

## Holotranscobalamin : A Newer Parameter in Diagnosis of Vitamin B<sub>12</sub> Deficiency States

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### Abstract

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*Background and objectives:* Vitamin B<sub>12</sub> is an essential nutrient that plays crucial roles in the human body. B<sub>12</sub> 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 B<sub>12</sub> deficiency are non specific and can be irreversible. Holotranscobalamin (Holo-TC) also called as active B<sub>12</sub> has been suggested as sensitive marker for early diagnosis of B<sub>12</sub> deficiency; therefore measurement of Holo-TC may be useful for taking therapeutic measures before neurological damage of vitamin B<sub>12</sub> deficiency develops. *Methods:* We tested serum samples of 50 cases for hemoglobin levels, MCV and total vitamin B<sub>12</sub> 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 B<sub>12</sub> in the range of 200-300 pmol/L. *Group:* 2) Hemoglobin less than 11.0 gm % and serum B<sub>12</sub> 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 B<sub>12</sub> level in normal range but Holo-TC value of these cases was found decreased suggestive of early vitamin B<sub>12</sub> deficiency. Group 2 cases were having a low value of serum B<sub>12</sub> with markedly low value of Holo-TC suggesting that value of Holo-TC decreases with decrease in serum B<sub>12</sub> levels. *Conclusions:* Data present in this study show correlation between Serum Vitamin B<sub>12</sub> 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 B<sub>12</sub> deficiency.

**Keywords:** Hemoglobin; MCV; Vitamin B<sub>12</sub>; Holo-TC.

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## Introduction

Vitamin B<sub>12</sub> also known as cyanocobalamin is a water soluble hematopoietic vitamin that is required for the maturation of erythrocytes.

Vitamin B<sub>12</sub> 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 B<sub>12</sub> status in pregnancy is associated with poor maternal and child health outcomes [1].

Vitamin B<sub>12</sub> deficiency is wide spread and availability of B<sub>12</sub> 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 B<sub>12</sub> deficiency is still an important nutritional problem worldwide as subclinical deficiency affects well defined risk groups. Vitamin B<sub>12</sub> levels decreases with age which means that deficiency risk increases in parallel with age. Deficiency of B<sub>12</sub> 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 B<sub>12</sub> [4].

B<sub>12</sub> deficiency can affect bone metabolism and can stimulate osteoclasts [5].

Vitamin B<sub>12</sub> deficiency can also cause hyperhomocysteinaemia, which has been related to the risk of vascular and cerebral diseases.

High maternal folate and low maternal B<sub>12</sub> concentrations was associated with greater adiposity and insulin resistance in children and post-partum mothers.

Maternal B<sub>12</sub> 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 B<sub>12</sub> 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 B<sub>12</sub> deficiency in the general population is unclear and prevalence of

a sub-clinical functional vitamin B<sub>12</sub> deficiency in the general population is higher than previously expected.

Vitamin B<sub>12</sub> 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 B<sub>12</sub> 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 B<sub>12</sub> < 148 pmol/L (i.e. chronic B<sub>12</sub> deficiency) present with true megaloblastic anemia [9-10].

If left untreated vitamin B<sub>12</sub> 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 B<sub>12</sub> is quite common in the general population. The gold standard for the diagnosis of Vitamin B<sub>12</sub> deficiency is a low blood level of serum vitamin B<sub>12</sub> estimation.

Total serum vitamin B<sub>12</sub> may not reliably indicate vitamin B<sub>12</sub> status.

To get more specificity and sensitivity in diagnosing vitamin B<sub>12</sub> deficiency the concept of measuring Holo-TC a sub-fraction of vitamin B<sub>12</sub> 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 B<sub>12</sub> 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 B<sub>12</sub> with total vitamin B<sub>12</sub>.
2. To investigate clinical utility of the parameter and

- To establish analytical validity of the active B<sub>12</sub> assay.

Vitamin B<sub>12</sub> 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 B<sub>12</sub>, it would take between 2-10 years for deficiency to develop even when a diet is completely lacking the vitamin.

