**Effect of peritoneal dialysis on antioxidant defense system and oxidative stress**

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Available online 23 October 2012

**Summary**

Objective: In this study we investigated the effect of peritoneal dialysis (PD) on some antioxidant markers and lipid peroxidation.

Methods: The levels of malondialdehyde (MDA), erythrocyte superoxide dismutase (SOD), glutathione peroxidase (GPx), vitamins A, E, and C, and total antioxidant capacity (TAC) were determined in 23 chronic ambulatory PD patients and 34 healthy controls.

Results: The results showed MDA level in patients were not significantly different from those in controls. Erythrocyte SOD and GPx activities and vitamin C level were significantly decreased in the chronic ambulatory PD group \((p < 0.0001)\), while the levels of TAC \((p < 0.01)\), uric acid, and vitamins A \((p < 0.0001)\) and E \((p > 0.05)\) were enhanced with respect to the control group.

Conclusions: The findings suggest that despite slightly increased MDA level, patients on PD are exposed to oxidative stress, as evidenced by the decreased levels of SOD, GPx, and vitamin C. Depletion of these antioxidants may contribute to accelerated atherogenesis in these patients.

**KEYWORDS**

Antioxidant defense system; Chronic ambulatory peritoneal dialysis; Oxidative stress

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背 景：本研究旨在調查腹膜透析對抗氧化指標及脂質過氧化的影響。

方 法：我們分別在23位腹膜透析患者(CAPD組)及34位健康人士(對照組)間，測量了malondialdehyde (MDA)、紅血球superoxide dismutase (SOD)與glutathione peroxidase (GPx)(酵素指標)、vitamin A、E、與C (非酵素指標)、及總抗氧化能力(TAC)水平。

結 果：結果顯示，相比於對照組，CAPD組病人的MDA水平並無明顯差異，但具明顯較低的SOD與GPx活動及vitamin C數值 \((P < 0.0001)\)。而與對照組相比，CAPD組具較高的TAC \((P < 0.01)\)，uric acid、及vitamin A \((P < 0.0001)\)與vitamin E \((P > 0.05)\)數值。

結論：本研究發現，即使具稍高的MDA數值，腹膜透析患者暴露於較高的氧化壓力中(反映於較低的SOD、GPx及vitamin C水平)。相關抗氧化物的損耗可能加速這些病人的動脈粥樣化進程。
Introduction

End stage renal disease patients are reported to have high mortality unless renal replacement therapy is administrated in the form of hemodialysis, peritoneal dialysis or kidney transplantation. Peritoneal dialysis (PD) is an established supportive treatment; almost 15% of patients on dialysis in the developed world and half the dialysis patients in the UK are treated in this way but PD therapy duration is limited because of progressive atrophy of the peritoneum within a relatively short period. Oxidative stress is a common phenomenon in end stage renal disease patients treated by dialysis. Patients on PD are perhaps at particular oxidative risk due to decreased antioxidant levels and increased production of reactive oxygen species. On the other hand, increased oxidative stress has been suggested as one possible mechanism of peritoneum structural and functional changes resulting from prolonged exposure to PD solutions containing high concentrations of glucose, obstruction of catheter and high incidence of peritonitis. The most important antioxidants that protect from oxidant damage include: erythrocyte superoxide dismutase (SOD) and glutathione peroxidase (GPx); and nonenzymatic antioxidants vitamins A, C, and E. The importance of oxidative stress in the pathogenesis of cardiovascular complications has recently been emphasized. Thus, the present study was undertaken to investigate some antioxidant markers and potential effects PD on oxidative stress.

Patients and methods

Patients

Twenty-three patients (12 female, age 26–70 years, mean 46.1 ± 14.5 years) on continuous ambulatory PD (CAPD) were enrolled from the center of hemodialysis at Khatam-Al-Anbia Hospital, Zahedan Medical University, Iran. They received three or four daily exchanges of 2 L of peritoneal dialysis solution with either 1.5% or 2.5% glucose. Duration of dialysis was 3 to 48 months (mean 24.6 months). Dialysis adequacy was defined by Kt/V of 1.2 or greater. The causes of end stage renal failure included diabetes (n = 5), blood pressure (n = 7), diabetes and blood pressure (n = 2), glomerulonephritis (n = 3), lupus erythematosus (n = 1), polycystic kidney (n = 1) and unknown (n = 4). They had no history of cardiovascular diseases or recent occurrence of peritonitis. None of them took antioxidant supplements and none were smokers. Thirty-four aged-matched healthy individuals (18 female, age 25–72 years, mean 49.4 ± 15 years) as control were included in this study. They had no medical problems, no cigarette smoking, and were on neither lipid lowering drugs nor vitamin supplements. The protocol of study was approved by the ethical committee of Zahedan University of Medical Sciences and informed consent was obtained from all patients and healthy individuals.

