Basal and Post-Methionine Serum Homocysteine and Lipoprotein Abnormalities in Patients With Chronic Liver Disease

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ABSTRACT

Background: **Lipoprotein abnormalities are commonly found in chronic liver diseases (CLDs), particularly hypercholesterolemia in primary biliary cirrhosis (PBC). However, affected patients may not be at increased risk of coronary heart disease. Cirrhotic patients display impaired methionine clearance, and an increased level of homocysteine, a methionine metabolite, is an independent risk factor for coronary heart disease. Thus, we hypothesized that the low risk of coronary heart disease in patients with CLD may be related to low serum levels of homocysteine. The aim of this study was to test this hypothesis after methionine load and to describe the serum lipoprotein profile in patients with PBC and in patients with hepatocellular liver disease.**

Methods: **Fifteen female patients (mean age, 58.2±11.7 years) with PBC, 15 female patients (mean age, 54.5 ± 9.6 years) with other causes of CLD, and 15 healthy sex- and age-matched controls were given L-methionine (50 mg/kg of ideal body weight). Basal fasting serum homocysteine level and 2, 4, and 6 hours of post–methionine load were determined using high-performance liquid chromatography with a fluorometric detector. Levels of fasting serum cholesterol, triglycerides, high-density lipoprotein (HDL), low-density lipoprotein (LDL), lipoprotein (a) (Lp(a)), and apoprotein B were also determined.**

Results: **Results showed that mean basal and post–methionine load (6 hours) serum homocysteine levels were statistically significantly higher in the patients with PBC and with CLD than in the control group ($P=0.04$) and that levels of serum cholesterol, LDL, HDL, and apoprotein B were significantly higher in the PBC patients than in the other two groups ($P\leq0.05$). There was no correlation between any of these parameters and the severity of liver disease. Serum HDL was significantly lower in the CLD group ($P\leq0.05$) and correlated with severity of liver disease. There was no significant difference in serum cholesterol, LDL, or apoprotein B between the CLD group and the controls. Serum triglyceride and Lp(a) levels were similar for all three groups.**

Conclusions: **In contrast to previous reports, the site of the methionine metabolic impairment was found to be below the homocysteine synthesis level. For most patients with CLD, factors other than serum homocysteine or Lp(a) are responsible for the reduction in the risk of coronary heart disease. Further studies with larger samples are needed. (J Investig Med 2001;49:325–329) Key Words: homocysteine • lipoprotein • chronic liver disease**

INTRODUCTION

The liver plays a key role in serum lipoprotein synthesis and metabolism. Changed lipid and apoprotein composition of serum lipoproteins is commonly found in patients with chronic liver disease (CLD). However, primary biliary cirrhosis (PBC), a chronic slowly progressive cholestatic liver disease,[1] is often associated, for reasons not well understood,[2] with lipoprotein abnormalities, particularly

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marked elevations in serum cholesterol levels, and is different from parenchymal diseases.[3–5] Nevertheless, although elevated serum cholesterol is an important risk factor for atherosclerosis in the general population, patients with PBC do not have higher than normal rates of atherosclerosis-related death[6,7] and, apparently, patients with hepatic cirrhosis seem less liable to develop coronary heart disease.[8]

A high serum level of homocysteine, a methionine metabolite, is an independent risk factor for vascular disease, thrombosis, and atherosclerosis, including coronary disease.[9–11] Methionine is an essential sulfur-containing amino acid that is catabolized mainly via the transsulfuration pathway, located principally in the liver, according to the following sequence: methionine→S adenosylmethionine→S-adenosylhomocysteine→homocysteine.[12] Previous studies have shown that cirrhotic livers are characterized by a markedly reduced activity of S-adenosyl-L-methionine synthetase, leading to impaired clearance of methionine[13,14] and, consequently, low homocysteine levels.

The aim of the present study was to determine whether the low risk of cardiac disease in patients with CLD is attributable to the concomitant presence of low levels of serum homocysteine. We also sought to investigate the serum lipoprotein changes in patients with PBC (a cholestatic liver disease) and in patients with other causes of CLD (a hepatocellular liver disease).