Vitamin B<sub>12</sub> has multiple binding proteins that facilitate its transport.

There are four known proteins involved in the absorption and transport of vitamin B<sub>12</sub>:

- R-protein (Transcobalamin I)
- Intrinsic factor
- Transcobalamin II
- 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 B<sub>12</sub> in the distal ileum.

While it has no known function vitamin B<sub>12</sub> bound to R-protein accounts for 80% of total plasma B<sub>12</sub> and may contribute to falsely high B<sub>12</sub> measurements.

Transcobalamin II also known as Holo-transcobalamin (Holo-TC) when bound to B<sub>12</sub> is found in plasma.

Holo-TC is the only biologically active form of B<sub>12</sub> and is responsible for the transport of B<sub>12</sub> to cell membrane receptors (part of receptor mediated endocytosis).

Holo-TC accounts for 6-25% of total plasma B<sub>12</sub> and is believed to be the most sensitive marker to depletion or repletion of B<sub>12</sub> [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 B<sub>12</sub>.

Several cut-points of vitamin B<sub>12</sub> have been suggested: 125 pmol/L, 148 pmol/L, 150 pmol/L, 184 pmol/L and 258 pmol/L.

A cut of point of B<sub>12</sub> 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 B<sub>12</sub> in the range of 200-300 pmol/L.

*Group: 2)* Hemoglobin less than 11.0 gm %, serum B<sub>12</sub> 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.

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 (B<sub>12</sub> 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 B<sub>12</sub> level in normal range but Holo- TC value of these cases was found decreased suggestive of early vitamin B<sub>12</sub> deficiency. (Fig. 2).

Group 2 included 24 cases having total serum B<sub>12</sub> 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 B<sub>12</sub> decreased value of Holo-TC also decreased markedly and is suggestive of B<sub>12</sub> deficiency. (Fig. 3).

It was observed that Group 2 cases were having a low value of serum B<sub>12</sub> with markedly low value of Holo-TC suggesting that value of Holo-TC decreases with decrease in serum B<sub>12</sub> levels.

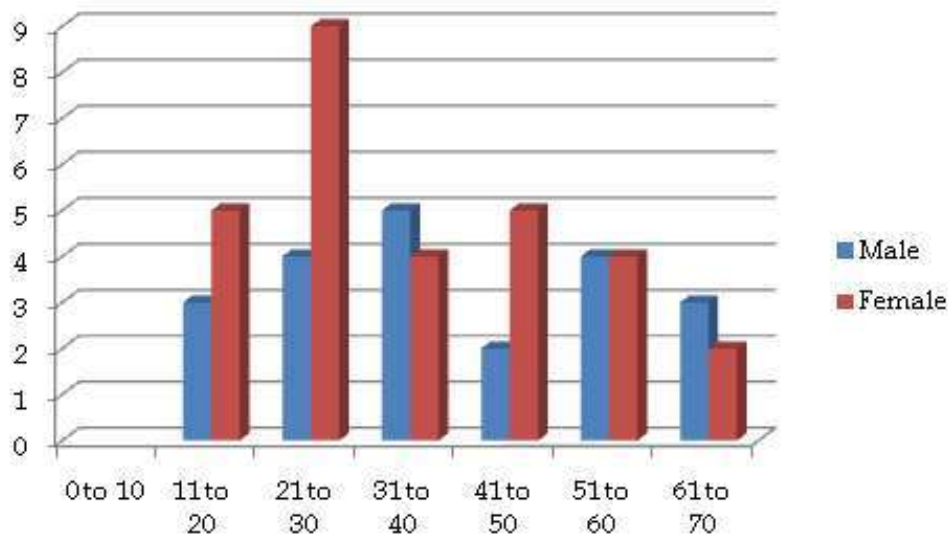


Fig. 1: Age and gender wise distribution of Serum B<sub>12</sub> value.