Biochemical analysis

After an overnight fast, venous blood samples were collected from patients and healthy individuals in tubes with and without anticoagulant. The anticoagulants used were heparin for measurement of enzymatic antioxidants, vitamin C, total antioxidant capacity (TAC), and malondialdehyde (MDA); and ethylenediaminetetra-acetic acid for hemoglobin measurement. Blood samples were protected from light by aluminium foil and centrifuged immediately at 3000 × g for 10 minutes and plasma or serum collected respectively. All samples were aliquoted and stored in a freezer at −80°C until analysis. Serum samples were used for biochemical analysis (blood urea nitrogen, uric acid, creatinine, total protein, albumin) using commercial colorimetric assay kits (Parsazmun company, Tehran, Iran). Hemoglobin was measured using a Sysmex KX-21 (Sysmex, Kobe, Japan). Low-density lipoprotein values were calculated by the Friedwald formula.

Antioxidants assay

Plasma total antioxidant status was determined by the ferric reducing/antioxidant power assay at 593 nm according to the method of Benzie and Strain. Erythrocyte SOD and GPx activity were measured using commercial colorimetric assay kit (Randox, Crumlin, County Antrim, UK) as described previously. Serum vitamin A and E were extracted with hexane and measured by using high pressure liquid chromatography with absorption at 325 nm and 292 nm, respectively. Plasma total vitamin C concentration was measured spectrophotometrically by the 2,4 dinitrophenylhydrazine method. Plasma MDA level was assayed as the end product of lipid proxidation by thiorbarbituric acid method at 530 nm.

Statistical analysis

Data were analyzed using SPSS 15.0 for Windows (SPSS, Chicago, IL, USA) and are expressed as mean ± SD. The different variables between PD patients and control subjects were compared using independent samples Student t test. The relationship between variables was checked by Pearson’s correlation coefficient. A p-value <0.05 was considered significant.

Results

Clinical and biochemical parameters

As shown in Table 1, creatinine, blood urea nitrogen, and uric acid concentrations were higher in PD patients compared to age-matched healthy controls. Cholesterol and high-density lipoprotein concentrations were lower, and triglyceride and low-density lipoprotein concentrations higher in patients than in controls. Patients on CAPD showed markedly lower hemoglobin and lower total protein and albumin concentrations than those in controls.

Biomarkers of oxidative stress

According to Table 2, MDA levels in patients were not significantly different from those in controls. Significantly higher levels of TAC and vitamin A of the CAPD patients
were found when compared to the control group (p < 0.01 and p < 0.0001, p < 0.0001, respectively). No significant difference was shown in the levels of serum vitamin E in CAPD patients and the control group, while the levels of plasma vitamin C (p < 0.0001) and erythrocyte activities of SOD and GPx (p < 0.0001) were markedly lower in CAPD patients than in controls. A significant correlation was observed between TAC and uric acid (r = 0.65, p < 0.001), but there was no correlation between MDA or TAC with other clinical parameters (blood pressure, serum albumin, and total protein). There were also an nonsignificant negative correlation between MDA with vitamin C (r = -0.29, p > 0.05) and vitamin E (r = -0.16, p > 0.05).

Discussion

As previously reported, dialysis treatment contributes to oxidative stress and to modifications of the balance between antioxidant and oxidant-generating systems that could be a primary event triggering the atherogenic complications in uremic patients. In CAPD patients, prolonged exposure to conventional PD fluid can lead to structural and functional alterations of the peritoneal membrane and enhanced oxidative stress. Our study showed that in CAPD patients, the high levels of MDA were no different from those observed in the healthy controls, in conflict with other studies. Ozden et al also showed that CAPD patients have elevated MDA, but at a lower level than hemodialysis patients; this suggests that uremic patients maintenance on dialysis are at a high risk of oxidative stress, which worsens with hemodialysis treatment. MDA is a good marker of lipid oxidation in biological systems. Increase in the MDA levels is a reflection of oxidative stress, which worsens with hemodialysis treatment. MDA is a good marker of lipid oxidation in biological systems. Increase in the MDA levels is a reflection of oxidative stress, which worsens with hemodialysis treatment. MDA is a good marker of lipid oxidation in biological systems. Increase in the MDA levels is a reflection of oxidative stress, which worsens with hemodialysis treatment. MDA is a good marker of lipid oxidation in biological systems. Increase in the MDA levels is a reflection of oxidative stress, which worsens with hemodialysis treatment.

