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Research Article

REASSESSING THE ROLE OF HOMOCYSTEINE AND HOLOTRANSCOBALAMIN LEVELS IN DIAGNOSING VITAMIN B12 DEFICIENCY ANEMIA

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ABSTRACT

Objective: The objective of the study was to assess homocysteine (Hcy) and holotranscobalamine (HoloTC) levels among patients with Vitamin B12 deficiency and to see if Hcy and HoloTC level assay can help us in diagnosis of Vitamin B12 deficiency.

Methods: We carried out a cross-sectional observational study on 60 patients of Sr. B12 deficient male and female patients between the ages of 18 and 65 years in IPD and OPD patients at multispecialty hospital. Data were collected from predefined pro forma and were asked about their diet, socioeconomic status, and history. Then, these patients further undergone anthropometric measurements and investigated for Hcy and HoloTC level. The statistical analysis was done using Statistical Package for the Social Sciences (SSPS) software (version 11).

Results: About 60% of cases were vegetarian and 40% of cases were non-vegetarian. Mean age of study participants was 43.67 years, mean of mean cellular volume was 90.7 fl, mean of B12 was 138 pmol/L, mean of HoloTC was 60.84 pmol/L, and mean of Hcy was 34.17 umol/L. Out of 60 patients, 10 patients had anemia, 21 patients had neurological manifestation, and 29 patients had gastrointestinal (GI) manifestation. In male group, out of 32, 11 patients had HoloTC <8.9, 19 patients had value between 8.9 and 128, and two patients had HoloTC more than 128. In female group, out of 28, seven patients had HoloTC <8.9, 14 patients had value between 8.9 and 128, and seven patients had HoloTC >128. In group of 32 male patients, none of male patients showed Hcy value <5.9, four patients showed Hcy between 5.9 and 16, and 28 patients showed Hcy value >16. Out of 28 female patients, none of female patients showed Hcy <3.36, nine patients showed Hcy between 3.36 and 20.4, and 19 patients showed Hcy >20.4. p <0.001 is highly statistically significant.

Conclusion: In our study, we found that 31.33% of cases also showed decreased HoloTC along with B12 deficiency, but this correlation was statistically insignificant. We also found that 78.33% of cases showed increased Hcy along with serum B12 deficiency, which was statistically significant, so we concluded that there is a strong association between serum B12 and Hcy. We found that all patients with elevated Hcy also had low HoloTC except in two cases, but this correlation was not found to be statistically significant.

Keywords: Homocysteine, Holotranscobalamin, Vitamin B12.

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INTRODUCTION

There are many patients with Vitamin B12 deficiency where it is difficult to pinpoint the etiology of Vitamin B12 deficiency. Most of the times, the etiology of B12 deficiency is multifactorial and the diagnosis is challenging in many patients. The diagnosis depends on many investigations and at times even after an exhaustive series of investigations, we fail to detect the exact cause of Vitamin B12 deficiency. Vitamin B12 is essential for the synthesis of S-adenosyl methionine and is involved in the metabolism of proteins, phospholipids, and neurotransmitters. Its deficiency leads to several neurological manifestations and affects all age groups [1]. Its deficiency can take 3–6 years to develop after absorption of dietary B12 had ceased and the initial clinical manifestations are subtle and non-specific or are attributed to the normal aging process. This problem was also underestimated in the past because of the belief that deficiency is unlikely except in strict vegetarian and pernicious anemia patients are known to be cobalamin deficient and that it usually takes about 20 years for stores of the vitamin to become depleted. Since a deficiency in this vitamin can lead to irreversible neurological damage [2] (subacute combined degeneration of the spinal cord and cognitive impairment in the elderly), megaloblastic anemia, osteoporosis [3], cerebrovascular, and cardiovascular diseases [4], early diagnosis is essential. In recent years, new and sensitive diagnostic markers to determine a person's cobalamin status have become available.

