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Plasma Trace Elements, Vitamin B_{12}, Folate, and Homocysteine Levels in Cirrhotic Patients Compared to Healthy Controls

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Abstract—Increased serum homocysteine (Hcy) can induce liver diseases and can play a remarkable role in hepatic disorders. The purpose of the present study therefore was to investigate the relationship between serum vitamin B_{12}, folate, zinc and copper, cysteine, and Hcy level differences between cirrhotic patients and healthy subjects. We studied 32 cirrhotic patients (12 females and 20 males) aged 45 ± 11 years and 32 control subjects (12 females and 20 males) aged 39 ± 9 years. There was an inverse correlation between Hcy and vitamin B_{12} in controls ($r = -0.442$, $p < 0.011$) but not in cirrhotic patients ($r = -0.147$, not significant). Also, mean plasma folate was decreased in cirrhotic patients compared to controls ($p < 0.001$). Copper increased whereas zinc decreased significantly in cirrhotic patients. A positive correlation was seen between the Cu/Zn ratio and Cu in controls ($r = 0.690$, $p < 0.01$), but the correlation between the Cu/Zn ratio and Cu was not significant in the cirrhotic group. Negative correlations were seen between plasma concentration of zinc and the Cu/Zn ratio in controls and cirrhotic patients ($r = -0.618$, $p < 0.01$ and $r = -0.670$, $p < 0.01$, respectively). Cirrhotic patients displayed multiple abnormalities, including changes in cysteine metabolism and in zinc and copper levels. Although hyperhomocysteinemia is known as an atherogenic and thrombogenic risk factor for cardiovascular disease, it might also be a risk factor for cirrhotic patients. Plasma Hcy, vitamin B_{12}, and folic acid measurement may be useful in the evaluation of cirrhotic patients.

Key words: cirrhosis, homocysteine, folate, vitamin B_{12}

Homocysteine (Hcy) is a sulfur-containing amino acid that is formed as an intermediary in methionine metabolism [1]. Plasma Hcy is a marker of folate or cobalamin deficiency states, while elevated plasma Hcy is an atherogenic and thrombogenic risk factor for cardiovascular diseases [2, 3]. Increased plasma Hcy in liver diseases may play an important role in hepatic disorders [4].

Homocysteine is metabolized through the trans-sulfuration and trans-methylation pathways, which require vitamin B_{12} (methylcobalamin), 5-methyltetrahydrofolate, and vitamin B_6 (pyridoxal phosphate, PLP) (figure). Three enzymes utilize Hcy as a substrate: methionine synthase and betaine-homocysteine methyltransferase, which convert Hcy back to methionine, and cystathionine β-synthase [1]. Changes in Hcy concentrations are likely to occur in liver disease, since many of the enzymes involved in Hcy metabolism have a high activity in liver. The liver possesses about 75% of the total body capacity for trans-methylation and hence production of Hcy [5].

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Zinc is an essential nutrient with a wide range of biological functions and is necessary for the growth and multiplications of cells [6]. Copper is an essential trace element. It is required in the diet because it is the metal cofactor for a variety of enzymes (e.g., amine oxidase, copper-dependent superoxide dismutase, cytochrome oxidase, and tyrosinase) [7].

Progressive liver fibrosis is observed in chronic alcoholic liver diseases. Liver fibrogenesis is a complex process in which collagen fibers are formed together with a protein matrix composed of proteoglycans, glucosaminoglycans, and other protein as laminin [8]. Some trace elements, like zinc and iron, play important roles as cofactors for a variety of enzymes involved in collagen synthesis [8, 9]. Low liver zinc and high liver copper concentrations have been reported in alcoholic cirrhosis [9].

The aim of this study was to measure and compare the levels of the trace elements Cu and Zn, vitamin B_{12}, folate, and homocysteine in the plasma of cirrhotic patients and healthy control subjects.

