

Clinical follow-up of children with high vitamin B12 values: should we worry?

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ABSTRACT

Background. Requests of Vitamin B12 test increased with the widespread use of autoanalysers. Although the cause of requests was deficiency suspicions, an important ratio of high levels of Vitamin B12 were reported to physicians by laboratory. Ratios of values of high Vitamin B12 among test request in adults are reported as 14-20% in present three monocentre studies and one multicentre study. There is no report on children with high vitamin B12 for both ratio in lab requests or clinical follow up.

Methods. We evaluated the records of 40 children (23 male /17 female) with high B12 values (>1000 pg/ml) retrospectively. Children were otherwise healthy children and were seen at outpatient pediatric clinics. Additionally, vitamin B12 values of 13 acute lymphoblastic leukemia patients at diagnosis time were retrieved to enlighten possible role of lymphocytes.

Result. Children did not have any malign or chronic diseases causing the high Vitamin B12 values. Holotranscobalamin levels were normal or slightly above. Two patients did develop leukemia later. Our follow up showed that high vitamin B12 values slightly decreased at 3 months and then remained unchanged later. The high numbers of T and B cells are not the source of vitamin B12 elevation.

Conclusions. Our study suggests that high-vitamin B12 values are usually benign in children but some patients may develop leukemia later. We suggest that patients should be followed up for some time after testing for severe hematological diseases.

Key words: vitamin B12, cobalamin, children, pediatric, holotranscobalamin.

Vitamin B12 (cobalamin, Cbl) is one of the most important vitamins that can be dissolved in water. It is absorbed in terminal ileum, transported by haptocorrins and stored in the liver. It is involved in methyl transfer and nucleotide synthesis. In children, deficiency leads to megaloblastic anemia and affects neurologic development. The importance of deficiency in children is well understood. There are many studies about vitamin B12 deficiency.¹⁻³

The widespread use of autoanalysers in hospital laboratories has made vitamin B12 testing,

like many tests, widespread, inexpensive, fast and accessible everywhere. Thus, all doctors, especially pediatricians, internist and neurologists, have started to request vitamin B12 tests from every patient in which the deficiency might have an impact or relation. Important data have accumulated in laboratory recording systems due to the increased requests. Analysis of these data showed that a significant proportion of the vitamin B12 tests were higher than normal.¹⁻³ Thus, a new research area related to vitamin B12 levels was opened. The new questions are what is the meaning of this high vitamin B12 (hCbl) level found incidentally high in a patient and how to manage such a patient. These questions are very important, because the high levels of Cbl are seen in malign or proliferative diseases of myeloid and lymphoid

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series diseases such as promyelocytic leukemia, chronic myeloid leukemia (CML), autoimmune lymphoproliferative syndrome (ALPS), systemic inflammatory syndrome, polycythemia vera, hypereosinophilic syndrome, solid cancers, liver diseases such as acute hepatitis, cirrhosis, hepatocellular carcinoma, kidney disease, autoimmune disease and some infection diseases such as HIV and malaria.¹⁻⁴

Elevation of Cbl may be one finding of these diseases or is likely to be the first presenting finding of a developing disease. This possibility makes it clinically important.

The ratio of hCbl among vitamin B12 samples that reached the laboratory is large enough to attract attention. Its ratio is 14%, 18.5% and 20% in three monocentre studies, and 18.5% a multicentre study. They also examined the relationship between high levels of Cbl with holotranscobalamin levels in the blood samples of these patients. They reported that 1% of samples are from patients with myeloproliferative disease.⁴⁻⁷ However, there is no report on clinical follow up of adult or child patients with high vitamin B12 values.

The management of children with a hCbl value is still an important and unanswered question. There is no publication in children in terms of laboratory and clinical short and long-term follow-up. There is no report on clinical follow up of adult or child patients with hCbl values.

In this article, we present clinical and laboratory follow-up of patients who were referred to a pediatric hematology outpatient clinic by pediatricians because of vitamin B12 elevation. We also point to possible causes of hCbl and research subject areas for patients with hCbl levels.

