WHAT INFLUENCES THE LEVEL OF OXIDATIVE STRESS AS MEASURED BY 8-HYDROXY-2'-DEOXYGUANOSINE IN PATIENTS ON HEMODIALYSIS?

Oxidative stress is at play in the progression of chronic renal failure (CRF) and in the genesis of atherosclerosis. The aim of the present study was to evaluate the factors that might influence the oxidative-antioxidative balance in patients on hemodialysis. The study group consisted of 71 hemodialysis patients due to CRF. Sixteen healthy subjects constituted a control group. The levels of 8-hydroxy-2'-deoxyguanosine (8-OHdG), C-reactive protein (CRP), and the blood lipid profile were measured in both groups. The results showed significantly higher mean levels of both 8-OHdG and CRP in the hemodialysis patients compared with that in the control subjects. The highest level of 8-OHdG was found in the subgroups of the patients with CRF primarily caused by diabetes (16.4 ng/ml) and with hypertensive nephropathy (15.8 ng/ml). More than a 2.5-fold higher level of 8-OHdG in the hemodialysis patients compared with the control subjects points to the presence of intensive oxidative stress in the patients.

Key words: C-reactive protein, hemodialysis, 8-hydroxy-2'-deoxyguanosine, oxidative stress
INTRODUCTION

The role of oxidative stress in chronic renal failure (CRF) progression and in the genesis of atherosclerosis was revealed in a number of experimental and clinical studies (1-3). During dialysis the oxidative stress hazard increases due to activation of inflammatory mediators, elimination of low molecular antioxidants, and the contact of blood with different types of dialysis membranes (4, 5). The intensity of oxidative stress in hemodialysis patients due to CRF can be influenced by many factors among which are age, duration of dialysis therapy, primary cause of CRF, lipid disturbances, intensity of chronic inflammation, type of diet, smoking, or environmental toxins (6-8).

The oxidative–antioxidative system is a complex structure dependent on enormous number of substances. On the one side of this system, there is oxygen, the molecule necessary for the life of each organism, and oxygen derivatives, free radicals which arise during the process of cellular respiration. On the other side, there is a pool of plenty antioxidants: low- and high-molecular compounds and enzymatic systems, whose aim is to protect the cell structure against an attack from toxic oxygen metabolites. The oxidative-antioxidative system’s imbalance leads to the pathology called oxidative stress (9).

CRF is a disease caused by damage to renal parenchyma by chronic pathologic processes leading to decreased glomerular filtration rate (GFR). Causes of CRF are multiple, with the predominance of primary glomerulonephritis (26.4%), diabetic nephropathy (19.2%), tubulointerstitial nephritis (16.5%), hypertensive nephropathy (8.9%), and polycystic kidney disease (8.9%) (10).

Oxidative stress is a factor in a spate of metabolic disturbances occurring in the course of CRF (11). One of the methods to measure oxidative stress is to measure the serum level of 8-hydroxy-2’-deoxyguanosine (8-OHdG) which is formed deoxyguanosine during DNA oxidation (12). The aim of this study was to evaluate factors which might have an influence on 8-OHdG, such as age, duration of dialysis, causes of CRF, coexistence of lipid disturbances and inflammation.

MATERIAL AND METHODS

The study was approved by a local ethics Committee and the subjects participating in the study gave informed consent to study procedures.

The study group consisted of 71 hemodialysis patients due to CRF. All patients were hemodialyzed with a carbohydrate solution. The weekly duration of dialysis was 12 h in 3-4-h sessions, performed with a polysulfone dialyzer. No patient was receiving any permanent pharmacological treatment known to influence lipid balance for 6 months before the onset of the study or antioxidant vitamins during the study course. Smoking in anamnesis was an exclusion criterion from the study. A control group consisted of 16 healthy persons, with no clinical symptoms of any disease and with the markers of renal function in the norm.
All blood samples were collected from the ulnar vein, in the morning before a dialysis session. The analysis of the following markers in the serum was performed: 8-OHdG, C-reactive protein (CRP), and a lipid profile: total cholesterol (TC), HDL fraction, and triglycerides (TG). The latter was assessed with an automatic Cobas Integra analyzer (Roche, Diagnostics, Graz, Austria). Friedewald’s formula was used to calculate LDL fraction. If the level of TG exceeded 400 mg%, the LDL fraction was additionally evaluated with an immunoenzymatic method (Behringer, Mannheim, Germany). 8-OHdG was measured using a Bioxytech 8-OHdG-EIA Kit manufactured by Oxis International (Portland, OR, USA) and CRP using an immunoturbidimetric method (X-Pand, Dade Behring, France).

