J Am Soc Nephrol 11: 134–137, 2000

Serum Total Homocysteine and Cardiovascular Disease Occurrence in Chronic, Stable Renal Transplant Recipients: A Prospective Study

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Abstract. Renal transplant recipients have disproportionately high rates of arteriosclerotic outcomes, and recent studies provided controlled evidence that clinically stable renal transplant recipients have an excess prevalence of hyperhomocysteinemia. Few studies suggest that hyperhomocysteinemia may be a cardiovascular risk factor in renal transplant recipients. In the study presented here, the association between atherosclerotic events and homocysteine concentrations was examined in 207 stable renal transplant recipients. The role of hyperhomocysteinemia was analyzed with respect to other known cardiovascular risk factors. The mean follow-up was 21.2 ± 1.9 mo (range, 14 to 26). Mean total homocysteine (tHcy) was 21.1 ± 9.5 μmol/L and median concentration was 19 μmol/L. Seventy percent of patients ($n = 153$) were hyperhomocysteinemic (values >15 μmol/L). tHcy correlated negatively with folate concentration ($r = -0.3$; $P < 0.01$). tHcy was closely related to creatinine concentration ($r = 0.54$; $P < 0.001$). Cardiovascular disease events (CVE) including death were observed in 30 patients (14.5%; 7.34 events per 1000 person-months of follow-up). Fasting tHcy values were higher in patients who experienced CVE (31.5 ± 10.3 *versus* 17.8 ± 7.5; $P < 0.001$). Cox regression analysis showed that tHcy was a risk factor for cardiovascular complications (relative risk [RR] 1.06; 95% confidence interval (95% CI), 1.04 to 1.09; $P < 0.0001$). This corresponds to an increase in RR for CVE of 6% per μmol/L increase in tHcy concentration. Age (RR 1.55; 95% CI, 1.09 to 2.19; $P < 0.01$) and creatinine concentration (RR 1.34; 95% CI, 1.08 to 1.66; $P < 0.01$) were also independent predictors for CVE. This study demonstrates that elevated fasting tHcy is an independent risk factor for the development of CVE in chronic stable renal transplant recipients. Randomized, placebo-controlled homocysteine studies of the effect of tHcy lowering on CVE rates are urgently required in this patient population.

Homocysteine is a sulfur amino acid formed from methionine during transmethylation, and either salvaged to methionine by a folate- and cobalamin-dependent remethylation reaction or directed toward degradation by the vitamin B_6-dependent enzyme cystathionine β-synthase (1).

Large studies have demonstrated that moderate hyperhomocysteinemia is an independent risk factor for cardiovascular disease (2–4). Stable renal transplant recipients have disproportionately high rates of arteriosclerotic outcomes (5), and recent reports provided controlled evidence that clinically stable renal transplant recipients have an excess prevalence of hyperhomocysteinemia (6). Some studies suggest that hyperhomocysteinemia may be a cardiovascular risk factor in renal transplant recipients (7–9). Nevertheless, their relevance is hampered by the small population size enrolled and the absence of control for confounding risk factors.

We examined the association between homocysteine concentration and cardiovascular disease events (CVE) in a large population of stable renal transplant recipients.

Materials and Methods

Patients

The aim of the study was to evaluate the relevance of a single determination of fasting total homocysteine (tHcy) concentration in a large population of stable renal transplant recipients.

Participants in the study were 207 chronic stable renal transplant recipients (*i.e.*, transplant duration >6 mo; no acute rejection; serum creatinine concentration <400 μmol/L).

We previously reported the prevalence and determinants of hyperhomocysteinemia in 227 renal transplant recipients (9). Twenty were excluded because they did not meet the inclusion criteria described above. We followed 207 of these patients for the occurrence of arteriosclerotic events. None of the patients of the study population received folic acid, vitamin B_{12}, or vitamin B_6 supplementation.

Twenty patients were treated with azathioprine and prednisone, and 187 patients were treated with azathioprine and prednisone in similar doses as well as cyclosporine. Because we recently demonstrated the absence of influence of cyclosporine on homocysteine metabolism (9), the two immunosuppressive regimens were studied together.

Received June 22, 1999. Accepted July 14, 1999.

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1046-6673/1101-0134

Journal of the American Society of Nephrology

Total Plasma Homocysteine, Folates, Vitamin B_{12}, Creatinine

Serum homocysteine concentration and factors known to influence homocysteine metabolism (serum folate, cobalamin, and creatinine concentrations) were measured between January and June 1996. Blood samples were drawn after an overnight fast for analysis of plasma concentrations of homocysteine, serum concentrations of creatinine, vitamin B_{12}, and folate.

