

## Holotranscobalamin (HoloTC) – Role of Holotranscobalamin In Early Diagnosis of Vitamin B12 Deficiency State.

Asha Purohit\*, Kalpen Patel\*\*, Prashant Jadav\*\*\*, Sneha Babaria\*\*\*\*, S.M.Patel\*\*\*\*\*

### Abstract :

**Introduction :** Vitamin B12 deficiency is widespread. Among the population, groups at risk are older people, vegetarians, pregnant women and renal patients. The neurological symptoms of vitamin B12 deficiency are non-specific and can be irreversible. Early detections are therefore important. The diagnostic use of HoloTC (active B12) allows the initiation of therapeutic measures before irreversible neurological damage develops. The present study was carried out, (i) to establish analytical validity of the active B12 assay, (ii) to investigate clinical utility of the parameter and (iii) to compare active B12 with total vitamin B12. **Material and Methods:** We tested 50 serum/plasma samples for active B12, total vitamin B12 assay, hemoglobin levels and mean corpuscular volume (MCV). **Results :** The HoloTC levels were lower than normal range in the subjects. A significant positive correlation was observed between HoloTC levels and total vitamin B12 levels. **Conclusion :** HoloTC measurement is a useful option for assessing vitamin B12 status, particularly in the subjects with borderline total vitamin B12 values and may be considered an early marker of vitamin B12 deficiency.

**Key Words :** Hemoglobin, Holo-TC, Mean corpuscular volume, Vitamin B12.

### Introduction :

Vitamin B12 or Cobalamin, a micronutrient supplied by meat and dairy products, is essential for mammalian intracellular metabolism, particularly metabolism of one-carbon groups and cell proliferation and differentiation. <sup>(1, 2)</sup> Vitamin B12 deficiency is wide spread and availability of B12 is very scarce in vegetarian diets. Among population at risk are older people. <sup>(3)</sup> Vegetarians, <sup>(4)</sup> pregnant women and patients with renal and intestinal diseases. Moreover, vitamin B12 deficiency can cause hyperhomocysteinaemia, which has been related to the risk of vascular and cerebral diseases. <sup>(5)</sup> 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. B12 deficiency can affect bone metabolism and can stimulate osteoclasts. <sup>(6)</sup> The prevalence of vitamin B12 deficiency in the general population is unclear.

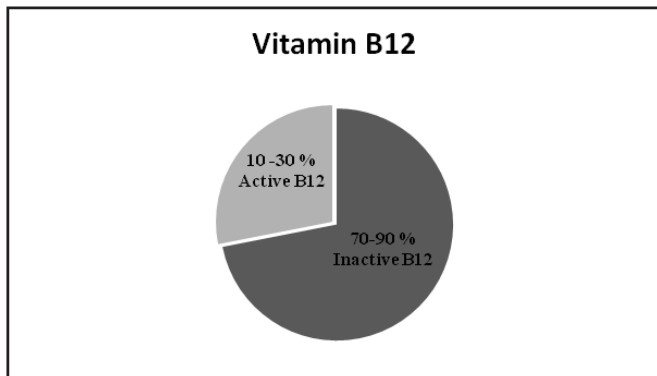
The greatest proportion of cobalamin in plasma is bound to heptocorrin and transcobalamin. Turnover of heptocorrin is very slow and function is also not known. Approximately, 20% of cobalamin is bound to transcobalamin which has rapid turnover and is biologically active fraction that can be delivered to all tissues of body. For this reason, holoTC is also referred to as active vitamin B12 (Figure: 1). However, serum total vitamin B12 (tB12) concentrations are a dubious marker of actual functional B12 status because measurement of total vitamin B12 serum levels might be misleading in alcoholics, and a tissue metabolic deficiency is possible even with normal serum cobalamin levels. In some cases it correlates poorly with hematological indices. <sup>(7)</sup> Therefore, measurement of Holo-TC (Active B12) has been suggested as a sensitive marker for early diagnosis of B12 deficiency. <sup>(8 - 13)</sup> Gujarat being a predominantly vegetarian state, clinical and subclinical deficiency of B12 is very common in the general population. The diagnostic use of Holo-TC, if proven, will allow the initiation of therapeutic measures before irreversible neurological damage develops because the clinical manifestations of vitamin B12 deficiency are nonspecific and people at risk should be identified and should be regularly monitored.

\* Associate Professor, \*\*Assistant Professor, \*\*\*\*Tutor, \*\*\*\*\*Professor & Head, Pathology Department, GCS Medical College, Ahmedabad, Gujarat, India

\*\*\* Assistant Professor, Biochemistry Department, GCS Medical College, Ahmedabad, Gujarat, India

Correspondence : ashapurohit54@gmail.com

**Figure 1: Percentage of active B12 in blood**



**Material and Methods :**

The study was approved by the Scientific Research Committee and Institutional Ethics Committee of the Gujarat Cancer Society (GCS) Medical College, Hospital & Research Centre, Ahmedabad. We selected 50 subjects retrospectively who were registered in various departments of a tertiary care level hospital who fulfilled the following criteria:

- Hemoglobin above 11.0 gm %
- MCV between 85-96 fl
- Serum B12 in the range of 200-300 mg/dl

Peripheral blood film of all subjects was examined for evidence of hyper segmentation.

