Homocysteine Levels in Pediatric Renal Transplant Recipients

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RENAL TRANSPLANTATION (RT) is an established method for the treatment of end-stage renal failure, but there is an associated increased risk of cardiovascular events. Long-term survival of renal transplant recipients (RTRs) appears to be highly dependent on the occurrence of cardiovascular complications. At present, cardiovascular disease (CVD) is the leading cause of mortality in these patients.[1,2]

Conventional CVD risk factors, such as hypertension, hyperlipidemia, glucose intolerance, and obesity, are probably involved in the pathogenesis of atherosclerosis in this setting. But traditional risk factors neither explain all of the cardiovascular events nor completely account for why CVD is the number one cause of death in RTRs.[1,2]

Increased plasma total homocysteine (tHcy) is considered to be a new and independent risk factor for CVD in adults with normal or impaired renal function, including stable RTRs; but little information exists about this condition in the pediatric age group.[3–10]

In the present study, we sought to: (1) analyze the distribution of fasting tHcy levels to estimate the prevalence of hyperhomocysteinemia in children adolescents bearing a kidney allograft; (2) examine the relation between tHcy levels and the vitamin cofactors (folic acid and vitamin B_{12}), renal function, immunosuppressive therapy, and other potential determinants; and (3) assess the association between hyperhomocysteinemia and conventional risk factors for CVD.

SUBJECTS AND METHODS

A cross-sectional study was conducted in 25 pediatric RTRs (10 girls), 4 to 18 years old (mean age, 14.7 ± 3.1 years). The eligibility criteria were as follows: (1) RTRs younger than 19 years of age; (2) minimum of 6 months since RT (3) stable plasma creatinine level during the preceding 3 months; and (4) no clinical or analytical evidence of liver disease.

The mean time after RT was 3.9 ± 2.8 years (range, 8 months to 10 years); the mean serum creatinine and creatinine clearance levels were 1.2 ± 0.2 mg/dL and 64.5 ± 8.9 mL/min/1.73m^2, respectively. No patient was on a dietary restriction or vitamin supplementation. All kidney allografts were from cadaver donors. The immunosuppression consisted of triple therapy with prednisolone, cyclosporine, and mycophenolate mofetil (60%); prednisolone, cyclosporine, and azathioprine (36%); or prednisolone and cyclosporine (4%). One child received tacrolimus. Mean daily prednisolone dosage was 0.14 mg/kg of body weight (range, 0.06 to 0.2) with 68% of patients on alternate-day regimens. The mean daily cyclosporine dosage was 4.5 mg/kg of body weight (range, 0 to 6.4). Antihypertensive drugs were mainly angiotensin-converting enzyme inhibitors and β-blocking agents.

The study was approved by the Ethical Committee of Santo Antonio Hospital. Written informed consent was obtained from the parents of each participating child or adolescent.

Biochemical Determinations

After an overnight fast, venous blood samples were drawn into EDTA-containing tubes. The sample intended for homocysteine analysis was immediately put on ice, centrifuged within 30 minutes, frozen, and stored at −20°C. Plasma tHcy levels were measured using a fluorescence polarization immunoassay kit (IMx, Abbott Laboratories, Axis Biochemical's ASA, Oslo, Norway). The normal range for tHcy was 5 to 15 μmol/L. Vitamin B_{12}, serum, and erythrocyte folate concentrations were measured using commercial chemiluminescence assay (ACS, Chiron Diagnosis). In our laboratory, normal levels of plasma vitamin B_{12}, of plasma, and of erythrocyte folate were 211–911 pg/mL, 2.6–20 ng/L, and 130–1102 ng/mL, respectively. Other biochemical parameters also were determined on plasma using standard procedures, namely, creatinine, urea, uric acid, total cholesterol, low-density lipoprotein (LDL) and high-density lipoprotein (HDL) cholesterol, triglycerides, apolipoproteins A and B, and lipoprotein(a). Creatinine clearance was calculated using a 24-hour urine collection. All blood samples were analyzed at the Clinical Chemistry Department of the Santo Antonio Hospital.

