

The Effect of Chronic liver diseases on homocysteine and vitamin B12 in patients serum

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Summary:

Background: Homocysteine (Hcy) is a sulfur containing amino acid that is formed as an intermediary in methionine metabolism. Raised plasma homocysteine levels, which may contribute to the increased risk of chronic liver disease.

Patients and Methods: Sixty two patients with chronic liver disease and 26 healthy individuals were included as normal controls for the study. The HPLC system was used for the determination of Hcy and vitamin B12.

Results: A highly significant Hcy concentrations were noted in all patients with cirrhosis, chronic hepatitis or liver mass. There was a tendency towards higher Hcy concentrations in more advanced stages of liver disease. The study showed that the concentrations of total Hcy were significantly higher in the patients than in the normal control group, irrespective of the age or gender. Mean serum concentration of vitamin B₁₂ were significantly increased in cirrhotic patients and those with liver cancer compared to the control subjects and chronic hepatitis symptomatic patients.

Conclusion: The serum levels of both homocystein (Hcy) and vit. B12 were significantly increased in cirrhotic patients and those with liver cancer compared to chronic hepatitis symptomatic patients.

Key words: chronic liver disease, homocysteine, vitamin B12.

J Fac Med Baghdad
Vol. 51, No.4, 2009
Received: Jan., 2009
Accepted: July 2009

Introduction:

Hcy, a normal breakdown product of the essential amino acid Methionine, is believed to exert several toxic effects. A growing body of evidence suggests that an elevated Hcy levels is a risk factor for heart disease, independent of other known risk factors such as elevated plasma cholesterol and hypertension (1). Hcy is an intermediate in Methionine metabolism, which takes place mainly in the liver (1). Elevated Hcy levels are observed in cirrhotic patients (2)

Hcy is slowly eliminated from plasma with a half-life of 3-4 hours; hence an elevated total Hcy would be expected within 12-20 hours after a protein-rich meal. Samples for the measurement of total Hcy should be taken in the morning, after a light breakfast and following a light meal in the evening before the test (3). Hcy concentrations are positively correlated with that of fibrinogen in hepatitis and liver cirrhosis and with C- reactive protein and leukocyte counts in cirrhosis. These latter indices are part of the inflammatory response. It is tempting to speculate that chronic tissue damage resulting from ischemia, autoimmune processes, viral infection or alcohol will induce cell repair and proliferation concomitantly, accelerating specific methylation reactions, that generate S-Adenosyl homocysteine

and releasing Hcy (4). This may explain the elevated Hcy concentrations seen after myocardial infraction (5), in hyper-proliferative disorders and malignancy (6). Clarke and Stanbie, (2001) (7) have found an association between elevated Hcy levels and impaired cognitive performance and dementia. There is also much focus on the association between carcinogenesis and impaired Hcy metabolism.

Deficiency in vit B₁₂ leads to an increase in serum methylmalonyl- CoA, and its metabolic product, methyl malonic acid (MMA). The second reaction uses cobalamin as a cofactor in the synthesis of methionine from homocysteine. Hcy levels increase in vitamin B₁₂ deficiency. Hcy levels are also increased in folic acid deficiency (8). Homocysteine occurs in plasma or serum; the major part is conjugated to protein through disulphide bonding (more than 80%) as the symmetrical disulphide homocysteine-cysteine; the free thiol (less than 20%). The free thiol can undergo a reversible conversion to homocysteine thiol-ketone but it is present in a very minor amount in plasma, probably at nano molar levels due to non specific enzymatic hydrolysis (11)

The term total homocysteine refers to the sum of the concentrations of the free and oxidized forms, measured after the reduction of the disulphide bond to liberate the free form. The main source of homocysteine is probably the liver and proliferating cells. Only a very minor fraction of the homocysteine produced by the cells is excreted in the urine, degradation in the renal tissue after tubular re-absorption seems to account for the major part of

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the homocysteine clearance (11). In addition, the intake of vitamins B₆, B₁₂ and folate in the diet level will influence the serum Hcy levels. Factors determined by life style do play a role e.g. coffee consumption, cigarette smoking and intake of specific items of food. Because the essential amino acid Methionine is the only source of homocysteine; food intake would be expected to influence homocysteine. The aim of this study was to evaluate the levels of Hcy and vitamin B₁₂ in patients with chronic liver diseases cirrhosis and liver cancer patients compared with normal individuals.

Materials and Methods:

This study was carried out in the specialized Center of Gastroenterology and Hepatology in Baghdad, during the period from October 2003 up to the end of November 2004. Sixty two patients selected; all of them fulfilled the criteria of chronic liver disease (cirrhosis, chronic viral hepatitis and hepatocellular carcinoma).

From each patient, a detailed history was taken concerning illness, age at the onset of illness (age at onset of symptoms) and age at presentation (age at which the patient consulted his physician), complications of the disease, other associated diseases, residency, occupation, drug, alcohol or smoking history, family history of the same illness. These patients were subdivided to 3 groups:

- 1) Those with liver cirrhosis: includes 25 patients (15 males and 10 females), ages (7-73) years, (mean ± SD: (44.92 ± 15.411) years).
- 2) Those with chronic viral hepatitis (B & C) (CVH) includes 24 patients (16 males and 8 females) ages (17-73) years; mean ± SD: (43.00 ± 14.679).
- 3) Those with hepato-cellular Carcinoma (HCC); 13 patients (5 males and 8 females), with an age range of 27-66 years, (mean ± SD: (47.62 ± 13.95) years).

