Serum Homocysteine Levels in Renal Transplant Recipients with and without Hypercholesterolemia

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Summary

Background: Hyperhomocysteinemia seems to be frequent after renal transplantation. No study so far has assessed the role of homocysteine (Hcy) associated with dyslipidemia in Brazil.

Objective: To determine the prevalence of hyperhomocysteinemia (serum Hcy >15 mmol/l) in stable renal transplant recipients and to evaluate the role of serum lipids and graft function in serum Hcy levels.

Methods: One hundred and five stable renal transplant recipients were evaluated, considering age, post-transplant time, cholesterol levels, graft function, proteinuria, and cyclosporine (analyzed using multiple linear regression). The prevalence of hyperhomocysteinemia was 74.3%. Patients were further divided into two groups, hyper (total cholesterol >200mg/dl, LDL-cholesterol >130mg/dl) and normocholesterolemic.

Results: Hypercholesterolemic recipients were older, had shorter post-transplant time, lower endogenous creatinine clearance, and higher proteinuria and Hcy serum levels. Patients with hyperhomocysteinemia had statistically significantly higher serum triglycerides and poorer graft function, and their LDL-cholesterol also tended to be higher. A positive correlation was found between serum creatinine and Hcy levels (r = 0.32, P = 0.01). Multiple regression analysis revealed that both dyslipidemia and renal function independently affect Hcy values.

Conclusion: We observed a high prevalence of hyperhomocysteinemia in renal transplant recipients, especially in hypercholesterolemic, suggesting that worse graft function may influence serum Hcy and cholesterol levels negatively. Further studies should investigate if this adverse metabolic profile is associated with higher cardiovascular mortality in the long term. (Arq Bras Cardiol 2007;89(3):154-159)

Key words: Dyslipidemia, hyperhomocysteinemia, renal transplantation.

Introduction

Cardiovascular disease is an important cause of morbidity and mortality after renal transplantation. Distinct etiological factors lead to endothelial dysfunction. Generally, more than one of these factors (systemic arterial hypertension, diabetes mellitus, obesity, dyslipidemia, smoking, and family history) are found in renal transplant recipients[1-3]. Elevated serum levels of total cholesterol and LDL-cholesterol are frequent after renal transplantation, affecting more than 60% of these patients[4,5].

Homocysteine (Hcy), as a cardiovascular risk factor, was studied over 30 years ago, through the observation of extensive atherosclerotic lesions during autopsies of patients affected by certain genetic variants of homocystinuria. Thereon, Hcy has been investigated as a factor in the genesis of atherosclerosis[6].

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Today, hyperhomocysteinemia is a well-established cardiovascular risk factor in the general population, and some studies suggest that this association is also present among renal transplant recipients. In 50-70% of the patients, serum Hcy concentration is increased[1,2,7-9].

Recent studies have suggested mechanisms through which hyperhomocysteinemia may be an additional factor for the development of atherosclerosis and cardiovascular disease in patients with other risk factors, such as dyslipidemia[2,10,11]. Ducloux et al[3] showed a positive correlation between serum Hcy and LDL-cholesterol in clinically stable renal transplant recipients. In this context, endothelial damage occurs due to the predominance of oxidized forms of Hcy in plasma, thus generating reactive oxygen species and tissue toxicity[6,10,12,13].

Factors associated with hyperhomocysteinemia are age, smoking, systemic arterial hypertension, folate and vitamin B12 levels, elevated cholesterol, sedentary lifestyle and, especially, renal function[3,7,14-20]. Some studies have shown that there is no correlation between blood cyclosporine and Hcy levels[11,15,17].

Serum Hcy levels are inversely related to renal function,

which, in turn, is the major determinant of the former[10,21]. However, after renal transplant, the decrease in serum Hcy levels seems to be lower than expected with the improvement in renal function. Other factors, such as chronic graft dysfunction, dyslipidemia or the effect of immunosuppressive drugs, may influence Hcy, thus increasing cardiovascular risk in these individuals[14,17,22].

The purpose of this study was to determine the prevalence of hyperhomocysteinemia in stable renal transplant recipients with or without dyslipidemia, and to assess the effect of clinical variables (graft function, serum cholesterol and cyclosporine use) on serum Hcy.

Methods

The clinical and demographic data that we included in another study[4] had been previously collected from 67 renal transplant recipients with hypercholesterolemia. Sera were collected and stored at -80ºC from January 2000 to December 2001. This study was approved by the Ethics Committee of the institution, and the patients were included after signing an informed consent.