Even though the human body can store Vitamin B12 to last for up to 5 years, its deficiency is not very uncommon. The diagnosis is frequently made based on a low serum Vitamin B12 level or megaloblastic bone marrow or both [5]. Vitamin B12, apart from causing neuropsychiatric symptoms, leads to hyperhomocysteinemia and methylmalonic acidemia which can have serious health implications. Low serum Vitamin B12 levels have low sensitivity and specificity in terms of tissue deficiency [6]. Homocysteine (Hcy) and methylmalonic acid estimations are adjunct and aid in diagnosis of B12 deficiency but still serum Vitamin B12 measurement is the extensively applied standard method by practical purposes. To obtain a more sensitive marker of Vitamin B12 status, a new test involving measurement of the levels of holotranscobalamine (HoloTC)-transcobalamin-Vitamin B12 complex has been introduced [7]. HoloTC promotes global cellular uptake of cobalamin by specific receptors; therefore, it may be more sensitive than serum Vitamin B12 levels in indicating Vitamin B12 status [8].

The racial, religious, ethnic, and socioeconomic heterogeneity of the people in India greatly influences their dietary habits. This study was carried out to assess Hcy and HoloTC levels among patients with Vitamin B12 deficiency and to see if Hcy and HoloTC level assay can help us in diagnosis of Vitamin B12 deficiency.

METHODS

We carried out a cross-sectional observational study among inpatients and outpatients of tertiary care center for a period of 6 months. Age group of the study population was between 18 and 65 years. We selected a convenient sample of 95 patients with B12 deficiency, randomly from laboratory of patients with B12 less than 200 pg/dl and after retrogradely seeing patients, we excluded patients with renal, hepatic, and heart diseases due to possibility of falsely elevated Hcy level. Out of 95, a total of 60 patients were included in the study. Data were collected from predefined pro forma and were asked about their diet, socioeconomic status, and history. Then, these patients further undergone anthropometric measurements and investigated for Hcy and HoloTC level. B12 was estimated by the electrochemiluminescence immunoassay "ECLIA." Holotranscobalamin assay was a precision test for the AxSYM microparticle enzyme immunoassay (MEIA) technology. Informed consent was taken, and identity of the patients was kept confidential. The statistical analysis was done using Statistical Package for the Social Sciences (SSPS) software (version 11). Chi-square was used to test the significance between groups and Pearson's correlation coefficient was used, to test the correlation between different variables with $p<0.05$ which was accepted as statistically significant. The test results were tabulated and graphically represented using Microsoft Word 2007.

RESULTS

This study was conducted on 60 patients of Sr. B12 deficient male and female patients between the ages of 18 and 65 years in IPD and OPD patients at multispecialty hospital. About 60% of cases were vegetarian and 40% of cases were non-vegetarian. Mean age of the study participants was 43.67 years, mean of mean cellular volume (MCV) was 90.7 fl, mean of B12 was 138 pmol/L, mean of HoloTC was 60.84 pmol/L, and mean of Hcy was 34.17 umol/L (Table 1). Out of 60 patients, 10 patients had anemia, 21 patients had neurological manifestation, and 29 patients had gastrointestinal (GI) manifestation (Figs. 1 and 2). It showed that out of 32 male patients, 28 patients had normal MCV and four patients had MCV more than 100. Out of 28 female patients, 25 patients showed normal MCV and two patients showed MCV more than 100. In male group, out of 32, 11 patients had HoloTC <8.9, 19 patients had value between 8.9 and 128, and two patients had HoloTC more than 128. In female group, out of 28, seven patients had HoloTC <8.9, 14 patients had value between 8.9 and 128, and seven patients had HoloTC >128. In group of 32 male patients, none of male patients showed Hcy value <5.9, four patients showed Hcy between 5.9 and 16, and 28 patients showed Hcy value >16 (Table 2). Out of 28 female patients, none of female patients showed Hcy <3.36, nine patients showed Hcy between 3.36 and 20.4, and 19 patients showed Hcy >20.4. $p<0.001$ is highly statistically significant. Table 3 shows relation of MCV, HoloTC, and Hcy with clinical manifestations among male and female patients. Table 4 shows distribution of different laboratory values among different age groups of study participants.

In our study, we found that 31.33% of cases also showed decreased HoloTC along with B12 deficiency, but this correlation was statistically insignificant. We also found that 78.33% of cases showed increased Hcy along with serum B12 deficiency, which was statistically significant, so we concluded that there is a strong association between serum B12 and Hcy. We found that all patients with elevated Hcy also had low HoloTC except in two cases, but this correlation was not found to be statistically significant.

