

Blood Cobalamin Level Pre-and Post-liver Transplantation

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Received: April 4, 2013 Revised: April 29, 2013

Accepted: May 1, 2013

Published online: June 21, 2013

ABSTRACT

AIM: To evaluate the cobalamin (vitamin B12) blood level pre- and post-liver transplantation in patients with liver diseases.

METHODS: Cobalamin level was measured in 30 patient's blood samples pre- and post-liver transplantation. The majority of patients 21 (70%) had viral hepatitis either B or C, whereas the rest 9 (30%) had other liver diseases. Cobalamin level was analyzed with the Advia Centure immunochemiluminometric assay (Siemens, USA) with normal range of 155-672 pmol/L and compared to other blood liver tests such as alanine transaminase (ALT), aspartate transaminase (AST), and bilirubin levels. The *p*-values were analyzed using the Wilcoxon signed ranks test and the statistical analysis was done by SPSS software.

RESULTS: The mean of blood cobalamin level was decreased (26%) from 692±220 pmol/L to 508±248 pmol/L post-liver transplantation (*p*=0.002). A decrease in cobalamin level by 23% was observed in hepatitis B and hepatitis C when grouped together (531±287 pmol/L;

p=0.019), by 21% in hepatitis C (559±302 pmol/L, *p*=0.039), and by 31% in non-viral hepatitis (459±125 pmol/L; *p*=0.021). The cobalamin level was normalized earlier with a mean of 7.6±1.7 days compared to other routine liver function tests (ALT, AST and bilirubin) 27.4±3.9 days (*p*<0.0001).

CONCLUSION: Blood cobalamin level has been normalized earlier than other routine liver markers in post-liver transplantation; However, larger study is needed to evaluate the benefit of measuring blood cobalamin level in combination with other liver tests for monitoring graft function recovery post liver transplant.

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Key words: Cobalamin; Vitamin B12; Liver; Transplantation; Hepatitis

Munshi M, Ameer EA, Abdulkareem A, Aljumah A, Hasanato R, Alfawaz H, Tamim H, Alothaim A, Balwi MA, Altraif I, Tamimi W. Blood Cobalamin Level Pre-and Post-liver Transplantation. *Journal of Gastroenterology and Hepatology Research* 2013; 2(6): 642-645 Available from: URL: <http://www.ghrnet.org/index.php/joghr/article/view/406>

INTRODUCTION

Cobalamin (Vitamin B12) is an important water-soluble vitamin. In contrast to other water-soluble vitamins, it is not quickly excreted in the urine but rather accumulates and stored in the liver, kidney and other body tissues. As a result, a cobalamin deficiency may not manifest itself until after 5 or 6 years of a diet that supplies inadequate amounts. Cobalamin functions as a methyl donor, works with folic acid in the synthesis of DNA and the production of red blood cells and is vitally important in maintaining the health of the insulation sheath (myelin sheath) that surrounds nerve cells^[1].

Cobalamin is not manufactured in the body and must be absorbed from dietary or supplemental intake. The main sources of cobalamin include meat, eggs, and dairy products. For cobalamin to be absorbed by the body, it must bind to intrinsic factor (IF), which is a protein that is secreted by Parietal cells in the intestine. Cobalamin is attached to protein when ingested. It can be complexed to the protein haptocorrin (HC) to form holohaptocorrin (HoloHC) and is transported to the small intestine where the cobalamin is complexed

to IF and absorbed. Alternatively, cobalamin can be complexed to the protein transcobalamin (TC) to form active-B12 (HoloTC), which is then taken up by specific cell receptors. The liver is a major storage site for cobalamin^[2-4]. In 1959, elevated blood cobalamin levels were reported in patients with hepatic congestion associated with mitral stenosis, congenital heart disease, or constrictive pericarditis^[4]. Hepatocyte necrosis has been implicated in the origin of elevated serum cobalamin involving these noncardiac and cardiac disorders of the liver^[5]. Additionally, reduced cobalamin uptake by the diseased liver or failed cobalamin transporter protein synthesis have been implicated^[5].