Material and Methods

The records of 40 children with high Cbl value (>1000 pg/ml) were evaluated retrospectively. These patients were seen in our outpatient pediatric hematology clinic in the years of 2017-2020. Most of them were referred

from other pediatric outpatient clinics for hematologic evaluation. The vitamin B12 levels had been requested during routine outpatient examinations for suspicion of deficiency. After history and physical examination, vitamin B12 (Normal value 200-800 pg/ml), holotranscobalamin (Normal value 37-171 pmol/L), hemogram, ferritin, iron, ALT, AST, creatinine, folate values and abdominal ultrasonography of the patients were examined. They were also followed-up for any disease related with hCbl level such as myeloproliferative or lymphoproliferative disease. Cbl levels were measured initially and at third and sixth month. The patients were followed up clinically between 1 and 3 years.

In addition, vitamin B12 levels in 13 patients with acute lymphoblastic leukemia (ALL) having high vitamin B12 level at diagnosis were retrieved to discuss the possible lymphocyte origin of this high level.

Categorical data were expressed as number and percentage (%). Scale variables were expressed as mean± standard deviation (SD) and range. Student's t test was used for comparison of means. Categorical variables were compared with Chi-Square. P<0.05 was assessed as statistically significant. The statistical relationship between two variables was calculated by Pearson Correlation analysis.

Results

The records of 40 children with hCbl value (>1000 pg/ml) were evaluated retrospectively. Twenty three patients (58%) were male. The average age of patients was 3.12±1.43 year. Initial vitamin B12 are 1267.89±382.01 pg/ml and vitamin B12 in the third month are 1162.09±381.96 pg/ml, in the sixth month are 1169.64±351.74 pg/ml.

Demographic and laboratory findings are represented in Table I. Cbl levels decreased slightly in the third month. Difference of Cbl values between initial and in third month and sixth month was p=0.03 and statistically significant. But difference of values between

Table I. Demographic and laboratory findings of children with high Cobalamin (Cbl) values.

	Mean	SD	Range
Male/Female	23/17		
Age (year)	3.18	±2.11	1-6.5
Hemoglobin (g/dL)	12.11	±0.88	9.6-13.6
White Blood Cell (x10 ⁹ /L)	9.52	±3.53	3.75-15.49
Lymphocyte (x10 ⁹ /L)	4.63	±1.59	2.65-8.64
C-reactive protein (mg/L)	0.23	±0.4	0.01-1.39
Ferritin (ng/ml)	40.74	±17.6	9-77
Holotranscobalamin (pmol/L)			
(normal values 25-165 pmol/L)	147.07	±60.06	82.80-630
Initial Cbl (pg/ml)	1267.89	±382.01	1000,9-2001
At third month Cbl (pg/ml)	1162.09	±381.96	606.8-2000
At sixth month Cbl (pg/ml)	1169.64	±351.74	466.2-2000

third and sixth month was not significant ($p>0.05$). Holotranscobalamin levels are 147,07±60,06 pmol/L (normal values 25-165). Holotranscobalamin levels were normal %67 and slightly above in 33%. Correlations between vitamin B12 and holotranscobalamin levels was $r=0.24$. There was no correlation between vitamin B12 and white blood cells, neutrophil, lymphocyte, platelet, hemoglobin and ferritin levels.

The follow-up period of the patients lasted from one year to three years. Two patients developed leukemia at follow-up. The first patient was a 2-year-old female patient and she developed ALL after two months of first admission. The second patient was a 6 year old male and he developed chronic myeloid leukemia (CML) after two years of the first hCbl level.

Characteristics of children with ALL are given in Table II. Cbl levels of ALL patients were normal range excluding one B cell ALL patient whose vitamin B12 level was 1154 pg/ml. In two patients with delta-gamma T cell ALL, Cbl levels were normal.

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Table II. Characteristics of children with acute lymphoblastic leukemia.

