Holotranscobalamin as an Indicator of Vitamin B₁₂ Deficiency in Gastrectomized Patients

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Abstract. Transcobalamin-bound vitamin B₁₂, or holotranscobalamin (HoloTC), a biologically active form, is believed to be a sensitive marker of vitamin B₁₂ deficiency. We investigated the prevalence of vitamin B₁₂ deficiency in gastrectomized patients using HoloTC and total vitamin B₁₂ to determine the diagnostic utility of HoloTC. We enrolled 128 gastrectomized patients and measured serum HoloTC, total vitamin B₁₂, homocysteine (Hcy), and complete blood count (CBC). HoloTC values were also obtained from 100 healthy controls. The precision of HoloTC measurement was good and the normal range of HoloTC was set at ≥42.48 pmol/L. Among the 128 gastrectomized subjects, HoloTC was low (<42.48 pmol/L) in 32 patients (25.0%) and total vitamin B₁₂ was low (<189 pg/ml) in 10 patients (7.8%). Among the patients who had total vitamin B₁₂ concentrations in the borderline range (189~400 pg/ml), 44% had low HoloTC concentrations. Clinical findings as well as Hcy concentrations suggested vitamin B₁₂ deficiency in this subset of patients. The patients with both low total vitamin B₁₂ and low HoloTC had significantly higher Hcy concentrations than those with either normal total vitamin B₁₂ or normal HoloTC. Although a quarter of gastrectomized patients had low HoloTC, some of these had normal total vitamin B₁₂ concentrations. Our study suggests that serum HoloTC is a more sensitive marker than total vitamin B₁₂ in diagnosing vitamin B₁₂ deficiency.

Keywords: holotranscobalamin, vitamin B₁₂ deficiency, gastrectomy, homocysteine

Introduction

Patients who have undergone gastrectomy are at high risk of vitamin B₁₂ deficiency, and regular monitoring of vitamin B₁₂ status is recommended for them [1,2]. Vitamin B₁₂ deficiency can lead to macrocytic anemia and progressive neurological disease. Macrocytic anemia is relatively easy to diagnose and can be treated with vitamin B₁₂ supplementation. However, neurological problems can occur at early stages of vitamin B₁₂ deficiency without macrocytic anemia, and are irreversible unless treated soon after their onset [3]. Thus, early diagnosis and treatment of vitamin B₁₂ deficiency are important to prevent irreversible effects.

Currently, no entirely adequate test is available to diagnose vitamin B₁₂ deficiency in the routine clinical laboratory. Serum vitamin B₁₂ is not a sensitive marker, and neurological complications can develop despite normal vitamin B₁₂ concentrations [3,4]. Accumulated metabolites, eg, serum homocysteine (Hcy) and methylmalonic acid (MMA), are commonly used as markers of vitamin B₁₂ deficiency. However, Hcy increases are seen in many conditions other than vitamin B₁₂ deficiency and MMA is difficult to measure because it requires HPLC or gas chromatography/mass spectrometry. Additionally, both Hcy and MMA concentrations are affected by renal function [5-7].

Vitamin B₁₂ in serum is bound to two proteins, transcobalamin (TC) and haptocorrin (HC). TC-bound vitamin B₁₂, holotranscobalamin (HoloTC), is a biologically active form that can enter into cells requiring vitamin B₁₂; it has a shorter half-life than...
HC-bound vitamin B_{12} (HoloHC). Thus, HoloTC is expected to be a sensitive and specific indicator of vitamin B_{12} status [8,9]. A few studies support the clinical utility of HoloTC determinations and have reported the distribution of serum HoloTC concentrations in patients at high risk of vitamin B_{12} deficiency, such as older adults and those consuming a vegetarian diet [9-11].

