

Holotranscobalamin : A Newer Parameter in Diagnosis of Vitamin B₁₂ Deficiency States

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Abstract

Background and objectives: Vitamin B₁₂ is an essential nutrient that plays crucial roles in the human body. B₁₂ deficiency primarily affects the hematopoietic system, but its effects extend to other tissues and organs, most notably the nervous system. The neurological symptoms of vitamin B₁₂ deficiency are non specific and can be irreversible. Holotranscobalamin (Holo-TC) also called as active B₁₂ has been suggested as sensitive marker for early diagnosis of B₁₂ deficiency; therefore measurement of Holo-TC may be useful for taking therapeutic measures before neurological damage of vitamin B₁₂ deficiency develops. *Methods:* We tested serum samples of 50 cases for hemoglobin levels, MCV and total vitamin B₁₂ assay. We divided them into two groups and also tested for Holo-TC. *Group:* 1) Hemoglobin above 11.0 gm %, MCV between 85-96 fl and serum B₁₂ in the range of 200-300 pmol/L. *Group:* 2) Hemoglobin less than 11.0 gm % and serum B₁₂ less than 200 pmol/L. MCV and hemoglobin was measured by Sysmex KX-21 five part differential cell counter. PBF of all subjects was examined for evidence of hyper segmentation. Cases having history of any disease were not included in the study. *Results:* Group 1 cases were having total serum B₁₂ level in normal range but Holo-TC value of these cases was found decreased suggestive of early vitamin B₁₂ deficiency. Group 2 cases were having a low value of serum B₁₂ with markedly low value of Holo-TC suggesting that value of Holo-TC decreases with decrease in serum B₁₂ levels. *Conclusions:* Data present in this study show correlation between Serum Vitamin B₁₂ and Holo-TC results. In majority of cases significant correlation was observed between the two indices. We predict that Holo-TC is a good marker for early diagnosis of vitamin B₁₂ deficiency.

Keywords: Hemoglobin; MCV; Vitamin B₁₂; Holo-TC.

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Introduction

Vitamin B₁₂ also known as cyanocobalamin is a water soluble hematopoietic vitamin that is required for the maturation of erythrocytes.

Vitamin B₁₂ is an essential nutrient that plays crucial roles in the human body ranging from supporting a healthy nervous system to synthesis of DNA and red blood cells (RBC).

It has been established that impaired maternal B₁₂ status in pregnancy is associated with poor maternal and child health outcomes [1].

Vitamin B₁₂ deficiency is wide spread and availability of B₁₂ is very scarce in vegetarian diets. Among population at risk are older people, vegetarians, pregnant women and patients with renal and intestinal diseases [2,3].

Vitamin B₁₂ deficiency is still an important nutritional problem worldwide as subclinical deficiency affects well defined risk groups. Vitamin B₁₂ levels decreases with age which means that deficiency risk increases in parallel with age. Deficiency of B₁₂ in older people may lead to neurological symptoms like confusion, depression, memory loss and balance problems (gait ataxia) which are irreversible even after treatment with vitamin B₁₂ [4].

B₁₂ deficiency can affect bone metabolism and can stimulate osteoclasts [5].

Vitamin B₁₂ deficiency can also cause hyperhomocysteinaemia, which has been related to the risk of vascular and cerebral diseases.

High maternal folate and low maternal B₁₂ concentrations was associated with greater adiposity and insulin resistance in children and post-partum mothers.

Maternal B₁₂ deficiency has also been linked to preeclampsia and various health outcomes for the offspring such as growth inhibition, developmental regression, neurological symptoms, cognitive impairment, behavioral symptoms, and an increased risk for development of chronic diseases later in life.

In addition impaired B₁₂ and folate status can affect in utero methyl nutrient availability. This imbalance has been suggested to affect long-term risk for insulin resistance type-2 diabetes and cardiovascular diseases like ischemic heart disease, deep vein thrombosis and stroke [6,7,8].

The prevalence of vitamin B₁₂ deficiency in the general population is unclear and prevalence of

a sub-clinical functional vitamin B₁₂ deficiency in the general population is higher than previously expected.

Vitamin B₁₂ deficiency can arise for many reasons.

1. Inadequate intake.
2. Dysfunction of food-cobalamin absorption.
3. Dysfunction of transport.

Megaloblastic anemia, progressive neurological impairment and several gastrointestinal symptoms can result as symptoms of chronic B₁₂ deficiency.