Male/female	6/7
T cell	2
B cell	9
deltagamma T cell	2
Mean cobalamin±SD (pg/ml)	504.59±333.91
Median cobalamin, range (pg/ml)	429.4 (166.2-1154)
Mean White Blood Cell ±SD (x10 ⁹ /L)	30.35±52.57
Median White Blood Cell, range (x10 ⁹ /L)	10.9 (1.03-184)

Discussion

There is no report on clinical follow up of adult or child patients with Cbl values, although clinicians need to frequently decide about these patients. There is not enough publications about the meaning, importance, management and reasons of hCbl in a person who looks healthy in other respects and does not use vitamin B12 drugs. Our study is the first study to show what can be seen with clinical follow-up of patients. It has the potential to trigger other clinical studies.

Clinicians have been encountering more patients with hCbl results in the last decades. Patients and parents read the reasons for the hCbl from the internet and come to the physician with true or false information, fear and questions. Fear is due to the fact that the hCbl is associated

with many hematological and chronic diseases such as juvenile myelomonocytic leukemia, CML, polycythemia vera, hypereosinophilic syndrome, chronic lymphocytic leukemia, acute leukemias, lymphomas, myelodysplastic leukemia, autoimmune lymphoproliferative syndrome, liver and kidney diseases.¹⁻³ They want satisfying and detailed information. Patients live through a referral chain starting from their own doctor through a pediatrician to pediatric hematologist.

There have been many developments in recent years that have caused us to see more patients with hCbl. This increased our need for a management algorithm. We know better the relationship of Cbl deficiency and diseases.^{1-3,8} Ideas are being developed about how vitamin B12 deficiency changes the clinics of other diseases. Autoanalysers offer fast, inexpensive, and results available on the same day and then we can use these results to decide patient treatment. Doctors are very concerned about the consequences of Cbl deficiency and use auto analyzer facilities abundantly. They request measurement of plasma Cbl for their patients and intend to find a deficiency. But, see both high and low Cbl test results.^{4,7} Our cases are also determined by test request for deficiency suspicion.

To understand the cause, significance, possible side effects and possible new research areas of Cbl elevation, we must understand the structure, synthesis by bacteria and archaea, transporting to human by food chain.⁹⁻¹²

Cbl is a haptocorrin compound with cobalt in its center. Only a limited number of prokaryotes (some bacteria such as *Pseudomonas denitrificans* and archaea such as *Thermosiphon africanus*) can synthesize cobalamins. These prokaryotes are a part of intestinal microbiota. Other living things take cobalamins from bacteria and archaea by a food chain. In the intestine, the synthesis of this molecule is by two pathways.¹³ The first path is anaerobic synthesis and studied in *Salmonella typhimurium*, the second pathway is aerobic synthesis and studied in *Pseudomonas denitrificans*.^{14,15}

If the bacteria in our gut synthesize Cbl, why is Cbl deficiency occurring? Cbl, are synthesized by the bacteria and archaea, but it remains in the living bacteria until it dies.

Most of bacteria are found in colons and it is after terminal ileum where the Cbl receptors are located.⁹⁻¹³ This is an important detail because the host may not absorb Cbl in its own bacteria. For this reason, animals usually do not use these sources except that very small part.

In humans, Cbl -containing proteins are hydrolyzed in the stomach and intestine. Free Cbl now binds to intrinsic factor secreted by parietal cells of the stomach. Cbl and intrinsic factor complex bind to cubulin receptors of epithelia of terminal ileum and enters epithelia cell by active transport by contributions of amnionless, megalin, serum paraoxonase/arylesterase and receptor-associated-protein.¹⁴ In addition, 1% of an oral vitamin B12 is also passively absorbed. Then Cbl is secreted to portal vessels. In plasma, cobalamin is bound to either transcobalamin II (TC) as active form (holo-TC) or other haptocorrins transcobalamin I, transcobalamin III (HC) as storage of Cbl. holo-TC constitutes 6 to 20% of plasma Cbl but HC binds to 80–94% of them. Transcobalamin II is produced by mainly hepatocytes and additionally enteric epithelia, endothelia and monocytes. Haptocorrins are a part of secondary granules. Filtered holotranscobalamin are reabsorbed by megalin receptors in renal tubules. Cells receive Cbl by holotranscobalamin II receptor CD 320. It is converted to methyl Cbl in cytoplasm and adenosyl Cbl in mitochondria. Methyl Cbl is a co-factor of methyl transferases, including methionine synthase (MetH) and corrinoid iron-sulfur protein (CFeSP). It is required for methionin synthesis from homosistein. Methionin is a methyl donor. In the methylation deficiency, thymidine synthesis and methylation of nucleotides are affected. Cbl deficiency also effect uracil synthesis. In bacteria, new molecules and enzymes are affected by methyl cobalamin levels. In mitochondria, Adenosyl cobalamin are required as a cofactor to convert methylmalonyl CoA to succinyl CoA.