DISCUSSION

Deficiency of Vitamin B12 is very common because of inadequate dietary intake and/or malabsorption. The deficiency state has a very wide presentation and can cause or exacerbate neuropsychiatric and other vague symptoms. It has been observed that Vitamin B12 deficiency is far more prevalent than expected and majority of the cases remain undiagnosed. In early stage, Vitamin B12 deficiency might present with subtle and slight cognitive impairments. Hence, early recognition becomes crucial for preventing irreversible damage.

Dietary Vitamin B12 deficiency is a severe problem in the Indian subcontinent [9] as seen in this study. The mean Vitamin B12 level was observed to be 138 pmol/L which itself was on a lower side. The findings from several studies performed in different clinical settings have confirmed that serum Vitamin B12 is a relatively poor marker with low sensitivity and specificity in predicting Vitamin B12 status and that HoloTC is a useful diagnostic indicator for this purpose [7]. In addition, a few studies performed in different countries and ethnic groups have shown differences in the prevalence of Vitamin B12

Table 1: Demographic data and laboratory results of study participants (*n*=60)

Variables	Patients
Age (years)	Mean 43.67
Sex (male/female)	32/28
Results	**Mean values**
Hemoglobin (g/dl)	12.705
MCV	90.7 fl
Vitamin B12 (pmol/L)	138
HoloTC (pmol/L)	60.84
Hcy (umol/L)	34.17
Laboratory parameter	**Number of patients with low S. B12 (*n*=60)**
HoloTC	19
Hcy	47

MCV: Mean corpuscular volume, HoloTC: Holotranscobalamine, Hcy: Homocysteine

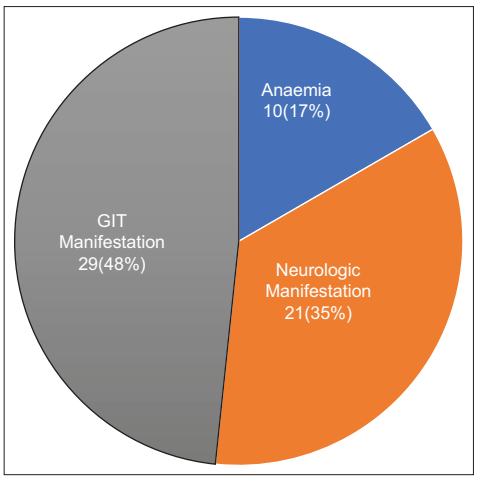

Fig. 1: Distribution of clinical presentation of study participants

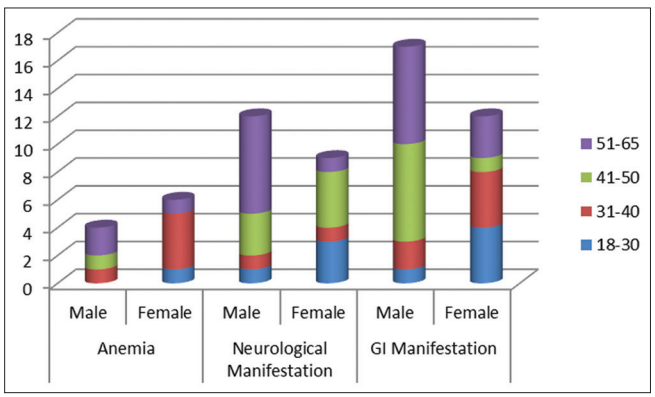

Fig. 2: Distribution of clinical presentation and age and sex of study participants

Table 2: Distribution of mean corpuscular volume, holotranscobalamine, and homocysteine levels in male and female patients

Laboratory values	Male (*n*=32)			Female (*n*=28)			*P*
	<80	80–100	>100	<80	80–100	>100	
MCV	0	28	4	1	25	2	>0.05
Percentage	0	46.67	6.67	1.67	41.67	3.33	
Laboratory values	**Male (*n*=32)**			**Female (*n*=28)**			***P***
	<8.9	**8.9–128**	**>128**	**<8.9**	**8.9–128**	**>128**	
HoloTC	11	19	2	7	14	7	>0.05
Percentage	18.33	31.67	3.33	11.67	23.33	11.67	
Laboratory values	**Male (*n*=32)**			**Female (*n*=28)**			***P***
	<5.9	**5.9–16**	**>16**	**<3.36**	**3.36–20.4**	**>20.4**	
Hcy	0	4	28	0	9	19	<0.001
Percentage	0	6.67	46.67	0	15	31.67	