All data are presented as means ±SD and were statistically analyzed using a commercial Statistica 5.0 package.

RESULTS

The levels of 8-OHdG and CRP were, on average, 2.5-2.7-fold greater in the hemodialysis patients than those in the healthy control subjects (Table 1). Moreover, the mean concentration of CRP in hemodialysis patients was almost two times higher than the laboratory norm for healthy people (up to 5 mg/l), which points to the presence of chronic inflammation in this group of subjects.

The 8-OHdG level was increasing with progressing age of the hemodialysis patients, but the correlation was weakly positive (r=0.25) (Fig. 1). To get a deeper insight into the relationship between age and 8-OHdG level in the hemodialysis patients we stratified this group into 3 age-groups of <50, 51-65, and >65 years old. The highest level of 8-OHdG tended to be in the middle age-group, although the differences did not assume significance (Table 2).

There was no statistically significant relation between the duration of hemodialysis-therapy and the 8-OHdG serum concentration. (Table 3)

<table>
<thead>
<tr>
<th>Marker</th>
<th>Hemodialysis patients n=71</th>
<th>Control subjectss n=16</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>8-OHdG (ng/ml)</td>
<td>14.2±7.9</td>
<td>5.6±2.2</td>
<td>P=0.000003</td>
</tr>
<tr>
<td>CRP (mg/l)</td>
<td>9.6±7.5</td>
<td>3.5±0.7</td>
<td>P=0.000052</td>
</tr>
</tbody>
</table>

Table 1. Comparison of the mean serum concentration of oxidative stress markers in hemodialysis patients and healthy controls.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Patients up to 50 yr n=27</th>
<th>Patients aged 50-65 n=22</th>
<th>Patients aged &gt;65 n=22</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean age (yr)</td>
<td>41 ±7</td>
<td>55 ±5</td>
<td>69 ±3</td>
<td>—</td>
</tr>
<tr>
<td>8-OHdG (ng/ml)</td>
<td>13.8 ±8.3</td>
<td>16.5 ±9.0</td>
<td>12.4 ±5.7</td>
<td>0.31</td>
</tr>
<tr>
<td>CRP (mg/l)</td>
<td>8.3 ±6.3</td>
<td>11.0 ±7.5</td>
<td>8.0 ±7.0</td>
<td>0.31</td>
</tr>
</tbody>
</table>

Table 2. Mean values of oxidative stress and inflammatory markers in serum of hemodialysis patients, as stratified by age.
Analyzing the connection between the 8-OHdG level and the cause of CRF, we found that the highest mean level of 8-OHdG was present in the subgroup of

Table 3. Mean values of oxidative stress and inflammatory markers in serum of hemodialysis patients. The patients were grouped according to the duration of hemodialysis.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Hemodialysis up to 12 mo (n=17)</th>
<th>Hemodialysis for 12-36 mo (n=25)</th>
<th>Hemodialysis &gt;36 mo (n=29)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean duration of hemodialysis (mo)</td>
<td>7 ±3</td>
<td>23 ±7</td>
<td>69 ±29</td>
<td>—</td>
</tr>
<tr>
<td>Mean age (yr)</td>
<td>51 ±16</td>
<td>57 ±10</td>
<td>53 ±13</td>
<td>0.6</td>
</tr>
<tr>
<td>8-OHdG (ng/ml)</td>
<td>14.2 ±9.3</td>
<td>16.6 ±8.3</td>
<td>12.7 ±6.4</td>
<td>0.08</td>
</tr>
<tr>
<td>CRP (mg/l)</td>
<td>8.4 ±7.1</td>
<td>8.9 ±6.9</td>
<td>10.9 ±8.3</td>
<td>0.48</td>
</tr>
</tbody>
</table>

Fig. 1. Relation between age and 8-OHdG serum concentration in hemodialysis patients.