Physical and behavioral symptoms of megaloblastic anemia include fatigue, dyspnea, pallor, listlessness, and glossitis, which can be confirmed by clinical evidence of macrocytosis, reduced population of RBCs and hypersegmentation in granulocytes. Bone marrow shows megaloblastosis. It is diagnosed by an increased MCV (normal range 80-100 fl) or by assessment of a blood smear.

Less than ten percent of individuals with Serum B₁₂ < 148 pmol/L (i.e. chronic B₁₂ deficiency) present with true megaloblastic anemia [9-10].

If left untreated vitamin B₁₂ deficiency will result in the classical hematologic or neurologic complications, neurological complications include progressive sub-acute combined degeneration of the spinal cord, loss of proprioception, weakness of lower limbs, peripheral neuropathy, cerebral demyelination, optic atrophy and progressive cognitive decline and are irreversible [11].

Gujarat being predominantly vegetarian state clinical and subclinical deficiency of B₁₂ is quite common in the general population. The gold standard for the diagnosis of Vitamin B₁₂ deficiency is a low blood level of serum vitamin B₁₂ estimation.

Total serum vitamin B₁₂ may not reliably indicate vitamin B₁₂ status.

To get more specificity and sensitivity in diagnosing vitamin B₁₂ deficiency the concept of measuring Holo-TC a sub-fraction of vitamin B₁₂ has aroused great interest [12,13,14,15,16].

The diagnostic use of Holo-TC if proven will allow the initiation of therapeutic measures before irreversible neurological damage develops because clinical manifestations of vitamin B₁₂ deficiency are nonspecific and people at risk should be identified and should be regularly monitored.

The present study was carried out-

1. To compare active B₁₂ with total vitamin B₁₂.
2. To investigate clinical utility of the parameter and

3. To establish analytical validity of the active B₁₂ assay.

Vitamin B₁₂ is the only water soluble vitamin stored by the human body; liver is the primary storage site containing more than 1.5 mg of the vitamin B₁₂, it would take between 2-10 years for deficiency to develop even when a diet is completely lacking the vitamin.

Vitamin B₁₂ has multiple binding proteins that facilitate its transport.

There are four known proteins involved in the absorption and transport of vitamin B₁₂:

1. R-protein (Transcobalamin I)
2. Intrinsic factor
3. Transcobalamin II
4. Transcobalamin III.

R-protein which is also known as haptocorrin or transcobalamin I is found in most body fluid including saliva.

Intrinsic factor is secreted by the parietal cells of the stomach and is required for the intestinal absorption of vitamin B₁₂ in the distal ileum.

While it has no known function vitamin B₁₂ bound to R-protein accounts for 80% of total plasma B₁₂ and may contribute to falsely high B₁₂ measurements.

Transcobalamin II also known as Holo-transcobalamin (Holo-TC) when bound to B₁₂ is found in plasma.

Holo-TC is the only biologically active form of B₁₂ and is responsible for the transport of B₁₂ to cell membrane receptors (part of receptor mediated endocytosis).

Holo-TC accounts for 6-25% of total plasma B₁₂ and is believed to be the most sensitive marker to depletion or repletion of B₁₂ [13].

Lastly Transcobalamin III is made by granulocytes and has an unknown function. However it has clinical significance as elevated levels of Transcobalamin III as seen in chronic myelogenous leukemia may cause falsely high measures of B₁₂.

Several cut-points of vitamin B₁₂ have been suggested: 125 pmol/L, 148 pmol/L, 150 pmol/L, 184 pmol/L and 258 pmol/L.

A cut of point of B₁₂ value in our study of Group -1 is less than 200 pmol/L and Group - 2 is 200-300 pmol/L.

Materials and Methods

The study was approved by the scientific review committee and ethics committee of the Gujarat University, Ahmedabad.

We selected two groups of 50 subjects who fulfilled the following criteria;

Group: 1) Hemoglobin above 11.0 gm %, MCV between 85-96 fl, serum B₁₂ in the range of 200-300 pmol/L.

Group: 2) Hemoglobin less than 11.0 gm %, serum B₁₂ less than 200 pmol/L.

PBF of all cases was examined for evidence of hypersegmentation.

Cases having history of any disease were not included in the study.

Blood samples of 50 cases were collected in plain tubes and were stored at -20°C.