MCV: Mean corpuscular volume, HoloTC: Holotranscobalamine, Hcy: Homocysteine

Table 3: Distribution of mean corpuscular volume, holotranscobalamine, and homocysteine with clinical manifestations among male and female patients

	Number of patients (%)	
	Anemic patients (male *n*=4)	**Anemic patients (female *n*=6)**
MCV	3 (30)	2 (20)
HoloTC	2 (20)	1 (10)
Hcy	4 (40)	5 (50)
	Number of patients (%)	
	Neurological patients (male *n*=12)	**Neurological patients (female *n*=9)**
MCV	1 (4.7)	0
HoloTC	3 (14.3)	3 (14.3)
Hcy	8 (38.1)	5 (23.8)
	Number of patients (%)	
	GI patients (male *n*=17)	**GI patients (female *n*=12)**
MCV	0	0
HoloTC	6 (20.69)	4 (13.8)
Hcy	17 (58.62)	8 (27.58)

MCV: Mean corpuscular volume, HoloTC: Holotranscobalamine, Hcy: Homocysteine, GI: Gastrointestinal

Table 4: Distribution of different laboratory values among different age groups of study participants

	18–30 years (*n*=11)	31–40 years (*n*=14)	41–50 years (*n*=15)	51–65 years (*n*=20)	Total (*n*=60), *n* (%)
MCV	1	1	3	1	6 (10)
HoloTC	3	5	5	6	19 (31.66)
Hcy	8	11	12	16	47 (78.33)
Hb	1	5	2	2	10 (16.67)

MCV: Mean corpuscular volume, HoloTC: Holotranscobalamine, Hcy: Homocysteine, Hb: Hemoglobin

deficiency and have also revealed that HoloTC is a more sensitive marker than total serum Vitamin B12 for investigating Vitamin B12 status.

Vitamin B12 acts as cofactor, in Hcy metabolism [10], so elevated serum Hcy levels have been linked to Vitamin B12 deficiency [11]. Hcy levels have been considered to be more sensitive than serum Vitamin B12 levels for determining Vitamin B12 status [11]. This study was conducted to know the role of Hcy and HoloTC in serum B12 deficiency. Vegetarian diet has always been suspected to contribute toward the development of B12 deficiency. Although dietary data history recovered was partial in the present survey, it could be assessed that vegetarian dietetic practice offered considerable risk for developing B12 deficiency, rate being 60%. This is in consistence with several studies [12].

In the present study, Vitamin B12 measurement was used as the first-line test and the definition of Vitamin B12 deficiency was based on low level of serum Vitamin B12 although the measurements of metabolites such as methylmalonic acid and Hcy have been shown to be more sensitive in the diagnosis of Vitamin B12 deficiency [13]. Hcy is known as a sensitive functional marker of inadequate cellular Vitamin B12 concentration. Deficiency of this vitamin has important health consequences, in addition to role in Hcy metabolism [14]. In our study, we observed raised Hcy level in 78.33% of subjects along with Vitamin B12 deficiency ($p<0.001$). A weak direct correlation was found in between serum Vitamin B12 and HoloTC (panel A: $r=0.09723$, $p=0.45$) (Fig. 3). As shown in Fig. 4, a strong indirect correlation was found between Hcy with low serum Vitamin B12 (panel B: $r=-0.42313$, $p=0.00075$). A higher concordance was found between Hcy and HoloTC II concentrations ($r=-0.013885$, $p<0.9166$) than was found between HoloTC II and Vitamin B12 concentrations.

