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Relationship between the Levels of Holotranscobalamin and Vitamin B12

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To date, the determination of serum vitamin B12 levels has been the most common laboratory test for the assessment of vitamin B12 status; however, the diagnostic accuracy of this test is low. To obtain a more sensitive marker, a new test to measure holotranscobalamin (holoTC) levels has been introduced. In this study, we assessed 45 patients for whom a vitamin B12 test had been requested and 139 anemic patients. We investigated the associations between the levels of homocysteine (Hcy) and those of holoTC, serum vitamin B12, and folate and assessed the diagnostic value of holoTC levels as a marker for vitamin B12 deficiency. We also determined the precision of the AxSYM holoTC assay by calculating the coefficient of variance (CV). The within-run and between-run precision values were excellent, as all CV values were less than 3.5%. The holoTC levels were low (<35 pmol/L) in 7 samples, and 6 of these samples had normal total serum vitamin B12 levels. In 2 of these samples, high Hcy levels (>12 μmol/L) indicated vitamin B12 deficiency. Thus, the holoTC levels were more sensitive than the serum vitamin B12 levels for indicating vitamin B12 status. If the serum vitamin B12 level is 151-300 pmol/L, the levels of holoTC alone or in combination with serum vitamin B12 levels are likely to be more useful markers than serum vitamin B12 levels alone. *(Korean J Lab Med 2010;30:185-9)*

Key Words : *Holotranscobalamin, Vitamin B12 deficiency, Vitamin B12, Homocysteine*

Vitamin B12 is essential for cellular metabolism, particularly DNA synthesis and regulation. Vitamin B12 deficiency may cause severe and irreversible damage, and early detection of vitamin B12 deficiency is important. While vitamin B12 status is currently determined by performing blood tests to assess serum vitamin B12 levels, the diagnostic accuracy of these tests is low [1, 2].

To obtain a more sensitive marker of vitamin B12 status, a new test involving measurement of the levels of holotranscobalamin (holoTC)—transcobalamin-vitamin B12 complex—has been introduced [3-7]. 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]. We investigated the association between the levels of holoTC and serum vitamin B12, determined the diagnostic value of holoTC levels in populations susceptible to vitamin B12 deficiency, and performed a precision test for the AxSYM holoTC assay.

We included 45 samples from patients for whom a serum vitamin B12 test had been requested because of conditions such as dementia with Alzheimer's disease, Parkinson's disease, cancer, including adenocarcinoma of the stomach, unstable angina, and infarction. We also included 139 samples from patients with normocytic or macrocytic anemia who were admitted to Dong-A University Hospital between August 2007 and March 2008. The 5 patients with adenocarcinoma of the stomach had undergone total or subtotal

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gastrectomy. Informed consent was obtained from all the participants.

Blood samples were collected and sera were directly separated. Separated sera were frozen at −70℃ until further testing. Serum vitamin B12 levels were measured using Abbott Architect B12 (Abbott Laboratories, Abbott Park, IL, USA) with an Abbott Architect immunoanalyzer. HoloTC and homocysteine (Hcy) levels were measured by performing the AxSYM assay (Abbott Laboratories, Abbott Park, IL, USA), which is based on the microparticle enzyme immunoassay. Folate levels were determined using ADVIA (ADVIA Centaur system, Bayer AG, Leverkusen, Germany). The maximum detectable concentrations for vitamin B12, holoTC, folate, and Hcy were 2,000.0 pmol/L, 128.0 pmol/L, 20.0 μgl/L, and 50.0 μmol/L, respectively. The samples with high levels of serum biomarkers other than holoTC were diluted and their levels were re-assayed. The cutoff values of the analytes were determined on the basis of standard clinical reference intervals or values reported in the literature: vitamin B12, 150.0 pmol/L [9]; holoTC, 35.0 pmol/L [5, 10]; folate, 3.0 μgl/L (standard clinical reference interval); and Hcy, 12.0 μmol/L [5, 10].

The assay precision (which was defined in terms of the CV) of the holoTC test was evaluated according to CLSI protocol EP5-A2 [11]. We assayed the low- and high-concentration controls together. We measured the holoTC concentration twice daily for 5 consecutive days.

Statistical analysis for all the results was performed using MediCalc version 9.3 (MedCalc Software, Mariakerke, Belgium). Data were also analyzed using Analyse-it (Analyse-it Software). *P* values less than 0.05 were considered to be statistically significant.

We recruited 184 patients in the study. The demographic data of the patients are shown in Table 1.

The result of the precision study showed that the within-run and between-run CV values were excellent, and all values were less than 3.5% (Table 2). Low holoTC levels (<35 pmol/L) were observed in 7 samples; 1 sample had both low holoTC level (22.6 pmol/L) and low serum vitamin B12 level (<150.0 pmol/L). The other 6 samples had normal serum vitamin B12 levels; the serum vitamin B12 levels in 4 of these 6 samples were in the borderline range (151.0-300.0 pmol/L) (Table 3). Chi-square test was performed to determine the relationship between holoTC and vitamin B12, and the obtained *P* value was less than 0.0001. The serum vitamin B12 levels in 20 of the 157 samples with normal holoTC levels were in the low ranges, although not decreased. Further, elevated Hcy levels (>12.0 μmol/L) were observed in 55 patients. In 2 patients with low holoTC levels, the increased Hcy levels (>12.0 μmol/L) indicated vitamin B12 deficiency. In these 2 patients, the folate levels were normal. Decreased folate levels (<3.0 μg/L) were observed in 10 patients, but their holoTC levels were within the

Table 1. Demographic data and laboratory results of the 184 patients*

Variables	Patients (N=184)
Sex (female/male)	90/94
Age	61.5 (22-83)
Results of laboratory tests	
Vitamin B12 (pmol/L)	564.7 (115.0-1,475.0)
HoloTC (pmol/L)	22.0-128.0†
Hcy (μmol/L)	9.2 (3.0-50.0)
Folate (μg/L)	6.4 (0.8-48.0)

*Data are presented as median (range) unless otherwise noted; †Data are presented as ranges because samples with high levels of holoTC (>128.0 pmol/L) were not diluted.
Abbreviations: holoTC, holotranscobalamin; Hcy, homocysteine.

