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Hyperhomocysteinemia in Renal Transplantation: Preliminary Results

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CARDIOVASCULAR disease (CVD) is a major cause of morbidity and mortality after renal transplantation (RT).[1,2] The excess risk of CVD in RT is due in part to a higher prevalence of established atherosclerotic risk factors, including hypertension, dyslipidemia, diabetes, obesity, and physical inactivity.[1,2] However, some renal-related risk factors like immunosuppressive medication and residual renal insufficiency also contribute to this excess CVD risk and may complicate the management of dyslipidemia and hypertension in this population.[1,2] Accordingly, there is a compelling need to identify and safely manage other putative CVD risk factors among RT patients. Elevated plasma homocysteine is emerging as an important risk factor for cardiovascular disease in general populations.[3,4] Some studies have demonstrated that hyperhomocysteinemia is present in patients with impaired renal function and is associated with CVD.[5–7] Only a small number of studies are available on the prevalence and determinants of hyperhomocysteinemia in renal transplant recipients.[8–15] We undertook this study to

1. estimate the prevalence of hyperhomocysteinemia in renal transplant recipients;
2. examine the relationships between plasma total homocysteine (tHcy) and its metabolic determinants vitamin B_6, vitamin B_{12}, and folic acid; and
3. identify other determinants of tHcy.

MATERIALS AND METHODS

A cross-sectional study was conducted in 202 stable RT recipients (113 male, 89 female), selected from 633 RT patients with functioning allografts of our Renal Transplant Unit. All recipients received grafts from cadaver donors. The eligibility criteria were: age over 18 years, first renal allograft, time since RT of at least 6 months, and stable plasma creatinine values during 3 months prior to study. Patients with diagnosis of any kind of cancer, clinical or analytical evidence of liver disease, and chronic alcoholism were also excluded. None of the selected RT recipients were taking B vitamin.

The mean age was 44 ± 11 years (range, 21 to 71; median, 43). The mean duration after renal transplantation was 58.5 ± 37.2 months (range, 17 to 192; median, 50.6). Patients had been on dialysis for an average of 42.7 ± 37.5 months (range, 0 to 221; median, 30.5) before transplantation. Mean serum creatinine value was 1.5 ± 0.6 mg/dL (range, 0.6 to 4.7; median, 1.4) at the time blood was drawn for this study. Immunosuppression protocol of the 202 RT patients is described on Table 1.

Table 1. Immunosuppression Protocol

Immunosuppression Protocol	Total (n = 202) (%)	Male (n = 113) (44.1%)	Female (n = 89) (55.9%)
Prednisolone + azathioprine	1 (0.5)	—	1 (0.5)
Prednisolone + cyclosporine	86 (42.6)	53 (46.9)	33 (37.1)
Prednisolone + cyclosporine + azathioprine	71 (35.1)	39 (34.5)	32 (36.0)
Prednisolone + cyclosporine + MMF	25 (12.4)	14 (12.4)	11 (12.4)
Prednisolone + tacrolimus	3 (1.5)	1 (0.9)	2 (2.2)
Prednisolone + tacrolimus + MMF	1 (0.5)	—	1 (1.1)
Cyclosporine	4 (2.0)	1 (0.9)	3 (3.4)
Cyclosporine + azathioprine	10 (5.0)	5 (4.4)	5 (5.6)
Cyclosporine + MMF*	1 (0.5)	—	1 (1.1)

*MMF: mycophenolate mofetil.

The Ethics Committee of Santo Antonio Hospital approved the study protocol, and all participants provided written informed consent.

Biochemical Determinations

Overnight fasting blood samples were collected from each participant. Total fasting homocysteine levels were determined by polarized immunofluorescence on an automated Abbott IMx analyzer. Hyperhomocysteinemia was considered if plasma levels of fasting tHcy were higher than 15 μmol/L. Plasma pyridoxal 5′-phosphate was determined by high performance liquid chromatography with fluorescence detection. Plasma vitamin B_{12}, plasma, and erythrocyte folate were measured on automated analyzer ACS: Centaur (Chiron Diagnostics) by chemioluminescence. Laboratory tests performed included serum measurements of creatinine, urea, uric acid, albumin, total protein, HDL, LDL, total cholesterol, and apolipoproteins A and B-100.