Holo-TC is a stable analyte and no specific precaution is to be taken.

Serum Holo-TC was measured undiluted by micro plate immunoassay method (Axis Shield Diagnostics) with low and high controls.

Calibration range was 1.00-128 pmol/L. To minimize analytical variations single technician assayed all the samples. A Holo-TC value below 25.0 pmol/L was chosen for cut-off threshold for assessing cobalamin deficiency in the subjects.

MCV and hemoglobin was measured by Sysmex KX-21 Five part differential cell counter.

Statistical analysis was performed by Microsoft excel 2007.

Results

A total of 50 cases were enrolled in the department of pathology, GCS Medical College Hospital and Research Centre, Ahmedabad.

Age of the cases varied from 14 years to 65 years. Maximum numbers of patients were seen between the age group of 20 to 30 years (Fig. 1).

Female cases are more common than males in our study.

Holo-TC value from 10-24 pmol/l was considered as low.

Group 1 included 26 cases (B₁₂ in range of 200-300 mg) from which 13 cases had Holo- TC value less than 25 pmol/l, 6 cases ranged between

25-30 Pmol/l and remaining 7 cases were found near to border line level of Holo- TC that is around 30 pmol/l.

It was observed that Group 1 cases were having total serum B₁₂ level in normal range but Holo- TC value of these cases was found decreased suggestive of early vitamin B₁₂ deficiency. (Fig. 2).

Group 2 included 24 cases having total serum B₁₂ level less than 200 mg/dl out of these 24 cases

19 cases had Holo-TC value of less than 10 Pmol/l remaining 5 cases had Holo-TC value below 25 Pmol/l hence it was observed that as the value of B₁₂ decreased value of Holo-TC also decreased markedly and is suggestive of B₁₂ deficiency. (Fig. 3).

It was observed that Group 2 cases were having a low value of serum B₁₂ with markedly low value of Holo-TC suggesting that value of Holo-TC decreases with decrease in serum B₁₂ levels.

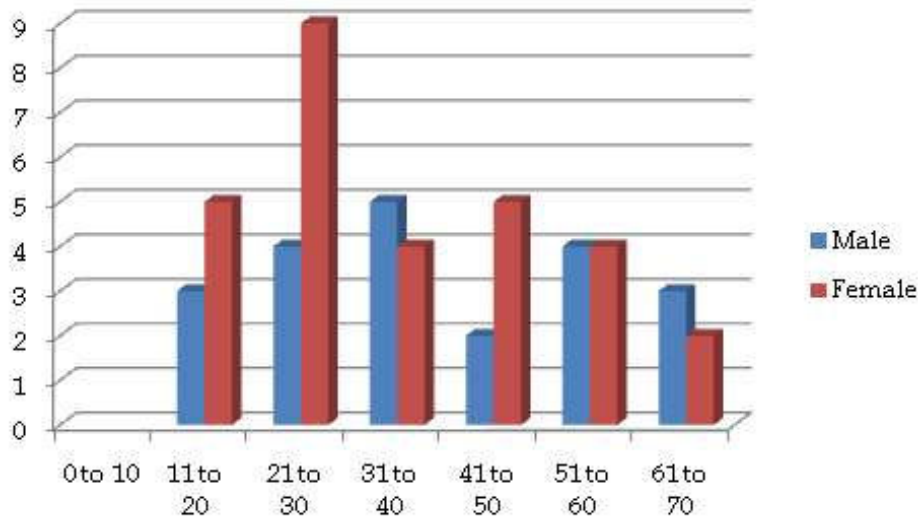


Fig. 1: Age and gender wise distribution of Serum B₁₂ value.