Fig. 2. Comparison of serum 8-OHdG concentrations with respect to the primary cause of chronic renal failure. Values are means ±SD. The SD is illustrated by the top cross-squared parts of the bars.
patients with CRF primarily caused by diabetes (16.4 ng/ml), with the hypertensive nephropathy subgroup coming in second (15.8 ng/ml) (Fig. 2). There was no relationship between the level of 8-OHdG and lipid concentration in the hemodialysis patients.

**DISCUSSION**

There are a great number of metabolic derangements in the course of CRF which become intensified in end-stage renal disease, when dialysis is required (13). Zwolinska (14) has reported impairments of enzymatic systems, such as glutathione peroxidase (GSH-Px) or superoxide dismutase (SOD), deficiency of selenium, zinc, copper, vitamins A, C, and E, and also diminished glutathione concentration in CRF.

Oxidative stress is another accompaniment of CRF. Gerardi et al (13) and Marnett (15) have found increased serum concentrations of lipid the peroxidation products malondialdehyde and 4-hydroxynonenal in hemodialysis patients. Mezzano et al (16) have revealed increased concentration of thiobarbituric acid reactive substances (TBARS), which is a product of lipid peroxidation, and a higher level of advanced oxidation protein products (AOPP) in patients with uraemia. Nguyen-Khoa et al (7) have shown, in addition to high levels of TBARS and AOPP, a decreased activity of CuZn-SOD and GSH-Px.

In present study, the level of 8-OHdG was 2.5-fold greater in hemodialysis patients compared with that in healthy subjects, which may be taken as evidence of intensive oxidative stress in these patients. Therefore, we corroborated the results of Kato et al (17) who have shown an even higher disproportion in the level of 8-OHdG between the hemodialysis and normal subjects; 21.3 ng/ml vs. 2.7 ng/ml, respectively. Tarng et al (18) have measured 8-OHdG in peripheral blood leukocytes, using HPLC, and revealed that the concentration of 8-OHdG was highest in patients on peritoneal dialysis; it was lower in CRF patients treated conservatively and was the lowest in healthy subjects. These authors also have found a positive relationship between the 8-OHdG concentration in leukocytes and serum concentration of iron and a negative one between 8-OHdG and α-tocopherol concentration. In another study Tarng et al (19) have shown that the 8-OHdG concentration in peripheral blood leukocytes is dependent on the gene hOGG1 1245C → G polymorphism. The hOGG1 inactivates glycosylation of 8-OHdG. The authors report that the 8-OHdG concentration is about two times higher in patients with the 1245GG genotype in comparison with the 1245CG and 1245CC genotypes. At the same time they point that the 8-OHdG concentration is not dependent on the duration of dialysotherapy and serum antioxidant or iron concentrations.

One of the results of the present study showed a positive, although weak, correlation between age and 8-OHdG concentration. Analyzing further this
problem, the highest intensity of oxidative stress tended to be in the group of patients aged 50-65 and the lowest in the oldest patients, over 65 years. These observations are in line with those of Kato et al (17).

Analyzing the influence on 8-OHdG of the cause of CRF we affirmed that the highest 8-OHdG level is present in patients whose CRF was triggered by diabetes and hypertension, which corroborates previous observations made by Kato et al (17). There are a number of studies which confirm the presence of oxidative stress in diabetes. In a rat model of experimentally induced diabetes Park et al (20) have found an increase in 8-OHdG in serum and in leukocytes of diabetic rats. Wu et al (21) have reported increased 8-OHdG concentration in the urine in patients with tumours, diabetes, and atherosclerosis. The presence of oxidative stress in both diabetes and hypertension has been confirmed in a number of other studies (22-24).

We conclude that the measurement of 8-OHdG may be used to estimate the level of oxidative stress in hemodialysis patients due to chronic renal failure. Oxidative stress and inflammation seem to be intensively present in such patients and the best markers for their assessment are yet to be found.

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REFERENCES


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