Original Article

A study of holotranscobalamine as sensitive marker in the diagnosis of megaloblastic anemia

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Abstract

Background: After introduction of the metabolic tests for plasma methylmalonic acid (MMA) and plasma total homocysteine (Hcy), attention has been drawn to vitamin B12 status in asymptomatic individuals. **Aims and Objectives:** To study holo trans cobalamine as sensitive marker in the diagnosis of megaloblastic anemia. **Methodology:** After approval from institutionalethical committee this study was carried out at tertiary health care hospital in the 50 patients during January 2016 to December 2016 with clinical features of Megaloblastic anemia The sensitivity, Specificity, Positive Predictive and Negative Predictive Value etc. of HoloTC test with respect to Serum cobalamin test was calculated by Med Cal software. **Results:** The majority of the Patients were from the age group of 1-10 i.e. 6% followed by 10 -20 - 10 %; in 20-30 was 8%; in 30-40 was 14 %; in 40-50 was 18% and in 50-60 Was 20 % and >60 was 24 %. The majority of the patients were Females i.e. 54% followed by Males i.e. 46. Sensitivity of the Holo TC test is 93.33% with 95 % CI (81.73% to 98.60%) Specificity was 80.00 % (28.36% to 99.49%) and Positive Likelihood Ratio was 4.67 (0.81 to 26.98) and Negative likely hood ratio was 0.08 (0.03 to 0.27) and Positive Predictive Value was 97.67% (87.71% to 99.94%) and Negative Predictive Value was 57.14 % (18.41% to 90.10%). **Conclusion:** From our study it can be concluded that holotranscobalamine is a sensitive marker with sensitivity of the test 93.33% and Specificity was 80.00 % so this test can be used as screening test us in conjugation with other test like Sr. Cobalamin, plasmamethylmalonic acid (MMA) and plasma total homocysteine (Hcy) etc. for accurate diagnosis of Megaloblastic anemia (Vit B12 Deficiency).
Keywords: HoloTC (Holo Trans Cobalamin), Megaloblastic anemia **(**Vit. B12 Deficiency), Sr. Cobalamin.

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INTRODUCTION

After introduction of the metabolic tests for plasma methylmalonic acid (MMA) and plasma total homocysteine (Hcy), attention has been drawn to vitamin B12 status in asymptomatic individuals[1]. Based on MMA and Hcy tests, vitamin B12 deficiency is common, especially among the elderly, with prevalence estimates of 10–20%[2–4]. However, the metabolic tests have some disadvantages, as Hcy is non-specific[5] and determination of MMA is quite complex and expensive. The diagnostic reliability of the third marker, plasma cobalamin, has been discussed for several years[6–8]. Thus an optimal test for diagnosing vitamin B12 before the onset of progressive neurological and psychiatric complications is still in demand. Vitamin B12 in plasma is bound to two proteins, transcobalamin (TC) and haptocorrin[9]. Holotranscobalamin (holo TC) contains the biologically available vitamin B12 because only TC promotes the uptake of vitamin B12 by all cells, and several authors have suggested holo TC to be an early marker of vitamin B12 deficiency.[10–14]

MATERIAL AND METHODS

After approval from institutional ethical committee this study was carried out at tertiary health care hospital in the 50 patients during January 2016 to December 2016 with clinical features of Megaloblastic anemia; Here the

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Megaloblastic anemia patients (Vitamin B 12 Deficiency patients) considered in whom the Serum cobalamin of less than 200 ng/L (148 pmol/L) and More than > 5 % Neutrophils with > 5 lobes and Oval macrocytes on Peripheral Blood Smear (PBS +ve) and for the a cut-off of 32pmol/L of Holo TC for screening for cobalamin deficiency both these levels were assessed by immune assay[15]. Written and explained consent to participate into study was done all necessary details like age and sex and demographic characteristics was taken. The sensitivity, Specificity, Positive Predictive and Negative Predictive Value etc. of Holo TC test with respect to Serum cobalamin test was calculated by Med Cal software[16].

RESULTS

Table 1: Age wise Distribution of the Patients

Age group	No.	Percentage (%)
1-10	3	6
10 -20	5	10
20-30	4	8
30-40	7	14
40-50	9	18
50-60	10	20
>60	12	24
Total	**50**	**100**

The majority of the Patients were from the age group of 1-10 i.e. 6% followed by 10 -20 -10 %; in 20-30 was 8%; in 30-40 was 14 %; in 40-50 was 18% and in 50-60 Was 20 % and >60 was 24 %.

