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Hyperhomocysteinemia in obese renal transplant patients

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SUMMARY
Hyperhomocysteinemia and obesity represent risk factors for the development of atherosclerosis and chronic transplant nephropathy. In a prospective metabolic study, we monitored, over a period of 36 months, a total of 138 obese renal transplant recipients (body mass index ≥ 30 kg/m^2) with hyperhomocysteinemia. We compared the findings of a new therapeutic regimen at 1 year (start of the study) and 3 years after renal transplantation. The new regimen consisted of a hypocaloric diet, corticoid withdrawal, folic acid (5 mg/day) and vitamin B_6 (50 mg/day).
During the follow-up period, significant decreases in total homocystein level (35.2 ± 12.4 μmol/l vs 14.7 ± 6.9 μmol/l, $P < 0.01$), body mass index (32.5 ± 3.2 kg/m^2 vs 28.4 ± 2.8 kg/m^2, $P < 0.025$) and triglycerides (3.8 ± 1.6 mmol/l vs 2.2 ± 1.2 mmol/l, $P < 0.01$) were noted. At the same time, serum levels of folate (17.8 ± 8.5 mg/l vs 32.9 ± 9.0 mg/l, $P < 0.01$) and vitamin B_6 (5.4 ± 1.5 μg/l vs 9.3 ± 2.2 μg/l, $P < 0.01$) were increased. Creatinine clearance, proteinuria, mean blood pressure, lipoprotein(a) and apolipoprotein E isoform did not differ significantly. We conclude that hyperhomocysteinemia and obesity as risk factors after kidney transplantation can be effectively treated.
Key words: atherosclerosis, hyperhomocysteinemia, hyperlipidemia, kidney transplantation, obesity.

Introduction

Hyperhomocysteinemia in chronic renal failure

Moderate hyperhomocysteinemia (HHcy) is present in the early stage of renal insufficiency and increases with deteriorating renal function. In patients with chronic renal failure (CRF), elevated plasma homocystein (tHcy) levels occur more frequently than any conventional risk factor and the prevalence of hyperhomocysteinemia is greater than 90% [1, 2].

Mechanisms by which tHcy levels increase in patients with CRF have not been conclusively identified because renal excretion accounts for less than 1% of homocysteine (Hcy) elimination, and a reduction in renal excretion alone therefore cannot explain the accumulation of Hcy. Other possible causes include the accumulation of toxic compounds in chronic renal failure, which can influence metabolism of vitamins (B_6, folate, and B_{12}), and/or abnormally high requirements for these vitamins. However, vitamin supplementation reduces, but does not normalize, tHcy levels in patients with end–stage renal disease (ESRD) [2, 3].

Studies using stable isotopes, showed that Hcy remethylation was decreased in hemodialysis (HD) patients, whereas transsulfuration did not differ from that in healthy subjects. However, it should be noted that after an overnight fast preceded by 3 days of a fixed protein intake, i. e., under such steady-state conditions, the flux through the transsulfuration pathway should balance the food intake of methionine (Met) and net release of Met from body proteins. Hence, it is conceivable that after an acute Met load, impairment of transsulfuration in HD patients might occur that is not seen in steady-state conditions [3].

A common genetic mutation of methylenetetrahydrofolate reductase (MTHFR), the C677T mutation, has a similar frequency of approximately 10% to 15% in uremic patients and the general population. This mutation has been shown to aggravate hyperhomocysteinemia in end stage renal disease (ESRD) patients. Hyperhomocysteinemia in patients with CRF is associated with various abnormalities in concentrations of other sulfur amino acids and their metabolites, such as elevated plasma levels of S-adenosylmethionine, S-adenosylhomocysteine, cystathionine, cysteine, cysteine-sulfinic acid, inorganic sulfate, and glutathione (but low blood levels of glutathione) and low plasma and muscle taurine levels. These abnormalities suggest that both the transsulfuration and remethylation pathways are impaired in CRF [4].

