ORIGINAL RESEARCH PAPER

Clinical Research

COMPARATIVE STUDY OF HOLOTRANSCOBALAMIN AND TOTAL VITAMIN B12 AS INDICATOR OF VITAMIN B12 STATUS

KEY WORDS: Cobalamin ,Vitamin B 12, HoloTC, HoloHC, Transcobalamin, Haptocorrin

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ABSTRACT

Vitamin B12 deficiency is common among the population and early detection is clinically important. HoloTC has been postulated to be the earliest marker of vitamin B12 deficiency. This study aims to evaluate the usefulness of holoTC compared to Total Vitamin B 12 in diagnosing B12 deficiency in patients investigated for Vitamin B12 deficiency. We randomly selected 65 patients visiting the outpatient department of KIMS Hospital Secunderabad and who had been referred to the Clinical Biochemistry Laboratory, for the assessment of vitamin B12 status, during September 2017. serum total vitamin B12 level was determined by the Electrochemiluminescence immunoassay (ECLIA) on cobas 6000 immunoassay analyzer. and holoTC (active vitamin B12) level was determined by micro particle enzyme immunoassay on Architect 2000i using Abbott kit Comparison of the data was performed to test the statistical relationship as reflected by the mean, median, standard deviation and correlation coefficient. The mean (SD) for serum holoTC and total vitamin B12 were 24.48(10.32) pmol/L and 131.46(41.76) pmol/L respectively. There was weak correlation between holoTC and total vitamin B12 (r= 0.443, P< 0.001) and the regression equation was expressed as; y = a + bx (i.e: holoTC = 11.08 + 0.1Total vitamin B12). holoTC and total vitamin B12, alone and in combination, have almost equal diagnostic efficiency in diagnosing vitamin B12 deficiency for the majority of patients. Even if there is weak correlation between the two majority of patients had agreement over either normal or low values of vitamin B12 as well as holoTC.

INTRODUCTION

Cobalamin (vitamin B12) is essential for 1-carbon metabolism and cell division. The clinical consequences of cobalamindeficiency include megaloblastic anemia and neurologic disease [1].

Vitamin B12 (cobalamin) is an important metalloenzyme which consists of a porphyrin-like macromolecule with a central cobalt atom that is bound to a nucleotide residue, the whole unit is called cobalamin. It is required as coenzymes for the metabolism of the amino acids methionine, threonine and valine, as well as for the transformation of methyl-tetrahydrofolate to tetrahydrofolate which is necessary for DNA synthesis [2].Plasma vitamin B12 is bound to 2 proteins, transcobalamin and haptocorrin. Transcobalamin carries a minor part of the circulating vitamin B-12, and only approximately 10% of the protein is saturated with vitamin B12. Transcobalamin transports vitamin B12 into all cells of the body and is responsible for the transport of about 4 nmol of vitamin B12 every day. Haptocorrin is an almost fully saturated vitamin B12 binding glycoprotein of unknown function that carries the major part of circulating vitamin B12 and in addition, the inactive forms of the vitamin, the so-called analogs. The metabolism of the protein is slow, with a turnover of approximately 0.1 nmol vitamin B12 every day[3]. Genetic absence of HC is rare but not a serious condition and is usually discovered accidentally. On the other hand, the genetic absence or an abnormality of TC manifests as the typical hematologic and neurologic pathologies of vitamin B12 deficiency[4].The shorter half life for holoTC compared to holoHC makes a decrease in holoTC as one of the earliest markers of cobalamindeficiency[5]. Measurement of total vitamin B12 suffers from some limitations, in particular, most of the cobalamin measured is that bound to HC [6]. HoloTC has been postulated to be the earliest marker of negative vitamin B12 balance, and in the last few years, reliable methods for estimating holoTC have become available [7].The objective of present study is to compare the results of serum holoTC and total vitamin B12 in patients investigated for vitamin B12 disorders and to determine the diagnostic value of holoTC levels.

MATERIALS & METHOD:

We randomly selected 65 patients visiting the outpatient department of KIMS Hospital Secunderabad and who had been referred to the Clinical Biochemistry Laboratory, for the assessment of vitamin B12 status, during September 2017.These patients included 30 females and 35 males with an age range of 14-70 years. Blood samples were collected and sera were separated. A separated serum was frozen at -70°until further testing. For each patient, serum total vitamin B12 level was determined by the Electrochemiluminescenceimmunoassay (ECLIA) on cobas6000 immunoassay analyzer and holoTC (active vitamin B12) level was determined by micro particle enzyme immunoassay on Architect 2000i using Abbott kit. Comparison of the data was performed to test the statistical relationship as reflected by the mean, median, standard deviation and correlation coefficient [8]. Further we evaluated the data concerning any disagreement of the results by comparing holoTC versus total vitamin B12 values, in terms of whether either or both values agree or disagree for classifying the patients as being normal (results within the reference range) or abnormal (results lower or higher than the reference range). The cut-off thresholds for the data were assessed using the kit's quoted reference range: Total vitamin B12-145-569 pmol/L and HoloTC 19.1 to 119.3 pmol/L.