For the present analysis, 38 renal transplant recipients without hypercholesterolemia (serum cholesterol < 200mg/dl and LDL-cholesterol < 130 mg/dl) were selected using the same inclusion and exclusion criteria (with the exception of serum cholesterol levels) as in the aforementioned study. Such criteria were: age over 18 years, post-transplant time greater than six months, and endogenous creatinine clearance greater than 20 ml/min. Patients with nephrotic syndrome, severe congestive heart failure (class III or IV) or recent acute myocardial infarction (< 6 months), in use of anticoagulants and/or statins and with serum triglyceride concentration greater than 400 mg/dl were excluded. No patient was on folic acid or vitamin B supplementation.

The demographic and clinical variables studied were age, gender, systemic arterial hypertension, diabetes mellitus, smoking, use of oral contraceptives, post-transplantation time, number of transplants, and immunosuppressive regimen. Serum urea, creatinine, and albumin, fasting blood glucose, complete blood count, platelets, liver enzymes, endogenous creatinine clearance, and 24-hour proteinuria were all measured. Serum samples were drawn after 12-hour fasting and stored at -80°C, from January to December 2003.

Serum Hcy was measured by polarized immunofluorescence (Abbot GmbH Diagnostika, Wiesbaden-Delkenheim, Germany). Hyperhomocysteinemia was defined as serum Hcy levels greater than 15 μmol/l[2].

Patients were initially divided into two groups, according to serum cholesterol levels, in normo- and hypercholesterolemic. In the second step, patients were further divided into two groups, now according to serum Hcy levels.

Student's *t* test and Mann-Whitney or Wilcoxon tests were respectively used for independent samples and continuous variables with normal or asymmetric distribution. Chi-square or Fisher exact tests were used for categorical variables. Pearson or Spearman coefficients were used for correlation analysis. As the distribution of Hcy serum levels was asymmetric, values underwent logarithmic transformation in order to reduce skewness. Median and interquartile range of log Hcy of the normo- and hypercholesterolemic groups were also presented. Multiple linear regression analysis was used to assess the independent effect of dyslipidemia, post-transplantation time, age, graft function, cyclosporine use, and proteinuria on serum Hcy levels. Statistical significance was established at $p < 0.05$.

Results

The demographic and clinical data of the patients included in the study, according to cholesterol levels, are shown in Table 1. Patients with hypercholesterolemia were considerably older and had shorter post-transplantation time. There were also differences in triglycerides, proteinuria and Hcy levels, which were higher in this group. Renal function was worse in these patients.

Seventy percent of the patients were maintained on a cyclosporine-based regimen. Fifty-two (70.2%) hypercholesterolemic patients and 20 (52.6%) normocholesterolemic patients received a triple regimen (cyclosporine, azathioprine and prednisone. Other immunosuppressive regimens were prednisone and azathioprine, given to 15 patients with hypercholesterolemia (29.8%) and seven patients without hypercholesterolemia (18.5%). Six patients used tacrolimus associated with mycophenolate or azathioprine. No difference was observed in either serum cholesterol or Hcy levels regarding cyclosporine use (Tables 1 and 2).

The prevalence of hyperhomocysteinemia was 74.3%. Serum Hcy levels were significantly higher in patients with hypercholesterolemia (Table 1). This difference is also illustrated in Figure 1.

It was also found that serum Hcy levels were not influenced by demographic factors, such as gender and ethnicity, or clinical variables, such as systemic arterial hypertension, diabetes mellitus, smoking or immunosuppressive regimen. However, patients with hyperhomocysteinemia presented higher triglyceride and serum creatinine concentrations (Table 2).

A positive and significant correlation was found between graft function and Hcy levels ($r = 0.32$, $p = 0.01$). Multiple linear regression revealed that cyclosporine use, proteinuria, age and post-transplant time were not confounding factors in the association between Hcy and serum cholesterol. These factors, after adjustment in the regression model, did not affect Hcy values. The only factors that independently affected Hcy serum levels (expressed as a logarithm) were dyslipidemia and renal function (Table 3).