Causes of hyperhomocysteinemia in uremia

Possible causes of hyperhomocysteinemia in renal failure include: (1) impaired renal excretion, (2) reduced renal metabolism, and (3) inhibition of enzymes related to Hcy metabolism by retained uremic toxins. It was previously believed that impaired renal excretion could be responsible, but it has been ascertained conclusively that Hcy excretion is negligible.

Therefore, it appears that if renal function was abolished, consequent Hcy accumulation could be prevented easily by its whole-body metabolic clearance [5].

Results obtained in a rat model for Hcy metabolism in the renal parenchyma showed a 20% decrease in Hcy level in the renal vein with respect to the renal

artery; however, in this experimental system, Hcy was present mostly in its free non-protein-bound form. There was no significant difference measured in the renal vein and renal artery of 20 patients with normal renal function undergoing coronary angiography, and there was no significant difference.

In CRF, a uremic toxin may accumulate, leading to impairment of one or more of the relevant enzymes of Hcy metabolism. Supporting the role of retained uremic toxins are the following considerations [1].

1. In uremia, folates (cosubstrates of methionine synthase, a key enzyme in Hcy disposal), even if used at high dosages, are not able to completely normalize Hcy levels, suggesting that still unidentified uremic toxins may inhibit these enzymes [6].
2. Superflux dialyzers are able to reduce Hcy levels in the long term. Conversely, conventional dialysis can reduce Hcy levels up to a point because only free Hcy is dialyzable, and most Hcy is bound. The suggested explanation is that uremic toxins are dialyzed more efficiently by superflux dialyzers, thus easing the inhibition of Hcy metabolizing enzymes.
3. Kidney transplant recipients are responsive to relatively high dosages of folate and can normalize their plasma Hcy levels after therapy.

Hcy, transplantation, obesity

After renal transplantation, mean tHcy levels decrease as renal function improves, but remain twice those of mean levels in general populations free of chronic renal disease. More than 60% of stable renal transplant recipients have tHcy levels greater than 12 μmol/l. In contrast to treatment for HHcy in CRF, treatment for HHcy with supraphysiological doses of folic acid and B vitamins is much more effective, presumably because renal transplant recipients have much less impairment in renal function relative to dialysis-dependent patients with CRF. Beaulieu et al. showed that low doses of folic acid (2.4 mg/d) in combination with vitamin B_6 (50 mg/d) and vitamin B_{12} (0.4 mg/d) decreased the mean tHcy level to the normal range in a cohort of renal transplant recipients; the majority of the group reached levels less than 12 μmol/l [7, 8, 9, 10].

It is possible that tHcy-lowering treatment may reduce the risk and severity of arteriosclerotic vascular disease in renal transplant recipients, who would likely achieve normal or near-normal tHcy levels with high-dose folic acid and B vitamin-based treatment [10].

Obesity, hyperhomocysteinemia and secondary hyperlipoproteinemia are found very frequently after kidney transplantation (Tx). They may independently represent risk factors for development of atherosclerosis and chronic allograft nephropathy [11].

The last decade has seen a marked increase in the survival times of functioning renal grafts. Improved pre- and post-transplant care has resulted in a growing number of recipients in the older age groups, many of whom suffer from associated diseases (hypertension and diabetes in particular) and had long been on dialysis treatment. Besides hyperlipidemia, development of atherosclerotic and/or nephrosclerotic lesions is enhanced by a variety of other factors, particularly arterial hypertension (in more than 80% of patients), primary vessel wall lesions (vasculitis, calcification), primary or secondary diabetes (insulin- or non-insulin-dependent diabetes mellitus), significant decrease in renal function, and proteinuria. A most important role is played by long-term administration of immunosuppressive therapy, in particular cyclosporin A and corticoids [12, 13, 14].

Our previous studies have demonstrated that transplant recipients do not differ, genetically, from the general Czech population. However, the body weight of the former is increased significantly.

The aim of our study was to assess serum levels of homocystein and to evaluate the effect of new regimen for the treatment of obese transplant patients with BMI ≥ 30 kg/m^2 with hyperhomocysteinemia in a long-term study.

Material and Methods

The study protocol was approved by the Ethics Committee of the Institute for Clinical and Experimental Medicine.