Circulating cobalamin, which is bound to its transporter protein, transcobalamin II (TCII), is taken up by the liver via a membrane-bound receptor-mediated transport process^[6]. The majority of these receptors are present on the endothelium of the hepatic vasculature, where endothelial cell cobalamin-TCII binding and internalization occurs^[7]. The endothelium therefore plays a major role in regulating cobalamin uptake by the liver^[8]. Although TCII is the essential blood protein for transferring cobalamin into the cells of the body, the amount of cobalamin on TCII is normally very low <50 ng/L^[8]. TCII deficiency causes megaloblastic anemia because of failure of the cobalamin to enter the bone marrow from the blood; in this clinical situation, the serum cobalamin level is normal. This is because most of the cobalamin in the blood is bound to another transport protein, TCI. TCI is a glycoprotein that is largely synthesized by granulocytes and macrophages. In myeloproliferative diseases in which granulocyte production is greatly increased, both the TCI and cobalamin levels in serum increase considerably. Cobalamin that is bound to TCI does not transfer readily to bone marrow; it appears to be functionally inactive.

We hypothesized that patients with liver diseases that were mainly due to viral hepatitis would exhibit depleted cobalamin pre- and post-liver transplantation, in addition to impaired cobalamin uptake into the liver, as evidenced by increased serum cobalamin. Therefore, our aim was to review the data from liver disease patients who underwent either living related or deceased donor liver transplantation, to analyze how the level of cobalamin changes pre and post transplant and to determine whether it has any association with a specific etiology of liver disease.

METHODS

The blood cobalamin levels were reviewed from 30 patients who underwent either living-related or deceased donor liver transplantation between 2006 and 2009 at King Abdulaziz Medical City (KAMC) in Riyadh, Saudi Arabia. We have randomly selected 30 patients whom have their serum cobalamin checked pre and post transplant.

The inclusion criteria included all Saudi adult patients whom have a liver disease and were a potential candidate for liver transplantation. Patients with any primary graft dysfunction were excluded. All patients have been signed the inform consent form. There were three children patients in this study. It was better to analyze their data separately but due to their low number they were excluded from the study.

We excluded patients with a history of chronic hematologic diseases that were not related to the current health problems: these diseases include cobalamin deficiency anemia and folate deficiency anemia as well as cardiac diseases such as decompensated biventricular failure, which leads to hepatic congestion and elevated cobalamin level that could affect our study. In addition, we compared all patients without sub-dividing them according to cause so that in

some diseases, their cobalamin level is not affected. We reviewed their data electronically with the coordination and assistance of the laboratory department at KAMC. The cobalamin serum level was analyzed with the Advia Centure assay (Siemens, USA) based on the principle of immunochemiluminometric assay with a normal range of 155-672 pmol/L. The blood was drawn 1 to 2 days immediately after liver transplantation and then 1 week after liver transplantation to ensure the stability of the cobalamin level in the blood. Other routine liver markers such as alanine transaminase (ALT), aspartate transaminase (AST), and bilirubin levels were measured in the blood on daily basis before and after transplant. The statistical analysis used SPSS software. The *p*-values were analyzed using the Wilcoxon signed rank test. The study was approved by our institutional review board.

RESULTS

The major diagnosis in our patients was hepatitis C (60%). Other liver diseases included hepatitis B, primary sclerosing cholangitis (PSC), vanishing bile duct syndrome, biliary atresia which mostly in children, autoimmune hepatitis (AIH), and cholestasis. Table 1 shows the demographic data for all the 30 patients who participated in this study (12 female; 18 male; mean age 45.7±9.6). It also shows that 21 patients (7 female; 14 male; mean age 54.8±6.8) had viral hepatitis with either HCV and/or HBV of which eighteen patients 60% (7 female; 11 male; mean age 55.4±6.0) were diagnosed with HCV and three (10%) patients had hepatitis B. It also shows that 9 (30%) patients (5 female; 4 male; mean of age 27.0±15.9) were diagnosed with other non-viral hepatitis liver diseases.