Out of that 13.33% in between 18 and 30 years, 18.33% in between 31 and 40 years, 20% in between 41 and 50 years and 26.66% in between 51 and 65 years age group. This is consistent with – Fenech *et al*. [15] reported that 75% of the cases of high Hcy concentration were associated with low B12 concentration. Campbell *et al*. [16] reported that high serum Hcy (≥13 μmol/L) was found in 50.9% of deficient plasma B12 (<200 pg/mL). We also observed decrease in HoloTC level along with Vitamin B12 deficiency in 31.66% of patients, ($p>0.05$) out of that, 5% of patients in 18–30 years age, 8.33% in 31–40 years, and in 41–50 years age and 10% in 51–65 years age group. This is consistent with – Al Aisari *et al*. [17] reported that low serum transcobalamin level was found in 40% of plasma deficient patient.

In group of 60 patients, we found 16.67% of patients with anemia symptoms, 35% of patients show neurological manifestation, and 48.33% of patients showed GI manifestations ($p<0.05$). According to laboratory evaluation, we found that in patients with anemia symptoms, 6.67% of patients were male (Hb <13 gm/dl) and 10% were female (Hb <12 gm/dl). Patients with neurological and GI manifestation (total 83.33% of patients) were having Hb >12 gm/dl in females and Hb >13 gm/dl in males. In anemic patients, 75% of male patients showed raised MCV but only 50% of female anemic patients show raised MCV. In total anemia group, we found 10% in between 18 and

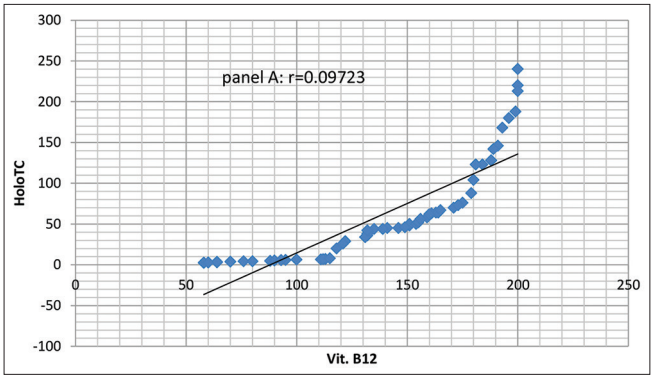

Fig. 3: The correlation between serum Vitamin B12 and holotranscobalamine II in Vitamin B12 deficiency state

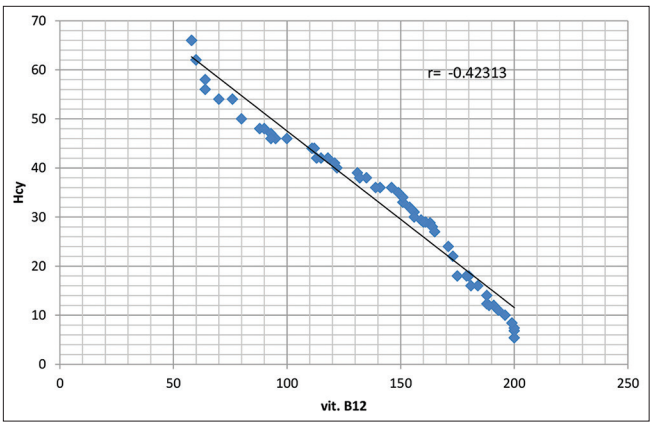

Fig. 4: The correlation between serum Vitamin B12 and homocysteine in Vitamin B12 deficiency state

30 years, 50% in between 31 and 40 years, 20% in between 41- and 50 years age group, and 20% in between 51- and 65 years age group. It showed that the prevalence of anemia with Vitamin B12 deficiency is highest in reproductive age group. In these groups, 90% of patients show raised Hcy level in blood but only 30% of patients show decreased HoloTC level in blood. This is consistent with Craig *et al.* [18] who did not find such a correlation between vitamin serum B12 deficiency and MCV. Stott *et al.* [19] found that only 23% of patients with serum B12 deficiency had raised MCV. Luong and Nguyen [20] told that he did not found any patient with macrocytosis in group of patients with anemia and Vitamin B12 deficiency.