Adenosyl Cbl is involved to lipid metabolism by this way.⁹⁻¹²

Cbl supply to cell may be deficient despite hCbl levels, because HC has higher affinity than TC. If intestinal supply of Cbl is insufficient, present Cbl remain in the high affinity HC pool, low affinity TC cannot find sufficient vitamin B12 to bind. Low holoTC levels cause Cbl deficiency even in hCbl levels.⁹⁻¹² Our cases have normal range holo-TC. There is no clinical deficiency. On the other hand holo-TC levels were normal although Cbl levels were high. That is, TC synthesis did not increase by some cause increasing HC levels

High levels of plasma Cbl in each disease are explained by different mechanisms.⁹⁻¹² Secretion of haptocorrin from leukocyte granules is responsible for high hCbl levels in lymphoproliferative diseases, such as, autoimmune lymphoproliferative disease (ALPS), myeloma and myeloproliferative diseases such as polycythemia vera and other cancers. In autoimmune disease, hCbl levels are due to production from leukocytes, decreased uptake and decreased filtration by TC autoantibodies. In ALPS, Cbl level increases 15-20 fold of normal. In a study, HC is in lymphocyte lysate of ALPS-FAS subjects but not that of controls. TC is not present in lymphocytes of both groups. HC is present in granulocytes of both ALPS-FAS patients and controls.¹⁶ This also explains why there is no increase of vitamin B12 in lymphocytosis patients. Our acute lymphoblastic leukemia patients with T, B and delta gamma T cells also show malign lymphocytes which were not usually the source of hCbl levels. Delta -gamma T cells have the same differences from alfa -beta T cells. Two delta gamma T cell leukemia/lymphoma patients have normal values. We saw hCbl levels in myeloid leukemias such as JMML, CML and acute myeloid leukemias.

In kidney disease, high levels may be caused by a decreased renal Cbl clearance. In hepatic disease, release from lysed hepatocytes and decreased uptake by hepatocytes cause increased levels of TC.¹⁻⁴

hCbl values in healthy children cannot be explained by suggested mechanisms hCbl associated disease. In our cases, kidney and liver diseases and abdominal tumors were excluded by abdominal ultrasonography and liver and kidney function test. Hematological disease was excluded by normal WBC, peripheral smear and clinical follow up. We need new explanations. For this reason, we look at the deficiency associated diseases and mechanism to see patterns and use it to explain the high levels in otherwise healthy children. The present list of variables are: oral intake by food or drugs, gastric intrinsic factor, consumption by intestinal parasites and bacterial overgrowth, bacterial flora, bacteriophages in flora, terminal ileum inflammation or resection, pH changes in gut, genetic disease of cubam receptor complex, transcobalamin II deficiency, haptocorrin releasing from leukocytes or malign cells, TC uptake by liver, TC reabsorption in kidney, holo-TC receptor deficiency and competition for limited Cbl between high affinity haptocorrins and TC.¹⁻⁴ Now thinking about these variables: oral intake was excluded initially. Intrinsic factor does not rate limiting for absorption although low levels cause deficiency. Cubilin expression is rate-limiting step with pH dependency because 5 pg daily absorption is saturated amount of oral diet.^{17,18} Increasing expressions of absorption receptor complex may be related with high vitamin B12 levels. Mutation studies of these genes may give a relation.