Statistical Analysis

Logarithmic transformations and geometric means were used for continuous variables with a positively skewed distribution. Means

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Table 2. Distribution of Vitamin B_6, Vitamin B_{12}, and Folate Concentrations and Normal Concentration Ranges

	Normal Range*	Under Lower Limit (%)**	Within Normal Limits (%)**	Over Higher Limit (%)**
Vitamin B_6 (n = 136)	3.6–18 μg/L	2.2	91.2	6.6
Vitamin B_{12} (n = 194)	211–911 pg/mL	1.5	93.8	4.6
Plasma folate (n = 197)	2.6–20 ng/mL	—	94.9	5.1
Erythrocyte folate (n = 183)	130–1102 ng/mL	6.6	93.4	—

*Accepted limits for normal fasting levels of erythrocyte folate and plasma vitamin B_6, B_{12}, and folate.
**Percentages were calculated after excluding missing cases.

of continuous variables were compared by Student's *t* test. Differences in proportions between groups were studied using the χ^2. Correlation between continuous variables was assessed by Pearson correlation.

RESULTS

The prevalence of fasting hyperhomocysteinemia was 48.7%, and it was significantly higher in male patients than in female (31.9% vs 16.8%; $P < .05$). Geometric mean of tHcy was 14.9 μmol/L and tHcy levels were significantly higher in male RT patients than in females (16.2 vs 13.4 μmol/L; $P < .001$).

Significant correlations were found between tHcy and plasma creatinine (r = .55; $P < .001$), urea (r = .49; $P < .001$), and uric acid (r = .51; $P < .001$).

No significant vitamin deficiencies were found in our patients. None of them had plasma folate concentrations under the lower limit of normal reference range. Plasma concentrations of vitamins B_6 and B_{12} were low only in three patients (2.2 and 1.5%, respectively) and erythrocyte folate was below lower limit in only 12 patients (6.6%) (Table 2). Nevertheless, we found a negative statistically significant correlation between tHcy and plasma folate (r = .36; $P < .001$), erythrocyte folate (r = .25; $P = .001$), and vitamin B_{12} (r = .27; $P < .001$). There was no significant relationship between fasting tHcy and vitamin B_6.

No significant linear correlation was found between tHcy and age, body mass index, serum albumin, total protein, triglycerides, total and LDL cholesterol, lipoprotein (a), and apolipoprotein B-100. In addition, there was no significant correlation between tHcy and dose or plasma levels of cyclosporine ($P > .05$).

DISCUSSION

Renal transplantation is a recognized treatment for end-stage renal failure. CVD represents a major cause of both morbidity and mortality.[1,2] Recent reports summarizing large collaborative studies show that increased tHcy is an important and independent risk factor for morbidity and mortality of CVD disease.[3,4] Several reports in RT also point out that hyperhomocysteinemia is present in RT patients,[9,10–15] but limited data are available on the prevalence of hyperhomocysteinemia in stable RT recipients.[9,12,14,15] We have confirmed the presence of increased levels of tHcy, showing that 48.7% of our stable RT patients had hyperhomocysteinemia, mainly in males.

We have found that tHcy is correlated both with concentrations of creatinine and of urea, indicating that the degree of renal impairment is a major influence on homocysteine levels. This finding agrees with other studies.[8,12–15]

In our study, even within their normal range, tHcy were negative and significantly correlated with folic acid and vitamin B_{12} concentrations. However, it is unlikely that elevated levels of tHcy are attributable to deficiencies of these vitamins, because RT patients can have a regular diet without the restrictions that characterize dialysis patients. We confirmed this finding: no significant deficiencies were found in our patients. More than 90% had normal or higher levels of those vitamins.

Like other studies in RT population,[9,12,14] a significant correlation between tHcy and age was not found. Also, a significant relationship was not found between tHcy and other risk factors for atherosclerosis like plasmatic lipids and body mass index. This analysis was not performed in any of the studies conducted in RT patients.

Arnadottir et al suggests the possibility that cyclosporine use is associated with increased tHcy levels among RT recipients.[9] But, as in other studies, we didn't find any correlation between tHcy levels and dose and plasma levels of cyclosporine.[10–15]

CONCLUSIONS

In this study we found that hyperhomocysteinemia is common in RT patients and occurs despite normal concentrations of folate and vitamins B_{12} and B_6. As in general population, folic acid and B_{12} were significant determinants of fasting tHcy. Serum creatinine, urea, and uric acid were also important determinants of tHcy. Plasma lipids and cyclosporine appear not to influence tHcy.

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