Total plasma homocysteine was measured using a previously described method (9). The normal range of values for tHcy ranged from 7 to 15 μmol/L using this method with a coefficient of variation $<3\%$. Cobalamin and folate in plasma were determined by radioassay using purified intrinsic factor and purified folate binding protein. The normal values for cobalamin and folate were 420 $\pm$ 250 pg/ml and 10 $\pm$ 5 ng/ml, respectively.

Risk Factors for Vascular Disease

All of the traditional cardiovascular disease risk factors were ascertained at the same time as the determination of tHcy levels. Risk factors including age, hemodialysis duration before transplantation, past history of cardiovascular disease, BP, smoking status, obesity (Quetelet index), hypercholesterolemia, hypertriglyceridemia, diabetes mellitus, and immunosuppressive regimen were analyzed. A past history of cardiovascular disease was defined by:

- *Coronary Heart Disease*: Myocardial infarction documented by serial 12-lead electrocardiogram evidence or Q-wave infarction and appropriate myocardial enzyme elevations; coronary revascularization including coronary artery bypass surgery or percutaneous transluminal coronary angioplasty; typical history of angina with abnormal coronarography or myocardial scintigraphy
- *Stroke/Cerebrovascular Disease*: Both nonhemorrhagic and hemorrhagic strokes; carotid endarterectomy
- *Abdominal Aortic or Lower Extremity Arterial Disease*: Abdominal aortic repair; lower extremity revascularization via bypass surgery or angioplasty; lower extremity amputation

Cardiovascular risk factors were defined as the following:

- *Hypertension*: Use of antihypertensive therapy, or systolic BP >140 mmHg and/or diastolic BP >90 mmHg in patients not receiving antihypertensive medication
- *Hypercholesterolemia*: Total fasting cholesterol concentrations >7 mmol/L
- *Hypertriglyceridemia*: Total fasting triglycerides concentrations >2 mmol/L
- *Diabetes Mellitus*: Fasting glycemia exceeding 7.8 mmol/L and the need for oral antidiabetic or insulin therapy
- *Smoking*: Patients reported a dose–time smoking >5 packs per year
- *Obesity*: body mass index >30

Arteriosclerotic Events

- *Coronary Heart Disease*: Myocardial infarction documented by serial 12-lead electrocardiogram evidence or Q-wave infarction and appropriate myocardial enzyme elevations; coronary revascularization including coronary artery bypass surgery or percutaneous transluminal coronary angioplasty; typical history of angina with abnormal coronarography or myocardial scintigraphy
- *Stroke/Cerebrovascular Disease*: Both nonhemorrhagic and hemorrhagic strokes confirmed by neurologic examination findings consistent with new-onset focal neurologic deficits, with or without computed tomography or magnetic resonance imaging evidence of cerebral infarction; symptomatic extracranial carotid artery stenoses resulting in carotid endarterectomy
- *Abdominal Aortic or Lower Extremity Arterial Disease*: Abdominal aortic repair; lower extremity revascularization via bypass surgery or angioplasty; lower extremity amputation; new onset of intermittent claudication confirmed by Doppler or arteriography findings

Two physicians independent of the study were responsible for CVE ascertainment. This analysis was performed without knowledge of tHcy levels or other cardiovascular risk factor information. The study ended in February 1998.

Statistical Analyses

Arithmetic mean was calculated and expressed as $\pm$SD. The relationship between tHcy and folate, cobalamin, and serum creatinine was examined using Spearman correlation coefficients. Univariate Cox proportional hazards analysis was carried out to select among traditional risk factors that were linked to CVE. The time of entry in the study was tHcy determination. We first performed a univariate Cox analysis keeping covariates with P values <0.20 to include in the model. tHcy was tested as a continuous variable. The selected covariates ($P < 0.20$) were then included in the Cox model, and a backward stepwise selection process was performed. Because the time from kidney transplant to tHcy dosage varied among patients, transplant duration was forced into the Cox model. This way, we took this variation into account. We also forced baseline past history of CVE into the model.

Patients with CVE (CVE+) and patients without CVE (CVE−) were compared using t test for continuous variables and χ^2 for categorical variables. Finally, the patients were separated into three tertiles according to tHcy concentration. Relative risk of CVE was calculated in the two upper tertiles of tHcy compared with the lowest tertile. Trend was assessed by scoring the tHcy tertiles 1 to 3 and testing this continuous variable.