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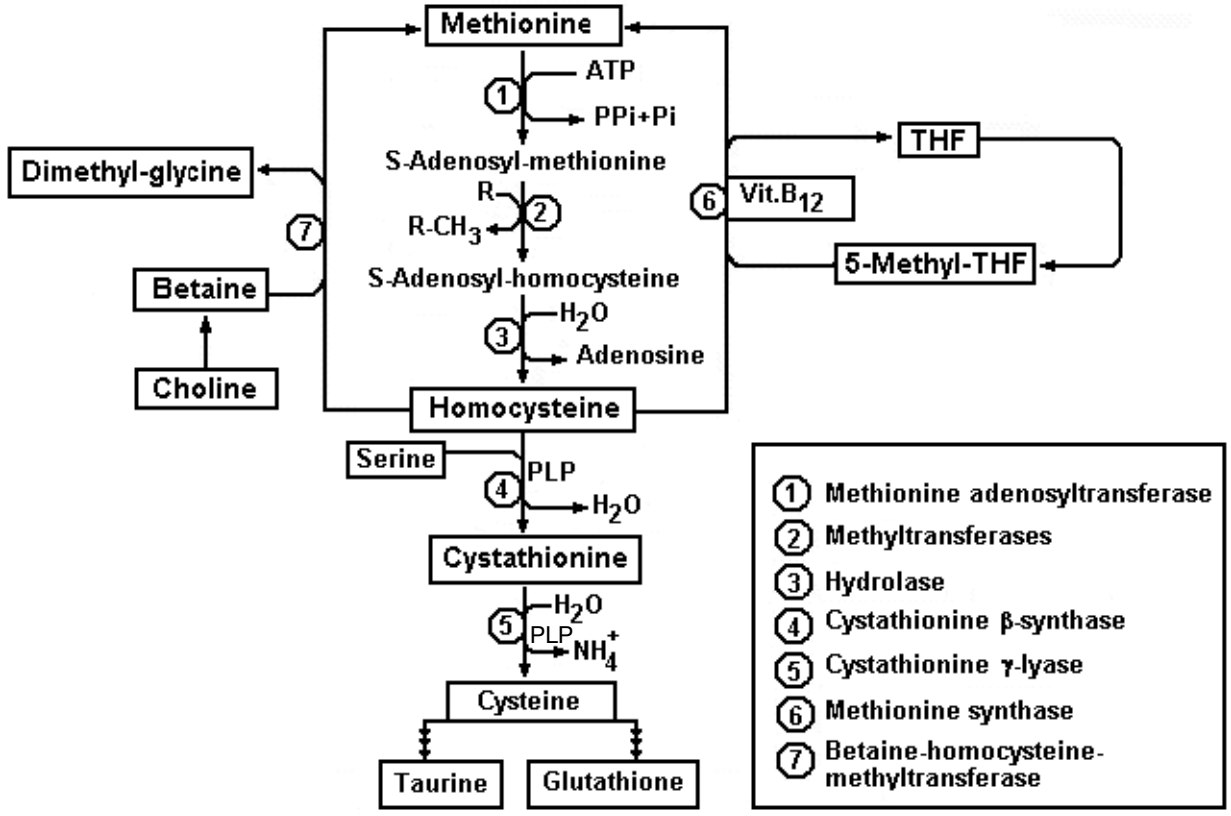

Metabolic pathways of homocysteine redrawn from Bosy-Westphal et al. [5] with minor modifications. THF) tetrahydrofolate; ATP) adenosine triphosphate; PLP) pyridoxal phosphate; PP_i) pyrophosphate

MATERIALS AND METHODS

Subjects. This study was carried out on 32 patients with liver cirrhosis (12 females and 20 males, aged 45 ± 11 years old) who were being treated in the gastroenterology clinic of the Medicine Center of Firat University. The etiology of cirrhosis was 19 alcoholic patients (59%), and 13 nonalcoholic patients (41%). Using Child–Purgh classification [10], 18 subjects were classified as grade A, nine as grade B, and five as grade C. The control group consisted of 32 healthy volunteers (12 females and 20 males, aged 39 ± 9 years old). All subjects underwent a standard clinical and biochemical evaluation (Table 1). After an overnight fast whole blood specimens were collected in sterile silicone-coated vacutainer tubes. The samples were then centrifuged at 4°C and the plasma was stored at −20°C until analyzed. This study was performed with the approval of the ethics committee and all subjects volunteered for the studies with informed consent.

