Cystatin C Is an Independent Predictor of Fasting and Post-Methionine Load Total Homocysteine Concentrations among Stable Renal Transplant Recipients

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Background: An increased prevalence of hyperhomocysteinemia with an increased incidence of cardiovascular disease events has been reported among stable renal transplant recipients (RTRs). Preliminary studies in a small number of these individuals have shown that serum creatinine and cystatin C, both markers of kidney function and glomerular filtration rate, are independent determinants of fasting tHcy concentrations; however, determinants of tHcy concentrations after a methionine load have not been studied.

Methods: We determined the prevalence of both fasting and 4-h post-methionine load (PML) tHcy concentrations in 78 stable RTRs and compared the role of cystatin C with the role of serum creatinine as determinants of fasting and PML tHcy.

Results: Of the 78 RTRs, 21 (26.9%) had fasting and PML tHcy within the respective reference intervals, and 57 (73.1%) had increased plasma tHcy. Of these 57 RTRs, 22 had fasting hyperhomocysteinemia, 9 had PML hyperhomocysteinemia, and 26 had combined hyperhomocysteinemia (both fasting and PML). Unadjusted Pearson correlations showed that fasting plasma tHcy correlated with both cystatin C (r = 0.564; P < 0.001) and creatinine (r = 0.519; P < 0.001) and that increases in PML tHcy modestly correlated with cystatin (r = 0.205; P = 0.072), but not creatinine (r = 0.057; P = 0.624). General linear regression modeling with stepwise analysis of covariance showed that both cystatin C (partial R = 0.554; P < 0.001) and creatinine (partial R = 0.535; P < 0.001) were independent predictors of fasting tHcy, but of the two, only cystatin C (partial R = 0.242; P = 0.035) was an independent predictor of increased PML tHcy.

Conclusions: Clinically stable RTRs have an excess prevalence of moderate hyperhomocysteinemia, and additional cases can be detected by methionine loading. Both creatinine and cystatin C are independent predictors of fasting tHcy in these individuals; however, only cystatin C is a determinant of tHcy concentration after a methionine load, probably because cystatin C is a more sensitive marker of glomerular filtration rate than serum creatinine.

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In recent years, hyperhomocysteinemia has been shown to be an independent risk factor for occlusive vascular diseases (1–5). An increased prevalence of hyperhomocysteinemia has been observed in renal transplant recipients (RTRs) (6–12), and it has been reported that hyperhomocysteinemia may contribute to the disproportionately high incidence of cardiovascular disease events experienced by RTRs (7, 12–15). In a recent prospective study of 207 chronic, stable RTRs, Ducloux et al. (16) demonstrated that increased fasting total homocysteine (tHcy) is an independent risk factor for the development of cardiovascular disease events in stable RTRs.

Hyperhomocysteinemia is characterized by mild to moderately increased concentrations of plasma tHcy, as measured during fasting or 2–6 h after a methionine load.

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5 Nonstandard abbreviations: RTR, renal transplant recipient; tHcy, total homocysteine; PML, post-methionine load; and GFR, glomerular filtration rate.
Boushey et al. (3) showed, in a metaanalysis of 27 studies, that both fasting and post-methionine load (PML) hyperhomocysteinemia are risk factors for coronary artery disease. Additionally, results from our laboratory (17), as well as from the European Concerted Action Project (5) and the National Heart, Lung, and Blood Institute Family Heart Study (4), showed that PML and fasting hyperhomocysteinemia are independent of each other in the majority of individuals and that without methionine loading, ~27–40% of the cases of increased plasma tHcy could be missed. Despite the importance of measuring tHcy concentrations after a methionine load, only one study, involving 29 RTRs, has documented that there is an increased prevalence of both fasting and PML hyperhomocysteinemia in RTRs (9).

Moderately increased tHcy, whether measured during fasting or after a methionine load, can be caused by genetic factors (18, 19), nutritional deficiencies of the B vitamins (20–22), and/or renal insufficiency (23, 24). Although successful kidney transplantation lowers tHcy concentrations, it is unclear why a majority of stable RTRs still have hyperhomocysteinemia (12). Serum creatinine, a widely used marker in predicting kidney function and glomerular filtration rate (GFR), has been shown to be an independent determinant of tHcy concentrations in chronic, stable RTRs (25). It has been suggested that plasma tHcy is related to serum creatinine both as a result of renal function and as a result of the close relationship between Hcy production and creatine– creatinine formation (26).