PATIENTS AND METHODS

The study population included 15 women of mean age 58.2±11.7 years with a diagnosis of PBC based on clinical, biochemical, immunological, and histological criteria.[15] Histological staging was performed according to Scheuer[16] (stage 1, n=4; stage 2, n=3; stage 3, n=3; stage 4, n=5). All patients were treated with ursodeoxycholic acid (URSO) 10 mg/kg/d; none was receiving any other cholesterol or lipid-altering medication. None of the patients had clinical evidence of coronary artery disease. Findings were compared with those in 15 patients with CLD resulting from other causes (chronic hepatitis C infection, n=9; chronic hepatitis B infection, n=3; alcohol-induced hepatitis, n=2; and autoimmune hepatitis, n=1), who were matched for sex (all females), age (mean, 54.5±9.6 years), and severity of disease (by Child-Pugh criteria: A, n=6; B, n=3; C, n=6).[17] The control group consisted of 15 healthy, drug-free, sex- and aged-matched volunteers (mean age, 56.5±11.1 years) with normal liver function tests. None of the women received hormone replacement therapy. Informed consent was obtained from each patient, and the study protocol conformed to the ethical guidelines of the 1975 Declaration of Helsinki.

Methionine Load

All study participants were maintained on an isoenergetic diet containing 40 g of protein. The caloric intake was calculated from the Harris and Benedict equation,[18] which adjusts for height, present weight, and age. On day 4, after an overnight fast, venous plasma samples were obtained for the determination of homocysteine, and an oral dose of L-methionine 50 mg/kg of ideal body weight was administered at 0800 hours. A protein-free breakfast was given 30 minutes after the methionine load. Carbohydrate and fat sources were substituted for protein calories during lunch (150 kcal). Blood samples were obtained at 2, 4, and 6 hours after methionine loading, placed on ice, and centrifuged for 10 minutes. These specific time points were chosen according to the study of Horowitz et al,[13] wherein peak plasma methionine level was reached at 30 minutes after methionine load in patients with cirrhosis and control subjects. Thereafter, the decline in plasma concentration followed first-order kinetics, and at 6 hours after methionine load, plasma methionine did not change significantly.[17] The plasma was separated and immediately frozen at −80°C until analysis. Plasma samples were analyzed for homocysteine by high-performance liquid chromatography with a fluorometric detector, as described by Ubbink et al.[19] The method is based on reduction with tri-n-butyl-phosphine and derivatization with SBD-F (ammonium 7-fluorobenzo-2-oxa-1,3, diazole-4-sulfonate). Hyperhomocystinemia was diagnosed when fasting plasma homocysteine levels or absolute increments of homocysteine after methionine load exceeded the upper limit of the normal range (mean±SD) of the control group.

Lipoprotein Measurements

Blood samples were collected in plain tubes after an overnight fast (14 hours) and centrifuged within 1 hour. Aliquots of serum were stored at −20°C before lipoprotein (a) (Lp(a)) and apolipoprotein B (apoB) analysis. All other tests were performed on fresh samples. Serum total cholesterol was measured with a cholesterol oxidase method kit (Reagents Applications, Inc, San Diego, Calif), and serum triglyceride (TG) with the lipase-glycerol kinase end point reaction method (kit supplied by Raichen, San Diego, Calif). Serum high-density lipoprotein (HDL)-cholesterol level was assayed with the heparin-manganese precipitation method. Serum low-density lipoprotein (LDL) was calculated with Friedewald's formula.[20] Serum Lp(a) was determined with a cardio-check Lp(a) kit (Alerchek, Inc, Portland, Me), and serum apoB was measured

according to our local protocol.[21] Serum folate and vitamin B_{12} levels were also measured.

Statistical Analysis

Results are given as mean±SD. The Pearson correlation coefficient (r) and the significance for it (P) were calculated between the variables. Analysis of variance with the Duncan multiple comparison option was performed to determine significant differences in mean continuous variables (cholesterol, TG, HDL, LDL, Lp(a), homocysteine) among the three groups of patients and by severity of disease (Child-Pugh for CLD, staging for PBC). $P \leq 0.05$ was considered statistically significant.