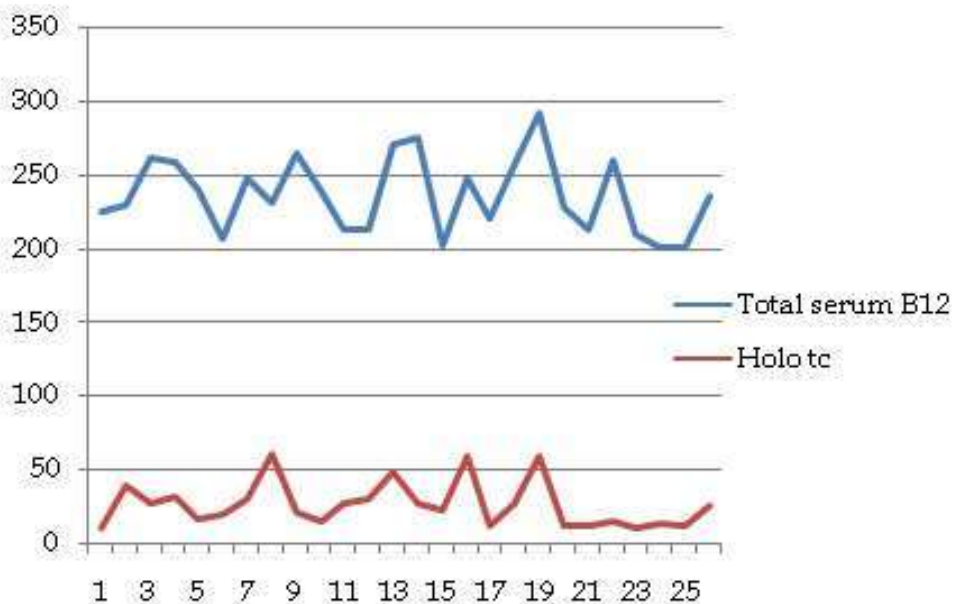


Fig. 2: Group 1-Serum B<sub>12</sub> level in 200-300 range with decreased Holo-TC values