A recently developed automated assay for HoloTC has made it possible to use it as a screening test in the routine clinical laboratory [12]. The purpose of this study was to evaluate the clinical utility of serum HoloTC measurements to diagnose vitamin B_{12} deficiency in gastrectomized patients. We investigated the vitamin B_{12} status of gastrectomized patients using HoloTC in addition to 3 markers of vitamin B_{12} deficiency: total vitamin B_{12}, Hcy, and mean corpuscular volume (MCV). We compared the diagnostic performance of serum HoloTC and total vitamin B_{12} levels in assessing vitamin B_{12} status.

Materials and Methods

Subjects. We enrolled 128 patients diagnosed with gastric cancer who had undergone gastrectomy (55 women and 73 men). The mean age of the patients was 60.3 ± 12.1 yr (mean ± SD; range, 29-86 yr). The mean period after gastrectomy was 30.3 ± 24.0 mo (mean ± SD; range, 1-100 mo). We measured serum HoloTC, total vitamin B_{12}, homocysteine (Hcy), and complete blood cell count (CBC). We excluded the patients with estimated glomerular filtration rate (eGFR) calculated using the MDRD equation [13] <30 ml/min/1.73 m² because renal function may affect serum HoloTC and Hcy concentrations. We also analyzed serum samples of 100 healthy controls recruited during routine hospital visits and determined to have normal MCV.

Assays. Serum HoloTC concentration was measured using the AxSYM Active-B_{12} kit (Abbott Diagnostics, Abbott Park, IL, USA), which is a quantitative microparticle enzyme immunoassay (MEIA). Serum HoloTC binds to microparticles coated with mouse anti-HoloTC monoclonal antibody and alkaline phosphatase conjugated anti-TC antibody binds to HoloTC-anti-HoloTC complex. Antigen-antibody complex-bound alkaline phosphatase catalyzes the substrate, 4-methylumbelliferyl phosphate, yielding a fluorescent product, 4-methylumbelliferone. The fluorescence intensity is directly related to serum HoloTC concentration.

Total vitamin B_{12} was measured using the Architect B_{12} kit (Abbott) and serum Hcy was measured using the AxSYM homocysteine kit (Abbott). The cut-off values for abnormal were <189 pg/ml for total vitamin B_{12}, >14.05 umol/L for Hcy, and >95 fl for MCV. We designated serum total vitamin B_{12} concentrations of 189 - 400 pg/ml as a borderline range.

We evaluated the precision of HoloTC measurement by the AxSYM Active-B_{12} kit (Abbott), using 2 control materials according to CLSI EP-5A [14]. The control materials were assayed in replicates of 2 at 2 separate times/day for 10 days.

Statistics. Statistical analyses were carried out using MedCalc (v. 9.4.2.0, MedCalc Software, Mariakerke, Belgium) and Microsoft Excel 2003 (Microsoft Corp., Seattle, WA, USA) Statistical significance was defined as p < 0.05.

Results

Precision of serum HoloTC assay. The mean HoloTC value was 23.05 pmol/L for the low-level control and 54.56 pmol/L for the high-level control. CVs of within-run precision were 6.4% for the low-level control and 4.3% for the high-level control. The CVs of total precision were 9.4% and 7.9% for the low- and high-level controls, respectively.

Reference values for serum HoloTC levels. We measured serum HoloTC in 58 healthy males and 42 healthy females with an age range of 24-74 yr. Serum HoloTC concentrations were not normally distributed and were above the assay range (>128 pmol/L) in 43 people (Fig. 1). The lower limit of normal of HoloTC was 42.48 pmol/L, as defined by the central 95% of observations.

Serum HoloTC and vitamin B_{12}-related parameters in gastrectomized patients. In the sample of 128 gastrectomized patients, serum HoloTC was low in 32 (25.0%), and serum total vitamin B_{12} was low in 10 (7.8%) and borderline in 50 (39.1%). Serum Hcy was high in 40 patients (30.3%) and MCV was high in 35 patients (27.3%).