Results

The patients were followed for a mean duration of 21.2 $\pm$ 1.9 mo (range, 14 to 26). Their mean age was 48.3 $\pm$ 14.1 yr (19 to 76) and 131 (63.3%) were men. Hypertension and diabetes were present in 132 patients (63.8%) and 19 patients (9.1%), respectively. Fifty-four patients (26%) had a history of current or past smoking. Hypercholesterolemia and hypertriglyceridemia were present in 79 (38%) and 62 (30%) patients, respectively.

Mean transplant duration and hemodialysis duration were 73 $\pm$ 48 (4 to 244) and 27 $\pm$ 40 (0 to 204) mo, respectively.

Concentrations of tHcy, Folate, and Vitamin B_{12}

Mean tHcy was 21.1 $\pm$ 9.5 μmol/L (8 to 63) and median concentration was 19 μmol/L. Seventy percent of patients ($n = 144$) were hyperhomocysteinemic (values >15 μmol/L). Mean folate and cobalamin concentrations were, respectively, 6.3 $\pm$ 2.8 ng/ml (1 to 20) and 411 $\pm$ 280 pg/ml (35 to 2000). Mean serum creatinine concentration was 145 $\pm$ 62 μmol/L (61 to 447).

tHcy correlated negatively with folate concentration (Spearman coefficient $= -0.3$; $P < 0.001$). There was no relationship between tHcy and cobalamin. tHcy was closely related to serum creatinine concentration (Spearman coefficient $= 0.54$; $P < 0.001$).

Relationships between Homocysteine and Vascular Disease

Among 207 patients, 30 (14.5%) had one or more CVE during the follow-up period (cerebrovascular disease, 3; coronary disease, 14; peripheral vascular disease, 13). Nine patients (4.3%) died, four (1.9%) of cardiovascular causes. Fasting tHcy values were higher in patients who experienced CVE (33.3 ± 11.3 μmol/L *versus* 19 ± 7.5 μmol/L; $P < 0.001$).

Tertiles were defined according to tHcy (Table 1). There was a significantly increased risk of CVE in the upper tertile of homocysteine concentrations (relative risk [RR] 20.0; 95% confidence interval [CI], 2.6 to 157). There was a trend toward an increase in risk from tertile 1 to 3 ($P < 0.001$).

In monovariate analysis, baseline tHcy (continuous variable), age, gender, serum creatinine concentration, folate, hypertriglyceridemia, hypertension, and smoking status were associated with the subsequent development of CVE ($P < 0.2$).

To test a possible interaction between folate and tHcy, serum folate was converted into a categorical variable (two classes, separated by the median value = 6 ng/ml), and RR for tHcy was calculated separately for each folate class. This RR was equal to 1.10 (95% CI, 1.06 to 1.14) for folate ≥6 ng/ml and 1.07 (95% CI, 1.02 to 1.09) for folate <6 ng/ml. This interaction was minor because RR did not change much with folate level, and this did not modify the interpretation of the global RR 1.06 (95% CI, 1.04 to 1.09).

Transplant duration varied greatly among our population subjects, but transplant was not related to CVE. We checked the assumptions of Cox models (log-linearity, proportionality of the risk in time), which were met in the study.

Cox regression analysis showed that tHcy (RR 1.06; 95% CI, 1.04 to 1.09) was an independent risk factor for CVE. This corresponds to an increase in risk of 6% for each μmol/L increase in tHcy. Age (RR 1.55; 95% CI, 1.09 to 2.19 for a 10-yr increase) and serum creatinine concentration (RR 1.34; 95% CI, 1.08 to 1.66 for a 50 μmol/L increase) were also independent predictors for cardiovascular complications. Gender was not far from significance ($P = 0.07$), and men appeared at a higher risk of CVE than women (RR 3.03; 95% CI, 0.88 to 11.11) (Table 2).

Discussion

There is a high incidence of cardiovascular complications as well as high prevalences of hypertension, dyslipidemia, and diabetes in the transplant population. In our patients, the cumulative risk for developing CVE was 5.2% within 1 yr, which is comparable to a previous report (10).

