



Plasma homocysteine levels in patients with liver cirrhosis

Nivo homocisteina u plazmi bolesnika sa cirozom jetre

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Abstract

Background/Aim. Homocysteine (2-amino-4-mercapto-butyric acid) is an amino acid that may be found in small quantities in all cells, and is quantitatively the major methionine metabolite. The most prevalent form is protein-bound homocysteine (about 80%), mostly to albumins. If catabolism of homocysteine is impaired either due to enzyme defect or deficiency of required intracellular cofactors, homocysteine accumulates in cells and reaches the circulation. The aim of our study was to determine homocysteine values and factors affecting homocysteine metabolism in patients with liver cirrhosis. **Methods.** The prospective study included 35 patients with liver cirrhosis and 30 age and sex matched healthy controls. All the examinations were based on: medical history, physical examination, laboratory tests including serum homocysteine levels and liver biopsy. The degree

of liver failure was assessed according to the Child-Pugh classification. **Results.** The mean plasma homocysteine levels were much higher in the patients with liver cirrhosis than in the healthy controls (t -test, $p < 0.001$). There was no significant difference between the plasma homocysteine concentration and etiology of liver cirrhosis (ANOVA, $p > 0.05$). Correlation analysis showed a positive correlation between the homocysteine and creatinine concentrations and between the serum albumin and homocysteine values, (Pearson's correlation, $p < 0.01$, and $p < 0.05$ respectively). **Conclusion.** In liver cirrhosis, the genesis of homocysteinemia is multifactorial, influenced significantly by impaired catabolic liver function, renal failure and hypoalbuminemia.

Key words:
homocysteine; liver cirrhosis; hypoalbuminemia;
creatinine.

Apstrakt

Uvod/Cilj. Homocistein (2-amino-4-merkaptobuterna kiselina) je aminokiselina koja se nalazi u malim količinama u svim ćelijama, i predstavlja glavni metabolit metionina. Homocistein se u serumu nalazi najčešće vezan za albumin (oko 80%). U stanjima u kojima dolazi do poremećaja katabolizma homocisteina, zbog enzimskog defekta ili nedostatka intracelularnih kofaktora neophodnih za njegov metabolizam, homocistein se skladišti u ćelijama i dospeva u cirkulaciju u slobodnom obliku. Cilj ove studije bio je utvrđivanje nivoa homocisteina kod bolesnika sa cirozom jetre i faktora koji mogu da utiču na njegov metabolizam. **Metode.** Ovom prospektivnom studijom bilo je obuhvaćeno 35 bolesnika sa cirozom jetre i 30 zdravih osoba koje su činile kontrolnu grupu. Grupe zdravih ispitanika i bolesnika bile su podudarne prema polu i uzrastu. Kod svih ispitanika ispitivanje je obuhvatilo anamnestičke podatke, fizikalni pregled, laboratorijske analize uključujući i nivo

homocisteina u serumu, kao i biopsiju jetre u grupi bolesnika sa cirozom jetre. Step en oštećenja jetre procenjen je primenom Child-Pugh klasifikacije. **Rezultati.** Bolesnici sa cirozom jetre imali su u proseku više vrednosti homocisteina nego zdravi ispitanici (t -test, $p < 0,001$). Etiološki faktori za nastanak ciroze jetre (alkohol, virusi, autoimunski, kriptogena ciroza) nisu uticali na nivo homocisteina u serumu (ANOVA, $p > 0,05$). Takođe, utvrđeno je postojanje pozitivne korelacije između nivoa homocisteina i kreatinina, odnosno albumina u krvi kod bolesnika sa cirozom jetre (Pearson-ov koeficijent korelacije, $p < 0,01$ i $p < 0,05$ respektivno). **Zaključak.** Bolesnici sa cirozom jetre imaju više vrednosti homocisteina u krvi, što je posledica brojnih faktora kao što su oštećena funkcija jetre, bubrežna insuficijencija i hipoalbuminemija.

Ključne reči:
homocistein; jetra, ciroza; hipoalbuminemija;
kreatinin.

Introduction

Homocysteine (2-amino-4-mecarpto-butinic acid) is an amino acid that may be found in small quantities in all cells, and it is quantitatively the major methionine metabolite. Moreover, homocysteine can be found either free in a body or in the form of disulfide and proteins. In relation to a total homocysteine quantity, free or reduced one accounts for only 1%–2%. However, the most prevalent form is protein-bound homocysteine (about 80%), mostly to albumins¹.

It is well-known, that a high level of blood serum homocysteine is a powerful risk factor for cardiovascular disease². On the other hand, elevated levels of homocysteine have been linked to increased fractures in elderly persons³.

