Journal of Advanced Laboratory Research in Biology
E-ISSN: 0976-7614
Volume 12, Issue 4, October 2021
PP 38-43
https://e-journal.sospublication.co.in

Research Article | Open Access

Clinical utility of serum holotranscobalamin in the assessment of Vitamin B_{12} deficiency in patients with Hypothyroidism

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Keywords

Case-Control Studies
Hypothyroidism
Vitamin B_{12} Deficiency
Transcobalamins
Thyroid disorder

Abstract

Thyroid disorder is the second most frequently encountered endocrinological condition after diabetes mellitus. When vitamin B_{12} deficiency coexists with hypothyroidism, neurological symptoms and signs are more pronounced. Holotranscobalamin (Active B_{12}) may be a more sensitive marker in the early diagnosis of Vitamin B_{12} deficiency than total B_{12}. The study aimed to evaluate the serum levels of active B_{12} in patients with clinical hypothyroidism and to correlate active B_{12} and thyroid profiles. The case-control study was carried out in a tertiary hospital on 80 study subjects, comprising 40 confirmed hypothyroidism patients and 40 age- and gender-matched healthy controls. Serum thyroid profile and active B_{12} assays were performed by Chemiluminescent Microparticle Immunoassay. Statistical methods such as independent t-test and Pearson's correlation were used to compare and correlate quantitative data. A significant percentage (90%) of hypothyroid patients had vitamin B_{12} deficiency, with a mean value of 17.39 ± 5.73 pmol/L. Active B_{12} showed a positive correlation with T_3 ($r = 0.818$; $P < 0.001$) and T_4 ($r = 0.851$; $P < 0.001$) and a negative correlation with TSH ($r = -0.930$; $P < 0.001$). Vitamin B_{12} deficiency was found in patients with hypothyroidism. This vitamin B_{12} deficiency may be caused by inadequate malabsorption, as seen in hypothyroidism. HoloTC (Active B_{12}) may be a promising marker for early detection and management of B_{12} deficiency, which may be beneficial in preventing irreversible neurological damage at an early stage.

1 Introduction

The thyroid is an important endocrine gland that releases thyroid hormones (T_3 and T_4), these hormones are involved in the metabolism, growth, and development of the human body. Thyroid hormones are regulated by thyroid-stimulating hormone or thyrotropin (TSH), which is secreted by the anterior pituitary gland. Currently, thyrotropin (TSH), thyroxine (T_4), or T_4 in combination with triiodothyronine (T_3) are suggested to be used as indicators in laboratory testing to clinically evaluate thyroid function [1,2]. Hypothyroidism is defined as low T_4 levels with elevated serum TSH levels. The most common cause of hypothyroidism is iodine deficiency. Hypothyroidism may also occur as a result of radioactive iodine treatment, thyroid surgery, thyroid cancer, after external beam radiotherapy for head and neck malignancy, and certain medications (such as lithium, amiodarone, tyrosine kinase inhibitors, thalidomide, interferon-alpha, monoclonal antibodies, and antiepileptic drugs) [3].

Vitamin B_{12} (cobalamin), a water-soluble vitamin, which was originally discovered as an anti-pernicious anemia factor, has two biologically active forms, namely methylcobalamin (MeCbl) and adenosylcobalamin (AdoCbl), AdoCbl also known as coenzyme B_{12} [4]. Vitamin B_{12} is essential for the synthesis of myelin, which is required for the formation of the myelin sheath, a protective layer for the spinal, cranial, and peripheral nerves. These functions are explained by the role of vitamin B_{12} as a cofactor for two enzymes methionine synthase and l-methylmalonyl-CoA mutase. Thus, vitamin B_{12} acts as a cofactor for the synthesis of methionine by transferring the methyl group to homocysteine (Hcy). Further methionine metabolism to S-adenosyl methionine (SAM) is required for the synthesis of myelin, maintenance of neuronal integrity,

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Received on: 24 September 2021
Accepted on: 25 October 2021

and regulation of neurotransmitters. Vitamin B_{12} deficiency causes either the incorporation of abnormal fatty acids into myelin sheaths or destruction of myelin sheaths, thus leading to impaired neural function and/or transmissions. This may be the root cause of the neurological symptoms associated with vitamin B_{12} deficiency [5].

