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THE IMPORTANCE OF HOLOTRANSCOBALAMIN MEASUREMENT

ZNAČAJ MERENJA HOLOTRANSKOBALAMINA

Velibor Čabarkapa, Zoran Stošić, Radmila Žeravica, Branislava Ilinčić, Ana Filipović

Institute of Laboratory Medicine, Clinical Center, Novi Sad, Serbia

Summary: Clinically significant vitamin B_{12} deficiency can occur even with total vitamin B_{12} levels apparently within normal range. There is an indeterminate zone between approximately 154 and 300 pmol/L of vitamin B_{12} where there is likely misclassification of B_{12} status if relying on total serum B_{12}. The aim of the present study was to assess the usefulness of holotranscobalamin in diagnosis of B_{12} deficiency. Blood samples were collected and subjected to assays for vitamin B_{12} and holotranscobalamin. We examined the levels of holotranscobalamin in 32 subjects (n=32, f=18, m=14) with vitamin B_{12} values within interval 154–300 pmol/L. These subjects were compared with control group with vitamin B_{12}>300 pmol/L (n=31, f=17, m=14). 25% of subjects with vitamin B_{12} of 154 to 300 pmol/L had low levels of holotranscobalamin. Holotranscobalamin levels of patients with vitamin B_{12} of 154 to 300 pmol/L were significantly lower than those of control subjects (38.55 ± 23.0 vs. 61.35 ± 31.81 pmol/L, p<0.01). The obtained results also show a positive significant relationship between levels of holotranscobalamin and vitamin B_{12} (r=0.53, p<0.01). In conclusion, holotranscobalamin is a better indicator of early vitamin B_{12} deficiency than total serum cobalamins and it is a useful tool in the diagnosis of vitamin B_{12} deficiency.

Keywords: vitamin B_{12}, holotranscobalamin

Kratak sadržaj: Klinički značajan deficit vitamina B_{12} može da se pojavi i pri normalnim vrednostima vitamina B_{12}. Postoji jedna nedefinisana zona gde su vrednosti vitamina B_{12} 154–300 pmol/L, i gde je moguće pogrešno proceniti status vitamina B_{12} oslanjajući se samo na vrednosti ukupnog serumskog B_{12} vitamina. Cilj ove studije je da se utvrdi značaj određivanja holotranskobalamina u dijagnostici deficita vitamina B_{12}. U uzorcima krvi analizirani su vitamin B_{12} i holotranskobalamin. Utvrđen je nivo holotranskobalamina kod 32 ispitanika (n=32, ž=18, m=14) sa vrednostima vitamina B_{12} od 154–300 pmol/L. Ovi ispitanici su upoređeni sa kontrolnom grupom kod koje je vitamin B_{12}>300 pmol/L (n=31, ž=17, m=14). 25% ispitanika sa vitaminom B_{12} između 154 i 300 pmol/L je imalo hipoholotranskobalaminemiju. Nivo holotranskobalamina kod grupe ispitanika sa vrednostima vitamina B_{12} unutar intervala 154–300 pmol/L bio je značajno niži od nivoa u kontrolnoj grupi (38,55 ± 23,0 vs. 61,35 ± 31,81 pmol/L, p<0,01). Dobijeni rezultati takođe pokazuju pozitivnu, statistički značajnu korelaciju između holotranskobalamina i vitamina B_{12} (r=0,53, p<0,01). Može se zaključiti da je holotranskobalamin bolji indikator ranog deficita B_{12} vitamina nego ukupni serumski kobalamin i da predstavlja korisno sredstvo u dijagnostici deficita vitamina B_{12}.

Ključne reči: vitamin B_{12}, holotranskobalamin

Introduction

A number of rapid fully-automated B_{12} assays are now used widely (1). The assay of total serum B_{12} remains the first-line investigation in the assessment of B_{12} status (2), but many patients with low cobalamin levels are not cobalamin-deficient (i.e. have »false« low values), while significant clinical impairment may occur despite normal cobalamin values (i.e. »false« high values) (3, 4). »False« low values are accompanied with folate deficiency, pregnancy, oral contraceptives, multiple myeloma, HIV infection, low haptocorrin levels. »False« high/normal values are accompanied with high haptocorrin levels, myeloproliferative disorders, renal disease, increased tissue release of cobalamin, liver disease, low/absent cobalamin, low affinity transcobalamin polymorphisms, inherited disorders of cobalamin metabolism, recent cobalamin therapy, high dose vitamin C. The measurement of serum vitamin B_{12} has the following limitations: it

Address for correspondence:
Dr Velibor Čabarkapa
Institute of Laboratory Medicine
Clinical Center-Novi Sad
Hajduk Veljkova 1–9
21000 Novi Sad, Serbia
e-mail: veliborcabarkapa@nspoint.net

measures total, not metabolically active B_{12}, the levels are not clearly correlated with clinical symptoms, there is a large »grey zone« or indeterminate range between normal and abnormal levels, and clinically significant vitamin B_{12} deficiency can occur with total vitamin B_{12} levels apparently in the normal range.