Twenty six healthy individuals were also included as normal controls for the study (13 males and 13 females) with an age of (15-60) years: mean ± SD (37.92 ± 12.881) years.

The HPLC system which was used in this study consisted of the following components:

Column model Shimpack MRC-ODS with a flow rate of 1 ml/min, mobile phase A (Methanol: Acetonitrile: 0.02 M Sodium Acetate pH 5.9; 80:2.5:17.5), and mobile phase B (Methanol: Acetonitrile: 0.02 M Sodium acetate pH 5.9; 80:2.5:17.5). The detection wave length was 338 nm.

Determination of Homocysteine: Two-hundred micro-liters of the frozen sample, after complete thawing at 4 C^o, were de-proteinized by 25 µl of 15% 5-Sulphosalicylic acid, mixed and centrifuged at 8000 r.p.m. for 10 minutes at 4 C^o. Derivatization of homocysteine: 25µl aliquots of standard or 30 µl of the clear supernatant of the de-proteinized samples were added to 20 µl of OPA (Ortho-Phenyl-Alanine) reagent. After 60 sec, 60 µl of 0.02 M Sodium Acetate (pH 5.9) was added. The solution then was mixed and 1 minute later, 20 µl of this mixture was injected where to be analyzed.

Determination of vitamin B₁₂ by HPLC: Injection of 5 µl of serum or plasma (frozen sample) after complete thawing at 4 C^o into the same system of HPLC for homocysteine determination, Methanol and aq. 0.2% NH₃ (9:1) were used as diluents (2.6 ml min⁻¹) permits separation of Hydroxo-cobalamin (I) from Methyl cobalamin (II) and Cyano-cobalamin; the retention times being 39, 111 and 115 sec., respectively, with absorbance measurements on the eluate at 280 nm.

(Separation of serum B₁₂ on reversed phase column (250*4.6 mm I.D.), mobile phase (5 mmol octane sulfonate: methanol (50/50 vol/vol)), flow rate 1.7 ml/min, UV at 254 nm ambient temperature (B₁₂ concentration 200 picomole/L).

Results:

Examination of total serum Hcy level in patients with chronic liver diseases showed the following: mean serum concentration of homocysteine (mean SD) values in the studied groups: (25.111±8.0252) µmol/L with cirrhosis, (11.550±1.4103) µmol/L with chronic viral hepatitis, (26.696±7.7174) µmol /L with HCC), While that of the control group was (9.923±0.4563) µmol /L.

The differences were statistically highly significant (p=0.0001) as shown in table 1, 2 and figure 1. This study showed that the homocysteine concentrations were elevated in all patient groups, (cirrhosis, chronic hepatitis and HCC). There was a trend towards higher Hcy concentrations in more severe stages of liver disease. This was reflected by significantly higher Hcy concentrations in cirrhosis than in chronic viral hepatitis, (25.111±8.0252) µmol /L; versus (11.550±1.410) µmol /L P= 0.0001. The highest levels were obtained from HCC cases (26.696±7.7174 µmol /L) is P<0.0001.

Table (1): Serum levels of homocysteine (µmol/L) in control and patients groups.

GROUP	N	Minimum	Maximum	Mean	SD	SEM
Cirrhosis	26	17.0	49.9	25.111	8.0252	1.574
Chronic viral hepatitis	24	9.4	14.3	11.550	1.4103	0.288
Hepatocellular carcinoma	13	18.0	42.7	26.696	7.7174	2.14
Control	26	9.2	10.8	9.923	0.4563	0.089
F=58.941, P=0.0001 (Significant)						

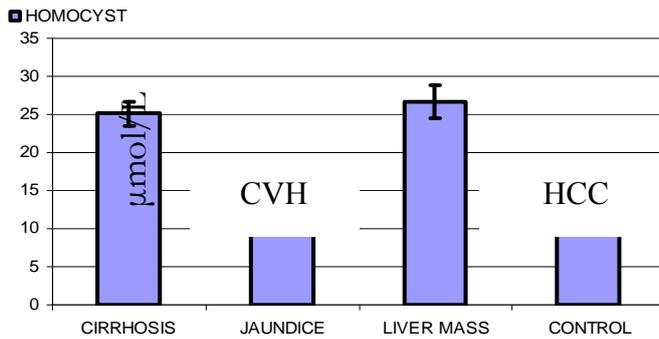


Figure (1): Auditory means for determination in blood sera of control and patients groups.

Serum homocysteine levels were significantly higher in reference cirrhosis patients than in the control subjects shown in table (2).

