Hyperhomocysteinemia in Liver Transplant Recipients: Prevalence and Multivariate Analysis of Predisposing Factors

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Liver transplant recipients have an increased risk for cardiovascular disease because of a high incidence of obesity, arterial hypertension, diabetes mellitus, and hyperlipidemia. Hyperhomocysteinemia has been found to be an important risk factor for cardiovascular disease in large studies. Fasting serum levels of homocysteine were measured in 105 liver transplant recipients, and hyperhomocysteinemia was defined as a fasting serum homocysteine level greater than 13 μmol/L. Patients with versus without hyperhomocysteinemia were compared. The possible association of hyperhomocysteinemia with age, sex, cause of liver disease, time elapsed since liver transplantation, immunosuppressive therapy, folic acid level, liver function test results, renal function, and other cardiovascular risk factors was investigated. Patients with serum homocysteine levels greater than 15 μmol/L were treated with folic acid, 10 mg/d, and serum homocysteine levels were measured again 1 to 3 months later in 10 patients. Hyperhomocysteinemia was detected in 28 patients (27%). In univariate analysis, it was associated with hepatitis C virus infection, treatment with mycophenolate mofetil, and greater serum levels of alkaline phosphatase, γ-glutamyl transpeptidase, urea, and creatinine. In multivariate analysis, only greater serum levels of creatinine ($P = .006$) were associated with hyperhomocysteinemia. Treatment with folic acid resulted in a decrease in fasting serum homocysteine levels in 9 of the 10 patients tested ($P = .01$). Hyperhomocystinemia, associated with renal dysfunction, is a frequent finding in liver transplant recipients. Treatment with folic acid may reduce fasting homocysteine levels. (*Liver Transpl 2000;6:614-618.*)

Cardiovascular disease is a major cause of morbidity and mortality in long-term survivors after liver transplantation. It is the most frequent nonimmune, non–immunosuppression-related cause of death in such patients.[1] Cardiac and neurological complications are more frequent in patients aged older than 60 years,[2] a subgroup of transplant recipients that is increasing during the last years. Some cardiovascular risk factors, such as obesity, arterial hypertension, hypercholesterolemia, and diabetes mellitus, are frequent after liver transplantation,[3] thus increasing their cardiovascular risk.

Homocysteine is a sulfur-containing amino acid formed in the metabolism of methionine. In 1969, McCully[4] found extensive arterial thrombosis and atherosclerosis in 2 children with high plasma homocysteine concentrations and homocystinuria. Since then, there has been a growing evidence of the association of high plasma levels of homocysteine and cardiovascular disease.[5] In the mechanism of this vascular damage, the rapid auto-oxidation of homocysteine in plasma, producing such potent reactive oxygen species as superoxide and hydrogen peroxide, may be involved.[6] Homocysteine is metabolized by methylation and transsulfuration. In the first pathway, the methyl donor is N_5-methyltetrahydrofolate. For this reason, folic acid dietary supplements have been shown to reduce fasting homocysteine levels.[7]

On these basis, we studied the incidence of hyperhomocysteinemia in liver transplant recipients, factors possibly associated with hyperhomocysteinemia, and its response to treatment with folic acid.

Patients and Methods

One hundred five adult liver transplant recipients were prospectively studied at different times posttransplantation. After overnight fasting (>10 hours), a complete blood count and serum levels of alanine transferase, aspartate aminotransferase, alkaline phosphatase, γ-glutamyl transpeptidase, total bilirubin, urea, creatinine, cholesterol, high-density lipoprotein cholesterol, low-density lipoprotein cholesterol, triglycerides, homocysteine, and folic acid were measured. Serum homocysteine levels were determined using a fluorescent polarized immunoassay run on an IMX system (IMX Homocisteina; Axis Biochemicals ASA, Oslo, Norway). Serum concentrations of folic acid were measured by chemiluminescent enzyme immunoassay (Immulite Folic Acid; Diagnostic Products Corp, Los Angeles, CA). Hyperhomocysteinemia was

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Table 1. General Characteristics of the Patients

Age (yr)	58.34 ± 0.89
Sex (male/female)	73/32
Cause	
Alcoholic cirrhosis	42 (40)
Hepatitis C cirrhosis	30 (29)
Other*	33 (31)
Hepatocellular carcinoma	29 (28)
Current immunosuppressive therapy	
Cyclosporine-based	71; monotherapy 43 (61)
Tacrolimus-based	29; monotherapy 16 (55)
Mycophenolate mofetil–based	5; monotherapy 5 (100)
Time after liver transplantation (mo)	45 ± 3 (1-144)
<1 yr	22 (21)
1-3 yr	25 (24)
>3 yr	58 (55)
Cardiovascular risk factors	
Arterial hypertension	79 (75)
Hypercholesterolemia	40 (38)
Obesity	35 (33)
Diabetes mellitus	12 (11)