Clinical signs and symptoms of cobalamin deficiency include megaloblastic anemia, paresthesias and neuropathy, and psychiatric symptoms such as irritability, dementia, depression, or psychosis. While hematological abnormalities disappear promptly after treatment, neurologic disorders may become permanent if left untreated.

Tests for cobalamin deficiency include measurements of (a) total cobalamin; (b) methyl malonil acid and homocysteine, as indices of functional deficiency; and (c) holotranscobalamin as a measure of the metabolically active fraction of circulating cobalamin.

Vitamin B_{12} (cobalamin) in serum is bound to two proteins, transcobalamin and haptocorrin. The transcobalamin-cobalamin complex is called holotranscobalamin. Only 6–20% of the cobalamin in serum is bound to transcobalamin II; the remainder is bound to transcobalamin I (haptocorrin I) whose function is uncertain (2). Transcobalamin II is a plasma protein that binds cobalamin and facilitates the cellular uptake of cobalamin by receptor-mediated endocytosis. Transcobalamin II, a non-glycosylated secretory protein of molecular mass 43 kDa, and its plasma membrane receptor are essential components of plasma cobalamin transport into all cells.

Humans are unable to synthesize cobalamin: *de novo* synthesis seems to be restricted exclusively to some bacteria (5), and so exogenous cobalamin must be efficiently absorbed in the intestine and then transported to cells (6).

Cobalamin is first released from proteins through peptic digestion in the stomach, and then bound to the protein ligands haptocorrins or transcobalamin I. These haptocorrins are digested by pancreatic proteases in the duodenum, thus allowing the subsequent binding of cobalamin to intrinsic factor. Finally, intrinsic factor-cobalamin complex binds to the receptor on the brush border of the ileal enterocytes and undergoes endocytosis. This receptor is known as cubilin (7, 8). Enterocyte uptake of the intrinsic factor-cobalamin complex is followed by the degradation of intrinsic factor, after which vitamin binds transcobalamin II transport protein and is finally secreted in this form into plasma. Cells other than enterocytes can only take up the cobalamin bound to transcobalamin (holotranscobalamin). About 80% of total cobalamin in serum is bound to haptocorrin and is not available to the cells. Transcobalamin does not deliver cobalamin to tissues. The holotranscobalamin enters the portal vein and is rapidly recognized by, and bound to, specific receptors distributed on the cell surfaces of a variety of organs. Cobalamin is first dissociated from transcobalamin II at low pH existing in lysosomes, then reduced, and finally converted to the two coenzyme forms (9).

Transcobalamin II deficiency leads to disturbed function of the two cobalamin requiring enzymes, methylmalonil coenzyme A mutase and methionine synthase, and to methylmalonic aciduria and homocystinuria. The aim of the present study was to assess the usefulness of holotranscobalamin in the diagnosis of B_{12} deficiency and the incidence of true hypoholotranscobalaminemia in a group of patients with vitamin B_{12} values within 154–300 pmol/L.

Material and Methods

In this cross-sectional study sixty three subjects (random sample) from different departments of the Clinical Centre Novi Sad (35 females and 28 males) were studied. Thirty subjects were hematological patients, fourteen ambulatory patients, ten gastroenterologic patients, three nephrologic, two endocrinologic, two surgical and two neuropsychiatric patients. They were examined on the basis of the vitamin B_{12} status. These subjects were divided into two groups: first group–subjects with vitamin B_{12} values within interval 154–300 pmol/L (n=32, 18 females and 14 males, mean age 45.57±17.2 years), and control group–subjects with vitamin B_{12} value above 300 pmol/L (n=31, 17 females and 14 males, mean age 54.7±13.9 years). After the vitamin B_{12} measurement, we measured the holotranscobalamin values in all patients.

Vitamin B_{12} and holotranscobalamin were measured by microparticle enzyme immunoassay–MEIA (AxSYM analyzer, Abbott reagents). Axsym holotranscobalamin direct binding assay is based on two well-characterised binders: mouse mAb to holotranscobalamin (that does not recognise transcobalamin) and mouse mAb to transcobalamin. The assay directly quantitates holotranscobalamin and avoids all pretreatment steps common to all B_{12} assays.

Statistical analyses were performed using Microsoft Office Excel program package 2003. Results are expressed as mean ± S.D. The following statistic methods were performed: f-test, t-test, correlation and single linear regression.