In a prospective metabolic study, we evaluated, for a period of 36 months, a total of 136 (65 M/71F) patients after their first cadaveric renal transplantation at 24–76 years of age. The patients were transplanted at the Transplant Center of the Institute for Clinical and Experimental Medicine and collection of patient data was completed by 31 March 2005. We compared the findings of 138 patients with BMI ≥ 30 (kg/m^2) on a new regimen one year (start of the study) and 3 years after renal transplantation.

Based on a Subjective Global Assessment Scoring Sheet, experienced dietetic nurse performed individualized dietetic intervention using DIETA computer software to prepare a individualized hypoenergic-hypolipidemic diet (IHHD) with energy intake < 25 kcal/kg BW. Subsequently, after corticoid withdrawal, IHHD was supplemented with folic acid 5 mg/day, and vitamin B_6 50 mg/day for a follow-up period of 2 years.

The patients were on follow-up at the Department of Nephrology, Transplant Center of the Institute for Clinical and Experimental Medicine where their laboratory, anthropometric, and dietetic profiles and therapeutic interventions were regularly evaluated at a three-month interval.

Long-term immunosuppressive therapy included cyclosporin A with effective levels of 150–250 ng/ml or tacrolimus 8–10 ng/ml. In addition, the patients received mycophenolate mofetil 1–2 g/day not shown to affect lipid metabolism.

The following biochemical investigations were performed using standard biochemical procedures:

– Total homocysteine (tHcy), i. e., the sum of all homocysteine derivatives present in plasma in free or protein-bound form after reduction was estimated by enzymatic FIA method on an Abbott Axsym under ERDNIM quality control.

– Vitamins were determined as follows: folate by chemiluminiscence EIA on Immulite and vitamin B_6 on HPLC Gold Beckman by Chromsystems.
– Leptin and Ob-Re were determined by ELISA kit BioVendor.
– Specific cyclosporin A and tacrolimus levels were determined by homogenous enzyme immunoassay technique (Emit).
– Apo E isoforms by PCR and restricted isotyping.
– DNA for MTHFR C677T polymorfism was isolated from whole blood using QIA amp midi kit (Qiagen) and analyzed by PCA.
– Dietary profile using ASTRIS and DIETA software.
– Body mass index by body weight and height (kg/m^2).
– Routine biochemical, hematological and immunochemical analyses performed on Synchron CLX2O, Advia 120 and Beckman Array analyzers.

Statistical analysis was performed using two-way analysis of variance complemented with multiple comparison and analysis of variance with repeated measures and grouping factor.

Results

During the study period, there was a significant decrease of tHcy level ($P < 0.01$) and BMI ($P < 0.025$). A decrease in BMI on long-term therapy was associated with a significant decrease in serum leptin ($P < 0.01$) and lipid metabolism parameters ($P < 0.01$, $P < 0.025$).

The mean values of serum folate and vitamin B_6 increased significantly ($P < 0.01$), creatinine clearance, mean blood pressure, proteinuria, Lp(a) and apoE isoforms did not differ significantly (Table 1).

Discussion

Hyperhomocysteinemia is an independent risk factor for the development of cardiovascular disease. Stable renal transplant recipients have disproportionately high rates of arteriosclerotic processes and recent reports provide hard evidence that clinically stable transplant patients have a high prevalence of hyperhomocysteinemia. Major determinants of plasma tHcy are renal function, folate levels and, to a lesser extent, vitamin B_6 concentration. Treatment for hyperhomocysteinemia with supraphysiological doses of folic acid and vitamin B could be effective because renal transplant patients have less impairment in renal function relative to dialysis patients. It is possible that tHcy-lowering treatment may reduce the risk for and severity of atherosclerotic vascular disease in renal transplant patients, who would likely achieve normal or near normal tHcy levels with high-dose folic acid and vitamin B-based treatment [15, 16].

Obesity represents a risk factor in patients after renal transplantation. It is characterized by the abdominal (visceral) type of obesity in men and women alike. The prevalence is high, ranging between 25 % and 35 % in the first post-transplant year. Obesity is associated with other risk factors, primarily hyperlipidemia [11, 17].