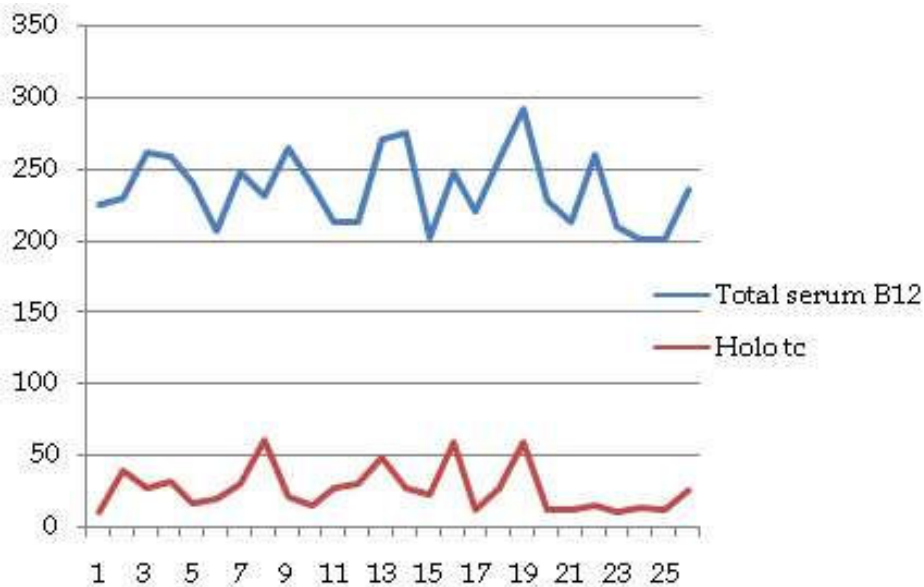


Fig. 2: Group 1-Serum B₁₂ level in 200-300 range with decreased Holo-TC values

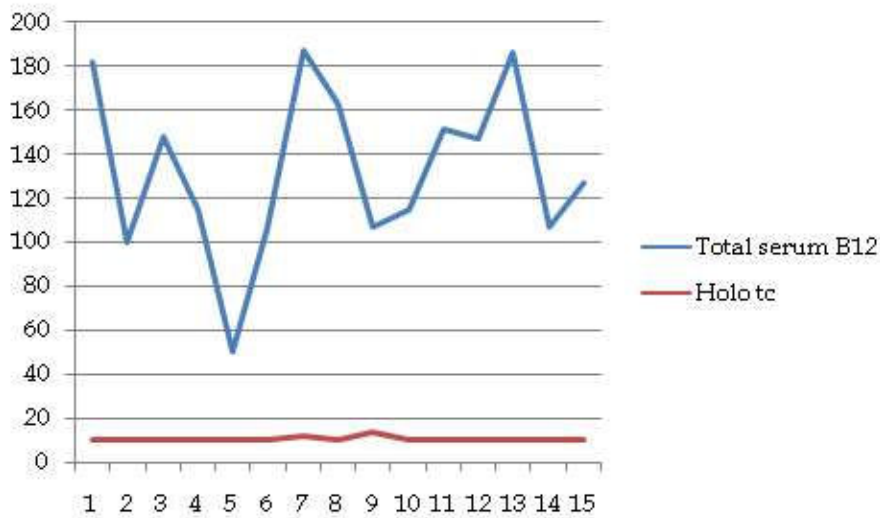


Fig. 3: Group 2-Serum B₁₂ level in 200 with decreased Holo-TC values

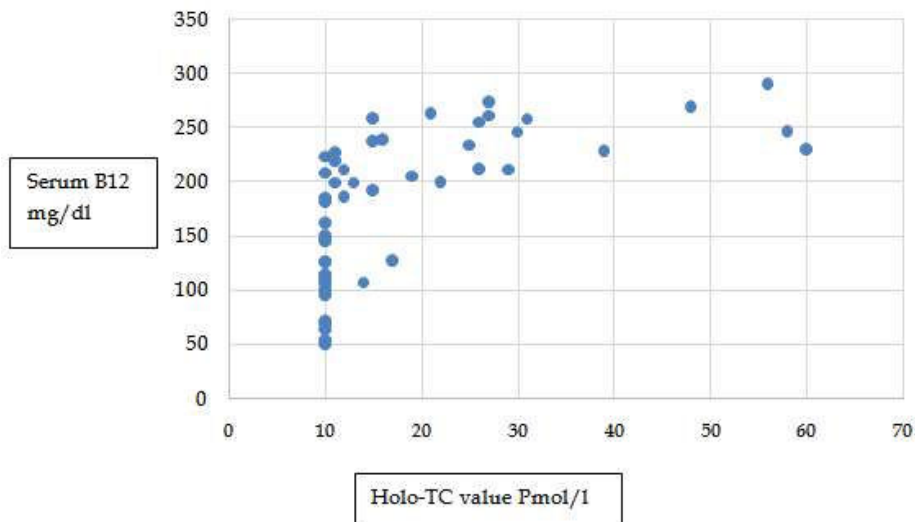


Fig. 4: shows correlation between Holo-TC and Serum Vitamin B₁₂

Discussion

Vitamin B₁₂(cyanocobalamine) is a water soluble hematopoietic vitamin that is required for the maturation of erythrocytes.