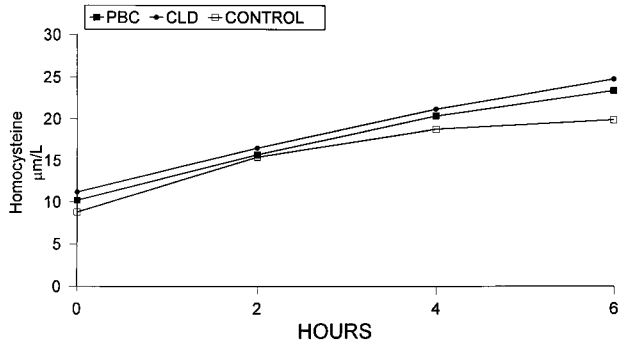
RESULTS

Basal and Post–Methionine Load Serum Homocysteine Level

Mean basal serum homocysteine levels (Figure) were statistically significantly higher in the PBC group compared with the controls (16% increase, $P=0.04$) and in the CLD group compared with the controls (27% increase, $P=0.04$) (Figure); there was no significant difference between the PBC and CLD groups. These findings were sustained at 6 hours after methionine loading: 17.5% increase for PBC group versus controls ($P \leq 0.05$) and 20% increase for CLD group versus controls ($P \leq 0.05$). Levels at 6 hours were higher than the 2- and 4-hour levels, but these differences did not reach statistical significance (Figure). There was no statistically significant correlation between serum homocysteine level and severity of liver disease in the PBC ($r=-0.36$, $P=0.19$) or CLD group ($r=0.05$, $P=0.8$).

Lipoprotein Measurements

The lipid and lipoprotein results are given in the Table. The PBC group had significantly higher levels of serum cholesterol, LDL, and apoB than either the CLD or the control group, which were similar ($P \leq 0.05$). They also had a significantly higher serum HDL level than the CLD group ($P \leq 0.05$). Serum HDL level was significantly lower in the CLD group compared with the control group ($P=0.05$). There was no significant statistical difference in serum cholesterol, LDL, or apoB between the CLD group and the controls. No between-group differences were noted on serum TG and Lp(a). Serum HDL level decreased with increasing severity of disease in the CLD group ($r=-0.63$, $P=0.01$), but not in the PBC group. None of the other parameters (cholesterol, LDL, apoB, TG, Lp(a)) correlated with severity of liver disease. All patients and controls had normal serum levels of vitamin B_{12} and folic acid (data not shown).



Mean basal and peak (6 hours) post–methionine load serum homocysteine levels were significantly higher (P≤0.05) in both patient groups (PBC and CLD) than in the control group.

DISCUSSION

Our findings failed to support our hypothesis. Mean basal and peak (6 hours) post–methionine load serum homocysteine levels were significantly higher ($P \leq 0.05$) in both patient groups (PBC and CLD) than in the control group. Mild to moderate hyperhomocystinemia either on fasting or after oral methionine loading has been found to be an independent risk factor for coronary disease.[9,10] Nevertheless, as previously reported, patients with PBC[6,7] and hepatic cirrhosis[8] are apparently less liable to develop coronary heart disease. Moreover, in contrast to previous studies wherein the site of impairment in the hepatic transsulfuration pathway was reported to be upstream of homocysteine synthesis,[13,14] our findings suggest that the site is downstream. The present study is limited by its small sample size, which decreases its statistical power. This is also true of prior studies of the risk of cardiovascular events in PBC patients. Furthermore, PBC patients are mostly women, who have a lower risk of coronary heart disease than the average population, which also includes men. Therefore, further large-scale, case-control studies are needed to confirm these results.

Patients with cholestatic liver disease show differences in serum lipids and lipoprotein patterns from patients with hepatocellular disease and healthy controls.[2–6] Jahn et al[3] noted that PBC patients show a significant increase in mean cholesterol and LDL levels with disease progression, in mean serum TG level in stages 1, 2, and 4 disease, and in mean serum HDL in stages 1 and 2 disease (but significantly decreased in stage 4). However, no control group was included in that study. In the study by Crippin et al[6] of a larger group of PBC patients and normal controls, serum cholesterol and LDL increased progressively with increasing histological stage, whereas serum HDL was

Serum lipid and lipoprotein levels (mg/dL) in the three disease groups.