History was taken from hospital records of all subjects and subjects having history of any disease were not included in the study. Out of total 50 cases, 32(64%) were females in age group of 26-80 years and 18(36%) were males in age group of 18-74 years. Blood samples 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 35.0 pmol/L cut-off threshold was chosen for Holo-TC for assessing cobalamin deficiency in the subjects<sup>(10)</sup> MCV and hemoglobin was measured by Sysmex KX-21 three part differential cell counter.

**Statistical analysis :**

Nonparametric statistical calculations were performed for this study. The equality of variance assumption was

verified using the Levene test. Correlations were evaluated using Spearman’s rank correlation coefficient. Differences of at least p<0.05 (2-sided) were considered statistically significant. Results are presented as median and range. Box and whisker diagram were plotted to show the median levels of various parameters. The ends of the vertical lines (whiskers) indicate the minimum and maximum data values. The symbols outside the ends of the whiskers indicate outliers (circles) and extreme observations (asterisks). (MedCalc, version 8.2.0.1, Belgium)

**Results :**

Amongst 50 subjects included in this randomized study, 32 (64%) were females and 18 (36%) were males. The median age of the subjects in this study was 45 years, ranging from 18 to 80 years. Out of 50 subjects, 34 had MCV in range of 85 to 90 fl and 16 subjects had MCV in range of 90 to 96 fl. Hemoglobin was ranging from 11 to 14.6 gm/dl. Median serum total vitamin B12 levels were in the normal range, though towards lower borderline. However, median Holo-TC levels were below the normal levels (Table-1, Figure 2 and 3).

**Table 1: Details of the subjects according to age, sex and laboratory parameters**

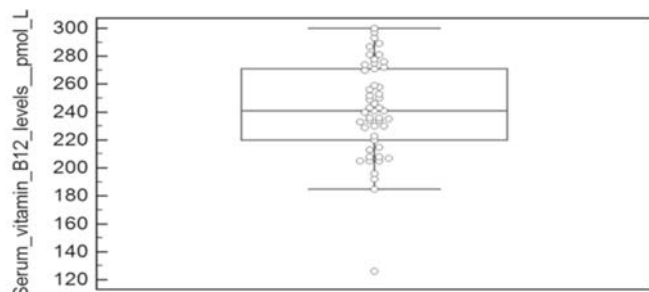
Characteristics of subjects	Number % 50 (100%)
Sex Male	18 (36.0%)
Female	32 (64.0%)
Age Male (median; range)	42.5 (20-80 years)
Female (median; range)	45.0 (18-74 years)
Hemoglobin (median; range)	12.45 gm% (11-14.6 gm%)
Serum vitamin B12 (median; range)	241pmol/L (200-300 pmol/L)
Holo TC (median; range)	25.21 pmol/L (10.3-50.5 pmol/L)*
MCV (median; range)	87.4 fl (84-96 fl)

\*normal value: >35pmol/L

We also compared holoTC levels, with the tests currently used for diagnosis of vitamin B12 deficiency, i.e., total vitamin B12, and hematological parameters. The frequency of low HoloTC value (<35 pmol/L) in

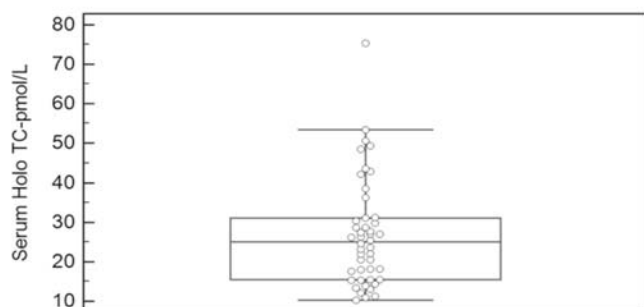
the subjects with low tB12 concentrations was 86% as compared to 14% in subjects with normal B12 levels.

**Figure 2: Box and whisker plot showing the median levels of serum vitamin B12 levels**



As shown in figure 2, the median value of serum vitamin B12 is indicated with a vertical line in the interior of the box, and the maximum and minimum levels are at the ends of the whiskers. The circles outside the ends of the whiskers indicate outlier. A significant positive correlation was found between serum total vitamin B12 and HoloTC levels ( $r = 0.424$ ,  $P = 0.003$ ) (Figure: 3). No correlation was found with level of hemoglobin or with RBC parameter (MCV) because patients with normal hemoglobin of 13 to 15.5 showed lower Holo-TC levels, reduction was not proportionate to increase in MCV.

**Figure 3 : Box and whisker plot showing the median levels of serum Holo TC levels**



As shown in figure 3, the median value of serum Holo TC is indicated with a vertical line in the interior of the box, and the maximum and minimum levels are at the ends of the whiskers. The circles outside the ends of the whiskers indicate outlier.

