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Plasma cobalamin level as a considered tumor marker for hepatocellular Carcinoma

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ABSTRACT

Hepatocellular carcinoma (HCC) is one of the most common cancer among men and women. There are many serological tumor markers for the diagnosis of HCC. These are alpha-fetoprotein (AFP), des-gamma-carboxyprothrombin, vitamin B12 binding protein and HCC associated alkaline phosphatase. The aim of this study was to evaluate the possibility of using vitamin B12 as a tumor marker for HCC.

This cross sectional study was performed during a 2 year period, and serum samples were obtained from 38 HCC, 57 non-cancerous cirrhotic and 82 healthy control groups. Vitamin B12 levels were determined by using an automated chemiluminescence system test kit.

All HCC patients also had an underlying cirrhotic pattern. The period of the previous liver disease was 30.7±26.3 month in cirrhotic patients and 15.4±10 month in the HCC group. AFP and vitamin B12 levels in HCC patients were significantly higher (median AFP: 219 ng/ml, median B12:1106 ng/ml) than cirrhosis patients (median AFP:9,7 ng/ml, median B12:445 ng/ml) and control group (median B12:442 ng/ml) ($p<0,001$). In the HCC group, there was a good positive correlation between level of vitamin B12 and AFP (p:0.002) but this correlation was not appeared in cirrhosis group. We also examined whether the correlation between the tumor size and vitamin B12 levels and AFP levels. There was no correlation between these parameters ($p>0.05$).

Vitamin B12 levels can be useful as tumor marker in addition to other tumor markers and imaging modalities. Additional studies should be performed related to this subject and the other liver masses without malignancies.

Key Words: Cobalamin, vitamin B12, tumor marker, HCC, hepatocellular carcinoma

Introduction

Hepatocellular carcinoma (HCC) is the fifth most common cancer in men and ninth most common in women, however the second most common cause of death from cancer worldwide (1). HCC is a neoplasm the incidence of which is increasing worldwide, but striking geographical differences are observed for both risk factors and occurrence. The incidence of HCC is the highest in those with hepatitis B (HBV) and hepatitis C (HCV) related cirrhosis, however even those with non-cirrhotic HBV or HCV are at increased risk for HCC (2).

The mainstay for the diagnosis for HCC includes serological tumor markers, such as alpha-fetoprotein (AFP), des-gamma-carboxyprothrombin, vitamin B12 binding protein and HCC-associated alkaline phosphatase, as well as imaging modalities. They do not correlate, but complement each other (3). Using screen tests are really important for high risked individuals because HCC are detected at an early stage have a 5-year survival that exceeds 50% with appropriate treatment. Therefore American Association for the Study of Liver Diseases (AASLD) guidelines recommend screening at risk populations for HCC every 6 months with an ultrasound, although other societies recommend AFP in addition to ultrasound for improved sensitivity of screening (1). However Plasma levels of AFP is increased in approximately 80% of patients with HCC but may show normal levels, especially in patients with small tumors' size (<4 cm). In addition, falsely elevated levels are seen in patients suffering from inflammation, such as in patients with viral hepatitis (4).

Vitamin B12 contains a cobalt complex and is therefore also called cobalamin (5). Vitamin B12 is a coenzyme for two physiologically important functions in humans: 1- the synthesis of

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methionine, and 2- the conversion of methylmalonic acid to succinic acid. Also, the synthesis of methionine requires methylcobalamin. Therefore all living cells require vitamin B12, rapidly dividing tumor cells have a highly increased need for this vitamin (6,7). Three proteins such as intrinsic factor (IF), transcobalamin (TC), and haptocorrin (HC) are involved in the uptake and transport of cobalamin. Vitamin B12 is known to accumulate at high levels in the liver. Therefore, the concentration of vitamin B12 in the blood rises in the presence of acute or chronic liver disease (8). Therefore when the liver is injured, stored vitamin B12 leaks out into the blood, which causes a severe B12-deficit in the liver.

Most of the studies indicate that patients with chronic liver disease, cirrhosis, HCC have higher level of serum cobalamin, HC ve TC II than normal patients (9). These high level results can be considered as marker of tumor and also can be directly associated with progression of disease or size of tumor (10). Therefore B12 vitamin screen tests are considered as really important for diagnosis of liver disease.

The present study evaluated level of vitamin B12 and possible tumor marker in patients with HCC and cirrhosis.

Materials and methods

Ethics: All patients provided written informed consent to participate. The study protocol conforms to the ethical guidelines of the 1975 Declaration of Helsinki as reflected in a priori approval by the institution's human research committee. The protocols used in this investigation were approved by the ethics committee.