The relationship between Cbl levels with genetic factors was investigated in deficient and normal persons. Heritability was estimated to be 59% in a study of monozygotic and dizygotic twins. This suggests that genetic effect is significant.¹⁹ Recent updates on whole genome SNP in deficient and control samples give a list of genes related to Cbl deficiency.²⁰ A counterpart of these studies in patients with hCbl values may give a candidate gene list. Passive absorption rate is 1% of oral high doses and 500mcg give 5 pg of passive absorption.²¹ But there was no oral b12 treatment in our patients. Dietary intake is also important. The mean serum Cbl in vegans

was 33% lower than in vegetarians and 57% lower than in omnivores.²² Diets of our patients were typical children's diets.

In children with hCbl level, all known diseases should be excluded first by hematologic, biochemical and radiologic investigation. As in our cases, hCbl levels did not have any other abnormalities. In these patients, we should think of new causes increasing levels of Cbl. One of them may be microbiota.²³ Horses do not eat soil or meal but their vitamin B12 levels are normal. Their normal values are found to be associated with helicobacter colonization because helicobacter can synthesize Cbl and its upper intestinal colonization is appropriate for the absorption of vitamin B12 by dead bacteria. In humans helicobacter colonization is common and associated with peptic ulcer and antibiotic is used extensively. Does Cbl deficiency deepen after helicobacter therapy in a vegetarian? We did not know the role of helicobacter colonization in our patients. Another possibility is an increase of intestinal colonization. But there is opposite clinical finding present in intestinal duplication by Cbl consumption by increased bacterial colonization. Other possibilities are feeding habits may cause preferential increase of Cbl synthesis in bacteria and archaea by ingredients. Pickles, vinegar, butyrate foods may be examples of ingredient sources.²³

In our study, leukemia developed in two of 40 children. The ratio 2/40 may be a coincidence. Annual risk of developing new leukemia in children is 4/100.000 in the literature.²⁴ That is 8/100000 new cases in two years. The difference is significant when we compare 2/40 and 8/100000 with the chi-square test ($p=0,000$). This important association should be validated with larger series.

We recommend that the following examinations be performed initially in a patient presenting with hCbl: WBC, peripheral smear, vitamin B12, holotranscobalamin, ferritin, folic acid, liver and kidney function tests and abdominal ultrasound. If there is cytopenia, tests should include direct coombs, autoantibodies

and double negative ratio in alpha beta T lymphocytes for ALPS and ALPS like diseases. In next visits, a limited test may be sufficient such as WBC, peripheral smear and Cbl. If there is a new abnormality in WBC or additional complaints, the test panel should be expanded appropriately. Patients should be followed up for two years for the development of leukemia and myeloproliferative disease. hCbl is not toxic. Meat restriction was not recommended because it is the main source of vitamin B12, iron and zinc. We are not afraid of Cbl elevation itself, but we do fear that it may be the first sign of a hematological disease such as leukemia.

Future clinical studies may be conducted in groups that can conduct laboratory research on pathways from oral intake to final clearance of Cbl and full genome analysis. This cooperation will provide sufficient material to find the causes leading to hCbl and to establish a cause and effect relationship.

In conclusion, a significant number of children seen in outpatient clinics due to other reasons have hCbl. Our study suggests a few patients may develop leukemia later. We suggest that patients should be followed up for two years after initial investigations for hematological, liver and kidney diseases showing hCbl levels. On this subject, longer and more detailed studies with larger case series are needed.

Author contribution

The authors confirm contribution to the paper as follows: study conception and design: DA; data collection: DA, CA; analysis and interpretation of results: DA, CA; draft manuscript preparation: DA, CA. All reviewed the results and approved the final version of the manuscript.

Ethical approval

The research was reviewed and approved by institutional review board of Ondokuz Mayıs University on clinical studies

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Conflict of interest

The authors have no conflict of interest to inform.

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