	PBC (n=15)	CLD (n=15)	Controls (n=15)	P*		
				PBC vs Controls	PBC vs CLD	CLD vs Controls
Cholesterol	281.2±161.3	180.4±51.4	201.0±41.2	0.05	0.05	NS
TG	166.2±106.3	137.7±41.1	148.8±89.5	NS	NS	NS
HDL	49.2±21.0	35.0±10.5	47.7±11.0	NS	0.05	0.05
LDL	198.6±151.4	117.9±43.6	123.5±34.0	0.05	0.05	NS
Lp(a)	19.3±6.4	18.6±6.9	18.1±3.8	NS	NS	NS
apoB	163.0±46.7	112.2±32.4	128.2±46.6	0.05	0.05	NS

NOTE. All laboratory values are mean±SD.
Abbreviation: NS, not significant.
** Analysis of variance with Duncan multiple comparison.*

elevated and serum TG was either normal or slightly elevated in all stages.

Ours is the first study to compare serum lipoprotein levels in PBC with those in age- and disease severity–matched CLD patients, in addition to healthy controls (all females). The PBC patients had significantly higher serum levels of cholesterol, LDL, and apoB than the CLD patients and the controls ($P \leq 0.05$) and a significantly higher serum HDL level than the CLD patients but not than the controls. None of these parameters correlated with disease stage. There was also no statistically significant difference in serum cholesterol LDL and apoB levels between the CLD patients and the controls. Serum HDL level was significantly lower in the CLD group than the controls, and it correlated with severity of liver disease (Child-Pugh).

Gregory et al[22] found that 14.3% of their PBC patients and 17.5% of their CLD patients had coronary heart disease, compared with 4.6% of the control group, whereas Howel and Manion[8] reported that patients with liver cirrhosis are less liable to acquire coronary heart disease than the general population. The latter study was supported by Crippin et al[6] and Propst et al,[7] who claimed that the hypercholesterolemia associated with PBC did not expose these patients to an increased risk of atherosclerotic-related deaths,[6,7] although the reason for this is not yet understood. One explanation is that PBC affects predominantly middle-aged women, as in our study, a subgroup known to be characterized by approximately one half the rate of coronary heart disease as middle-aged men.[23] Secondly, PBC patients have an elevated level of serum HDL, which plays a crucial role in the removal of cholesterol from peripheral tissues.[24,25] The decreased hepatic TG lipase levels in patients with PBC may account for their high serum HDL.[26]

High serum Lp(a) has also been associated with atherosclerotic disease and is considered to be a strong independent risk factor for cardiovascular disease.[27–30] However, studies in patients with CLD have reported controversial results. Gregory et al[22] found that PBC patients had lower serum Lp(a) levels than CLD patients and controls,[22] whereas Alessandri et al,[31] who investigated only patients with CLD, found that serum Lp(a) levels were not only reduced, but that this reduction was directly correlated with the severity of liver disease (Child-Pugh). They suggested that the low serum Lp(a) level in patients with cirrhosis[32] and PBC[22] exerts a cardioprotective effect. In our study, serum Lp(a) levels were not statistically different between the three groups, and it did not correlate with severity of disease.

High serum apoB levels have been implicated in the pathogenesis of myocardial infarction.[33] We found elevated serum apoB levels in the PBC group compared with the CLD group and controls, although previous reports found lower serum apoB levels in PBC patients.[22]

Although ursodeoxycholic acid (URSO) has been shown to have cholesterol-lowering effects in patients with PBC[34,35] the mean serum cholesterol level in our study group was high (281.2 mg/dL) despite URSO treatment for at least 2 years. However, the mean serum cholesterol level before URSO administration was not always available, so that the drug's cholesterol-lowering effect could not be determined. There may be a correlation between serum homocysteine level and URSO administration, but this needs to be further investigated.

Our findings show that neither serum homocysteine level nor serum Lp(a) is responsible for the reduced risk of coronary heart disease in patients with CLD (cholestatic and hepatocellular). Other unknown factors almost cer-

tainly play an important role in this complex interaction. These need to be sought in future studies with larger samples, and they warrant the assessment of the incidence and causes of atherosclerosis in patients with PBC and CLD.

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