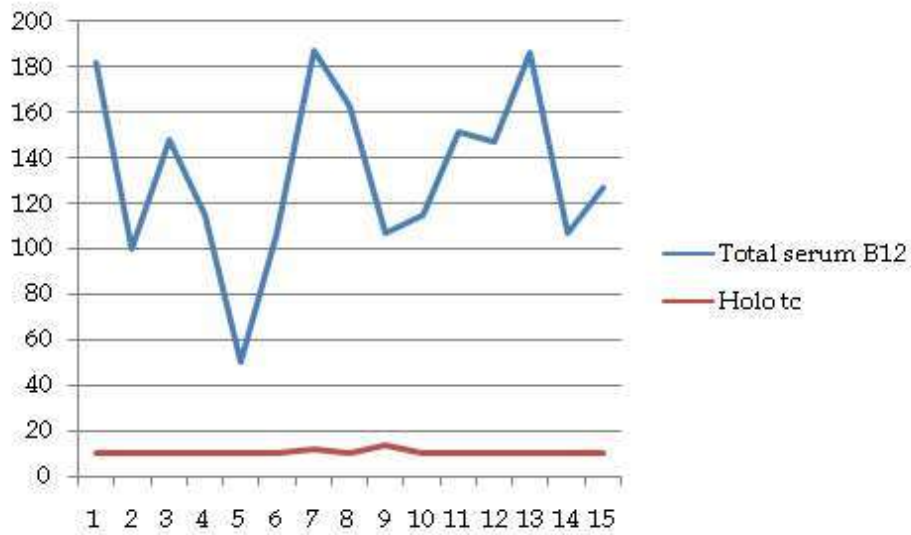


Fig. 3: Group 2-Serum B<sub>12</sub> level in 200 with decreased Holo-TC values

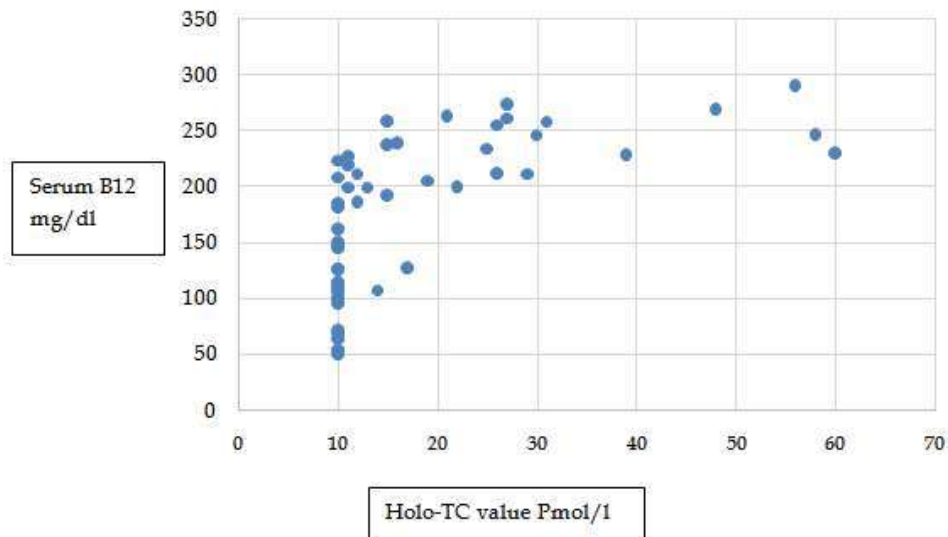


Fig. 4: shows correlation between Holo-TC and Serum Vitamin B<sub>12</sub>

**Discussion**

Vitamin B<sub>12</sub> (cyanocobalamin) is a water soluble hematopoietic vitamin that is required for the maturation of erythrocytes.

Vitamin B<sub>12</sub> 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, B<sub>12</sub> dependent processes and gastrointestinal mucosa.

Availability of B<sub>12</sub> 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 B<sub>12</sub> status in pregnancy is associated with poor maternal and child health outcomes.

Deficiency of B<sub>12</sub> 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 B<sub>12</sub>. By the time clinical symptoms appear the subjects are already severely deficient. Megaloblastic anemia can be reversed by replenishment of vitamin B<sub>12</sub> but neurological

symptoms are frequently irreversible even with replenishment of vitamin B<sub>12</sub>.

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 B<sub>12</sub> is a late biomarker of vitamin B<sub>12</sub> deficiency.

Holo-TC is a new marker which declines even before vitamin B<sub>12</sub> deficiency becomes apparent clinically or biochemically thus showing considerable potential for early diagnosis of vitamin B<sub>12</sub> 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 B<sub>12</sub> in the range of 200-300 pmol/L.

*Group (2):* Hemoglobin less than 11.0 gm % and serum B<sub>12</sub> 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 B<sub>12</sub> 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 B<sub>12</sub> 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 B<sub>12</sub> deficiency. The relative cost of Holo-TC and limited availability may be a barrier for its use.

## Conclusion

Vitamin B<sub>12</sub> 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, B<sub>12</sub> dependent processes and gastrointestinal mucosa.

More than 20 years ago looking to physiology of vitamin B<sub>12</sub> deficiency led to the suggestion that Holo-TC might be a sensitive marker of early vitamin B<sub>12</sub> 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 B<sub>12</sub> for early diagnosis of vitamin B<sub>12</sub> deficiency.

Data present in this study show correlation between serum vitamin B<sub>12</sub> 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 B<sub>12</sub> status.

In a few cases Holo-TC appeared to be a better reflector of vitamin B<sub>12</sub> status.

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## Key Message

Subclinical vitamin B<sub>12</sub> deficiency is difficult to diagnose and Holotranscobalamin (Holo-TC) being new parameter in early diagnosis of vitamin B<sub>12</sub> deficiency may be useful to take therapeutic measures before irreversible neurological damage occurs.

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