Relationship Between the Levels of Holotranscobalamin and Vitamin B₁₂ in Children

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Abstract The purpose of this study was to evaluate the relationship between the plasma holoTC and serum vitamin B₁₂ in children and to identify a cutoff cobalamin values according to holoTC. One hundred and fifty-five children were enrolled into the study. All children were evaluated for hemoglobin, vitamin B₁₂, folate, ferritin and holoTC levels. Children were grouped as with low vitamin B₁₂ level (≤ 200 pg/mL, group I) and normal vitamin B₁₂ (> 200 pg/mL, group II). Serum vitamin B₁₂, and holoTC levels were performed in each patient in the study. In 101 patients with low vitamin B₁₂ (group I) the mean holoTC was 21.74 ± 1.14 pmol/L. In 54 children with normal vitamin B₁₂ (group II) mean holoTC was 44.0 ± 2.7 pmol/L ($p < 0.01$). A ROC curve analysis was performed to delineate the optimum cut-off point for vitamin B₁₂ level and it was found to be 165 pg/mL with a sensitivity of 70% and specificity of 74%; the area under curve was 0.783 ($p < 0.01$). Our study displayed a positive correlation between vitamin B₁₂ and holoTC, and defined an optimum cutoff value for vitamin B₁₂ as 165 pg/mL. Further studies using the markers both MMA, tHcy and holoTC to confirm the findings are needed.

Keywords Vitamin B₁₂ · Holotranscobalamin · Children

Abbreviations

HoloTC	Holotranscobalamin
MMA	Methylmalonic acid
tHcy	Total plasma homocysteine

Introduction

Vitamin B₁₂ (cobalamin) is essential vitamin for many cellular metabolisms, particularly DNA synthesis and regulation, and plays a role in cell division and proliferation. Its deficiency may cause subtle developmental delay to severe life threatening conditions [1]. There is no single marker, which can reliably diagnose vitamin B₁₂ deficiency. Total serum vitamin B₁₂, quantitates both the inactive forms (transcobalamin I- and transcobalamin III-bound) and active form (transcobalamin II-bound), may not accurately reflect vitamin B₁₂ status and also low vitamin B₁₂ levels may not always reflect real vitamin B₁₂ deficiency. On the other hand some neuropsychiatric and metabolic abnormalities can occur within normal vitamin B₁₂ reference interval [2]. Most low concentrations might be considered to be meaningless and artificial if classical signs like megaloblastic anemia or neuropsychiatric symptoms do not accompany it. Furthermore various cutoff levels for vitamin B₁₂ indicating deficiency make a diagnostic challenge.

In vitamin B₁₂ deficiency, the concentration of both total plasma homocysteine (tHcy) and methylmalonic acid (MMA) increase while in folate deficiency only tHcy concentration elevated. Elevated concentrations of MMA are the most reliable marker of metabolic vitamin B₁₂ insufficiency, and the best diagnostic combination is

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currently serum vitamin B₁₂ and MMA assays [1, 3, 4]. However poor assay availability limits MMA assay's clinical utility. To get more specific and sensitive method for diagnosis of vitamin B₁₂ deficiency, the measurement of holotranscobalamin II (holoTC), the cobalamin-TC complex, has become available. HoloTC as a biologically available, active vitamin B₁₂ fraction promotes a specific uptake of its vitamin B₁₂ by all cells [5, 6]. Although there are several reports including cobalamin, holoTC, MMA and tHcy association in cobalamin deficiency, those study groups are mostly elderly subjects [7]. The aim of this study is to evaluate the relationship between the plasma holoTC and serum vitamin B₁₂ in children and to identify a cutoff cobalamin values according to holoTC. To our knowledge there is only one study in the literature that is assessed the association between plasma holoTC and cobalamin in children [8].