Results

Group of patients with vitamin B_{12} values within the range 154–300 pmol/L had mean plasma B_{12} value 215.03±38.23 pmol/L and mean holotranscobalamin level 38.55±23.0 pmol/L. Control group of patients with vitamin B_{12}>300 pmol/L had mean plasma B_{12} value 451.83±139.73 pmol/L and mean holotranscobalamin level 61.35±31.81 pmol/L. Blood

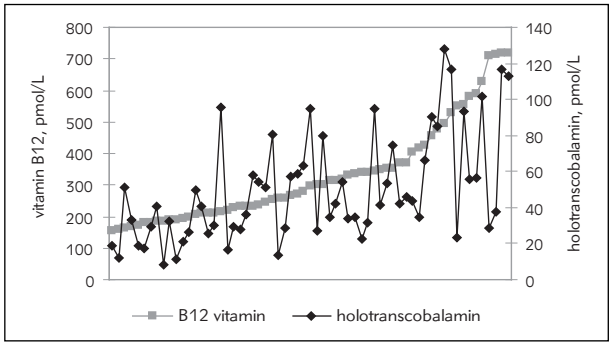

Figure 1 Correlation of vitamin B_{12} and holotranscobalamin ($r = 0.53$, $p<0.01$).

holotranscobalamin level of subjects with vitamin B_{12} values within 154–300 pmol/L were significantly lower than those of controls ($p<0.01$).

Twenty five percent of patients with vitamin B_{12} value within 154–300 pmol/L had a low level of holotranscobalamin. All subjects in the control group had normal values of holotranscobalamin.

Serum vitamin B_{12} and holotranscobalamin levels showed a statistically significant positive correlation by linear regression ($r=0.53$, $p<0.01$).

Age of subjects and holotranscobalamin levels did not show statistically significant correlation.

Discussion

Vitamin B_{12} deficiency may take decades to develop, and affected patients may be asymptomatic or may present with a wide spectrum of hematologic and neuropsychiatric manifestations (10). Herbert (11) outlined four stages in the development of vitamin B_{12} deficiency. Stages 1 and 2 represent the condition in which the biochemical depletion of vitamin B_{12} occurs prior to any obvious clinical damage; serum levels of B_{12} may still be normal. Stages 3 and 4 represent the condition of true deficiency with obvious metabolic components, clinical components, or both; serum B_{12} levels are low. Any hematological and neurologic features tend to occur in the later states. True challenge is to identify vitamin B_{12} deficiency in a preclinical stage, when treatment can avert complications. Early diagnosis of vitamin B_{12} deficiency is crucial because of the latent nature of this disorder and the possible risk of irreversible neurological damage (12). The plasma vitamin B_{12} concentration does not reliably rule out vitamin B_{12} deficiency. Thus, the finding of a normal serum B_{12} level does not completely exclude the possibility of B_{12} deficiency.

In plasma, vitamin B_{12} is bound to two proteins, haptocorrin and transcobalamin. Vitamin B_{12} attached to transcobalamin, holotranscobalamin, represents the biologically active fraction that can be delivered into all DNA-synthesizing cells. Therefore, measurement of holotranscobalamin has for years been suggested as a sensitive marker of vitamin B_{12} deficiency, and this measurement would be expected to provide a more reliable measure of B_{12} availability to tissues than total serum B_{12}. Deficit of transcobalamin II results in failure of immunoglobulin production, megaloblastic anemia, granulocytopenia, thrombocytopenia, and intestinal villous atrophy, all correctable with vitamin B_{12} therapy. However, the clinical usefulness of holotranscobalamin has not yet been evaluated thoroughly, and only limited knowledge exists of other factors influencing the level of holotranscobalamin (12).

Cobalamin deficiency occurs in the 3% to 40% of the general population (13–15). Dhamarajan et al. (16, 17) in studies of community, hospital, and nursing home subjects found the prevalence to be 15% to 25%. In our study, in subjects (random sample) with vitamin B_{12} values within the range 154–300 pmol/L we found 25% in which we can expect functional cobalamin deficiency because of their holotranscobalamin deficiency. This deficiency of holotranscobalamin occured in subjects with vitamin B_{12} level between 154.9–259.8 pmol/L. In study of Hvas (12) from 937 individuals with increased methyl malonic acid, in 242 (26%) the concentration of holotranscobalamin was below the reference interval. In Herrmann and Obeid (18, 19) studies with vitamin B_{12} risk population they found low levels of holotranscobalamin in 22% of elderly and 12% of subjects with normal diet. In subjects with $B_{12}>300$ pmol/L we did not find hypoholotranscobalaminemia. The holotranscobalamin mean values of these two groups were significantly different. For this reason, we considered additional holotranscobalamin measurement efficient only in subjects with borderline total vitamin B_{12}.

The concentration of holotranscobalamin in our study was significantly associated with plasma vitamin B_{12} levels ($r=0.53$, $p<0.01$). That is slightly lower than in other studies–Hvas (12) found stronger association $r=0.71$, $p<0.001$, Loikas et al. (20) found $r=0.80$, $p<0.0001$.

We did not find a significant correlation between aging and the level of holotranscobalamin.

The present study indicates that holotranscobalamin shows promise as a first-line test for excluding vitamin B_{12} deficiency, and that holotranscobalamin is a more sensitive marker in the diagnosis of this deficiency when compared with plasma vitamin B_{12}, especially for subjects with borderline vitamin B_{12} concentrations (154–300 pmol/L).

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