Table 2 shows the mean, standard deviation (SD), 95% confident interval (CI), and *p*-values of the blood cobalamin level in all pre- and post-liver transplantation patients. As seen in table 2 and figure 1, the mean blood cobalamin level for all pre-liver transplantation patients was 692 pmol/L, SD±220 with a 95 % CI (608-776). On the other hand, the mean blood cobalamin level in the post-liver transplantation patients was found 508 pmol/L, SD 248 with a 95% CI (413-602) in which a decrease of 26% in the blood cobalamin level was observed post-liver transplantation patients. The cobalamin level was normalized earlier than liver function tests with a mean

Table 1 Demographic and characteristic data of all patients.

Patients type	Total (n)	Female	Male	Age mean±SD
All Patients	30	12	18	45.7±9.6
Hepatitis (B & C)	21	7	14	54.8±6.8
Hepatitis C	18	7	11	55.4±6.0
Other Liver diseases	9	5	4	27.0±15.9

Table 2 Mean, standard deviation (SD), 95% confident interval (CI), and *p*-values of cobalamin in patients pre- and post-liver transplantation.

	Mean±SD Cobalamin pmol/L	95% CI	<i>p</i> -value
Pre-liver transplantation (all patients) n=30	692±220	609-776	0.002
Post-liver transplantation (all patients) n=30	508±248	413-602	
Pre-liver transplantation (hepatitis B & C) n=21	687±217	328-1288	0.019
Post-liver transplantation (hepatitis B & C) n=20	531±287	207-409	
Pre-liver transplantation (hepatitis C) n=18	711±218	630-840	0.039
Post-liver transplantation (hepatitis C) n=17	559±302	405-709	
Pre-liver transplantation (other liver diseases) n=9	664±260	455-870	0.021
Post-liver transplantation (other liver diseases) n=9	459±125	340-566	