Laboratory analyses. Using an Olympus 600 automated analyzer the following parameters were analyzed by standard chemical and enzymatic commercial methods: aspartate amino transferase (AST), alanine amino transferase (ALT), alkaline phosphate (ALP), γ-glutamyl transferase (GGT), lactate dehydrogenase (LDH), prothrombin activity, total bilirubin, direct bilirubin, total protein, and albumin. The concentrations of Hcy and cysteine were determined by high-performance liquid chromatography (HPLC) with a fluorescence detector (Model 10AD system, Shimadzu Corporation, Japan). Plasma Zn and Cu levels were determined by flame atomic absorption spectrophotometry (Model 6701F, Shimadzu Corporation). Folate and vitamin B_{12} concentrations were measured by microparticle immunoassay using Elecsys Moduler Analitics 170 (Roche Diagnostics, Germany).

Statistical methods. Analyses were performed using the SPSS 10.0 statistical package. The data are presented as arithmetic means ± standard deviations (SD). Pearson correlation coefficients were used to test the correlation between each of the two biochemical variables. Multiple linear regression analysis was used between homocysteine and possible determinants such as plasma concentrations of cysteine, Cu, Zn, Cu/Zn ratio, vitamin B_{12}, and folic acid. The difference in the means of two groups were evaluated with Student's *t*-test and significant levels were set at $p < 0.05$.

RESULTS

Table 1 summarizes some biochemical data from the control subjects and cirrhotic patients. As expected, mean plasma bilirubin, AST, ALT, ALP, GGT, LDH activities were higher in cirrhotic patients than in control subjects ($p < 0.001$), and total protein and albumin levels were decreased ($p < 0.01$ and $p < 0.001$, respectively). The activity of prothrombin in the cirrhotic group was significantly lower than in the control group ($p < 0.001$).

Mean plasma Hcy, vitamin B_{12} and Cu concentrations, and Cu/Zn ratio were significantly increased in cirrhotic patients ($p < 0.001$), but mean plasma folate and Zn levels were decreased in cirrhotic patients compared to the control subjects ($p < 0.001$). There was no difference between plasma cysteine concentrations in cirrhotic patients when compared to the control subjects (Table 2).

There was a significant inverse correlation between Hcy and vitamin B_{12} in controls ($r = -0.442$, $p < 0.011$) but not in cirrhotic patients ($r = -0.147$, not significant). Positive correlations were seen between plasma concentrations of Cu and vitamin B_{12} ($r = 0.450$, $p < 0.01$) in cirrhotic patients, but there was no correlation in controls ($r = -0.095$, not significant). An inverse correlation was seen between Hcy and Cu in cirrhotic patients ($r = -0.374$, $p < 0.035$) but there was no correlation in control subjects ($r = -0.120$, not significant). Plasma Zn values were slightly variable when compared to controls ($p < 0.001$) but there was no correlation between Hcy and Zn in cirrhotic patients ($r = -0.114$, not significant). A positive correlation was also observed between the Cu/Zn ratio and Cu in controls ($r = 0.690$, $p < 0.01$) but the correlation between the Cu/Zn ratio and Cu was not significant in the cirrhotic group. Negative correlations were observed between plasma concentration of zinc and the Cu/Zn ratio in controls and cirrhotic patients ($r = -0.618$, $p < 0.01$ and $r = -0.670$, $p < 0.01$, respectively).

Table 1. Biochemical characterization of controls and cirrhotic patients (mean ± SD)

Biochemical characteristics	Controls ($n = 32$)	Cirrhotic patients ($n = 32$)
AST, U/liter	23 ± 6	78 ± 66*
ALT, U/liter	20 ± 8	48 ± 16*
ALP, U/liter	92 ± 24	120 ± 67*
GGT, U/liter	22 ± 9	29 ± 12*
LDH, U/liter	350 ± 56	500 ± 31*
Prothrombin activity, %	83.12 ± 14.27	53.73 ± 13.10*
Total bilirubin, μmol/liter	8.67 ± 4.40	51.16 ± 33.24*
Direct bilirubin, μmol/liter	3.12 ± 1.46	20.73 ± 19.23*
Total protein, g/dl	7.40 ± 0.4	6.84 ± 0.97**
Albumin, g/dl	4.63 ± 0.30	2.90 ± 0.67*

Note: AST, aspartate amino transferase; ALT, alanine amino transferase; ALP, alkaline phosphate; GGT, γ-glutamyl transferase; LDH, lactate dehydrogenase.
* $p < 0.001$ and ** $p < 0.01$ with respect to healthy controls.

