases of the liver, both alcoholic liver disease and non-alcoholic liver disease. Despite different etiology, the pathogenesis leading to high Cbl levels is thought to be high HC levels because of impaired clearance together with high holoTC levels because of Cbl release fromhepatocytes. Several studies have suggested Cbl levels and/or levels of Cbl-bindingproteins as markers for disease progression, treatment response, and prognosis. Also, alcoholism without apparent liver disease seems to be associated with high Cbl levels, but liver disease linked with intestinal failure was not associated with high Cbl levels in one study.[4]

Chronic renal failure, acute renal failure, and diabetic renal disease have been associated with high Cbl levels. However, low Cbl levels and other markers showing Cbl deficiency have also been reported in patients with renal disease. The pathogenesis causing high Cbl levels in renal disease patients is not well characterized. Areekul et al. showed elevated TC levels in patients with acute renal disease due to malaria and typhus, and Carmel et al. (reported high HC levels in renal disease patients. One author group speculated that impaired renal tubular reabsorption of holoTC causes high holoTC levels.[4]

In autoimmune diseases, elevated plasma Cbl levels and levels of Cbl-binding proteins have been studied in some detail. For rheumatoid arthritis and systemic lupus erythematosus, elevated unsaturated TC levels have been identified, but without concurrent elevated Cbl levels. Another form of arthritis, Still's disease, has been associated with elevated Cbl levels. Macrophage activation is a central component of this disease, and TC correlates with macrophage activity. Thus, elevated Cbl levels in Still's disease could be caused by high TC release related to macrophage activity. Elevated Cbl levels are a part of the diagnostic criteria for autoimmune lymphoproliferative syndrome and are caused by high HC production from proliferating leukocytes. Also, high Cbl levels have been reported in autoimmune neutropenia.[4]

Different infectious diseases have been associated with elevated Cbl levels or elevated levels of Cbl-binding proteins in studies other than those on viral hepatitis and by Areekul et al., noted above. Scrub and murine typhus have been linked to elevated Cbl levels), and patients with HIV have been reported to have elevated levels of Cbl, TC, and HC. In HIV and AIDS, a high prevalence of low Cbl and holoTC has also been reported

STUDY	N	Prevalence of elevated cbl levels	Cut-off(pmol/L)	OTHER BIOMARKERS	Disease Assosciations
Camel et al.	135	14%	664	HC and TC, both Cbl- saturated and unsaturated; serum creatinine and albumin	Renal failure
chic he et al	411	18.5%	701	Not measured	Haematological and solid tumour cancer, liver and renal diseases.
Jeffery et al.	16,497	9%	664	IgG immune complex	not assessed
Jamal et al.	2,943	18%	605	Not measured	Haematological and solid tumour cancer, liver and renal diseases.
brah et al.	380	18%		Not measured	haematological cancer, liver and renal diseases
Remecha et al	10,325	1.30%	2500	immune complex with IgG or IgA+Cbl-binding protien(only in some patients	haematological and solid tumour cancer, liver and autoimmune diseases

CONCLUSION

Our study confirmed that abnormal elevation of B12 is significantly associated with the presence of kidney diseases with an odds ratio of 4.2. Liver diseases, leukaemia, and autoimmune diseases, showed high serum vitamin B12 levels.

Hypercobalaminemia is not specific for any one particular disease. Hence the present results do not advocate the use of serum vitamin B12 as a biomarker for any specific disease. High serum vitamin B12 are as frequent as low serum vitamin B12 levels in clinical practice. A systematic approach is needed to determine the potential indications of the search for high serum vitamin B12 levels. As in many fields of

medicine, Further studies are needed more than ever to better understand the clinical data related to high serum vitamin B12.[2]

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