Definition of Hyperhomocysteinemia

Levels of tHcy were compared with pediatric reference values for tHcy, obtained from a healthy pediatric population near our geographical area. Hyperhomocysteinemia was considered if tHcy levels were greater than the 95th percentile of that population.[11]

Statistical Analysis

Results are reported as mean ± SD and range (in parentheses). Normality was tested by the Kolmogorov-Smirnov test. Because the

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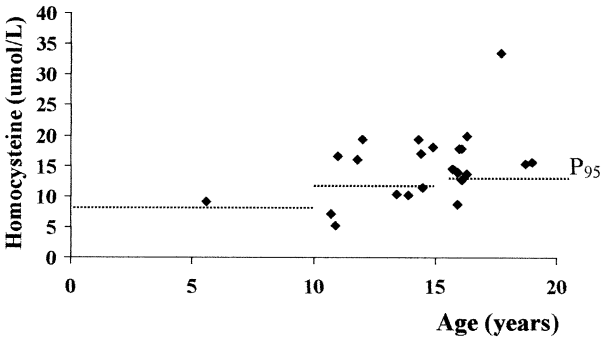

Fig 1. Distribution of tHcy (using the 95th percentile cut-off value of the published[11] pediatric reference values for tHcy).

data were normally distributed, Pearson's correlation was used to evaluate the relationship between continuous variables. Differences between means were analysed by Student t test or Mann-Whitney U test. Frequencies of categorical variables were compared using χ^2 test or Fisher exact test. All analyses were performed using SPSS software for Windows, version 10.0, and a P value of less than .05 was considered to indicate statistical significance.

RESULTS

Mean fasting tHcy level was 14.9 ± 4.3 μmol/L (range, 5.2 to 33.5 μmol/L), and 14 patients (56%) had tHcy levels 15 μmol/L. Seventeen patients (68%) exhibited levels of tHcy greater than the 95th percentile cut-off of published pediatric reference values for tHcy[11] (Fig 1).

No significant differences were observed between boys and girls in the mean tHcy concentrations, namely, 15.5 ± 7.1 μmol/L versus 14.0 ± 2.7 μmol/L ($P = .556$). A positive significant linear correlation was observed between tHcy and age ($r = 0.49$; $P < .05$). There was no significant correlation between tHcy and time post-RT, nor with the length of hospitalization after RT.

None of the RTRs displayed a vitamin deficiency for any of the evaluated vitamins. All patients had vitamin B_{12} levels within the normal range. Concerning plasma and erythrocyte folate, 21 (84%) and 23 (92%) patients, respectively, were within the normal range, and the remaining patients showed plasma and erythrocyte folate levels greater than the upper reference limit, namely, 3.3– 21 ng/L in plasma, and 158–744 pg/mL in red blood cells. Levels of tHcy were positively and significantly correlated with serum creatinine ($r = .45$; $P < .05$), urea ($r = .44$; $P < .05$), and uric acid ($r = .47$; $P < .05$), and inversely with creatinine clearance ($r = -.46$; $P < .05$).

None of the immunosuppressive (prednisolone, azathioprine, and mycophenolate mofetil) or antihypertensive therapies (angiotensin-converting enzyme inhibitors and β-blocking agents) appeared to be significantly associated with the occurrence of hyperhomocysteinemia.

The evaluated conventional risk factors for CVD were widely prevalent in our pediatric sample: hypertension was observed in 60% RTRs, obesity in 28%, hypercholesterolemia in 64%, and hypertriglyceridemia in 40%. One patient had posttransplantation diabetes mellitus and only one adolescent reported smoking.

None of the conventional risk factors was significantly associated with hyperhomocysteinemia, and none of the evaluated lipid parameters (total cholesterol, triglycerides, LDL, HDL cholesterol, apolipoproteins A and B, and lipoprotein(a)) was significantly correlated with tHcy levels. Furthermore no correlation was found between body mass index, blood pressure, and tHcy concentration.