It has been suggested recently that hyperhomocysteinemia could be a cardiovascular risk factor in the transplant population (7–9). Our group and others had previously reported that hyperhomocysteinemia was associated with a past history of posttransplant cardiovascular disease (8,9). In 1994, Massy *et al.* (7) conducted a prospective study on the influence of tHcy concentration and other cardiovascular risk factors in 42 long-term stable renal transplant recipients. Using deep-frozen sera, tHcy concentration was measured in samples drawn 1 to 6 mo before the first cardiovascular event. The authors observed a trend toward a higher mean tHcy concentration in male patients with cardiovascular disease than in men without cardiovascular disease, but not in women. This difference did not reach statistical significance, perhaps because of the small group of patients. Our study confirms in a large population that tHcy predicts cardiovascular complications in renal transplant recipients. The RR for the occurrence of cardiovascular complications increased by 6% for each μmol/L increase in tHcy.

Several studies have demonstrated that tHcy is closely related to renal function (11). Despite a close relationship between tHcy and renal function, we also identified serum creatinine concentration as an independent risk factor for CVE. It is possible that renal failure was a surrogate marker for the presence of other cardiovascular risk factors. An excess of prevalence of traditional risk factors including hypertension, increased circulating levels of lipoproteins, and fibrinogen may contribute to the increased incidence of CVE in transplant patients with impaired renal function (12).

Our study has some limitations. First, the short follow-up duration (21.2 mo) and the small number of patients do not permit clear conclusions to be made about the real influence of some potential risk factors such as diabetes. Second, we studied the influence of a baseline tHcy determination on the occurrence of CVE in a random population of renal transplant recipients. Serial measurements of tHcy in a longitudinal cohort design would certainly provide a more precise estimate of cardiovascular disease risk conferred by tHcy in this population. However, the longitudinal association we observed with a less precise, single determination of baseline tHcy would tend to underestimate the true relationship.

Our study demonstrates that hyperhomocysteinemia is an independent cardiovascular risk factor in stable renal transplant recipients. Randomized, placebo-controlled homocysteine

Table 1. Relationships between tertiles of tHcy and CVD events[a]

CVD Events	Tertile (T) Comparison[b]	Relative Risk Estimate (95% confidence interval)	
		Unadjusted	Adjusted[c]
30 events	T2 *versus* T1	1.74 (0.15 to 19.2)	1.38 (0.12 to 15.6)
	T3 *versus* T1	29.1 (4 to 212.7)	20.0 (2.6 to 157)

[a] tHcy, total homocysteine; CVD, cardiovascular disease.
[b] T1 (8 to 15), T2 (15 to 22), T1 (22 to 63).
[c] Adjusted for creatinine, age, gender, previous cardiovascular disease, and transplant duration.

Table 2. Relative risk estimates for CVD ($n = 30$ events) during follow-up (median, 21.2 mo) derived from proportional hazards modeling with tHcy as a continuous variable, unadjusted, individually adjusted, and with multivariate-adjustment for potential confounding variables[a]

Category	Relative Risk Estimate (95% confidence interval)	*P* Value
tHcy (per μmol/L increase), unadjusted		
tHcy (per μmol/L increase), adjusted for		
previous CVD	1.07 (1.04 to 1.09)	<0.001
time on dialysis	1.08 (1.06 to 1.10)	<0.001
use of cyclosporin A	1.08 (1.05 to 1.10)	<0.001
diabetes mellitus	1.08 (1.06 to 1.10)	<0.001
gender	1.07 (1.05 to 1.09)	<0.001
age	1.08 (1.06 to 1.10)	<0.001
smoking	1.08 (1.04 to 1.09)	<0.001
hypertension	1.07 (1.05 to 1.10)	<0.001
total cholesterol	1.08 (1.06 to 1.11)	<0.001
triglycerides	1.08 (1.05 to 1.10)	<0.001
creatinine	1.07 (1.05 to 1.09)	<0.001
Final multivariate-adjusted model		
total homocysteine (per mmol/L increase)	1.06 (1.04 to 1.09)	<0.001
creatinine (per 50 mmol/L increase)	1.34 (1.08 to 1.66)	<0.01
age (per 10-yr increase)	1.55 (1.09 to 2.19)	=0.01
gender (male *versus* female)	3.03 (0.88 to 11.11)	=0.07
baseline CVD	1.28 (0.54 to 3.02)	=0.58
transplant duration	1.00 (0.99 to 1.01)	=0.71

[a] Abbreviations as in Table 1.

studies of the effect of tHcy lowering on cardiovascular disease event rates are urgently required in this patient population.

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