**Figure 4 : The correlation between serum Holo TC and vitamin B12 levels**

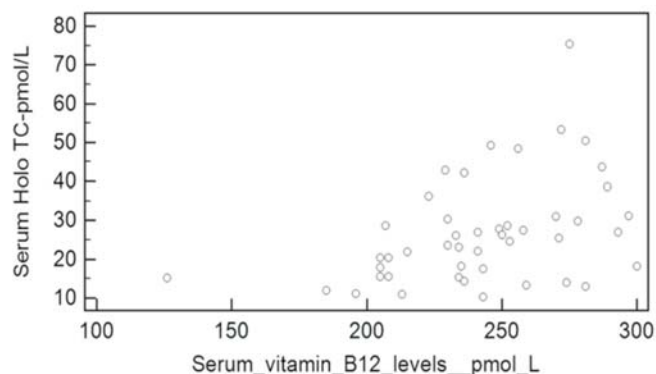


Figure 4 shows Scatter diagram showing correlation between serum Holo TC and vitamin B12 levels. The slope of the line is positive, showing positive correlation between the two sets of data ( $r = 0.424$ ,  $P = 0.003$ )

#### Discussion:

There is a concern about the feasibility of an early diagnosis of cobalamin deficiency in asymptomatic subjects, since the prevalence of sub-clinical functional cobalamin deficiency is higher than expected. The sequences of changes in developing B12 deficiency are

1. Early: low holoTC (Active B12)
2. Cellular: low serum B12, depletion of body stores
3. Metabolic: increased Homocysteine and MMA
4. Clinical: macrocytic anemia, neurological impairment

Measurement of serum B12 concentration, MCV with evidence of hyper segmentation has been the cornerstone for assessing suspected cases of vitamin B12 deficiency; however there are major limitations with this approach. Falsely increased cobalamin values are caused by myeloproliferative disorders, liver diseases, intestinal bacterial overgrowth, congenital TC II deficiency, nitrous oxide, and other specific clinical and laboratory circumstances.<sup>(14, 15)</sup> Increased MMA and homocysteine together can be found with primary metabolic defects, renal insufficiency and hypovolemia, while only homocysteine can increase in alcohol abuse, folate, and vitamin B6 deficiency. MMA is considered a sensitive marker of vitamin B12 deficiency, but the test has a limited availability. Furthermore, in the ambulatory care setting not only cobalamin, but also

MMA and homocysteine levels fluctuate with time and neither predicts nor precludes the presence of cobalamin-responsive hematologic or neurologic disorders. <sup>(16)</sup> HoloTC is the better index of B12 deficiency and is the earliest marker for vitamin B12

deficiency in populations at risk and in the elderly (Table: 2). Not all vitamin B12 in serum is active. 70-90% of vitamin B12 is biologically inert, around 20% of circulating B12 is carried on transcobalamin and is biologically active and is called as Active-B12 or holotranscobalamin (holoTC).

**Table 2 : Comparison of performance of various tests used to assess Vit. B12 deficiency <sup>(19)</sup>**

Laboratory Test	Rationale for Using the Test	Advantages & disadvantages
Cobalamin	Decrease in vitamin B12 deficiency	Easily accessible Sensitivity And specificity are questionable.
Methylmalonic acid(MMA)	Increases with vitamin B12 deficiency	High sensitivity Questionable specificity
Total homocysteine (Hcy)	Increases with vitamin B12 deficiency	High sensitivity Questionable specificity
Holo-TC	Decreases with B12 deficiency newer test	Early diagnosis high sensitivity specificity unclear.

In this study, we have evaluated the vitamin B12 status in 50 subjects by measuring not only total vitamin B12 concentrations, but also HoloTC levels, with the Axis Shield Diagnostics. In our study, median serum vitamin B12 levels and Holo TC levels were 241 pmol/L and 25.21 pmol/L, respectively. Interestingly, in our study technically a significant positive correlation, between vitamin B12 and Holo TC levels was found. A significant positive correlation was found between serum cobalamin and HoloTC levels, as reported by others using the same immunoenzymatic assay. <sup>(17, 18)</sup>

Our data, despite the small sample number, supports the assumption that HoloTC is an early marker for diagnosing B12 deficiency. This is a preliminary report; further confirmation needs to be clarified with more cases. Our findings suggest that in alcoholics caution is urged in the interpretation of these vitamin assays, particularly in the subjects with borderline-low cobalamin values. In this subset of patients, HoloTC measurement may be a useful option for assessing vitamin B12 status.

**Conclusion :**

About 20 years ago after understanding physiology of B12, it was thought that HoloTC is early marker of B12 deficiency; from that time Holo TC has come of age. On the basis of present study, we are also of view that Holo TC is early marker of B12 deficiency and can be

used as a marker for populations. However, its availability is limited because of its cost and limited availability.

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