Cystatin C, an inhibitor of cysteine proteinases, has the characteristic of an ideal marker to assess renal function (27). Cystatin C, a product of a housekeeping gene, is a nonglycosylated basic protein of low molecular weight (M, 13 260) synthesized by all nucleated cells at a constant rate (28); cystatin C is freely filtered by the glomerulus and is almost completely reabsorbed and catabolized by the proximal tubular cells (29). Previous studies have shown that the concentration of cystatin C in serum correlates with glomerular filtration, and it has been suggested that cystatin C may be a more sensitive marker of GFR than serum creatinine (30–32). Additionally, cystatin C has been shown to exhibit the highest predictive value for tHcy concentrations in healthy subjects as compared with age and serum creatinine (26). Preliminary investigations have also shown that there is an independent relationship between cystatin C and fasting tHcy concentrations in stable RTRs (33) and coronary artery disease patients (34) when these populations have clinically healthy renal function.

These previous studies have evaluated determinants of fasting tHcy concentrations in only a small number of RTRs, but there have been no reports on the relationship between cystatin C and PML tHcy. In the current investigation, we determined the prevalence of both fasting and PML hyperhomocysteinemia in 78 stable RTRs and studied creatinine and cystatin C with respect to the role of these two biochemical markers as determinants of plasma tHcy concentrations, both after fasting and 4 h after a methionine load.

**Materials and Methods**

**STUDY POPULATION**

We studied 78 stable RTRs (49 males and 29 females; age range, 27–73 years; mean ± SD, 51.6 ± 12.3 years). All participants had received a transplant at least 6 months before the study and had no clinical evidence of acute renal graft rejection. None had used vitamin B supplementation for at least 1 year before the time of examination. This study was approved by the Human Studies Committee of the University of Minnesota Institutional Review Board, and all subjects gave informed consent.

**BIOCHEMICAL ASSAYS**

Fasting blood samples were drawn and separated within 30 min for the measurement of fasting tHcy, creatinine, cystatin C, pyridoxal 5’-phosphate (vitamin B₆), vitamin B₁₂, and folate. Methionine (100 mg/kg of body weight) was administered orally, and a second blood sample was collected 4 h after loading for the determination of PML tHcy.

Both fasting and PML tHcy concentrations were measured in plasma from EDTA-anticoagulated blood by HPLC with fluorometric detection (35). Because the PML tHcy concentration can be confounded by an increased fasting tHcy concentration, we used the increased PML tHcy, calculated as the difference between the fasting and 4-h PML tHcy concentrations.

Serum creatinine was determined by rate reflectance spectrophotometry on the Vitros analyzer (Johnson & Johnson Clinical Diagnostics, Inc.). Cystatin C was measured in plasma by a particle-enhanced turbidimetric assay method (Dako Inc.) using the Roche COBAS FARA centrifugal analyzer (Boehringer Mannheim Diagnostics).

Vitamin B₆ was measured in plasma by a radioenzymatic assay (American Laboratory Products Company, Ltd.). Vitamin B₁₂ was determined in serum by the Access Immunoenzymatic Assay System (Sanofi Diagnostics Pasteur, Inc.), and serum folate was determined by the Access Chemiluminescent Immunoassay System (Sanofi Diagnostics Pasteur, Inc.).

**Table 1. Biochemical analytes in 78 stable RTRs.**

<table>
<thead>
<tr>
<th>Analyte</th>
<th>Mean ± SD</th>
<th>Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cystatin C, mg/L</td>
<td>1.71 ± 0.66</td>
<td>0.4–4.1</td>
</tr>
<tr>
<td>Creatinine, μmol/L</td>
<td>139.67 ± 49.50</td>
<td>61.9–371.3</td>
</tr>
<tr>
<td>Fasting tHcy, μmol/L</td>
<td>13.04 ± 5.49</td>
<td>5.8–36.9</td>
</tr>
<tr>
<td>PML increase in tHcy, μmol/L</td>
<td>26.39 ± 15.53</td>
<td>11.0–87.6</td>
</tr>
<tr>
<td>Vitamin B₆, nmol/L</td>
<td>48.7 ± 110.9</td>
<td>5–488</td>
</tr>
<tr>
<td>Vitamin B₁₂, pmol/L</td>
<td>315.9 ± 205.0</td>
<td>103–1108</td>
</tr>
<tr>
<td>Serum folate, nmol/L</td>
<td>24.0 ± 49.8</td>
<td>5–446</td>
</tr>
</tbody>
</table>

* Geometric mean.
Because the B vitamin values and increases in fasting and PML tHcy had a skewed distribution, these variables were natural log-transformed, and geometric means were used. The natural log-transformed variables and unadjusted correlations between these variables were assessed in a Pearson correlation matrix. Stepwise linear regression modeling (probability of F entry, \( \leq 0.05 \); probability of F removal, \( \geq 0.10 \)) was performed to determine the independent association between potential predictor covariables and increases in both fasting and PML tHcy concentrations. All statistical tests were two-tailed at 5% and 1%. The statistics were computed with SPSS for Windows (Ver. 10.0; SPSS).