The absorption, blood transport, and cellular uptake of vitamin B_{12} are dependent upon binding proteins such as haptocorrin (HC), intrinsic factor (IF), transcobalamin (TC), and cell surface receptors [6]. In serum, vitamin B_{12} is transported via two distinct binding proteins TC and HC. When vitamin B_{12} binds to TC and HC, the resulting complexes are known as holotranscobalamin (HoloTC or Active B_{12}), and holohaptocorrin (HoloHC - inactive form of vitamin B_{12}). The major fraction of circulating vitamin B_{12} (70-90%) is bound to HC. The actual function of this HC-bound vitamin B_{12} is unknown, but it is believed to be biologically unavailable to most cells. HoloTC binds the remaining 10–30% of vitamin B_{12} [7].

Vitamin B_{12} deficiency has been commonly reported in patients with thyroid disorders, especially in autoimmune thyroid diseases such as Hashimoto's. This association is probably due to impaired vitamin B_{12} absorption by atrophic gastritis and/or pernicious anemia associated with autoimmune thyroid disease [8]. Other causes of vitamin B_{12} deficiency in hypothyroid patients include inadequate dietary intake, altered intestinal absorption due to sluggish bowel motility, bowel wall oedema, and bacterial overgrowth. Non-autoimmune causes of B_{12} deficiency in hypothyroid patients have not been thoroughly investigated, and they may differ depending on dietary habits in different populations. Symptoms such as weakness, diarrhea, numbness, abdominal pain, paresthesia, memory loss, dizziness, dysphagia, and depression have been reported in hypothyroid patients. Patients with both hypothyroidism and vitamin B_{12} deficiency also have similar symptoms. Dysphagia, numbness, and paresthesia were the most commonly reported symptoms in hypothyroid patients with vitamin B_{12} deficiency [9].

Vitamin B_{12} deficiency has traditionally been diagnosed by measuring total serum vitamin B_{12} levels. The limitation of this biomarker is that it assesses total circulating vitamin B_{12}, of which 70–90% is bound to haptocorrin, which is not available to the cells and is unreliable to reflect cellular vitamin B_{12} status. According to the findings of studies on serum and cellular vitamin B_{12} levels, serum B_{12} levels do not always correspond to cellular B_{12} status. HoloTC is advantageous in determining vitamin B_{12} status as it reflects the metabolically active fraction of vitamin B_{12} in the serum [10]. Thus decreased concentrations of holoTC may be the earliest and most sensitive marker of vitamin B_{12} deficiency [11]. The present study was conducted to determine the clinical utility of serum holotranscobalamin (active B_{12}) in assessing vitamin B_{12} deficiency in hypothyroid patients and to correlate active B_{12} and thyroid profiles.

2 Materials and Methods

2.1 Study Duration and Setting

A prospective case-control study was carried out for a period of 6 months (December 2018 to May 2019) at a tertiary care hospital in Mangaluru, Karnataka, India. Ethical clearance was obtained from the Institutional Ethics Committee (AJEC/REV/70/2018).

2.2 Study Population

The current case-control study included 80 subjects, comprising 40 confirmed hypothyroidism patients (5 males and 35 females) (mean age: 34.58 ± 8.37) and 40 age- and gender-matched healthy controls (5 males and 35 females) (mean age: 33.28 ± 10.25). Written informed consent was obtained from all participants in the study. Patients with a history of kidney disease, diabetes mellitus, cardiac disease and liver disease, hypertension, pregnancy, and multivitamin supplementation were excluded from the study.

2.3 Sample Size

The sample size was estimated using the OpenEpi, version 3 (www.openepi.com). The sample size was calculated using the mean and standard deviation of vitamin B_{12} levels in hypothyroidism reported earlier by Khubchandani *et al.*, [12]. The estimated sample size was 40 subjects in each group (a total of 80 subjects), with a confidence interval (CI) of 95%, power of 80%, and a case-control ratio of 1:1.