NOTE. Values expressed as mean ± SEM (range) or number (percent).
*Other includes: primary biliary cirrhosis (8 patients), hemochromatosis (6 patients), hepatitis B cirrhosis (5 patients), cryptogenic cirrhosis (4 patients), Wilson's disease (3 patients), Budd-Chiari syndrome (2 patients), alpha$_1$-antitrypsin deficiency (1 patient), autoimmune hepatitis (1 patient), idiopathic adulthood ductopenia (1 patient), liver metastasis (1 patient), and polycystic liver disease (1 patient).

defined as a fasting homocysteine level greater than 13 μmol/L, according to Jacques et al.[7]

The incidence of other cardiovascular risk factors was recorded. Hypercholesterolemia was defined as a fasting serum cholesterol level greater than 220 mg/dL or the need for pharmacological therapy to maintain a lower level. Diabetes mellitus was defined as the need for pharmacological therapy to maintain normal glucose levels before and after meals. Arterial hypertension was defined as systolic blood pressure greater than 140 mm Hg, diastolic blood pressure of 90 mm Hg or greater, or the need for antihypertensive drugs to maintain blood pressure within normal limits. Obesity was defined as body mass index greater than 30 kg/m^2.

Other clinical data were recorded from the patients' medical histories, including age, sex, cause of liver disease, time elapsed since liver transplantation, and immunosuppressive therapy.

The possible association between hyperhomocysteinemia, the listed laboratory and clinical variables, and the incidence of other cardiovascular risk factors was investigated using Mann-Whiney *U* test for continuous variables and Chi-

Table 2. Clinical Predictive Factors for Hyperhomocysteinemia After Liver Transplantation by Univariate Analysis

	Hyperhomocysteinemia (n = 28)	No Hyperhomocysteinemia (n = 77)	*P*
Age (yr)	58 ± 2	59 ± 1	.99
Sex			
Men	20 (71)	53 (69)	1.00
Women	8 (29)	24 (31)	
Cause			
Alcoholic	8 (29)	34 (44)	.06
Hepatitis C	13 (46)	17 (22)	
Others	7 (25)	26 (34)	
Hepatitis C			
Yes	13 (46)	17 (22)	.03
No	15 (54)	60 (78)	
HCC			
Yes	6 (21)	23 (30)	.47
No	22 (79)	54 (70)	
Cyclosporine			
Yes	19 (68)	52 (68)	1.00
No	9 (32)	25 (32)	
Tacrolimus			
Yes	6 (21)	23 (30)	.47
No	22 (79)	54 (70)	
MMF			
Yes	9 (32)	6 (8)	.003
No	19 (68)	71 (72)	
Azathioprine			
Yes	5 (18)	18 (23)	.61
No	23 (82)	59 (77)	
Prednisone			
Yes	7 (25)	13 (17)	.40
No	21 (75)	64 (83)	
Follow-up (mo)	52 ± 7	44 ± 3	.34
Hypertension			
Yes	24 (86)	55 (71)	.20
No	4 (14)	22 (29)	
Obesity			
Yes	9 (32)	26 (34)	1.00
No	19 (68)	51 (66)	
Hypercholesterolemia			
Yes	12 (43)	28 (36)	.65
No	16 (57)	49 (64)	
Diabetes			
Yes	5 (18)	7 (9)	.30
No	23 (82)	70 (91)	

NOTE. Values expressed as number (percent) unless otherwise noted.
Abbreviations: HCC, hepatocellular carcinoma; MMF, mycophenolate mofetil.

Table 3. Predictive Laboratory Factors for Hyperhomocysteinemia After Liver Transplantation by Univariate Analysis

	Hyperhomocysteinemia (n = 28)	No Hyperhomocysteinemia (n = 77)	*P*
AST (IU/L)	27 ± 3	23 ± 2	.35
ALT (IU/L)	33 ± 4	32 ± 4	.17
AP (IU/L)	212 ± 24	164 ± 7	.04
GGT (IU/L)	90 ± 37	35 ± 5	.007
Total bilirubin (mg/dL)	1.24 ± 0.16	1.15 ± 0.08	.78
Hemoglobin (g/dL)	12.8 ± 0.3	13.2 ± 0.2	.34
White blood cell count ($10^3/\mu L$)	4.9 ± 0.3	5.1 ± 1.9	.60
Platelet count ($10^3/\mu L$)	132 ± 9.9	142 ± 5.1	.19
Cholesterol (mg/dL)	203 ± 9	194 ± 4	.22
HDL (mg/dL)	43 ± 3	45 ± 2	.40
LDL (mg/dL)	135 ± 7	126 ± 4	.24
Triglycerides (mg/dL)	135 ± 14	118 ± 7	.26
Urea (g/L)	0.64 ± 0.04	0.50 ± 0.02	.001
Creatinine (mg/dL)	1.47 ± 0.08	1.15 ± 0.03	<.001
Folic acid (ng/mL)	8.4 ± 0.8	8.8 ± 0.5	.60