Materials and Methods

In this prospective study, 155 children were investigated for serum vitamin B₁₂ and holoTC relationship in pediatric hematology department. One hundred and one children whom admit to our pediatric hematology clinics for the evaluation of low vitamin B₁₂ level (≤ 200 pg/mL) (group I) and 54 children whom admit to our pediatric hematology clinics with normal serum vitamin B₁₂ > 200 pg/mL (group II) were included in this study. Samples for plasma holoTC analysis were collected at the same day of serum vitamin B₁₂ assay and stored at -80 °C until analysis. Hemoglobin and mean corpuscular volume (MCV) were determined using Beckman COULTER[®] GEN-S system (COULTER[®]-Corp, Miami, FL, USA). Ferritin and folic acid levels were estimated by immunoassay system using Unicel DxI 800 Access Beckman COULTER[®] (Access Ferritin Reagent and Access Folate Reagent, Beckman Coulter, Ireland), US. Study procedures were approved by the Ethics Committee of

Ankara Children's Hematology and Oncology Hospital. Informed consent was obtained from all individual participants and/or parents included in the study.

Serum vitamin B₁₂ levels were analyzed by competitive-binding immunoassay on Beckman Coulter DXI 800.

Plasma holoTC was measured by microparticle enzyme immunoassay on AxSYM analyzer (Abbott, USA). The cutoff values of the holoTC were determined on the values reported in the manufacturer's book and 19.1–119.3 pmol/L were accepted as normal [6].

Statistical analyses were performed with Statistical Package for Social Science-SPSS version 17 software (SPSS Inc, Chicago, USA). Mann–Whitney U test was performed to compare the means of data. Spearman correlation analysis and ROC curve were used. A p value < 0.05 was considered statistically significant.

Results

The mean age of the patients was 11.59 ± 4.76 years in the low vitamin B₁₂ group and 9.15 ± 4.58 years in the normal vitamin B₁₂ group. Eighty-six of the patients were male and 69 of patients were female. Laboratory results of the patients are presented in Table 1. Folic acid levels were within normal range in both groups.

In 101 patients with low vitamin B₁₂ the mean holoTC was 21.74 ± 11.5 pmol/L, the minimum and the maximum values were 7.45 and 77.49 pmol/L, respectively. In 54 children with normal vitamin B₁₂ mean holoTC was 44.0 ± 27.3 pmol/L (min–max: 8.9–128 pmol/L). There was a statistically significant difference between two groups ($p < 0.01$). A significant positive correlation between serum vitamin B₁₂ and holoTC levels was found ($r = 0.648$; $p < 0.01$).

A ROC curve analysis was performed to delineate the optimum cut-off point for vitamin B₁₂ level taking the

Table 1 Laboratory data of patients

	Group I (n:101) (Vit B ₁₂ ≤ 200 pg/mL)	Group II (n:54) (Vit B ₁₂ > 200 pg/mL)	p value
Vitamin B ₁₂ (pg/mL)	149.70 ± 28.35^a (78.0–200.0) ^b	345.92 ± 166.76 (205.0–864.0)	$< 0.01^*$
HoloTC (pmol/L)	21.74 ± 1.14 (7.45–77.49)	44.0 ± 2.7 (8.93–128.0)	$< 0.01^*$
Folate (ng/mL)	7.32 ± 3.45 (2.45–20.0)	8.02 ± 3.55 (3.27–20.0)	0.16
Hemoglobin (g/dL)	12.88 ± 1.79 (6.3–17.5)	12.40 ± 1.89 (7.2–17.2)	0.15
MCV (fL)	83.25 ± 8.08 (53.5–101.5)	77.80 ± 12.58 (48.5–121.8)	$< 0.01^*$
Ferritin (ng/mL)	21.28 ± 18.6 (1.1–1633.0)	24.79 ± 20.56 (3.9–1741)	0.30

HoloTC Holotranscobalamin, MCV mean corpuscular volume

* $p < 0.05$, statistically significant (Mann–Whitney U)

^a Mean $\pm$ standard deviation

^b Minimum and maximum values

Table 2 Cut-off points for vitamin B₁₂ level taking the normal range holoTC as reference for the ROC curve