Discussion

The results of this study showed high prevalence of hyperhomocysteinemia (74.3%) in clinically stable renal transplant recipients. Patients with hypercholesterolemia showed higher serum Hcy levels, which is in agreement with previous studies[2,3,10,11,15]. Ducloux et al[10] conducted a study with 103 clinically stable renal transplant recipients and showed a positive association between Hcy and LDL-

Table 1 - Demographic and clinical data of renal transplant recipients according to cholesterol concentrations (n=105)

	Hypercholesterolemic (n=67)	Normocholesterolemic (n=38)	p
Age (years), mean ± SD	43.24±10.9	38±10.0	0.016
Gender (M/F)	30/37	24/14	0.07
Ethnicity (C/B)	61/6	34/4	0.79
Systemic arterial hypertension, N (%)	32 (47.8%)	20 (52.6%)	0.63
BMI, mean ± SD	27.09±4.23	28.19±4.74	0.72
Diabetes mellitus, N (%)	4 (6%)	3 (7.9%)	0.70
Smoking, N (%)	7 (10.4%)	4 (10.5%)	0.99
Oral contraceptive, N (%)	2 (5.4%)	4 (28.5%)	0.11
Cyclosporine use, N (%)	47 (70.2%)	20 (52.6%)	0,10
Post-Tx time (months), mean ± SD	57.51±38.22	86.08±59.61	0.01
Total cholesterol (mg/dl), mean ± SD	278.97±41.85	181.05±21.97	<0.001
LDL-cholesterol (mg/dl), mean ± SD	182.06±35.15	100.05±17.21	<0.001
HDL-cholesterol (mg/dl), mean ± SD	56.06±19.06	54.21±14.04	0.60
Triglycerides (mg/dl), mean ± SD	202.51±70.06	108.26±26.95	<0.001
Creatinine (mg/dl), mean ± SD	1.49±0.05	1.39±0.06	0.22
Endogenous creatinine clearance (ml/min), mean ± SD	68.08±18.67	75.25±14.42	0.03
Proteinuria (g/24h), mean ± SD	0.781±0.99	0.245±0.16	<0.001
Homocysteine (μmol/l), mean ± SD	22.79±8.6	18.66±8.5	0.03

BMI - body mass index; Tx - renal transplantation.

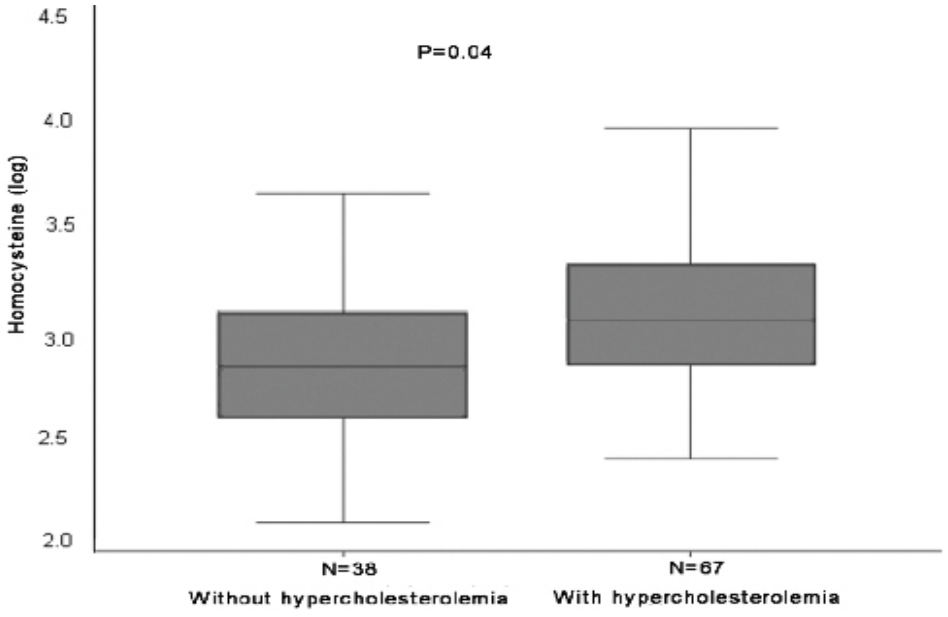

Fig. 1 *- Box-plot of log homocysteine levels (median and interquartile range) of hypercholesterolemic and normocholesterolemic patients.*

Table 2 - Demographic and clinical data of renal transplant recipients according to homocysteine levels (n=105)