**Results**

The analytes tested are listed in Table 1 and expressed as means ± SD and complete ranges on 78 stable RTRs. The mean fasting tHcy, cystatin C, and creatinine values were greater than the generally acceptable reference values for these analytes (17, 36, 37). Of the 78 individuals, 21 (26.9%) had fasting (<12 μmol/L) and PML (<30 μmol/L) tHcy within the respective reference intervals, and 57 (73.1%) had increased plasma tHcy concentrations. Of these 57 RTRs, 22 (38.6%) had fasting hyperhomocysteinemia, 9 (15.8%) had PML hyperhomocysteinemia, and 26 (45.6%) had combined hyperhomocysteinemia (both fasting and PML).

Unadjusted Pearson correlations between the continuous variables examined are shown in Table 2. Fasting plasma tHcy correlated with both cystatin C \((r = 0.564; P < 0.001)\) and creatinine \((r = 0.519; P < 0.001)\), whereas increased PML tHcy modestly correlated with cystatin C \((r = 0.205; P = 0.072)\), but not creatinine \((r = 0.057; P = 0.624)\). There was also an inverse correlation between fasting tHcy and vitamin B\(_{12}\) \((r = -0.247; P = 0.034)\). Overall, there was a significant correlation between cystatin C and creatinine \((r = 0.548; P < 0.001)\). This correlation was most significant when serum creatinine concentrations were >132.6 μmol/L \((r = 0.539; P = 0.001;n = 36)\), and the correlation was relatively weak when creatinine concentrations were <132.6 μmol/L \((r = 0.295; P = 0.058;n = 42); \) data not shown).

General linear regression modeling with stepwise (forward and backward) analysis of covariance was performed with both fasting and increased PML tHcy concentrations as the dependent variables and cystatin C or/and creatinine, vitamin B\(_{12}\), vitamin B\(_{6}\), folate, age, and sex as the independent variables. Table 3 shows that, when creatinine was excluded from the model, cystatin C was a strong independent predictor (partial \(R = 0.554; P < 0.001\)) of fasting tHcy concentrations after simultaneous adjustment for vitamin B\(_{12}\), vitamin B\(_{6}\), folate, age, and sex. When creatinine was included simultaneously with cystatin C in the model (data not shown), there was a similar but weaker relationship between fasting tHcy and cystatin C (partial \(R = 0.386; P = 0.001\)) after simulta-
Table 3. Linear regression of fasting plasma tHcy on potential independent predictor variables (cystatin C, creatinine, and vitamin B12).a

<table>
<thead>
<tr>
<th>Variable</th>
<th>Standardized β regression coefficient</th>
<th>Partial R</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cystatin C</td>
<td>0.555</td>
<td>0.554</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>B12</td>
<td>-0.229</td>
<td>-0.228</td>
<td>0.019</td>
</tr>
<tr>
<td>Creatinine</td>
<td>0.535</td>
<td>0.535</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>B12</td>
<td>-0.279</td>
<td>-0.278</td>
<td>0.005</td>
</tr>
</tbody>
</table>

*Model R²: 0.350, 0.005

Cystatin C excluded from general linearity model
- Full modelb 0.272, <0.001
- Full model minus B12 0.318, <0.001

Cystatin C and B12 as the independent variables.

Creatinine excluded in the general linear model
- Full modelb 0.350, 0.005
- Full model minus B12 0.272, <0.001

Cystatin C and creatinine as the independent variables.

Table 4. Linear regression of plasma PML increase in tHcy on potential independent predictor variables (cystatin C and gender).a

<table>
<thead>
<tr>
<th>Variable</th>
<th>Standardized β regression coefficient</th>
<th>Partial R</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cystatin C</td>
<td>0.245</td>
<td>0.242</td>
<td>0.035</td>
</tr>
<tr>
<td>Gender</td>
<td>-0.276</td>
<td>-0.273</td>
<td>0.018</td>
</tr>
</tbody>
</table>

*Model R²: 0.117, 0.035

Cystatin C and gender as the independent variables.

Creatinine excluded in the general linear model.
relationship between creatine–creatinine synthesis and Hcy production. With regard to the B vitamins, we show that only vitamin B_{12} is an independent predictor of fasting tHcy, thus illustrating that the B vitamins play a relatively minor role in predicting plasma tHcy in stable RTRs. This is in agreement with the recent findings of others (11, 25). The lack of correlation between serum folate and fasting tHcy may be partially explained by the fortification of cereal-grain products with folic acid in the last several years (40).

We conclude that methionine loading will detect additional cases of hyperhomocysteinemia among clinically stable RTRs and that, whereas our results show that both creatinine and cystatin C are independent predictors of fasting tHcy, only cystatin C, and not creatinine is a determinant of PML tHcy concentrations. More studies are needed to confirm our observation on the relationship between cystatin C and PML tHcy.

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References