Patients: This study was performed during a 2 year period, and serum samples were obtained from 38 HCC patients, 57 non-cancerous cirrhotic patients and 82 healthy control groups. We diagnosed with the HCC according to supporting findings of either based on AFP levels of >400 ng/ml and ultrasonography or computed tomography. The diagnosis of HCC was also supported by liver biopsy. Between July 2010 and October 2011 biopsy samples from 38 HCC and 57 cirrhotic patients were obtained through percutanous by using 16 to 18 gauge true-cut needles, or under diagnostic laparoscopy. HCC patients were naive. Cirrhosis was underlying liver disease in all of HCC patients. None of them had received treatment such as vitamin B12, and in none of the patient surgical resection, ethanol injection or chemoembolization had been performed. Also none of cirrhotic patients and control group had applied vitamin B12 treatment.

Tumor morphology and the appropriate HCC stage were classified according to the Okuda staging system and CLIP score (The cancer of the liver Italian Program) (11-14). Staging procedures included contrast-enhanced computer assisted tomography and serum AFP measurement performed by Standard RIA; AFP levels were categorized (≤400 or >400) according to the criteria of the CLIP. Data were collected regarding the HCC morphology (uninodular, multinodular or massive) and portal vein thrombosis (absent or present). Liver function and severity of liver disease were evaluated by Child–Pugh score and model of end-stage liver disease score (MELD score).

Laboratory procedure: Serum samples were collected at the time of diagnosis before the treatment. Vitamin B12 levels were measured by using automated chemiluminescence method with their commercial kits (ADVIA-Centaur; Bayer, New York, USA). The normal range for vitamin B12 was admitted as 180 to 710 pg/ml by the kit insert. Also all patients were evaluated for hemogram, biochemical parameters, folic acid and upper gastrointestinal endoscopy for portal hypertension.

Statistics: This study was designed as a cross sectional study, but control subjects were also used. Statistical tests were performed using the Statistical Package for the Social Sciences (SPSS) version 15.0 software (IBM; Armonk, NY, USA). Data are expressed as mean±SD, median and range. For grouped data multivariate analysis of variance (ANOVA) was applied, and, if a difference was found, this was followed by Student's unpaired t-test. The chi-square test or Fisher's exact test were used to compare qualitative variables. For quantitative variables, the normality of distribution was checked statistically. When distribution was detected normal, the t-test was used. When normal distribution was rejected, the Mann-Whitney test was used. For the correlation test, Spearman's rank correlation test was used. From this estimates, odds ratio (OR) with 95% confidence interval were computed. Significance was assumed at the $p< 0.05$ level.

Results

The various demographic, clinical, and laboratory data of the patients are shown in Table 1. Other

Table 1. Demographic, clinic and laboratory data of the patients

	HCC n=38 Mean±SD Median (Min-Max)	Cirrhosis n =57 Mean±SD Median (Min-Max)	p value
Gender (F/M)	4/34	17/40	0.04
Age	57.3±9.2	55.3±8.1	
	58	55	
	42-75	43-70	0.2
Folic acid (ng/ml)	10.8±5.6	13.8±9.9	
	10	11.8	
	1.1-24.5	2.2-37.7	0.2
ALT, IU/L	163±254	65±28	
	53	54	
	19-1020	21-162	0.3
AST, IU/L	201±237	71±34	
	11.5	60	
	41-844	33-154	0.000
ALP, IU/L	376±301	279±114	
	289	263	
	138-1468	164-595	0.3
GGT, IU/L	150±102	69±52	
	119	55	
	38-450	13-198	0.003
Haemoglobin (g/dl)	12.2±2.2	12.5±2.2	
	12.7	12.8	
	7-15.9	8-15.8	0.3
AFP	7677±13778	9.7±12.5	
	219	5.6	
	5.4-45852	2.3-56	0.000

ALT: Alanine transaminase, AST: Aspartate aminotransferase, ALP: Alkaline phosphatase, GGT: Gama Glutamil Transferase, AFP: Alpha-fetoprotein