Abbreviations: AP, alkaline phosphatase; GGT, γ-glutamyl transpeptidase; HDL, high-density lipoprotein cholesterol; LDL, low-density lipoprotein cholesterol; AST, aspartate aminotransferase; ALT, alanine aminotransferase.

square and Fisher's exact tests, as appropriate, for categorical variables. Those variables showing *P* less than .05 in univariate analysis were entered into logistic regression analysis to obtain relative risks with 95% confidence intervals adjusted for confounding factors. Correlations between serum homocysteine levels and continuous variables significantly associated with hyperhomocysteinemia were assessed by means of Spearman's rank-order test. All statistical work was performed using SPSS for Windows, version 8.0.0 (SPSS Inc, Chicago, IL).

Patients with fasting homocysteine levels greater than 15 μmol/L were treated with oral folic acid at a dosage of 10 mg/d. Fasting homocysteine levels were determined again 1 to 3 months later in 10 of these patients, and its evolution was studied using Wilcoxon signed-rank test. In a control group of 10 patients, homocysteine levels were determined again 3 months after their basal determination without changing pharmacological treatment or adding vitamin supplements.

Significance was recorded for *P* of .05 or less. Continuous variables are expressed as mean ± SEM.

Results

General characteristics of the patients are listed in Table 1. Induction immunosuppressive therapy combined a calcineurin inhibitor (either cyclosporine or tacrolimus), azathioprine, and prednisone. Long-term monotherapy with a calcineurin inhibitor was intended and was achieved in most patients between posttransplantation months 6 and 12. Patients with impaired renal function were treated with mycophenolate mofetil and partial or complete withdrawal of calcineurin inhibitors.

The mean serum concentration of homocysteine was 11.0 ± 0.4 μmol/L (range, 2.9 to 22.5 μmol/L). Hyperhomocysteinemia was found in 28 patients (27%). The study of predictive factors for hyperhomocysteinemia is listed in Tables 2 and 3. Patients with hyperhomocysteinemia had greater serum levels of al-

Table 4. Predictive Factors for Hyperhomocysteinemia After Liver Transplantation by Multivariate Analysis

	Relative Risk (95% confidence interval)	*P*
Creatinine (mg/dL)	41.81 (2.87-609.0)	.006
Urea (g/L)	1.98 (0.06-65.44)	.70
Alkaline phosphatase (IU/L)	1.00 (0.99-1.01)	.43
GGTP (IU/L)	1.00 (1.0-1.01)	.40
Treatment with MMF	2.49 (0.62-9.96)	.20
Hepatitis C virus infection	2.65 (0.89-8.20)	.09

Abbreviations: GGTP, γ-glutamyl transpeptidase; MMF, mycophenolate mofetil.

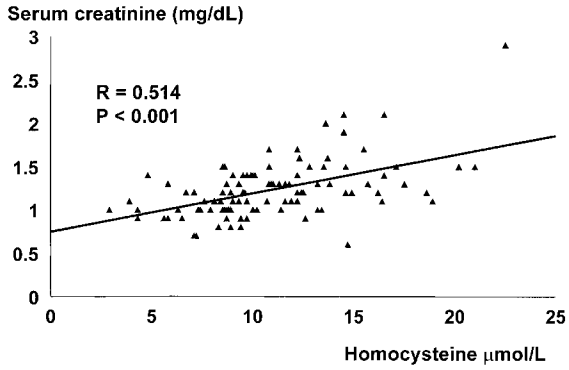

Figure 1. Correlation between serum creatinine levels and fasting serum homocysteine levels.

kaline phosphatase, γ-glutamyl transpeptidase, urea, and creatinine, and more frequently underwent transplantation for hepatitis C virus–related liver disease and were under treatment with mycophenolate mofetil. On multivariate analysis, the only factor associated with hyperhomocysteinemia was serum creatinine level (Table 4). There was a significant correlation between serum creatinine and homocysteine levels (Fig. 1). Only 8 of 60 patients (13%) with normal serum creatinine levels (<1.21 mg/dL) had hyperhomocysteinemia compared with 20 of 45 patients (44%) with high serum creatinine levels ($P = .001$). Patients with high serum creatinine levels had a relative risk for hyperhomocysteinemia of 5.20 (95% confidence interval, 2.01 to 13.43) compared with patients with normal renal function.