![Fig. 1.](image1)
Table 1. Distribution of serum HoloTC, serum homocysteine, and MCV levels according to serum total vitamin B12 concentrations in 128 gastrectomized patients.

<table>
<thead>
<tr>
<th>Total vitamin B12 (pg/ml)</th>
<th>Low (&lt;189)</th>
<th>Borderline (≥189 to &lt;400)</th>
<th>Normal (≥400)</th>
</tr>
</thead>
<tbody>
<tr>
<td>N</td>
<td>10</td>
<td>50</td>
<td>68</td>
</tr>
</tbody>
</table>

Low HoloTC (≤42.48 pmol/L)

<table>
<thead>
<tr>
<th>High Hcy (≥14.05 µmol/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>7 (70.0%)</td>
</tr>
<tr>
<td>21 (42.0%)</td>
</tr>
<tr>
<td>12 (17.6%)</td>
</tr>
</tbody>
</table>

High MCV (>95 fl)

| 0 (0.0%) |
| 20 (40.0%) | 15 (22.1%) |

The proportion of low HoloTC was 0%, 44%, and 100% in the patient groups with normal, borderline, and low total vitamin B12 concentrations, respectively. HoloTC was low in all patients with low total vitamin B12 and normal in all patients with normal total vitamin B12 (Table 1).

Among 50 gastrectomized patients with borderline total vitamin B12, 28 (56%) had normal HoloTC and 22 (44%) had low HoloTC (Fig. 2). Nine patients had high Hcy in addition to low HoloTC. Including 6 of these 9 patients, a total of 7 patients were clinically suspected of having vitamin B12 deficiency: 5 had decreased total vitamin B12 concentrations at follow-up, and 2 showed increased total vitamin B12 concentrations after vitamin B12 injection.

We grouped the patients based on total vitamin B12 concentration and HoloTC concentration and compared means of Hcy concentrations among the groups (Fig. 3). The mean Hcy concentrations were significantly different among the groups (p <0.001). Patients with both low total vitamin B12 and low HoloTC had significantly higher mean Hcy concentration than those with normal values for either total vitamin B12 or HoloTC, or both.
Patients with low HoloTC and normal total vitamin B₁₂ had an intermediate mean Hcy concentration.

We determined the correlation between each pair of vitamin B₁₂-related parameters. HoloTC was significantly but not strongly associated with total vitamin B₁₂ (r = 0.6591, p < 0.001). Both HoloTC and total vitamin B₁₂ were loosely associated with Hcy, although HoloTC showed better correlation with Hcy than total vitamin B₁₂ did (HoloTC: r = 0.4407, p <0.001; total vitamin B₁₂: r = 0.3599, p <0.001) (Fig. 4). Neither HoloTC nor total vitamin B₁₂ showed statistically significant association with MCV (HoloTC: p = 0.08; total vitamin B₁₂: p = 0.11).

Discussion

HoloTC has been measured by ELISA or RIA in previous studies; however these methods are not suitable to test large numbers of samples in usual clinical laboratory settings [15,16]. In this study, we measured HoloTC using a recently developed automated immunoassay method that can test a large number of samples in a short time and is more convenient than ELISA or RIA. This automated immunoassay showed good precision and the CV was comparable to measurements by RIA or ELISA [17]. HoloTC distribution in the healthy population was skewed, and many samples had HoloTC concentrations above the assay range (>128 pmol/L). The estimated lower reference interval of our study was 42.48 pmol/L, comparable with previous reports [15,18].