DISCUSSION

Numerous studies have suggested that hyperhomocysteinemia represents an independent risk factor for atherosclerosis and atherothrombosis.[4,12] These studies also have shown that the relationship between tHcy concentration and vascular disease risk appears to be continuous and graded: each 5 μmol/L increment in tHcy was associated with a 60% to 80% higher risk of coronary artery disease, a 50% higher risk of cerebrovascular disease, and a 6-fold higher risk of peripheral vascular disease.[12]

Compared with the general population, renal patients, including RTRs, display higher levels of tHcy.[6] Our results agree with other studies performed in adult and pediatric RTRs that show a higher prevalence of hyperhomocysteinemia after RT.[3–9] When compared with pediatric reference ranges for tHcy,[11] hyperhomocysteinemia was found in 68% of our pediatric RTRs, or 56% if we considered the threshold to be 15 μmol/L.

Homocysteine is a sulfur-containing amino acid, which is derived from the metabolism of methionine. Homocysteine itself is metabolized by one of two pathways. Through a vitamin B_{12}-dependent reaction catalyzed by methionine synthase, it may be remethylated to methionine, by 5-methyltetrahydrofolate donating a methyl group. Alternatively, homocysteine may enter the vitamin B_6-dependent transulfuration pathway, catalyzed by cystathionine β-synthase, where homocysteine is converted to cysteine.[4,5,10,13] Thus, folate, vitamin B_{12}, and vitamin B_6 status are important biological determinants of tHcy levels.[13] Increased Hcy concentrations have been correlated with low serum levels and low dietary intakes of vitamins B_{12}, B_6, and folate.[13] In fact, subclinical deficiencies of these cofactors have been shown to result in hyperhomocysteinemia.[13] In our pediatric RTRs, and in adult RTRs of our Unit,[3] folic acid and vitamin B_{12} levels were inversely correlated with tHcy concentration, but hyperhomocysteinemia occurred despite normal concentrations of folate and B_{12} vitamins. This finding suggests that, besides the absence of vitamin deficiencies, it is possible that higher vitamin concentrations are required to normalize tHcy levels in RTRs.

By measuring tHcy in Spanish children aged from 2 months to 18 years, Vilaseca et al. reported that tHcy concentrations increase with age.[11] This positive correlation was confirmed in our study. On the other hand, unlike adult population (both general[10,13] and RTRs[3–6]), where men

have higher tHcy levels than women, no significant differences were found between the genders in our pediatric RTRs. Possible explanations for the higher concentrations of tHcy in men may be the greater percentage of muscle mass, and the beneficial hormonal effect exhibited by women.[4–6] It is possible that these differences are attenuated in the pediatric population.

Like other studies that suggest that impaired renal function is strongly associated with increased Hcy levels,[3–6] we observed a positive and significant correlation between tHcy and serum creatinine, urea, and uric acid levels and, obviously, an inverse correlation with creatinine clearance.

Arnadottir et al. reported that cyclosporine might interfere with homocysteine metabolism.[6] Fonseca et al. found lower tHcy levels in adult RTRs treated with mycophenolate mofetil (submitted for publication). In pediatric RTRs, immunosuppressive (azathioprine, prednisolone, or mycofenolate mofetil) and antihypertensive therapies had no significant effect on tHcy levels. We could not draw a conclusion about the effect of cyclosporine on tHcy concentration because almost all patients were taking this agent.

At the time of study, our pediatric RTRs showed a high prevalence of traditional CVD risk factors, namely, hypertension, dyslipidemia, and obesity. Like adult RTRs, hyperhomocysteinemia was not significantly associated with the presence of any of these conventional CVD risk factors.[3] Furthermore, no significant correlation was observed between tHcy and plasma lipids, body mass index, or blood pressure in this pediatric group.

In summary, hyperhomocysteinemia was widely prevalent among our child and adolescent RTRs, which may contribute to an increased risk of CVD. Folic acid is known to be an effective factor to reduce elevated tHcy levels. These findings pose the question of whether an innocuous and inexpensive therapy, such as folate supplementation, may reduce the increased risk of CVD among pediatric RTRs. Further prospective studies are needed to answer this question.

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