Hyperhomocysteinemia, paraoxonase concentration and cardiovascular complications in Tunisian patients with nondiabetic renal disease

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Abstract

Objectives: Hyperhomocysteinemia is associated with an increased risk of cardiovascular diseases. We determine homocysteine levels (Hcy), paraoxonase (PON1) concentration and their relationship on cardiovascular complications in patients with chronic renal disease (CRD).

Design and methods: The study population included 100 CRD patients and 120 healthy controls. Renal function was assessed using the eGFR by the MDRD study equation. Patients were considered to have CRD when the eGFR was <60 mL/min/1.73 m². Hcy concentrations were determined by direct chemiluminescence assay. PON1 concentration was measured spectrophotometrically using phenylacetate as a substrate.

Results: We found an increased Hcy levels and a decreased eGFR and PON1 concentration in CRD patients compared to the control group (P<0.001, P<0.001, P<0.01 respectively). Patients with cardiovascular complications showed an increased Hcy levels and a lower PON1 concentration than patients without cardiovascular complications (P<0.001, P<0.01 respectively).

Conclusion: We showed that hyperhomocysteinemia and low PON1 concentration are associated with CRD and markedly associated in patients with cardiovascular complications. Additional effects contribute to the severity of renal disease and increase the incidence of cardiovascular disease.

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Keywords: Hyperhomocysteinemia; Paraoxonase concentration; Chronic renal disease; Cardiovascular complications

Introduction

Patients with chronic renal disease (CRD) represent an important segment of Tunisian population, and mostly because of the high risk of cardiovascular disease (CVD) associated with renal insufficiency, detection and treatment of chronic renal disease is now a public health priority [1–3]. The increased incidence of CVD is likely to be the result of a high prevalence of both traditional risk factors, such as diabetes mellitus, hypertension, dyslipidemia and smoking. Nontraditional risk factors, such as hyperhomocysteinemia, oxidative stress and inflammation have not been fully taken into account.

Homocysteine (Hcy) as a thiol-containing amino acid has gained great notoriety, since elevation of its plasma concentrations, a condition known as hyperhomocysteinemia, is associated with several diseases, in particular cardiovascular disease [4–7], neurodegenerative diseases [8], neural tube defects [9,10], and end-stage renal disease (ESRD) [11–15].

Serum paraoxonase 1 (PON1) is an oxidant-sensitive enzyme that inhibits the atherogenic oxidation of low density lipoprotein (LDL). PON1 activity is also implicated in protection against cardiovascular disease [16]. Some studies

Abbreviations: CRD, chronic renal disease; CVD, cardiovascular disease; eGFR, estimated glomerular filtration rate; ESRD, end-stage renal disease; Hcy, homocysteine; HeyT, homocysteine thiolactone; MRF, moderate renal failure; PON1, serum paraoxonase 1; SRF, severe renal failure.

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found that the paraoxonase protein (PON1), carried on high density lipoprotein (HDL), has homocysteine thiolactone (HcyT) hydrolase activity and protects against protein homocysteinylation in vitro [17,18]. Jakubowski et al. found that low homocysteine thiolactonase activity may be a risk factor in Hcy-cysteinylation in vitro [17,18]. Jakubowski et al. found that low HcyT hydrolase activity and protects against protein homocysteinylation in human vascular endothelial cells (HUVEC) [24,25]. The extent of HcyT synthesis and protein homocysteinylation in human vascular endothelial cells depends on levels of Hcy, methionine, folate and HDL that are linked to vascular disease [17]. In addition, Jakubowski showed that HcyT and N-Hcy-protein both contribute to the pathophysiological effects of Hcy on vascular atherosclerosis [26–28]. Recent studies showed that hyperhomocysteinemia and/or paraoxonase activity are involved in the development of cardiovascular complications in patients with chronic renal disease and in patients undergoing hemodialysis [29–32].