2.4 Sample collection and processing

Blood samples were collected after overnight fasting. 5 ml venous blood was collected from each study subject. These blood samples were allowed to stand at room temperature for clot formation before being centrifuged for 10 minutes at approximately 3500 rpm. Measurements of serum concentrations of Total T_3, Total T_4, TSH, and Vitamin B_{12} were done using Chemiluminescent Microparticle Immunoassay (CMIA) for the quantitative determination of holotranscobalamin (active B_{12}) in serum on the ARCHITECT iSystem (Abbott Diagnostics, Abbott Park, IL, USA). According to the applied reagent kit, the reference range for active B_{12} is considered to be 25.1 - 165.0 pmol/L.

2.5 Statistical Analysis

Statistical analysis was performed using IBM SPSS version 20.0 (SPSS, Inc., Chicago, IL, USA). Categorical variables were represented by frequency and percentage and continuous data were expressed as mean ± standard deviation (SD). A Chi-square test was performed to determine the association between categorical variables. An independent t-test can be used to compare means between cases and controls. Pearson correlation analysis was performed to analyze the association between age, thyroid hormones, and active B_{12}. $P < 0.05$ was considered statistically significant.

3 Results

The present study comprised 80 subjects, of which 40 were cases (5 males and 35 females) (mean age: 34.58 ± 8.37) and 40 were age- and gender-matched healthy controls (5 males and 35 females) (mean age: 33.28 ± 10.25). The demographic and biochemical parameters of the study subjects are shown in Table 1. According to the normal reference range, the cut-off for vitamin B_{12} deficiency based on HoloTC was considered at < 25 pmol/L.

The distribution of study subjects based on active vitamin B_{12} status is shown in Fig. 1. Symptoms of numbness, paresthesia, and dysphagia were seen in B_{12} deficient patients compared to B_{12} sufficient patients.

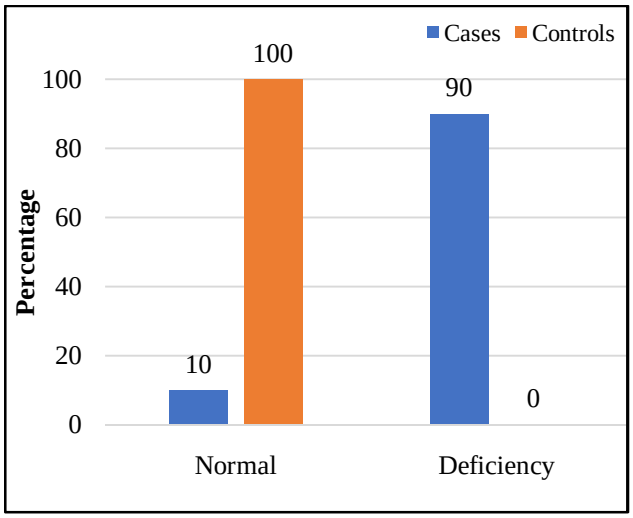

Fig. 1: Distribution of study subjects based on active Vitamin B_{12} status.

We obtained a significant decrease in serum T_3 level (0.60 ± 0.17 nmol/L; $P < 0.001$), a significantly decreased serum T_4 level (3.66 ± 0.85 µg/dL; $P < 0.001$) and a significantly increased TSH level (59.38 ± 8.09 mIU/L; $P < 0.001$) in patients with hypothyroidism as compared to controls. Patients with hypothyroidism had significantly lower serum vitamin B_{12} levels (17.39 ± 5.73 pmol/L; $P < 0.001$) as compared to controls (55.09 ± 7.69 pmol/L; $P < 0.001$). A significant percentage (90%) of hypothyroid patients had vitamin B_{12} deficiency, as shown by active B_{12} levels (Table 1).

Correlation of active B_{12} with age and thyroid profile is shown in Table 2. Active B_{12} shows a positive correlation with T_3 (r = 0.818; $P < 0.001$) and T_4 (r = 0.851; $P < 0.001$) (Fig. 2 & 3). There was a negative correlation between Active B_{12} and TSH (r = -0.930; $P < 0.001$) (Fig. 4).