The hyperlipidemia after kidney transplantation is of mixed etiology. After chronic renal disease with subsequent renal insufficiency and long-term dialysis therapy, patients presenting for transplantation often show various degrees of lipid metabolic disorders [16].

The mechanism of hyperlipidemia of recent onset or exacerbating after renal transplantation differs significantly in at least two aspects. If the function of the kidney transplant is good, resumption of the metabolic

Table 1. Basic metabolic parameters in obese patients at first and third years after renal transplantation (x ± SD)

Variable	1st year	3rd year	Statistical significance
Number of patients	138	136	
Gender (M/F)	65/73	65/71	NS
tHcy (μmol/l)	35.2 ± 12.4	12.7 ± 2.9	P < 0.01
BMI (kg/m^2)	35.5 ± 3.2	27.4 ± 28	P < 0.025
Folate (mg/l)	17.8 ± 8.5	32.9 ± 9.0	P < 0.01
Vit B_6 (μg/l)	5.4 ± 1.5	9.3 ± 2.2	P < 0.01
Cholesterol (mmol/l)	7.2 ± 2.4	6.1 ± 2.0	p < 0.01
LDL–cholesterol (mmol/l)	4.1 ± 1.2	3.0 ± 0.7	P < 0.01
HDL–cholesterol (mmol/l)	1.1 ± 0.2	1.0 ± 0.2	NS
Triglycerides (mmol/l)	3.8 ± 1.6	2.6 ± 0.6	P < 0.01
HDL–c/TG	0.28 ± 0.07	0.38 ± 0.06	P <0.01
Lp(a) (mg/l)	236 ± 209	204 ± 186	NS
apo E isoform	8:80:12	9:82:9	NS
ε 2: ε 3: ε 4 (%)			
MTHFR C 677T (%)	8.2	9.1	NS
Leptin (ng/l)	48.3 ± 20.7	16.8 ± 8	P < 0.01
Ob Re (U/ml)	16.2 ± 7.4	26.1 ± 13.1	P < 0.01
Proteinuria (g/24 hrs)	0.5 ± 0.2	0.3 ± 0.2	NS
Creatinine clearance (ml/s)	1.0 ± 0.5	0.9 ± 4	NS
Cyclosporine level (μg/l)	190 ± 30	205 ± 35	NS
Tacrolimus level (μg/l)	7.0 ± 2.5	8.8 ± 3.1	NS
Mean BP (torr)	135/85	130/85	NS

function of the kidney has a beneficial effect. An adverse effect is exerted by long-term use of immunosuppressive therapy (cyclosporin A, prednisone), previous hyperlipidemia associated with genetic predisposition, reduced kidney transplant function, repeat rejection episodes controlled by high doses of corticoids, major proteinuria, secondary diabetes mellitus, age, gender and obesity [18, 19, 20].

Very marked, in this respect, is also the increase in body weight, often associated with the development of secondary diabetes. In patients on immunosuppressive therapy who are, moreover, relatively often unwilling to cooperate, a more marked reduction of weight is difficult to achieve and requires long-term determined dietetic intervention. Our most remarkable finding not given adequate attention to date is that obese female transplant recipients over 60 years of age could be the most significant risk group in terms of developing atherosclerotic lesions. Their levels of total cholesterol, LDL-cholesterol, but, also, triglycerides appreciably exceeded those in men over 60. No doubt a role was played in this by their body weight (BMI). The parameters monitored through the diet-related questionnaire revealed a significant increase in energy intake (145 ± 12 kJ/kg b. w., $P < 0.01$) and reduced intake of fiber (0.7 g/kg; $P < 0.05$).

In conclusion, obesity with BMI ≥ 30 kg/m^2 associated with significant hyperhomocysteinemia should be treated effectively as a high-risk factor after renal transplantation.

Abbreviations:
BMI – body mass index
C_{Cr} – creatinine clearance
CRF – chronic renal failure
tHcy – total plasma homocystein
ESRD – end stage renal disease
met – methionine
Tx – transplantation
IHHD – individualized hypoenergic-hypolipidemic diet
ObRe – solubile leptin receptor

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