The liver has an important role in metabolism of homocysteine. It is condensed with serine and upon separation of molecule of water and cystathionine- β -synthetase (vitamin B6 dependent enzyme) it yields cystathionine. Cystathionine, breaks down to homoserine and cysteine, by the action of cystathionase (vitamin B6 dependent enzyme). Homoserine is transformed into α -ketobutyric acid under the action of homoserine desaminase, when ammonium hydroxide and hydrogen sulfide are being separated from it. Homocysteine may in one part, oxidize to homocystine. Methionine may be resynthesized from homocysteine and methyl-tetrahydrofolic acid⁴.

If catabolism of homocysteine is impaired either due to enzyme defect or deficiency of required intracellular cofactors, homocysteine accumulates in cells and reaches the circulation.

The aim of our study was to determine homocysteine values and factors affecting the homocysteine metabolism in patients with liver cirrhosis.

Methods

In the period from August 2008 to April 2009, the prospective study included 35 patients with liver cirrhosis and 30 age and sex matched healthy controls examined at the Clinic of Gastroenterology and Hepatology, Clinical Center of Serbia, Belgrade. Written informed consent was obtained from each subject.

Inclusion criterion was the patients' diagnosis of liver cirrhosis as an underlying disease. All the examinations were based on: medical history, physical examination, laboratory tests and liver biopsy. Laboratory tests included: liver functional tests as well as specific (etiologial) tests. Puncture liver biopsy was performed in 7 (20%) patients, using the 1.4 mm Menghini needle. The degree of liver failure was assessed according to Child-Pugh classification.

A day after an overnight fast, fasting blood samples were drawn to determine biochemical indices and homocysteine determination were collected in additive-free Vacutainers (BD), centrifuged at 3,500 rpm (\approx 2,000 g) 10 minutes, using the "Heraeus Digifuga GL" centrifuge. After separation, serum was stored at -20°C until measurement. Blood was drawn between 8 and 9 a.m. Homocysteine was measured by commercial test kits by means of Immulite, (Siemens, USA) and expressed in $\mu\text{mol/L}$.

The results were expressed as mean \pm SD or as stated. Distribution data were compared by χ^2 -test. The differences between the groups were analysed using a paired Student's *t*-test and correlation coefficients (Spearman¹, correlation and Person's correlation test). All statistical analyses were performed with the SPSS 10.0 for Windows package (SPSS Inc., Chicago, IL). The values at the $p = 0.05$ level were considered statistically significant.

Results

The most common cause of liver cirrhosis was alcohol – 21 (60%) patients. The incidence of posthepatitis liver cirrhosis was lower – 7 (20%) of the patients, autoimmune liver diseases were quite rare – 4 (11.4%) of the patients, while etiology was unknown in 3 (8.6 %) of the cases.

The patients were classified into 3 classes according to generally accepted Child's system. Child-Pugh class A included 19 (54.2%), class B 8 (22.9%), and class C 8 (22.9%) of the patients.

The mean plasma homocysteine levels were much higher in the patients with the cirrhosis than in healthy controls. A statistically significant difference was found between homocysteine plasma values in patients with cirrhosis and healthy subjects (14.85 ± 5.40 versus $9.17 \pm 1.99 \mu\text{mol/L}$, *t*-test, $p < 0.001$).

On the other hand, there was no significant difference between the plasma homocysteine concentration and etiology of the cirrhosis (ANOVA, $p > 0.05$).

In relation to creatinine concentration, the patients were divided into two subgroups, *i.e.* with normal ($< 120 \mu\text{mol/L}$) and higher creatinine levels ($> 120 \mu\text{mol/L}$). The normal creatinine values were reported in 28 (80%) of the patients and higher levels were recorded in 7 (20%) of the cases.

Correlation analysis showed a positive correlation between homocysteine and creatinine concentrations (Pearson's test, $r = 0.4622$; $t = 2.994$, $p < 0.01$). In addition, a positive correlation between the serum albumin and homocysteine values was also established (Pearson's correlation, $p < 0.05$).

Discussion

Patients with cirrhosis develop a hyperdynamic state of circulation, with high cardiac output, increased blood volume, reduced systemic vascular resistance, and they are prone to arterial hypotension. Increased hepatic and collateral resistances as well as portal blood flow maintain portal hypertension^{5,6}.

It is considered that bacterial lipopolysaccharide endotoxins cause multiple-hour release of nitric oxide (NO) from vascular endothelium, what leads to peripheral vasodilatation, hypotension and tachycardia. *In vitro* effect of endotoxin and cytokine on NO synthesis induction has been proved in endothelium and smooth muscles with progressive vascular relaxation and poorer response to vasoconstrictors⁷.