The results showed that there was no significant correlation (r = -0.140; $P = 0.217$) found between age and thyroid profile / active B_{12}.

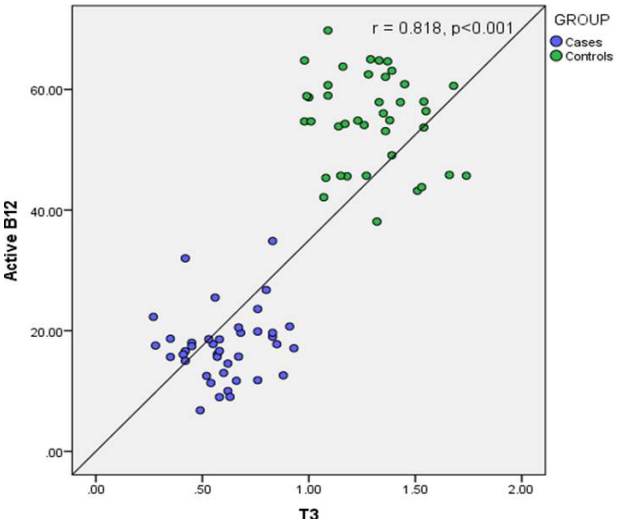

Fig. 2: Correlation between T_3 and Active B_{12}.

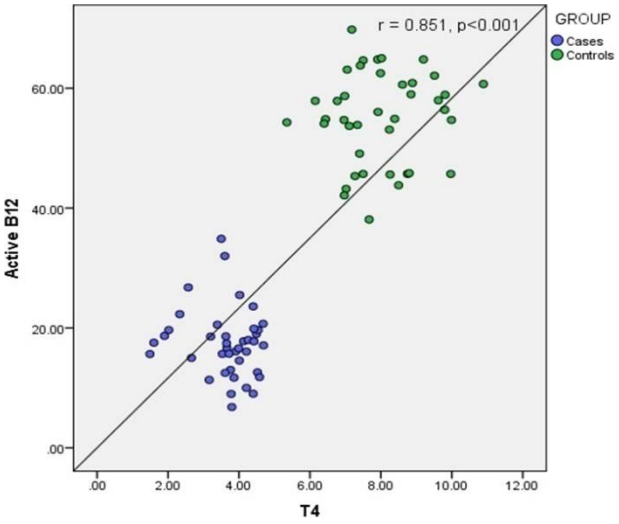

Fig. 3: Correlation between T_4 and Active B_{12}.

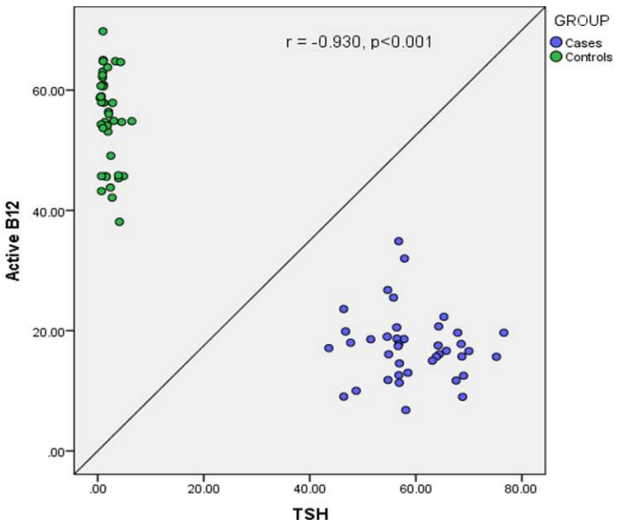

Fig 4: Correlation between TSH and Active B_{12}.

Table 1: Demographic and biochemical parameters of the study subjects.