Total vitamin B ₁₂ (pg/mL)	Sensitivity	Specificity
100	100	13
130	95	30
140	90	36
146	85	45
152	80	50
160	75	64
165	70	74

Table 3 HoloTC classification according to total vitamin B₁₂ levels

Total vitamin B ₁₂ (pg/mL)	Low HoloTC n (%)	Normal HoloTC n (%)	Total n
≤200	56 (55.4)	45 (44.5)	101
>200	5 (9.2)	49 (90.7)	54
≤165	45 (61.6)	28 (38.3)	73
>165	14 (17.1)	68 (82.9)	82

normal range holoTC as reference. The optimum cut-off point for vitamin B₁₂ level was found to be 165 pg/mL with a sensitivity of 70% and specificity of 74%; the area under curve was 0.783 ($p < 0.01$). Table 2 lists cutoff points for the ROC curve.

In 101 patients with low serum vitamin B₁₂, 55.4% patients had low serum holoTC, however in 44.6% holoTC was within normal range. Also in 54 children who had normal vitamin B₁₂, 87% had normal holoTC (Table 3). If vitamin B₁₂ cutoff value was accepted as 200 pg/mL, positive predictive value was 91.66% and negative predictive value was 50%. However if the cut-off point was accepted as 165 pg/mL positive predictive value was 76.2% and negative predictive value was 93.1%.

Discussion

Vitamin B₁₂ deficiency is considered if serum cobalamin levels are <200 pg/mL (<148 pmol/L), or <250 pg/mL with some assays [9]. Several patients with low vitamin B₁₂ concentration do not exhibit any clinical or biochemical evidence of deficiency [10]. The sensitivity of cobalamin concentration <200 pg/mL usually exceeded 95% in patients with megaloblastic anemia [11]. However it declines to 38–39% in nonclinical population compared against gold standard MMA or tHcy [12].

HoloTC II, the TCII fraction with attached cobalamin delivers cobalamin to all tissues. The vitamin B₁₂ associated with TC (holoTC) represents the functionally important fraction of plasma vitamin B₁₂. Some authors

interpreted holoTC abnormality as the earliest marker of cobalamin deficiency because it occurs in patients with normal concentrations of all other biomarkers [7, 13, 14].

Our objective was to evaluate the correlation between serum vitamin B₁₂ and plasma holoTC levels in children with vitamin B₁₂ deficiency and our study displayed a positive correlation between vitamin B₁₂ and holoTC. Rogers et al. [8] reported that plasma holoTC concentration has been correlated strongly with serum cobalamin, however less strongly with MMA and tHcy. Moreover holoTC levels of in our study group with vitamin B₁₂ deficiency were found to be similar as in cobalamin deficient elderly patients [15]. We also defined the optimum cut-off point for vitamin B₁₂ level as 165 pg/mL with a sensitivity of 70% and specificity of 74%. If vitamin B₁₂ cutoff value was accepted as 200 pg/mL, positive predictive value was 91.66% and negative predictive value was 50%. However if the cut-off point was accepted as 165 pg/mL positive predictive value was 76.2% and negative predictive value was 93.1%.

The main poverty of our study is lack of functional biomarker like serum MMA or tHcy assays. Measurement of MMA unfortunately could not perform routinely many countries like Turkey. Although measurement of tHcy is less expensive and easier to perform, is not as specific for vitamin B₁₂ status as MMA. In addition, tHcy levels influence from many conditions like folate and other vitamin B deficiencies, renal dysfunction and alcohol abuse [10]. In addition, MMA and tHcy were not elevated in some cases with both low serum Cbl and holoTC, like patients with pernicious anemia or iron deficiency [9, 16]. We planned to conduct further studies using the markers both MMA, tHcy and holoTC to confirm the findings in the current study.

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Compliance with Ethical Standards

Conflict of interest The authors declare no conflict of interest.

Ethical Approval All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

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