Table 2: Sex- wise distribution of the Patients

Sex	No.	Percentage (%)
Male	23	46
Female	27	54
Total	**50**	**100**

The majority of the patients were Females i.e. 54% followed by Males i.e. 46.

Table 3: Distribution of the Megaloblastic anemia patients with respect to HoloTCand Sr. Cobalamin

HoloTC< 32pmol/L	Sr. Cobalamin < 148 pmol/L with PBS +ve		Total
	Present (Megaloblastic anemia)	Absent (No Megaloblastic anemia)	
Yes	42	1	43
No	3	4	7
Total	**43**	**5**	**50**

From above table Sensitivity of the Holo TC test is 93.33% with 95 % CI (81.73% to 98.60%) Specificity was 80.00% (28.36% to 99.49%) and Positive Likelihood Ratio was 4.67 (0.81 to 26.98) and Negative likely hood ratio was 0.08 (0.03 to 0.27) and Positive Predictive Value was 97.67% (87.71% to 99.94%) and Negative Predictive Value was 57.14 % (18.41% to 90.10%).

DISCUSSION

In patients with classical megaloblastic anaemia, the presence of a low serum cobalamin level and objective assessment of response in terms of the rise in haemoglobin concentration clearly outlines the treatment pathway. However, the majority of patients do not have such a clear cut picture. Neurological presentation (peripheral neuropathy, sub-acute combined degeneration of the cord) may occur in the absence of haematological changes, and early treatment is essential to avoid permanent neurological disability. Low cobalamin levels of uncertain significance may occur with non-specific symptoms and no anaemia. Furthermore, patients with strong clinical features of cobalamin deficiency may have serum cobalamin levels which lie within the reference range (false normal cobalamin level). As a result, other tests may be used to try and determine an underlying functional or biochemical deficiency [reviewed in (Quadros 2010)[17]. These tests, plasma homocysteine, plasma methylmalonic acid, and serum holotranscobalamin may help but are not widely available currently and the cut-off points to indicate deficiency vary between different laboratories (Carmel 2011)[18]. In addition, their role is not clearly defined in the routine diagnostic setting. Therefore, there is currently no 'gold standard' test for the diagnosis of cobalamin deficiency (Herrmann and Obeid 2012)[19]. A serum cobalamin assay is currently the standard initial routine diagnostic test. It quantitates both the inactive forms (transcobalamin I- and transcobalamin III- bound, now referred to as holohaptocorrin) and the active form (transcobalamin II-bound, now referred to as holotranscobalamin) of cobalamin in serum. It is widely available, low cost, automated and based on intrinsic factor binding of cobalamin and immunechemiluminescence based assays. It is not entirely clear what should be regarded as a clinically normal serum cobalamin level, although it has been proposed that a serum cobalamin of less than 200 ng/L (148 pmol/L) would have a sensitivity of diagnosing 97% of true cobalamin deficiency (Carmel and Sarrai 2006, Snow 1999)[18]. Holotranscobalamin (HoloTC), the 'active' fraction of plasma cobalamin, may be more specific than serum cobalamin levels, and an immunoassay for this fraction is now available. In clinical research studies, the HoloTC assay performs better than serum cobalamin assay in assessing deficiency based on MMA levels(Miller, *et al* 2006)[20] and red cell cobalamin levels (Valente, *et al* 2011)[22] as reference assaysA recent multicentre study suggested a cut-off of 32pmol/L of HoloTC for screening for cobalamin deficiency based on MMA level of greater than 0.45 µmol/L. In our study we found that Sensitivity of the Holo TC test is 93.33% with 95 % CI (81.73% to 98.60%) Specificity was 80.00 %

(28.36% to 99.49%) and Positive Likelihood Ratio was 4.67 (0.81 to 26.98) and Negative likely hood ratio was 0.08 (0.03 to 0.27) and Positive Predictive Value was 97.67% (87.71% to 99.94%) and Negative Predictive Value was 57.14 % (18.41% to 90.10%). These findings are similar to Edward Valente et[22] al who found that Serum holoTC was the best predictor, with area under the ROC curve (95%CI) 0.90 (0.86 –0.93), and this was significantly better (P < 0.0002) than the next best predictors; serum cobalamin, 0.80 (0.75–0.85), and MMA, 0.78 (0.72–0.83).

CONCLUSION

From our study it can be concluded that holotranscobalamine is a sensitive marker with sensitivity of the test 93.33% and Specificity was 80.00 % so this test can be used as screening test our in conjugation with other test like Sr. Cobalamin, plasma methylmalonic acid (MMA) and plasma total homocysteine (Hcy) etc. for accurate diagnosis of Megaloblastic anemia (Vit B12 Deficiency)

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