There was a difference between the prevalence of low HoloTC and that of low total vitamin B₁₂ in our study. HoloTC was low in 25% of gastrectomized patients, whereas total vitamin B₁₂ was low in only 7.8%. All patients whose total vitamin B₁₂ concentrations were low also had low HoloTC, but 22 patients (44%) of 50 patients whose total vitamin B₁₂ concentrations were in the borderline range had low HoloTC concentrations. Among these 22 patients with borderline total vitamin B₁₂ and low HoloTC concentrations, 7 patients were clinically suspected of having vitamin B₁₂ deficiency, and except for one patient, they had high Hcy concentrations. Considering these findings, vitamin B₁₂ deficiency cannot be ruled out if total vitamin B₁₂ concentration is in the borderline range, and HoloTC appears to be more sensitive than total vitamin B₁₂ in diagnosing vitamin B₁₂ deficiency.

When we compared serum Hcy concentrations with total vitamin B₁₂ concentrations and HoloTC concentrations, the patients with normal total vitamin B₁₂ but low HoloTC had higher Hcy concentrations than those with both normal total vitamin B₁₂ and HoloTC. Assuming that higher Hcy concentration reflects a greater likelihood of vitamin B₁₂ deficiency, this suggests that low HoloTC can be an indicator of vitamin B₁₂ deficiency. We also suggest that patients with low
HoloTC but with normal total vitamin B$_{12}$ might have an early stage of vitamin B$_{12}$ deficiency.

Total vitamin B$_{12}$ concentration may not reflect vitamin B$_{12}$ status because the HoloTC fraction comprises only 20% of total vitamin B$_{12}$ and most vitamin B$_{12}$ (80%) is bound to HC, the inactive fraction [19]. If total vitamin B$_{12}$ is in the borderline range, vitamin B$_{12}$ deficiency can be masked by increased HC-bound vitamin B$_{12}$ fraction, and further tests are recommended to rule out vitamin B$_{12}$ deficiency [20].

Falsely low total vitamin B$_{12}$ level was not a problem in our series of gastrectomized patients. All patients with low total vitamin B$_{12}$ had low concentrations of HoloTC in our study. However, Loikas et al [20] reported that 1.3% of patients with low total vitamin B$_{12}$ had normal HoloTC concentrations. Mild HC deficiency can cause total vitamin B$_{12}$ deficiency without altering vitamin B$_{12}$ function [21]. However, because the incidence of HC deficiency is low and vitamin B$_{12}$ supplementation does not cause toxicity, falsely low total vitamin B$_{12}$ does not present a significant problem in clinical settings.

HoloTC concentration was not well-correlated with Hcy concentration, although the correlation coefficient was greater than that of total vitamin B$_{12}$ and Hcy. Hvas et al [22] reported a greater correlation coefficient between HoloTC and Hcy than that of our study. This may be due to a difference in study populations. Hcy is a metabolite increased by vitamin B$_{12}$ deficiency and is considered to be an indicator of vitamin B$_{12}$ deficiency [23]. But many other factors affect plasma concentrations of Hcy, including folate deficiency, increased age, and abnormal renal function [5]. In our study, Hcy concentration was increased in all of the cases with extremely low HoloTC concentration ($\leq$15.06 pmol/L), but was variable in cases with >15.06 pmol/L HoloTC concentration. The higher mean age and wide age range of our population may be factors in our findings of high Hcy concentrations without low HoloTC concentrations, despite the exclusion of patients with low eGFR.

A limitation of this study is that we didn’t compare HoloTC with MMA, a sensitive metabolic marker of vitamin B$_{12}$ deficiency along with Hcy [18,23]. Moreover, this study was not designed to look for false positive HoloTC results. HoloTC seems to be sensitive in diagnosing vitamin B$_{12}$ deficiency but studies are needed to evaluate the diagnostic specificity of HoloTC determinations.

In summary, we investigated for the first time the distribution of serum HoloTC concentrations in patients who had undergone gastrectomy to treat gastric cancer, and we compared the serum values of HoloTC with total vitamin B$_{12}$ and Hcy. Our results suggest that serum HoloTC is more sensitive than total serum vitamin B$_{12}$ to detect vitamin B$_{12}$ deficiency, and is therefore an effective diagnostic marker for vitamin B$_{12}$ deficiency.

References