Parameters	Cases (n = 40)	Controls (n = 40)	P value
	N (%) / Mean ± SD (Min. – Max.)		
Age (in years)	34.58 ± 8.37 (19.00 – 49.00)	33.28 ± 10.25 (18.00 – 49.00)	0.536
Age group			
18 – 26 years	9 (22.50)	14 (35.00)	0.466
27 – 35 years	12 (30.00)	10 (25.00)	
> 35 years	19 (47.50)	16 (40.00)	
Gender			
Males	5 (12.50)	5 (12.50)	1.000
Females	35 (87.50)	35 (87.50)	
Hormones			
Serum T_3 (nmol/L)	0.60 ± 0.17 (0.27 – 0.93)	1.29 ± 0.20 (0.98 – 1.74)	< 0.001
Serum T_4 (µg/dL)	3.66 ± 0.85 (1.49 – 4.69)	8.01 ± 1.22 (5.35 – 10.89)	< 0.001
Serum TSH (mIU/L)	59.38 ± 8.09 (43.60 – 76.60)	1.99 ± 1.45 (0.47 – 6.43)	< 0.001
Serum Vitamin B_{12} (pmol/L)	17.39 ± 5.73 (6.80 – 34.87)	55.09 ± 7.69 (38.09 – 69.78)	< 0.001

Table 2: Correlation between age, thyroid profile and active B_{12}.

Correlations						
		Age	**T_3**	**T_4**	**TSH**	**B_{12}**
Age	Pearson Correlation	1	-0.076	-0.077	0.103	-0.140
	Sig. (2-tailed)		0.500	0.497	0.361	0.217
	N	80	80	80	80	80
T_3	Pearson Correlation	-0.076	1	0.854**	-0.878**	0.818**
	Sig. (2-tailed)	0.500		0.000	0.000	0.000
	N	80	80	80	80	80
T_4	Pearson Correlation	-0.077	0.854**	1	-0.905**	0.851**
	Sig. (2-tailed)	0.497	0.000		0.000	0.000
	N	80	80	80	80	80
TSH	Pearson Correlation	0.103	-0.878**	-0.905**	1	-0.930**
	Sig. (2-tailed)	0.361	0.000	0.000		0.000
	N	80	80	80	80	80
B_{12}	Pearson Correlation	-0.140	0.818**	0.851**	-0.930**	1
	Sig. (2-tailed)	0.217	0.000	0.000	0.000	
	N	80	80	80	80	80

** Correlation is significant at the 0.01 level (2-tailed).

4 Discussion

Hypothyroidism (underactive thyroid) is defined as the failure of the thyroid gland to produce enough thyroid hormones (T_3 and T_4) needed for the body's metabolic demands. Untreated hypothyroidism can lead to cognitive impairment, hypertension, dyslipidemia, infertility, and neuromuscular dysfunction [13]. The prevalence of vitamin B_{12} deficiency in both hypothyroidism and autoimmune thyroid disease (AITD) reflects the nutritional status of the population. AITD can be independently associated with pernicious anemia and atrophic gastritis, which may lead to impaired absorption of vitamin B_{12} [14]. An immune response against the gastric H/K-ATPase in the parietal cells of the stomach occurs in an autoimmune disorder [15]. According to Centanni *et al*., (1999), in patients with AITD, atrophic gastritis was found in 22 cases (35%), with pernicious anemia in 10 cases (16%), and also indicated that about one-third of AITD patients have atrophic gastritis [16]. Intrinsic factor antibodies have also been found in AITD patients [17]. In the absence of AITD, the association between hypothyroidism and vitamin B_{12} deficiency has not been thoroughly evaluated. This association may differ depending on dietary habits among different population groups [9]. Direct biomarkers such as total B_{12} and holotranscobalamin (holoTC) and the two metabolic markers homocysteine (Hcy) and methylmalonic acid (MMA) are preferred for diagnosing B_{12} deficiency [18]. In our study, total B_{12}, homocysteine, and MMA were not studied.