We reported in previous studies that hyperhomocysteinemia, oxidative stress and paraoxonase activity are risk factors for cardiovascular disease and the severity of coronary artery disease in Tunisian population [7,33]. The association between chronic renal disease, homocysteine and paraoxonase concentration may not have been fully taken into account, and the development of cardiovascular complications related to end-stage renal disease (ESRD) remains unclear. The aim of this study is to determine the prevalence of hyperhomocysteinemia and paraoxonase concentration and their relationships with cardiovascular complications in patients with nondiabetic renal disease. Furthermore, we showed some possible mechanisms mediating hyperhomocysteinemia to induce the severity of renal disease.

Materials and methods

Study population

The control group gathered 120 healthy volunteers with no history of CVD, diabetes mellitus or renal disease. Their average age was 54±10 years and consisted of 87 males and 33 females.

Consecutive 100 patients with chronic renal disease were enrolled from University Hospital Fattouma Bourguiba (Nephrology department) in Monastir (Tunisia). The average age of this group was 51±15 years and consisted of 55 males and 45 females. All patients are nondialyzed chronic kidney disease, and with nondiabetic renal disease. We excluded patients with folate and/or vitamin B₁₂ treatments and patients with diabetic renal disease because diabetes do adequately account for the substantial increase cardiovascular risk and mortality observed in chronic renal disease (CRD). Kidney function was assessed using glomerular filtration rate (GFR) as estimated by the four-variable Modification of Diet in Renal Disease (MDRD) study equation as follows: eGFR=186.3×(serum creatinine/88.4)⁻¹.¹⁵⁴×(age)⁻⁰.²⁰³×(0.⁷⁴₂ if female)×(1.²¹² if black). eGFR was expressed in mL/min/1.73 m² [34], and patients were considered to have chronic kidney disease when the eGFR was <60 mL/min/1.73 m² [35]. Patients according to eGFR were subclassified as follows: Group (MRF) who had moderate renal failure (n=42), group (SRF) who had severe renal failure (n=35) and group (ESRD) who had end-stage renal disease (n=23). Etiologies of chronic renal disease in patients were chronic glomerular nephritis (n=41), chronic tubulointerstitial nephropathy (n=30), vascular nephropathy (n=23), and unknown cause (n=6). Anemia was defined as hemoglobin<12 g/dl [36]. Cardiovascular complications in all groups were diagnosed by echocardiography and electrocardiography. We found patients without cardiovascular complications (n=66), and patients with cardiovascular complications (n=34), included cardiac insufficiency (n=10), and left ventricular hypertrophy (n=24).

All participants were interviewed, and data on hypertension, dyslipidemia, smoking habits were recorded. Informed consent was obtained from each patient and healthy subject according to the guidelines of our ethics committee. For cardiovascular risk factors, the following definitions were used: individuals were defined as hypertensive if their blood pressure was >140/90 mm Hg or if they were receiving any antihypertensive treatment; individuals were defined as smokers if their total cholesterol concentration was ≥5.68 mmol/L, or their triglyceride concentration was ≥2.28 mmol/L, or they were receiving lipid-lowering drugs. Smoking history was coded as never and current smoker.

Measurements of lipids, creatinine, Hcy, folate, vitamin B₁₂ and paraoxonase concentration

Serum total cholesterol and triglyceride were measured with commercially available kits using AU 640 automate (Olympus Diagnostica GmbH, Clare, Ireland). After precipitation of serum chylomicron, LDL and VLDL using a precipitating reagent, the HDL-cholesterol left in the supernatant was measured by an enzymatic method. LDL-cholesterol was calculated with Friedwald formulas. Serum creatinine concentration was determined by Jaffe’s test. Plasma concentration of total homocysteine was determined by direct chemiluminescence (a competitive immunoassay) on ACS: 180 (Bayer Vital GmbH, Fernwald, Germany). Plasma folate and vitamin B₁₂ concentrations were determined by a competitive RIA method and dual assay on a Multi-Crystal Gamma Counter (LB 211) (Berthold, Germany) using a Simul TRAC-SNB Radio assay kit vitamin B₁₂ (⁵⁷Co)Folate (¹²⁵I) (MP, Orangeburg, NY, USA). Paraoxonase concentration was measured by adding serum to 1 mL of Tris–HCl buffer (100 mM, pH 8.0) containing 1 mM CaCl₂ and 1 mM of phenyl acetate (Sigma) as described previously [37]. The sodium acetate hydrolysis rate was determined spectrophotometrically (UVIKON 930) at 270 nm. Serum phenylacetate hydrolyase activity of PON1 was expressed in international units (U) per millilitre of serum.
Statistical analysis