	Homocysteine >15 (n = 78)	Homocysteine ≤15 (n = 27)	p
Age (years), mean ± SD	41.7 ± 10.9	40.2 ± 10.6	0.53
Gender (M/F)	41/37	13/14	0.69
Ethnicity (C/B)	68/10	27/0	0.08
BMI, mean ± SD	20.0 ± 4.3	26.8 ± 5.0	0.47
Post-Tx time (months), mean ± SD	62.3 ± 44.5	83.7 ± 57.5	0.08
Systemic arterial hypertension, N (%)	38 (48.7%)	14 (51.9%)	0.78
Smoking, N (%)	9 (11.5%)	2 (7.4%)	0.73
Diabetes mellitus, N (%)	6 (7.7%)	1 (3.7%)	0.67
Oral contraceptive, N (%)	3 (3.9%)	3 (11.1%)	0.18
Cyclosporine use, N (%)	56 (71.8%)	18 (66.7%)	0.62
Total cholesterol (mg/dl), mean ± SD	247.7 ± 59.5	231.4 ± 58.0	0.22
LDL-cholesterol (mg/dl), mean ± SD	157.5 ± 47.8	137.5 ± 52.4	0.07
HDL-cholesterol (mg/dl), mean ± SD	53.1 ± 14.2	62.0 ± 23.2	0.21
Triglycerides (mg/dl), mean ± SD	180.5 ± 72.6	133.1 ± 66.6	0.04
Creatinine (mg/dl), mean ± SD	1.5 ± 0.38	1.2 ± 0.44	0.01
Endogenous creatinine clearance (ml/min), mean ± SD	70.2 ± 16.2	71.9 ± 21.1	0.65
Proteinuria (g/24h), mean ± SD	0.58 ± 0.81	0.60 ± 0.92	0.92

BMI - body mass index; Tx - renal transplantation.

Table 3 - Multivariate analysis of log homocysteine correlations

Model	Effect on log Hcya	Beta coefficient	p
Constant	2.496	-	< 0.001
Age	-0.003	-0.073	0.47
Time post transplantb	-0.001	-0.160	0.16
Cyclosporine use	-0.005	-0.006	0.94
Serum creatinine	0.277	0.302	0.001
Hypercolesterolemia	0.182	0.23	0.014

[a] Beta coefficient expressing the effect on log homocysteine were obtained in a multiple linear regression model; [b] Time from transplant to last follow up.

cholesterol. However, such association was not found by other authors[18,21].

Renal transplant recipients with hyperhomocysteinemia showed higher serum creatinine levels, although the difference did not seem to be clinically relevant. However, graft function in these transplant recipients was evaluated by serum creatinine measurement and endogenous creatinine clearance, methods known as not very accurate in estimating glomerular filtration[23]. Poorer graft function may have affected serum Hcy levels, as suggested by regression analysis results.

A moderate elevation of Hcy levels is found in the early stages of renal failure, which increases linearly with the decrease in glomerular filtration[3,15,16,20]. Several studies have shown an inverse correlation between renal function and serum Hcy using different renal function measurement methods, such as serum creatinine, endogenous creatinine clearance, cystatin C, and Cr-EDTA[1,3,8,11].

Patients with hypercholesterolemia seem to have worse metabolic profile, as evidenced by higher Hcy serum levels.

Fonseca et al[19] reported that serum Hcy elevation was

associated with male gender, aging, smoking, systemic arterial hypertension, and high cholesterol levels. In the present study, only triglyceride levels were higher in the hyperhomocysteinemic group. LDL-cholesterol also tended to be higher.

Multiple linear regression analysis was performed in order to confirm the effect of clinical variables on serum Hcy levels. In this model, the only factors that independently influenced Hcy were serum cholesterol and graft function.

Hcy levels tended to be lower in patients using oral contraceptives (15.33 ± 5.28 μmol/l x 21.67 ± 8.98 μmol/l, p = 0.09). Estrogens might affect the activity of enzymes that participate in Hcy metabolism. This could explain our results[19]. Also, post-transplant time tended to be shorter in the hyperhomocysteinemic group.

Ducloux et al[24] showed a 6% increase in the relative risk of cardiovascular complications for each μmol/L increase in blood Hcy. It was not the purpose of this study to evaluate the association between ischemic heart disease and hyperhomocysteinemia, but according to previous studies[2,10,22] it is likely that the sum of risk factors found in transplant recipients - dyslipidemia, hyperhomocysteinemia, arterial hypertension, smoking, obesity – may result in greater cardiovascular morbidity and mortality.

No correlation between serum cyclosporine and Hcy levels was found. This finding was also described in other studies[11,15,17]. Lower Hcy values in patients using tacrolimus have been described. However, the lower creatinine levels found in these patients could, by itself, justify this finding[25-28].

Winkelmayer et al[11] found an association between elevated serum Hcy levels and risk for renal graft loss and death in renal transplant recipients.

In summary, our study shows high prevalence of hyperhomocysteinemia in renal transplant recipients, especially in the setting of hypercholesterolemia and poor graft function. Further studies should investigate the possibility that poorer graft function may negatively affect serum Hcy levels.

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