The incidence of other metabolic cardiovascular risk factors is listed in Table 1. Only 13 patients (12%) had none of the mentioned cardiovascular risk factors (arterial hypertension, obesity, hypercholesterolemia, diabetes mellitus, and hyperhomocysteinemia), 29 patients (28%) had 1 risk factor, 33 patients (31%) had 2 risk factors, 24 patients (23%) had 3 risk factors, and 6 patients (6%) had 4 risk factors. No patient had 5 risk factors. There was no association between hyperhomocysteinemia and other cardiovascular risk factors.

Treatment with folic acid in patients with fasting serum homocysteine levels greater than 15 μmol/L was followed by a reduction in homocysteine levels in 9 of 10 patients ($P = .01$). These results are shown in Figure 2. Mean fasting serum homocysteine levels in these 10 patients were 17.8 ± 0.7 μmol/L (range, 15.5 to 22.5 μmol/L) before treatment with folic acid and 13.0 ± 1.5 μmol/L (range, 5.9 to 21.4 μmol/L) after treatment. In the control (untreated) group, serum homocysteine levels did not change significantly (11.0 ± 0.9 *v* 12.0 ± 0.9 μmol/L).

Discussion

Hyperhomocysteinemia has been found to be an independent risk factor for cardiovascular disease.[8] Epidemiological studies have found that patients with hyperhomocysteinemia have a greater risk for coronary heart disease, cerebrovascular disease, and peripheral vascular disease.[9] In a large European series, patients with fasting homocysteine levels greater than 12 μmol/L had twice the risk for coronary heart disease as patients with lower levels, whereas the relative risk for coronary heart disease of a patient with a fasting cholesterol level of 275 mg/dL compared with a patient with 189 mg/dL was 1.4.[10] Fortunately, hyperhomocysteinemia may be corrected with folic acid supplements.[7] Whether folic acid supplements reduce the risk for cardiovascular disease is currently unknown, but it may be presumed because a greater intake of folate is associated with a lower incidence of cardiovascular disease.[11]

The results obtained in this study showing that more than 25% of liver transplant recipients have elevated fasting homocysteine levels add more evidence of the increased risk for cardiovascular events in these patients. In our series, hyperhomocysteinemia was more prevalent than diabetes mellitus and nearly as prevalent as hypercholesterolemia and obesity. Hyperhomocysteinemia powerfully increases the cardiovascular risk associated with other risk factors, such as arterial hypertension,[10] a very frequent secondary effect of immunosuppressive therapy.

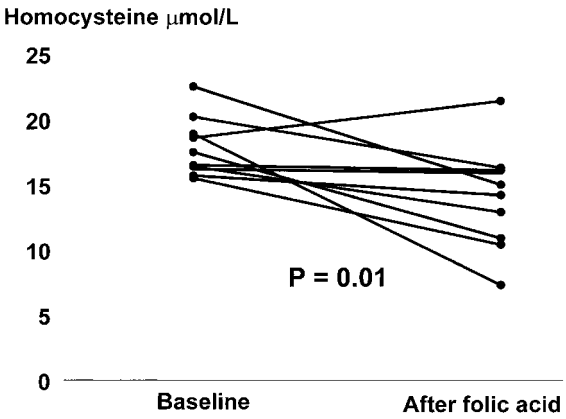

Figure 2. Evolution of fasting serum homocysteine levels after treatment with oral folic acid supplements (10 mg/d) in 10 patients with fasting serum homocysteine levels greater than 15 μmol/L.

In our series, the only factor predisposing to hyperhomocysteinemia was impairment of renal function. The association of hyperhomocysteinemia and chronic renal failure has been previously reported,[12] but it is unknown whether it is caused by impaired metabolism or reduced excretion. Hyperhomocysteinemia has been proposed as a partial explanation of the accelerated atherosclerosis of patients with end-stage renal disease.[6] The existence of hyperhomocysteinemia for a long time in patients with chronic renal failure may help explain why cardiovascular disease is the second leading cause of death in kidney transplant recipients[13] as opposed to liver transplant recipients, who do not have such a high rate of cardiovascular mortality. Furthermore, end-stage liver disease is associated with low peripheral vascular resistance and hypocoagulability, probably protecting patients with cirrhosis from atherosclerosis. In our study, the association between treatment with mycophenolate mofetil and hyperhomocysteinemia found in univariate analysis may be related to the use of this drug in patients with impaired renal function to allow cyclosporine reduction.

The reduction of homocysteine levels after treatment with folic acid shown in previous series and this report may reduce the cardiovascular risk of patients with hyperhomocysteinemia. Whether this intervention reduces the incidence of cardiovascular disease and cardiovascular mortality has to be answered by randomized, placebo-controlled trials that are currently under way. In conclusion, hyperhomocysteinemia is a cardiovascular risk factor frequently found in liver transplant recipients, usually in association with renal dysfunction. Its association with other posttransplantation cardiovascular risk factors may be an important cause of morbidity and mortality in long-term survivors.

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