Statistical analyses were performed using Statistical Package for Social Sciences (SPSS) for Windows 10.0. Comparisons between two groups were performed using unpaired two-tailed t tests. Comparisons between groups >2 were performed by One-way analysis of variance (ANOVA). Simple associations between variables were calculated as the Pearson correlation. The independent influence of various factors on the presence of CRD was assessed with a multiple linear regression model. Hyperhomocysteinemia was defined as a mild increased Hcy (fasting total Hcy >15 μmol/L). Results were expressed as mean±SD and P<0.05 value was considered statistically significant.

Results

Clinical characteristics of the study population

Clinical characteristics of CRD patients and control groups are shown in Table 1. There are no differences in the mean age, and BMI between the two groups, while the mean of triglyceride, total cholesterol, LDL-cholesterol, creatinine, and homocysteine were significantly higher in patients group than in the control group. An increased Hcy levels was observed in 94% of patients. However the mean of HDL-cholesterol, hemoglobin, PON-1 concentration and eGFR were significantly lower in patients group than in the control group. Plasma folate and plasma vitamin B12 were not significantly different between groups.

eGFR, creatinine, homocysteine and paraoxonase concentration in patient with MRF, SRF and ESRD groups

Patients with CRD (n=100) were classified into 3 subgroups (MRF, SRF, and ESRD) related to the eGFR (Table 2). eGFR and PON1 concentration were significantly decreased in patient groups (P<0.001, P<0.01 respectively). However, Plasma Hcy increased significantly with patient groups (P<0.01). The coexistence of a high level of Hcy and a low PON1 concentration increase the severity of renal disease.

### Table 1
Characteristics of patients with CRD and control groups

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Controls (n=120)</th>
<th>CRD (n=100)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>54±10</td>
<td>51±15</td>
<td>NS</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>27.6±5</td>
<td>29.4±5</td>
<td>NS</td>
</tr>
<tr>
<td>Hypertension, n (%)</td>
<td>7 (5.83)</td>
<td>62 (62)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Dyslipidemia, n (%)</td>
<td>5 (4.16)</td>
<td>38 (38)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Triglyceride (mmol/L)</td>
<td>1.19±0.68</td>
<td>1.63±1.18</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Total cholesterol (mmol/L)</td>
<td>4.20±1.03</td>
<td>5.94±1.41</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>HDL cholesterol (mmol/L)</td>
<td>0.90±0.24</td>
<td>0.66±0.23</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>LDL cholesterol (mmol/L)</td>
<td>3.14±0.92</td>
<td>3.87±1.03</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>eGFR (mL/min/1.73 m²)</td>
<td>91.9±8.4</td>
<td>28.4±10.4</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Creatinine (μmol/L)</td>
<td>80.88±15.7</td>
<td>611±296</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Hemoglobin (g/dL)</td>
<td>13.8±1.2</td>
<td>8.8±2.2</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Homocysteine (μmol/L)</td>
<td>11.9±3.25</td>
<td>36.86±13.63</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Folate (μmol/L)</td>
<td>19.85±8.06</td>
<td>21.11±10.31</td>
<td>NS</td>
</tr>
<tr>
<td>Vitamin B12 (pmol/L)</td>
<td>293±94</td>
<td>311±105</td>
<td>NS</td>
</tr>
<tr>
<td>Paraoxonase activity (U/mL)</td>
<td>181±73</td>
<td>105±78</td>
<td>&lt;0.01</td>
</tr>
</tbody>
</table>

NS: not significant.