High circulating endotoxin concentrations were found in cirrhosis, which may persist even without evident clinical

signs of infection. The endotoxins in cirrhosis are supposed to induce, directly or indirectly, the increase of NO release and synthesis, which causes the methionine-synthase inactivation, giving rise to accumulation of homocysteine in cells and extracellular space⁸.

Homocysteine and related biogenic thiols produce chemically and physiologically specific products in reactions with nitric oxides: nitrogen dioxide, dinitrogen trioxide and dinitrogen tetroxide. A tendency towards interaction with metal nitrosyl complexes is also manifested. In both cases, reaction products are S-nitrosothiol or thionitrites. These substances strongly activate the enzyme guanylate cyclase and are an important intermediary agent in metabolism of the endothelium-relaxing factor (EDRF)⁹.

Elevated homocysteine levels in cells and extracellular space, by inducing the synthesis of vasoactive EDRF, are involved in the pathogenesis of hyperdynamic circulation.

The study by Woitas et al.¹⁰ found significantly higher homocysteine concentrations in cirrhotic patients in relation to the controls ($p = 0.0002$), and a non-significant correlation between homocysteine concentration and degree of liver insufficiency according to the Child-Pugh classification ($p = 0.1$).

Ferre et al.¹¹ analyzed 76 patients with cirrhosis. Alcoholic cirrhosis was diagnosed in 48 (63%) of the patients and non-alcoholic in 28 (37%) of the cases. They verified higher homocysteine concentrations in cirrhotic patients in relation to the healthy controls which depended upon the extent of liver impairment. No difference in homocysteine concentrations between alcoholic and nonalcoholic cirrhosis was found.

Our studies are compatible with those of Woitas et al.¹⁰ and Ferre et al.¹¹. In our study as in the study by Woitas et al.¹⁰ there was no correlation between homocysteine concentrations and liver insufficiency according to the Child-Pugh classification ($p > 0.05$). It differs from the study by Ferre et al.¹¹ which noted that higher homocysteine concentrations correlated with more severe degree of liver insufficiency.

The kidneys are considered to have a major role in homocysteine metabolism and are involved in about 70%-elimination of homocysteine from plasma, by glomerular filtration and metabolism in tubular cells, breakdown through transsulfuration or remethylation into methionine. Some articles report that homocystinemia directly correlates with serum creatinine and glomerular filtration¹².

A significance of tubular metabolism of homocysteine has been corroborated by clinical studies. Renal posttrans-

plantation levels of homocysteine are much higher in comparison to patients with end-stage renal failure who had not undergone transplantation. During transplantation, a kidney is subjected to ischemic injury of tubular cells, which may be additionally damaged by immunological reactions and immunosuppressive drugs. Lower homocysteine metabolism in tubules causes higher homocysteine values¹³.

Our study found a positive correlation between homocysteine and creatinine levels ($p < 0.01$). Given that in our study only 7 (20%) of the patients had impaired renal function, evaluated on the basis of higher creatinine values, homocystinemia in cirrhosis could not be accounted for disordered glomerular filtration and tubular metabolism only.

Suliman et al.¹⁴ indicated that blood homocysteine level was lower in patients with end-stage renal failure with cardiovascular diseases and they associated such paradox with hypoalbuminemia.

It is well-known that plasma homocysteine may be found in several forms. The majority, about 70%, is bound to plasma proteins, *ie.* to albumin, mostly *via* cysteine, while the remaining free homocysteine is, due to high reactivity of the thiol group, susceptible to autooxidation and formation of disulfide bonds; the rest is composed of free, reduced form of homocysteine (only 1% of total plasma homocysteine)^{14,15}. Our study found a positive correlation between albumin and homocysteine, what is in compliance with the past results.

On the other hand, it is well known that vitamin B6 deficiency is usually the result of malabsorption syndrome, uremia, cancer, cirrhosis, alcoholism, old age, and pregnancy¹⁶. Moreover, it is showed that plasma levels of pyridoxal-5'-phosphate (PLP) in cirrhotic patients were significantly lower than in healthy control subjects¹⁷.

Nutrition regime significantly interferes with the level of homocysteine that may vary in relation to methionine intake by food. Animal food is richer in methionine than plant one. Meat and fish contain 2.7 g/100 g, eggs 3.2g/100 g, cow milk 2.9 g/100 mL of methionine versus fruit and vegetables containing only 0.9–1.2 g/100g^{15,18}. Although the nutritive status was not the subject of analysis in our study, it is well-known that cirrhotic patients have poor appetite and reduced intake of proteins in relation to healthy subjects¹⁹.

Conclusion

In liver cirrhosis, the genesis of homocystinemia is multifactorial, influenced significantly by impaired catabolic liver function, renal failure and hypoalbuminemia.

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