RESULTS:

Comparison of the data was conducted to reflect the mean, standard deviation (SD) **(Table 1)** and correlation coefficient between the two groups**(figure 1)**. The mean (SD) for serum holoTC and total vitamin B12 were 24.48(10.32) pmol/L and 131.46(41.76) pmol/L respectively. There was weak correlation between holoTC and total vitamin B12 (r= 0.443, P< 0.001) and the regression equation was expressed as; y = a +bx (i.e: holoTC = 11.08 + 0.1Total vitamin B12).

Table 1: Serum HoloTC and Total Vitamin B12 in the Study Group

	Holo TC (pmol/L)	Total VitaminB12 (pmol/L)
MEAN	24.48	131.46
MEDIAN	23	133.06
SD	10.32	41.76

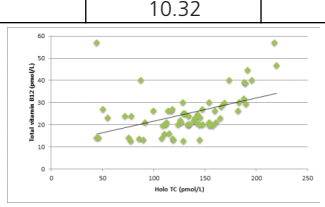

Figure 1: Relationship (Regression plot) between serum total vitamin B12 and holotranscobalamin (r= 0.443; y= 11.08 + 0.1X)

DISCUSSION:

In this study, comparable results with weak correlation (r= 0.443) were noted between holoTC and total vitamin B12. Using the kits' quoted reference range for Total vitamin B12 145-569 pmol/L and Holo TC 19.1-119.3pmol/L. In 70.03% of patients there was agreement in the classification of results, both being normal or abnormal (low or high). In 50.76% of cases, both results were within the reference ranges. However in 29.2% cases (19 patients) there was disagreement of results they had normal holoTC but low Total vitamin B12 whereas there was 1.5% cases (1 patient) that had low HoloTC and normal Total vitamin B12. However in 18.46% of cases, both results were lower than the reference ranges.

The correlation between HoloTC and total vitamin B12 concentrations varies widely between the previous studies. Similar results were found in previous study Bamonti *et al*.(2010) where they reported a weak correlation (r = 0.42). whereas other studies Al Aisari F, et al.(2010)and Golding (2016) reported strong correlation (r= 0.765) and (r^2=0.49) respectively [6,9,10]. On the other hand Woo KS et al (2010) stated significant correlation between holoTC and Total Vitamin B12, however in their study out of 7 samples that showed low holoTC levels, 6 showed normal serum vitamin B12 levels, and the serum vitamin B12 levels in 4 of those 6 were in the borderline range this finding is contrary to our finding where we had 19 (29.2%) samples with low Total vitamin B12 but holoTC was within the reference range similar findings were reported by Aisari FA, *et al*.(2010) where 6 (7.8.%) patients, holoTC was within reference range, however, total vitamin B12 was low[6,11]. The probable reason being when there is no short-term disruption to the enterohepatic cycle, HoloTC concentration will be maintained until there is insufficient holohaptocorrin remaining to sustain HoloTC production. There is a significant depletion period before the eventual failure of the enterohepatic cycle leads to the onset of Metabolic Deficiency. Where the cause of vitamin B12deficiency does not disrupt the enterohepatic cycle, HoloTC will not be an early responder to a deficiency. When the cause of vitamin B12 deficiency does disrupt the enterohepaticcycle, the HoloTC will be an early responder but there will be no depletion period; the metabolites will respond quickly when HoloTC falls below normal for the individual [12]. Therefore we cannot say that HoloTC will respond early to vitamin B12 depletion, before the onset of a clinical deficiency, it does not take into account how enterohepatic recycling regulates the HoloTC concentration and hence both Vitamin B12 and HoloTC both are equally efficient for diagnosis of Vitamin B12 Deficiency.

Our study had several limitations. Small sample number, possible ethnic differences and selection bias. In addition, the serum holoTC levels can be affected by several factors. Food intake, amount of absorbed vitamin B12, renal and hepatic function, and other disease conditions can influence the concentration of holoTCas well as vitamin B12 status [11]. Clinical data, including the symptoms and medical history and the data for other parameters of vitamin B12 status should also be determined for evaluating the diagnostic value of Architect holoTC assay .

CONCLUSION:

Our study concludes that holo TC and Total Vitamin B12 either individually or in combination have almost equal diagnostic role in vitamin B12 deficiency disorders. Even if a weak correlation was reported between the two indices it does not necessarily mean that HoloTC is a more sensitive marker. There was a disagreement in a minority of patients who had low total vitamin B12 but low-normal holoTC levels. Further studies with large sample size should be done to clarify the effectiveness of holoTC in the clinical setting.

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