The physiology of vitamin B_{12} suggested that holoTC may be a sensitive marker of early vitamin B_{12} deficiency. Currently, there are 3 methods for analyzing holoTC: direct measurement of the complex between transcobalamin and vitamin B_{12}, measurement of the amount of transcobalamin saturated with vitamin B_{12} or measurement of vitamin B_{12} attached to transcobalamin [19]. In clinical studies, the holoTC performs better than total vitamin B_{12} in assessing B_{12} deficiency based on the high concentration of methylmalonic acid [19] and red cell cobalamin concentrations [20]. In renal disease, plasma holoTC is increased. Therefore, holoTC cannot be used as an indicator of vitamin B_{12} status in patients with renal disease [21].

In our study, we obtained a significant decrease in serum T_3 level, a significantly decreased serum T_4 level, and a significantly increased TSH level in patients with

hypothyroidism as compared to controls. Li *et al*., (2014) also found a significant decrease in the serum T_3 levels (1.02 ± 0.984 nmol/L), a significant decrease in T_4 levels (34.66 ± 13.201 nmol/L), and a significantly increased TSH level (21.99 ± 24.418 mIU/L) in cases, which was consistent with our findings [22].

In the present study, patients with hypothyroidism had significantly lower serum vitamin B_{12} levels as compared to controls. A significant percentage (90%) of hypothyroid patients had vitamin B_{12} deficiency, as shown by active B_{12} levels. Similar findings were reported by Khubchandani *et al.*, (2015) who found that, serum vitamin B_{12} levels ($P < 0.05$) of hypothyroidism patients are significantly lower (187.38 ± 35.89) than normal subjects (365.17 ± 45.82). The prevalence of B_{12} deficiency was observed in hypothyroid patients and 32 out of 50 (64%) patients were found to have B_{12} deficient [12]. Lakho *et al*., (2018) studied the prevalence of B_{12} deficiency in hypothyroid patients and found that 105 (72%) of 145 patients had low B_{12} levels [23]. Similarly, Jabbar *et al*., (2008) reported the prevalence of B_{12} deficiency in hypothyroid patients and found that 47 (40.5%) of 116 patients had low B_{12} levels [9].

Vitamin B_{12} deficiency is a common condition with non-specific clinical symptoms. Manifestations of vitamin B_{12} deficiency range from subtle, nonspecific clinical features to severe neurological and neuropsychiatric complications [24]. The metabolic level at which a vitamin B_{12} deficiency should be classified varies depending on the method and laboratory [25]. The prevalence of vitamin B_{12} deficiency may be underestimated because people with diets rich in B_{12} may have food-cobalamin malabsorption issues due to atrophic gastritis and as a result of *Helicobacter pylori* infection [26].

In our study, Active B_{12} showed a positive correlation with T_3 and T_4. There was a negative correlation between Active B_{12} and TSH and there was no significant correlation between age and thyroid profile / Active B_{12}. According to a study conducted by Ranjan *et al.*, (2020) vitamin B_{12} has a positive correlation with serum T_3 ($r = 0.01$; $P = 0.94$) and T_4 ($r = 0.05$; $P = 0.77$) [27]. In another study, there was no correlation found between TSH and vitamin B_{12} ($r = 0.006$; $P = 0.935$) [28]. A recent study found no correlation between vitamin B_{12} with age and thyroid profile [17].

The present study revealed that HoloTC showed better diagnostic accuracy than vitamin B_{12} and can be used as a primary screening test in patients suspected of vitamin B_{12} deficiency. It is recommended that patients with autoimmune thyroid diseases should be periodically screened for vitamin B_{12} deficiency.

5 Conclusion

Vitamin B_{12} deficiency was observed in patients with hypothyroidism. Active B_{12} (HoloTC) may be a promising marker for the early detection and management of B_{12} deficiency, thus proving beneficial in preventing irreversible neurological damage at the earliest. To prevent complications associated with B_{12} deficiency, studies in different clinical settings are required to clarify the efficacy of holoTC in determining vitamin B_{12} status and to establish a causal relationship between active B_{12} and hypothyroidism. In addition, further research is needed to compare total B_{12} and active B_{12} in hypothyroid patients to identify which of the two is a sensitive marker of vitamin B_{12} deficiency.

Conflicts of Interest

The author declares that there is no conflict of interest.

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