Table 2. Baseline characteristics of HCC and cirrhotic patients

	HCC n (yes/no) (% of yes)	Cirrhosis n (yes/no) (% of yes)	p value
Smoking	25/13 (65.8)	17/40 (29.8)	0.001
Alcohol	14/24 (36.8)	12/45 (21.1)	0.1
Activation of disease	15/21 (41.7)	37/9 (80.4)	0.000
Esophageal varices	32/6 (84.2)	36/21 (63.2)	0.03
Ascites	20/18 (52.8)	25/32 (43.9)	0.4
Portal vein Thrombosis	16/22 (42.1)	5/22 (8.8)	0.000
Encephalopathy	6/32 (15.8)	11/46 (19.3)	0.7
Child-Pugh			
A	14 (36.8)	29 (50.9)	
B	14 (36.8)	9 (15.8)	
C	10 (26.3)	19 (33.3)	0.06
AFP			
≤10	2 (5.3)	49 (86.0)	
11-400 ng/ml	17 (44.7)	8 (14.0)	
>400 ng/ml	19 (50.0)	0 (0.0)	0.000

HCC: Hepatocellular carcinoma, AFP: Alpha-fetoprotein

baseline characteristics of cirrhotic and HCC patients are shown in Table 2. All HCC patients also had an underlying cirrhotic pattern.

The period of the previous liver disease was 30.7±26.3 month in cirrhotic patients and 15.4±10 month in the HCC group. The difference was statistically significant (p:0.008); that is, HCC patients had short history for cirrhosis. Of the twenty-six and 32 HBV infected patients with HCC and cirrhosis respectively, twelve were positive for HBV DNA in the HCC group, while 26 were positive for HBV DNA in the cirrhosis group (OR 3.7; 95% CI, 1.4-10.7; p:0.008).

Mean and median values of vitamin B12 were shown in table 3. HCC patients had highly increased vitamin B12 and AFP (chi-square, p<0.001) levels than cirrhosis, and differences were statistically significant (In all group, ANOVA test for vitamin B12: p<0.001, and accepted statistically significant p value in chi-square test was 0.0125).

If we accept AFP as a gold standard serological test, the sensitivity and specificity of vitamin B12 as a serological test was shown in table 4. In the HCC group, there was a good positive correlation between level of vitamin B12 and AFP. Spearman's correlation coefficient (r) was 0.485 (p:0.002). The similar correlation was not found between vitamin B12 and Aspartate aminotransferase (AST), Alanine transaminase (ALT) (p>0.05).

We also examined whether the correlation between the tumor size and vitamin B12 levels and AFP levels. We did not found any correlation between these parameters (p>0.05). We didn't also found any correlation between vitamin B12 levels and Okuda score (p>0.896). Despite mean of vitamin B12 levels were increased somewhat degree according to the CLIP score, p value was not statistically significant (Figure 1).

Discussion

In our study the levels of vitamin B12 appeared as significantly high for the patients with HCC rather than patients with cirrhosis and control group. Between size of tumor and levels of B12 no significantly associated statistical results were feund.

Between other biochemical parameters and levels of vitamin B12 no associationwas feund either.

DL Lildballe et al. (10) have indicated in their studies in the patients with HCC that vitamin B12 and also vitamin B12 binding protein were high in level and this high level value is directly associated with the progression of tumor. Also Lin et al. (15) have declared that vitamin B12 is high in level for patients with HCC and this high level value is directly associated with the size of tumor, liver damage, poor survival and also can be considered as tumor marker. Goel et al. (16) found vitamin B12 is high in level patients with cryptogenic cirrhosis than patients with idiopathic non-cirrhotic intrahepatic portal hypertension and also can be considered as marker. Nevertheless different studies have indicated that vitamin B12 and vitamin B12 binding protein increased in all of the patients with chronical liver disease, cirrhosis and HCC compared to control group and

Table 3. Vitamin B12 values in groups

	HCC n=38 mean±SD Median (Min-Max)	Cirrhosis n =57 mean±SD Median (Min-Max)	Control n=82 mean±SD Median (Min-Max)
Vitamin B12* (ng/ml)	1106.2±731.6 860 231-2620	445.8±184.1 399 210-933	442.1±167.6 395 241-880

HCC: Hepatocellular carcinoma

*p<0,001 in ANOVA and chi-square test for HCC compare with cirrhosis group.

Table 4. Sensitivity and specificity of vitamin B12 when AFP as a gold standard serological test.