Table 2. Results of the precision analysis for the AxSYM holoTC assay, which was performed using low and high controls

Material	Within-run		Between-run		Between-day		Total	
	SD	CV (%)	SD	CV (%)	SD	CV (%)	SD	CV (%)
Low (21.0 pmol/L)	0.5	2.9	0.5	2.5	0.7	3.8	1.0	5.5
High (48.0 pmol/L)	1.4	3.3	0.4	1.1	2.4	5.7	2.9	6.7

Abbreviation: holoTC, holotranscobalamin.

Table 3. Classification of patients (N=184) according to the holoTC levels and total serum vitamin B12 ranges (*P*<0.0001)

Total serum vitamin B12 (pmol/L)	HoloTC<35 pmol/L	HoloTC≥35 pmol/L
100-150	1	0
151-300	4	20
>301	2	157

Abbreviation: holoTC, holotranscobalamin.

Table 4. Number of patients (N=55) with hyperhomocysteinemia (>12 μmol/L), which was determined to the levels of holoTC and folate

	HoloTC<35.0 pmol/L	HoloTC>35.0 pmol/L	Total
Folate < 3.0 μg/L	0	10	10
Folate > 3.0 μg/L	2	43	45
Total	2	53	55

Abbreviation: holoTC, holotranscobalamin.

normal range (Table 4).

The symptoms of vitamin B12 deficiency are variable, and these symptoms may be easily missed, particularly if the serum vitamin B12 levels are within the normal range [12, 13]. In the absence of a gold standard to detect vitamin B12 deficiency, the current laboratory tests to measure serum vitamin B12 levels may not be able to diagnose vitamin B12 deficiency in the early stages [1, 12-15]. 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 [3, 5]. In addition, a few studies performed in different countries and ethnic groups have shown differences in the prevalence of vitamin B12 deficiency and have also revealed that holoTC is a more sensitive marker than total serum vitamin B12 for investigating vitamin B12 status [5, 12, 16-19]. However, most of the previous studies were performed using radioimmunoassays, and very few studies have been performed with the more recent microparticle enzyme immunoassays. Very few studies have analyzed the relationship between the levels of holoTC, serum vitamin B12, folate, and Hcy to investigate the effectiveness of holoTC for estimating vitamin B12 status.

Brady et al. [7] reported that the Abbott AxSYM holoTC assay allowed rapid, precise, sensitive, and specific measurement of holoTC. In this study, we have evaluated the precision of the Abbott AxSYM holoTC assay and obtained results that are concordant with those of Brady et al. [7].

As shown in Table 3 and 4, out of the 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 (151.0-300.0 pmol/L). In addition, in 2 of these samples, high Hcy level (>12 μmol/L), normal creatinine level (<1.2 mg/dL), and normal folate level (>3.0 μg/L) indicated the deficiency of vitamin B12. Neurological symptoms, including peripheral neuropathy, cognitive impairment, or dementia were not observed in any of the cases. The patients showed a range of symptoms such as fatigue, depression, and poor memory; however, these symptoms were nonspecific and did not facilitate the diagnosis of vitamin B12 deficiency. All the patients had cancer, with gastric cancer being the most common. Absorption of dietary vitamin B12 requires intact and functioning stomach, exocrine pancreas, intrinsic factor, and small bowel. Therefore, malfunctioning or abnormalities in any of these organs may cause malabsorption of dietary cobalamin, thereby resulting in vitamin B12 deficiency.

Further, folate and vitamin B12 act as cosubstrate and cofactor, respectively in Hcy metabolism [20]. As shown in Table 4, elevated serum Hcy levels have been linked to both vitamin B12 and folate deficiency [10, 21, 22]. Hcy levels have been considered to be more sensitive than serum vitamin B12 levels for determining vitamin B12 status [20]. However, hyperhomocysteinemia is also induced by other conditions, including renal insufficiency.

Our study had several limitations. Small sample number, possible ethnic differences, selection bias, and different methods used for evaluating holoTC levels may have influenced the differences in holoTC levels. 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 factors can influence the concentration of holoTC as well as vitamin B12 status [5]. Further, we have not considered the percentage of total vitamin B12 bound to transcobalmin (holoTC/B12 ratio) in the assessment of vitamin B12 status. Although this percentage typically ranges from 20% to 30%, it can vary from 10% to 70% among individuals, and this variation may influence the results. Clinical data, including the symptoms and medical history and the data for other parameters of vitamin B12 status such as methylmalonic acid levels, should also be determined for evaluating the diagnostic value of the

AxSYM holoTC assay [2, 23].

On the basis of these results, we concluded that the holo-TC levels may be more suitable than serum vitamin B12 levels to obtain information about vitamin B12 status, and the AxSYM holoTC assay is a reliable and reproducible technique for the measurement of holoTC levels. If the serum vitamin B12 level is between approximately 151-300 pmol/L, holoTC alone or in combination with the serum vitamin B12 is likely to be more useful than serum vitamin B12 levels alone to indicate vitamin B12 status.

However, more studies in different clinical settings are required to clarify the effectiveness of holoTC in determining the vitamin B12 status and thorough investigation of any conditions or medications that may cause vitamin B12 deficiency is required.

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