### Table 2
eGFR, serum creatinine, homocysteine, paraoxonase concentration and severity of CRD

<table>
<thead>
<tr>
<th>CRD (n=35)</th>
<th>CRD (n=23)</th>
<th>CRD (n=23)</th>
<th>CRD (n=23)</th>
<th>CRD (n=23)</th>
<th>CRD (n=23)</th>
</tr>
</thead>
<tbody>
<tr>
<td>eGFR</td>
<td>51.5±3.3</td>
<td>17.8±2.1</td>
<td>8.4±1.8</td>
<td>&lt;0.001</td>
<td></td>
</tr>
<tr>
<td>(mL/min/1.73 m²)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Creatinine (μmol/L)</td>
<td>154±73</td>
<td>571±130</td>
<td>1060±294</td>
<td>&lt;0.001</td>
<td></td>
</tr>
<tr>
<td>Hcy (μmol/L)</td>
<td>25.24±8.70</td>
<td>36.72±9.4</td>
<td>46.11±7.78</td>
<td>&lt;0.01</td>
<td></td>
</tr>
<tr>
<td>PON1 (U/mL)</td>
<td>133±43</td>
<td>104±25</td>
<td>82±29</td>
<td>&lt;0.01</td>
<td></td>
</tr>
</tbody>
</table>

### Table 4
Indicates Hcy levels and paraoxonase concentration in CRD patient with and without cardiovascular complications

Table 4 indicates Hcy levels and paraoxonase concentration in CRD patient (MRF, SRF, and ESRD) with and without cardiovascular complications. Patients with cardiovascular complications showed an increased Hcy levels and a lower PON1 concentration than patients without cardiovascular complications (P<0.001, P<0.01 respectively). Furthermore, in patients with cardiovascular complications, we showed a graded elevated Hcy levels and a graded lower PON1 concentration in ESRD patients than patients with moderate renal failure or patients with severe renal failure (P<0.001).

### Results of Pearson correlation and multivariate analysis

We studied the Pearson correlation between the presence of renal disease and some variables in CRD patients. We found that renal disease showed a positive correlation with eGFR.

<table>
<thead>
<tr>
<th>CRD (n=42)</th>
<th>CRD (n=35)</th>
<th>CRD (n=23)</th>
<th>CRD (n=23)</th>
<th>CRD (n=23)</th>
<th>CRD (n=23)</th>
</tr>
</thead>
<tbody>
<tr>
<td>eGFR</td>
<td>16.2±1.8</td>
<td>12.8±2.1</td>
<td>18.4±1.1</td>
<td>48.3±1.8</td>
<td></td>
</tr>
<tr>
<td>(mL/min/1.73 m²)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Creatinine (μmol/L)</td>
<td>630±190</td>
<td>855±203*</td>
<td>585±175</td>
<td>279±105</td>
<td></td>
</tr>
<tr>
<td>Hcy (μmol/L)</td>
<td>28.76±8.29</td>
<td>43.11±9.78</td>
<td>38.72±10.4</td>
<td>19.49±4.8</td>
<td></td>
</tr>
<tr>
<td>PON1 (U/mL)</td>
<td>132±23</td>
<td>92±29</td>
<td>104±14</td>
<td>143±16</td>
<td></td>
</tr>
</tbody>
</table>

* P<0.01 compared with G1 group.

\( a \) P<0.01 compared with G1 group.

### Notes for Tables

- **Table 2**: eGFR, creatinine, homocysteine and paraoxonase concentration in patient with etiologies of CRD

Patients were also classified into 4 subgroups with etiologies of CRD as follow: Group G1 with chronic glomerular nephritis, Group G2 with chronic tubulointerstitial nephropathy, Group G3 with vascular nephropathy, and Group G4 with unknown cause (Table 3). eGFR was significantly lower in each group. We showed an increased Hcy levels and a decreased PON1 concentration in each group except the unknown cause. In addition, G2 and G3 groups showed markedly a higher Hcy levels than G1 group (P<0.01).