	AFP level		Sensitivity	Specificity
	>400	<400	(%95 CI)	(%95 CI)
B12 vitamine level				
>710	17	6	89.4%	68.4%
<710	2	13	(75.6-100.0)	(47.5-89.3)

AFP: Alpha-fetoprotein

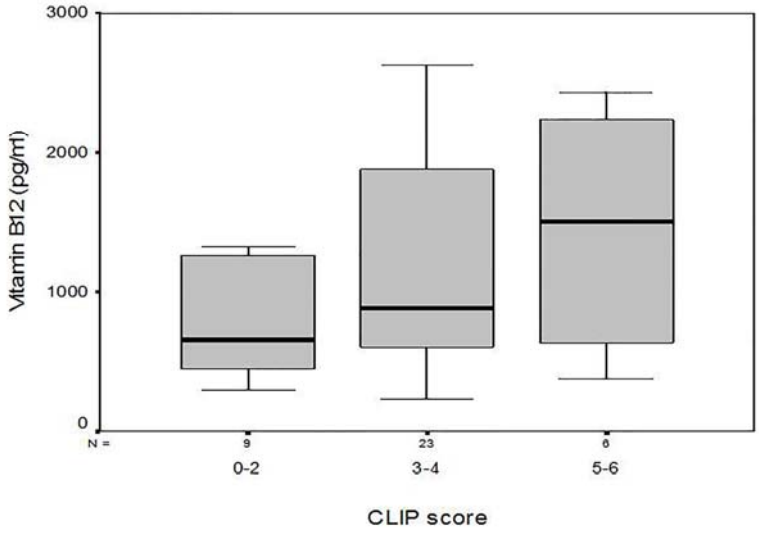

Fig. 1. Vitamin B12 values in HCC patients according to the CLIP score (p:0.068).

there was no significant difference between each other. For this reason high level of vitamin B12 is not only related to HCC but also related to all liver diseases therefore this symptom cannot be used as tumor marker (4). Our study is in comply with studies indicating that vitamin B12 can be used as tumor marker as in our study indicates that vitamin B12 is significantly high for the patients with HCC compared to control group and patients with cirrhosis. Our study is different with studies indicating suggestion between tumor progression and vitamin B12 levels. In the CLIP staging system, although mean values of vitamin B12 in each stage was different, there was no statistically significance in the ANOVA test because the median levels were close. That is, there was no relationship between levels of vitamin B12 and the increasing of tumour stages. Also, there was no correlation between the levels of vitamin B12 and the duration of underlying cirrhotic pathology in the HCC group. There were no differences between the cirrhosis group and the control group. Also, there was no correlation between the levels of vitamin B12 and the duration of underlying cirrhotic pathology in the HCC group.

HCC patients have a short history of previously known as liver disease such as cirrhosis. In this study, the main etiological factor for both HCC and cirrhosis was HBV. This result suggest that HCC patients probably had long subclinical liver disease. Despite the alcohol consumption was higher in the HCC group, difference was no statistically significant. We observed that 36% of the HCC patients used to consume alcohol. This observation suggests that if the patients with cirrhosis of whom etiological factor without alcohol, is used to consume alcohol, development of HCC may increase.

Some of the studies showed correlation between vitamin B12 and liver enzymes in the patients with HCC or liver disease (17). However there was no correlation between vitamin B12 and liver enzymes in our study. The increment might be caused by release from damaged liver or HCC cells (5,18), although the lack of correlation with plasma ALT and AST is not in favor of this hypothesis. Although AST and ALT levels might be changed because activity of the etiological agents (such as active replication of HBV or continuation of alcohol), correlation was not ascertained between these parameters and the activity of the etiological agent.

In the previous studies, increased serum vitamin B12 binding protein, TC were found in HCC patients (19-21). Hence, raised levels of TC might be reflected by rises in serum vitamin B12 levels. HC, one of the TC, is stored in hepatic cells and this storage is modified by hepatic carcinogenesis. Although HC is not as valuable as AFP, it may be used as a marker for detection of hepatoma in which patients do not have increased AFP levels (22). In this study, two patients with HCC had low levels of AFP. One of them had increased vitamin B12 level (1200 ng/ml), and the AFP levels of 17 patients were between 10 to 400 ng/ml. That is, nearly 50% patients with HCC have below the 400 ng/ml of AFP. Moreover, sensitivity and specificity of vitamin B12 for detection of HCC is also high, when AFP is accepted as a gold standard serological test. If a patient, who has a liver mass, has low level of AFP and has a

suspicious for the malignancy, serum vitamin B12 level can help to diagnosis of HCC. But about this subject, and the other liver masses without malignancies.

The main limitation of this study is to include only patients with HCC and cirrhosis. Detection of vitamin B12 level specificity on HCC will be more reliable if different cancer types can be tested.

In conclusion, our results confirm that patients with HCC constitute a heterogeneous group with respect to the levels of vitamin B12 and AFP. When the vitamin B12 concentration is increased in plasma, this may support the diagnosis. Therefore vitamin B12 detection could be used to substitute vitamin B12 binding protein as a tumor marker to detect HCC. However, vitamin B12 within the reference interval does not exclude HCC.

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