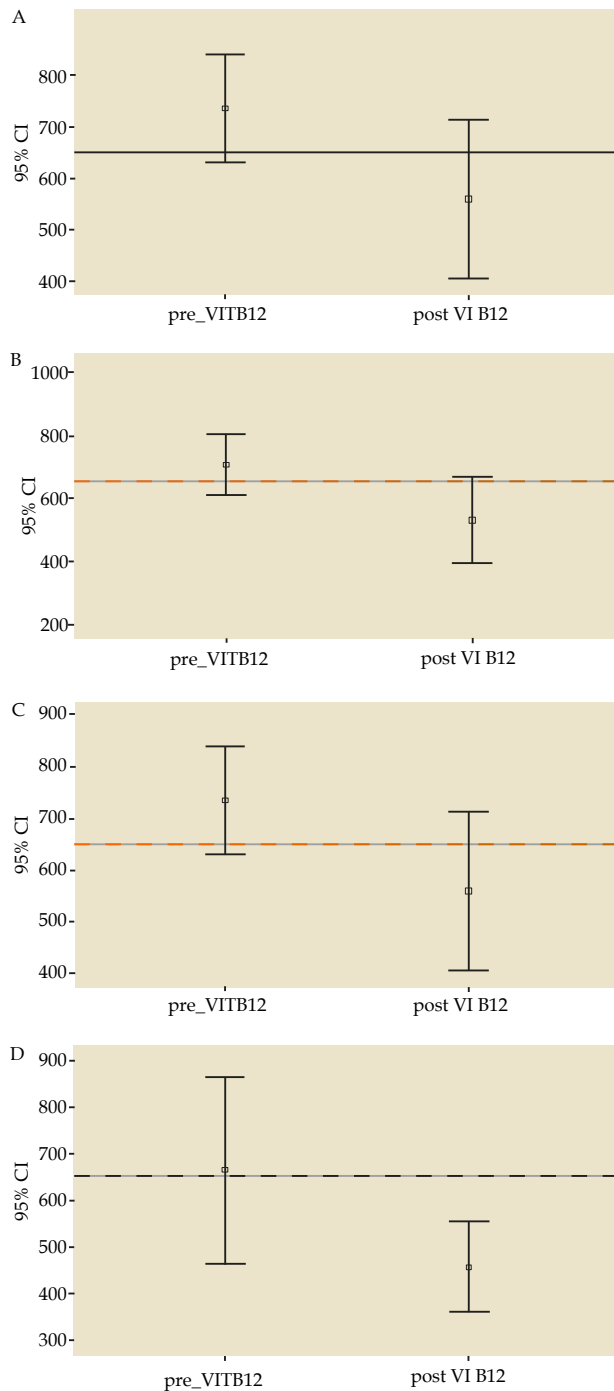


Figure 1 The 95% confident interval (CI) of the mean cobalamin level in patients pre- and post-liver transplantation for all patients (A), viral hepatitis patients (B), hepatitis C patients (C), and other patients with liver diseases (D). Pre_VITB12 and post VIT B12 indicate the cobalamin level pre- and post-liver transplantation respectively.

of 7.6 ± 1.7 days ($p < 0.0001$). Other routine liver function tests (ALT, AST and bilirubin) were normalized between 10 to 35 days post-liver transplantation with mean of 27.4 ± 3.9 days, depending on the clinical course in each case. The post-liver transplantation data from one patient were missing because of his death immediately after the operation. The difference between the pre and post blood cobalamin levels was significant $p < 0.002$. Table 2 and figure 1 also show that in patients with viral hepatitis (3 HBV and 18 HCV), the mean pre- and post-liver transplantation blood cobalamin levels were 687 pmol/L ($SD \pm 217$; 95% CI 328-1288) and 531 pmol/L ($SD \pm 287$; 95% CI

207-409), respectively, with $p = 0.019$ with a decrease of 23%. Table 2 and figure 1 show that the mean blood pre- and post-liver transplant cobalamin levels in patients with HCV were 711 pmol/L ($SD \pm 218$; 95% CI 630-840) and 559 pmol/L ($SD \pm 302$; 95% CI 405-709), respectively, with $p = 0.039$ with a decrease of 21%. These findings also showed that the mean of blood cobalamin levels pre- and post-liver transplantation in patients with other non-viral hepatitis liver diseases were 664 pmol/L ($SD \pm 260$; 95% CI 455-870) and 459 pmol/L ($SD \pm 125$; 95% CI 340-566), respectively, with $p = 0.021$ with a decrease of 31%.

DISCUSSION

The findings in this study showed that there was significant decrease in the blood level of cobalamin immediately post-liver transplantation from abnormal level to normal level in all subjects with end stage of liver diseases. The rate of decrease was 23% in viral hepatitis patients, who exhibited an increase above the upper limit of normal and a decrease back to normal levels after liver-transplantation with a significant difference ($p = 0.019$). When we compared the mean cobalamin level in hepatitis C patients with that in patients with the other non-viral hepatitis liver diseases, we found a highly significant increase in the blood cobalamin level above the upper limit of normal in the pre-liver transplantation patients and a significant decrease in the post-liver transplantation patients with a p -value of 0.039. In other non-viral hepatitis liver diseases patients, a highly significant decrease in blood cobalamin level was also observed despite the fact that most cobalamin values were within normal limits which may be due to the fact that these subjects were younger than other groups. The relationship of elevated serum vitamin B-12 concentration to alcoholism in general hospital patients was studied by Goldman *et al*^[9] both in a retrospective and in a prospective group of patients. They found that alcoholism was the most common disease correlate of vitamin B-12 elevations in females. They reported that about 61% of female alcoholics had a serum vitamin B-12 concentration larger than or equal to 1000 ng/liter at the time of admission and only 17% of the nonalcoholics, whatever their other disease, exhibited such high values. They found that the sensitivity of high B-12 levels for alcoholism in females paralleled that of high AST, GGT and MCV values. In contrast, males (approximately 70% of their alcoholics) were far less often exhibited B-12 elevations even when AST, GGT and MCV were abnormally high. They concluded that elevation of serum B-12 concentration should always raise the suspicion of alcohol abuse, especially in females. These findings were consistent with those of Lambert *et al* (1997), who found that the serum cobalamin concentration was positively correlated with blood aspartate transaminase (AST) levels, but the relationship between cobalamin and the other liver enzymes, such as GGT, GLDH and ALT, was not investigated^[10]. In our results, we demonstrated a substantial positive relationship between blood cobalamin level and viral hepatitis diseases, which is consistent with other studies^[11,12]. Possible explanations for this phenomenon may be the failure of the damaged liver to take up cobalamin and its analogues from the blood (Green, 1983; Kanazawa and Herbert, 1985)^[11,12]. Another explanation is that the hepatocellular damage, such as that in viral hepatitis, decreases the levels of transcobalamin TCII, which is the active form of transcobalamin, that is mainly synthesized in the liver sinusoidal tissues and causes cobalamin to leak out of the liver into the circulation^[2].