Table 2. Plasma Hcy and its determinants in healthy controls and cirrhotic patients (mean ± SD)

Concentration	Controls ($n = 32$)	Cirrhotic patients ($n = 32$)
Homocysteine, μmol/liter	10.15 ± 1.67	21.71 ± 4.93*
Cysteine, μmol/liter	140.42 ± 26.10	149.79 ± 4.34**
Vitamin B_{12}, pmol/liter	283.0 ± 97.63	812.07 ± 238.96*
Folate, nmol/liter	12.69 ± 3.50	7.70 ± 1.94*
Cu, μg/dl	130.08 ± 15.68	165.91 ± 22.62*
Zn, μg/dl	109.37 ± 10.58	81.83 ± 34.98*
Cu/Zn ratio	1.21 ± 0.19	2.29 ± 1.32*

* $p < 0.001$, ** $p > 0.05$.

DISCUSSION

In the present study, we found that plasma levels of Hcy, cysteine, vitamin B_{12}, and Cu and Cu/Zn ratio were significantly higher in cirrhotic subjects than in healthy controls. In spite of these parameters, levels of folate and Zn were lower than in control subjects.

Homocysteine, 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 level is a risk factor for heart disease, independent of other known risk factors such as elevated plasma cholesterol and hypertension [11, 12]. Homocysteine is an intermediate in methionine metabolism, which takes place mainly in the liver [13]. Elevated Hcy plasma levels are observed in cirrhotic patients [4, 5, 13].

In the present study, the Hcy level was significantly higher in cirrhotic subjects. Cysteine is a product of Hcy

breakdown and it is a constituent of glutathione. A slight decrease in folate [4, 5] concomitant with the significantly elevated levels of vitamin B_{12} [4, 5, 14] are in agreement with our results. Increases in vitamin B_{12} indicate that these individuals are not deficient in this vitamin. Increases in vitamin B_{12} in cirrhotic patients could be due to hepatocellular damage. Elevation in Hcy is probably the consequence of liver dysfunction [13] and decreased folate levels [4, 5]. Vitamin B_{12}-dependent methionine synthase activity could be diminished because of the lower folate levels and thus insufficient remethylation of Hcy to methionine would take place, and the level of vitamin B_{12} and Hcy was high. In the presence of pyridoxal phosphate (vitamin B_6) Hcy combines with serine and forms cystathionine and by the breakdown of this intermediate product cysteine first occurs, and then this product is converted to taurine and glutathione by the trans-sulfuration (figure). The level of vitamin B_6 is reduced in cirrhotic patients and chronic alcoholics [5, 15]. Vitamin B_6 is a coenzyme for cystathionine β-synthase which catalyses the formation of cystathionine. Cystathionine is then converted to cysteine.

We did not measure the level of vitamin B_6 in the present study. It is likely that significant changes had occurred in the level of vitamin B_6 like in those of folate and vitamin B_{12}. We did not observe a significant change in the level of cysteine. This could be taken as evidence that the trans-sulfuration route was active. However, an increase in the Hcy level could be due to diminished enzyme activity of methionine synthase as a consequence of the low concentration of folate.

In the present study, decreased plasma Zn and increased Cu levels and high Cu/Zn ratios were observed in cirrhotic subjects when compared to control subjects. The differences observed in Zn and Cu are in accordance with other reports [9, 16]. Low hepatic zinc would impair Cu/Zn superoxide dismutase function, thus allowing free radicals to damage hepatocyte function and structure, leading to hepatocyte necrosis and fibrosis [9]. Administration of Zn inhibits formation of malondialdehyde and decreases the accumulation of collagen in liver [6]. Zinc is a negative acute phase reactant [17, 18], and the Cu concentrations increase as a part of the inflammatory response [19, 20].

Taken together, the data obtained from the present study suggest that in addition to traditional biochemical tests, Hcy levels and the Cu/Zn ratio could be beneficial tools for management of cirrhotic patients.

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