### Homocysteine and paraoxonase concentration in CRD patient with and without cardiovascular complications

Table 4 indicates Hcy levels and paraoxonase concentration in CRD patient (MRF, SRF, and ESRD) with and without cardiovascular complications. Patients with cardiovascular complications showed an increased Hcy levels than patients without cardiovascular complications (P<0.001, P<0.01 respectively). Furthermore, in patients with cardiovascular complications, we showed a graded elevated Hcy levels and a graded lower PON1 concentration in ESRD patients than patients with moderate renal failure or patients with severe renal failure (P<0.001).

### Results of Pearson correlation and multivariate analysis

We studied the Pearson correlation between the presence of renal disease and some variables in CRD patients. We found that renal disease showed a positive correlation with eGFR.
Hyperhomocysteinemia and a low PON1 concentration were associated in patients with each etiology except the unknown cause. We showed an increased Hcy levels in patients with chronic tubulointerstitial nephropathy than patients with glomerular nephropathy. Hyperhomocysteinemia is able to promote glomerular damage and generate tubulointerstitial lesions. These findings were reported in experimental models that mild hyperhomocysteinemia promotes renal hemodynamic dysfunction [52,53]. Recently, O’Riordan et al. showed that chronic endothelial nitric oxide synthase (eNOS) inhibition actsuates endothelial mesenchymal transformation in chronic kidney disease [54]. Several studies have demonstrated that the bioavailability of NO is decreased in hyperhomocysteinemia [55,56]. In addition, we found that patients with vascular nephropathies showed an increased hyperhomocysteinemia than patients with glomerular nephropathy. Kassab et al. showed that hyperhomocysteinemia induced renovascular affect via activation of the rennin angiotensin system mediated by angiotensin II type 1 (AT1) receptors [57]. Minuz et al. found the increased oxidative stress was related to activation of the rennin angiotensin system in patient with hypertension and renovascular disease [58].

Hyperhomocysteinemia and a low PON1 concentration were markedly associated in CRD patient with cardiovascular complications including cardiac insufficiency and left ventricular hypertrophy. Recent studies have shown that hyperhomocysteinemia induced hypertension and ventricular hypertrophy.
[57,59]. Given the similarity of pathological changes between glomerular injury and Hcy induced arterial damages, such as endothelial injury, cell proliferation or growth, increased matrix formation, and aggregated proteoglycan, it is assumed that an increase in plasma Hcy levels may also directly act on glomerular and tubulointerstitial cells, resulting in glomerular damage and tubulointerstitial lesions. Moreover, an impaired renal function will lead to a further increase in plasma Hcy levels which in turn exaggerates the progression of glomerular injury, resulting in a vicious cycle and consequent glomerulosclerosis and ESRD.

The introduction of recombinant human erythropoietin (rhEPO) has marked a significant advance in the management of anemia associated with CRD. Marsillach et al. found that rhEPO and iron treatments of anemia promote significant changes in serum PON1 activity and concentration and have a beneficial effect on oxidative stress in predialysis patients with chronic renal disease [60]. Thus, EPO seems to be a very beneficial effect on oxidative stress in predialysis patients with changes in serum PON1 activity and concentration and have a role in anemia associated with CRD. Marsillach et al. found that rhEPO and iron treatments of anemia promote significant changes in serum PON1 activity and concentration and have a beneficial effect on oxidative stress in predialysis patients with chronic renal disease [60]. Thus, EPO seems to be a very beneficial effect on oxidative stress in predialysis patients with changes in serum PON1 activity and concentration and have a role in anemia associated with CRD.

In conclusion, this study shows that hyperhomocysteinemia and low PON1 concentration are associated with chronic renal disease and markedly associated in patients with cardiovascular complications. We postulate that with chronic toxicity induced by hyperhomocysteinemia may contribute to the severity of renal disease and increase the incidence of cardiovascular disease in patients. Some key targets in these pathogenic pathways must be identified to direct toward prevention or treatment of ESRD associated with hyperhomocysteinemia which is particularly important since so far there are no efficient Hey lowering and Hey detoxifying strategies being used in CRD patients.

Acknowledgments

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References