Vitamin B₁₂ deficiency is still an important nutritional problem worldwide as subclinical deficiency primarily affects the hematopoietic system, but its effects extend to other tissues and organs, most notably the nervous system, B₁₂ dependent processes and gastrointestinal mucosa.

Availability of B₁₂ is very scarce in vegetarian diets. Among population at risk are older people,

vegetarians, pregnant women and patients with renal and intestinal diseases.

Impaired maternal B₁₂ status in pregnancy is associated with poor maternal and child health outcomes.

Deficiency of B₁₂ in older people may lead to neurological symptoms like confusion, depression, memory loss and balance problems (gait ataxia) which are irreversible even after treatment with vitamin B₁₂. By the time clinical symptoms appear the subjects are already severely deficient. Megaloblastic anemia can be reversed by replenishment of vitamin B₁₂ but neurological

symptoms are frequently irreversible even with replenishment of vitamin B₁₂.

Historically the first widely used clinical assay for cobalamin deficiency was microbiologic assay. This assay utilized strains of *Lactobacillus leichmannii* or *Euglena gracilis* that depended on exogenously added cobalamin for growth. Bacterial growth can be affected by a number of interferences such as antibiotics. Subsequently a radio dilution assay was developed. Another cobalamin-related test is the Schilling test.

Total serum vitamin B₁₂ is a late biomarker of vitamin B₁₂ deficiency.

Holo-TC is a new marker which declines even before vitamin B₁₂ deficiency becomes apparent clinically or biochemically thus showing considerable potential for early diagnosis of vitamin B₁₂ deficiency.

We selected two groups of 50 cases for Holo-TC estimation who fulfilled the following criteria;

Group (1): Hemoglobin above 11.0 gm %, MCV between 85-96 fl and serum B₁₂ in the range of 200-300 pmol/L.

Group (2): Hemoglobin less than 11.0 gm % and serum B₁₂ less than 200 pmol/L.

PBF of all cases was examined for evidence of hyper segmentation.

Cases having history of any disease were not included in the study.

Out of 50 cases, 29 cases were females and 21 cases were males.

Age of the cases varied from 14 years to 65 years. Maximum numbers of patients were seen between the age group of 20 to 30 years In our study females had increased incidence of B₁₂ deficiency than males, age ranged from 11 to 70 years with common in age group of 20-40 years.

Holo-TC has been measured by ELISA or RIA in previous studies. In this study, we measured Holo-TC using a recently developed automated immunoassay method.

If a positive correlation between low Holo-TC & borderline deficiency of vitamin B₁₂ can be demonstrated; patients can be identified and treated even before overt deficiency occurs preventing progression to overt clinical disease.

There is a need for continuous awareness program for physicians, dietitians, and general population to identify risk factors, and to implement guidelines for prevention, early detection and

treatment of hidden vitamin B₁₂ deficiency. The relative cost of Holo-TC and limited availability may be a barrier for its use.

Conclusion

Vitamin B₁₂ deficiency is still an important nutritional problem worldwide as subclinical deficiency primarily affects the hematopoietic system and its effects extend to other tissues and organs like nervous system, B₁₂ dependent processes and gastrointestinal mucosa.

More than 20 years ago looking to physiology of vitamin B₁₂ deficiency led to the suggestion that Holo-TC might be a sensitive marker of early vitamin B₁₂ deficiency.

Since then Holo-TC measurement has come to the present position. Today we can conclude that Holo-TC seems more suitable marker than only estimation of total serum B₁₂ for early diagnosis of vitamin B₁₂ deficiency.

Data present in this study show correlation between serum vitamin B₁₂ and Holo-TC results.

In the majority of cases significant correlation was observed between the two indices. We predict that Holo-TC is an excellent marker for early diagnosis of vitamin B₁₂ status.

In a few cases Holo-TC appeared to be a better reflector of vitamin B₁₂ status.

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Key Message

Subclinical vitamin B₁₂ deficiency is difficult to diagnose and Holotranscobalamin (Holo-TC) being new parameter in early diagnosis of vitamin B₁₂ deficiency may be useful to take therapeutic measures before irreversible neurological damage occurs.

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