Table (2) Level homocysteine group in patients group % and control group

Homocysteine groups	Cirrhosis		Chronic viral hepatitis		Hepatocellular carcinoma		Control	
	No	%	No	%	No	%	No	%
<10 μmol/L	0	0	2	8.3	0	0	16	61.5
10-19	9	34.6	22	91.7	4	30.8	10	38.5
20-29	12	46.1	0	0	6	46.2	0	0
≥30	5	19.2	0	0	3	23.0	0	0
Total	26		24		13		26	

To examine the effect of renal function on homocysteine concentrations, a separate analysis was done for patients with cirrhosis, chronic viral hepatitis and Hepatocellular carcinoma with normal and elevated serum creatinine concentrations (Table 3). Patients with elevated creatinine concentrations had significantly higher Hcy concentrations in cirrhosis and liver mass and with some patients of jaundice group, Compared with the control group.

Table (3) Serum creatinine level (μmol/L) in control group and patients groups.

GROUP	N	Min.	Max.	Mean	SD	SEM
Cirrhosis	25	87.7	146.4	119.7	17.731	3.5462
Chronic viral hepatitis	24	73.9	129.4	99.3	17.985	3.6712
Hepatocellular carcinoma	13	118.8	148.9	130.9	10.341	2.8681
Control	26	63.9	104.6	84.3	12.112	2.3753

F=37.516 , P=0.0001 (Significant)

Mean serum concentration of vitamin B₁₂ in both the patients groups and the control subjects, given in (table 4 and figure 2), were significantly increased in cirrhotic patients and liver cancer (p<0.0001) compared to the control subjects and those with chronic viral hepatitis.

Table (4): Serum vitamin B₁₂ concentration in patients groups with liver disease and control subjects.

GROUP	N	Min.	Max.	Mean	SD	SEM
Cirrhosis	26	420.0	828.0	680.1	102.8075	20.16
Chronic viral hepatitis	24	210.0	450.0	328.5	72.2219	14.74
Hepatocellular carcinoma	13	490.0	810.0	695.0	86.7948	24.07
Control	26	230.0	376.0	282.52	39.6981	7.785

F=176.438 , P=0.0001 (Significant)

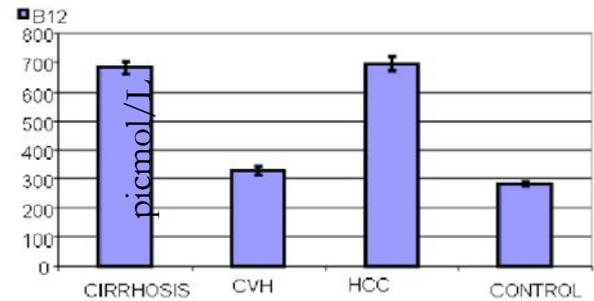


Figure (2): Histogram for data of table (4) showing vitamin B₁₂ determination in blood sera of control and patient groups.

Discussion:

The results showed that the total Hcy concentrations were significantly higher in all the patients groups irrespective of age or gender than in the control group. Other studies showed that the highest total Hcy concentrations were in patients aged (60-69) years, (both sexes). (Durand *et al.*)(9) who concluded that aging gradually increases Hcy blood concentration. It was also shown that Hcy concentration in men was not significantly different from that of women(10), this is similar to our findings. The elevated Hcy concentrations seen in patients with liver cirrhosis and hepatocellular carcinoma might be explained in part by tissue damage occurring directly through increasing Hcy leakage or indirectly by initiated cell repair (12). In this study serum concentrations of vitamin B₁₂ were elevated and increased with the severity of liver disease. Homocysteine was metabolized through the trans-sulfuration and trans-methylation pathways, which require vitamin B₁₂ (methyl cobalamin); changes in homocysteine concentrations are likely to occur in liver disease, concomitant with the significantly elevated levels of vitamin B₁₂. (Ferree *et al.*)(14) is in agreement with our results. The major finding of this study was a high prevalence of hyper homocysteinemia in the different patient groups (28.4% in the patients with cirrhosis, 14.8% in the patients with HCC). This effect was independent of the etiology of liver disease. Increases in vitamin B₁₂ in cirrhotic and liver mass patients could be due to hepato-cellular damage. A cellular leakage of vitamin B₁₂ with a subsequent intracellular vitamin B₁₂ deficiency has been

proposed for liver cirrhosis. This might lead to the so-called Folate trapping mechanism in which intracellular vitamin B₁₂ deficiency leads to an accumulation of missing (16). In hepatocellular damage, vitamin B₁₂ binding and storage is disrupted and causes vitamin B₁₂ to leak out of the liver into the circulation (17). The latter may have relevant clinical implications in a malnourished patient with primarily decreased peripheral vitamin B₁₂ levels; the deficiency of vitamin B₁₂ in the periphery may be temporarily masked by vitamin B₁₂, leaking out of the damaged hepatic cells in massive alcohol consumption (18).

Conclusion:

Homocysteine concentrations are constantly higher in patient with cirrhosis and hepatocellular carcinoma more than the chronic viral hepatitis and control groups. Elevations of tHcy in patients were in the age group (60-69) years in both sexes. The levels of vitamin B12 were significantly increased in cirrhotic patients and hepatocellular carcinoma compared to the control group and chronic viral hepatitis patients.

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