Another explanation of why cobalamin increases during liver diseases has been proposed by Baker H *et al*^[2], who indicated that severe alcoholic liver disease involves the leakage of cobalamin

from liver tissue into the blood. Holo TC I and III concentration increases in blood; and this increase prevents the high blood levels of cobalamin from being excreted. However, blood holo TC II B12 distribution is decreased, indicating that there is a decrease in the exogenous cobalamin entering the blood and tissues. In severe liver disease, liver tissue cobalamin binding and storage by TC is disrupted and causes cobalamin to leak out of the liver into the circulation. Eventually, liver disease could produce enough severe tissue cobalamin deficits to cause metabolic dysfunction despite elevated total blood cobalamin. The elevation of blood cobalamin accompanied by a decrease in holo TC II distribution seemed to be a useful index of liver disease severity that helps to guide preventative treatment^[2].

Ermens AA *et al*^[13] reported that the increase in circulating cobalamin levels was predominantly caused by enhanced production of haptocorrin. Several liver diseases, such as acute viral hepatitis, cirrhosis, hepatocellular carcinoma and metastatic liver disease, can also be accompanied by an increase in circulating cobalamin. This phenomenon is predominantly caused by cobalamin release during hepatic cytolysis and/or decreased cobalamin clearance by the affected liver^[13].

Because hepatic dysfunction affects cobalamin storage and is liberated into the blood stream during liver disease^[14,15], Retief & Huskisson explained that cobalamin was effectively retained by blood binders, resulting in significant urinary loss only when the serum cobalamin increases to substantially high levels^[16,17]. Fukuda *et al*^[18] reported that the serum cobalamin levels in acute hepatitis are significantly elevated compared with normal controls. However, in chronic hepatitis, the cobalamin levels were within normal limits, whereas a moderate increase in cobalamin levels were demonstrated in cirrhosis.

There were some limitations in this study which may strengthen our data if performed in future, for instance, this was a descriptive study that although with a relatively small sample size shows a significant decrease in cobalamin after liver transplantation. The conclusion that cobalamin may be a useful marker to monitor the prognosis after liver transplantation was however not possible to evaluate since that the outcome data was not enough and therefore larger multicenter studies were needed to draw this solid conclusion. In addition, the relationships between other outcome markers of liver transplantation and blood cobalamin for example, INR, bilirubin level, blood lactate, patient/graft survival and trends of post-liver transplantation in day 1, 3, 7 blood cobalamin should be established in any future study.

Our observations indicate blood cobalamin level has been normalized earlier than other routine liver markers in post-liver transplantation patients; however, larger study is needed to establish this conclusion and to evaluate the benefit of using blood cobalamin level with combination with other liver function tests in monitoring patients' pre- and post-liver transplantation.

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