In our study group, we found that 35% of patients show neurological manifestations, out of that, 57.14% of patients were male and 42.8% of patients were female. In total group of 21 patients, 4.76% of males and 14.28% of females were between 18 and 30 years age group, 4.76% of males and 4.76% of females were between 31 and 40 years age group, 14.28% of males and 19.05% of females were in between 41 and 50 years age group, and 33.33% of males and 4.76% of females were in between 51 and 65 years age group. It showed that association of Vitamin B12 deficiency with neurological manifestation is highest in 51–65 years age group male patients followed by 41–50 years age group female patients. In this group, we found that all patients with neurological manifestations showed Vitamin B12 deficiency but showed normal hemoglobin value, only 4.76% of patients show raised MCV value, 61.90% of patients show raised Hcy level but only 28.57% of patients show decreased HoloTC level. It is consistent with the study done by Mouallem *et al.* [21], showed that 87.72% of neurological cases had normal hemoglobin concentration while 12.28% had lower values. Lindenbaum *et al.* [22] have reported neuropsychiatric disorders caused by Vitamin B12 deficiency in the absence of anemia.

In our study, we found, 48.33% of patients showed GI manifestation, out of that, 58.62% of patients were male and 41.38% were female. In total 29 patients, 3.45% of males and 13.8% of females were between 18 and 30 years age group, 6.9% of males and 13.8% of females were between 31 and 40 years age group, 24.14% of males and 3.45% of females were in between 41 and 50 years age group, and 24.14% of males and 10.34% of females were in between 51 and 65 years age group. It showed that association of Vitamin B12 deficiency with GI manifestation is highest in male patients in more than 40 years age group. In this group, not a single patient showed raised MCV, 86.20% of patients showed raised Hcy and 34.48% of patients showed decreased HoloTC level. This is consistent with Andrès *et al.*, they observed that food-cobalamin malabsorption accounted for about 60%–70% of the cases among elderly patients [23].

After evaluation of all data, we found that out of 60 patients, only 16.67% of patients showed decreased hemoglobin level and only 10% of patients showed raised MCV ($p>0.05$). Hence, according to this, in our study, only 16.67% of patients were anemic, and 10% of patients showed megaloblastic anemia along with Vitamin B12 deficiency which is statistically insignificant. In our study group, we found that 31.66% of patients showed decreased HoloTC ($p>0.05$) which is statistically insignificant. However, 78.33% of patients showed raised Hcy level along with Vitamin B12 deficiency ($p<0.001$) which is statistically highly significant. Hence, according to this, Vitamin B12 deficiency is more associated with hyperhomocysteinemia. This is consistent with Haltmayer *et al.* [24] in a study involving 200 hospitalized patients at the Konvent hospital. Barmherzige Brueder reported that cobalamin (Vitamin B12) and folate deficiencies are related to both increased erythrocytes MCV and raised serum total Hcy values.

Limitations

First, ours was a small convenient sample. Hence, study findings cannot be extrapolated. Macrocytic anemia may be masked in vegetarians by excess folate intake or by concomitant iron deficiency. We did not check Sr. folate and Sr. iron level. Our study had possible ethnic differences and selection bias. Our study did not show significant correlation in between Vitamin B12 deficiency and serum HoloTC, the serum HoloTC levels can be affected by several factors, for example, food intake, amount of absorbed Vitamin B12, and subclinical renal and hepatic dysfunction. We have not considered the percentage of total Vitamin B12 bound to transcobalamin in the assessment of Vitamin B12 status. Although this percentage typically ranges from 20% to 30%, it can vary from 10% to 70% and this variation may influence the results.

CONCLUSION

Thus, we conclude that the vegetarians investigated in this study had more Vitamin B12 deficiency than non-vegetarians, which were related to the degree of animal product restriction. Hyperhomocysteinemia was linked to Vitamin B12 deficiency. According to our data, the assessment of HoloTC II, accompanied by that of metabolic markers such as Hcy, may offer sensitive and reliable tools for early diagnosis and hence proper intervention can be carried out in persons who are prone to Vitamin B12 deficiency. This study also suggests that Hcy levels can be early markers for tissue Vitamin B12 deficiency, even before hematologic manifestations occur.

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AUTHORS' CONTRIBUTIONS

All authors have contributed to the preparation of manuscript.

CONFLICTS OF INTEREST

Nil.

AUTHORS' FUNDING

Nil.

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