With regard to the nonsignificant elevated MDA level in the present study, it seems CAPD treatment by itself does not lead to a significant increase in MDA levels when compared to healthy controls. This finding suggests that other factors, such as the composition of the PD fluid or the individual patient's response to dialysis, may play a role in the development of oxidative stress in CAPD patients.

Table 1  Characteristics of peritoneal dialysis (PD) patients and control subjects.

<table>
<thead>
<tr>
<th></th>
<th>PD patients (n = 23)</th>
<th>Controls (n = 34)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (y)</td>
<td>46.2 ± 14.5</td>
<td>49.4 ± 15.7</td>
<td>NS</td>
</tr>
<tr>
<td>Sex</td>
<td>F/M: 12/11</td>
<td>F/M: 18/16</td>
<td></td>
</tr>
<tr>
<td>Duration of PD (mo)</td>
<td>20 ± 16.1(3-48)</td>
<td>–</td>
<td></td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>22.9 ± 3.5</td>
<td>25.6 ± 4.7</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Kt/V</td>
<td>2.01 ± 0.74</td>
<td></td>
<td></td>
</tr>
<tr>
<td>RRF (mL/min)</td>
<td>2.5 ± 1.7</td>
<td></td>
<td></td>
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<tr>
<td>Blood pressure (mmHg)</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Systolic</td>
<td>141.1 ± 23.4</td>
<td>113.1 ± 14.1</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Diastolic</td>
<td>83.9 ± 9.4</td>
<td>72.2 ± 10.2</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Creatinine (mg/dL)</td>
<td>7.32 ± 2.6</td>
<td>0.87 ± 0.18</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>BUN (mg/dL)</td>
<td>65.4 ± 30.3</td>
<td>13.1 ± 4</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Uric acid (mg/dL)</td>
<td>7.4 ± 1.8</td>
<td>5.3 ± 1.5</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Cholesterol (mg/dL)</td>
<td>172.8 ± 31</td>
<td>218.6 ± 58.1</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>LDL (mg/dL)</td>
<td>141 ± 65.1</td>
<td>94.5 ± 26.8</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>HDL (mg/dL)</td>
<td>41.1 ± 13.2</td>
<td>54.2 ± 11.6</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Triglycerides (mg/dL)</td>
<td>182.3 ± 78.1</td>
<td>112.4 ± 47.2</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Albumin (g/L)</td>
<td>3.1 ± 0.48</td>
<td>4.6 ± 0.5</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Total protein (g/L)</td>
<td>5.2 ± 0.27</td>
<td>6.9 ± 0.4</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Hemoglobin (g/dL)</td>
<td>10.1 ± 2</td>
<td>14.8 ± 1.5</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

Values are mean ± SD.

BUN = blood urea nitrogen; BMI = body mass index; HDL = high-density lipoprotein; LDL = low-density lipoprotein; NS = nonsignificant; RRF = residual renal function.

Table 2  Oxidant and antioxidant status of PD patients and control subjects.

<table>
<thead>
<tr>
<th></th>
<th>PD patients (n = 23)</th>
<th>Controls (n = 34)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>MDA (µmol/L)</td>
<td>0.132 ± 0.06</td>
<td>0.112 ± 0.02</td>
<td>NS</td>
</tr>
<tr>
<td>TAC (µmol/L)</td>
<td>969.4 ± 237.1</td>
<td>803 ± 184</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>SOD (U/g Hb)</td>
<td>1173.2 ± 362.8</td>
<td>2316.5 ± 493.1</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>GPx (U/g Hb)</td>
<td>16.3 ± 3.9</td>
<td>30.5 ± 8.2</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Vitamin A (µg/dL)</td>
<td>136.2 ± 54.2</td>
<td>58.3 ± 11.4</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Vitamin E (µg/dL)</td>
<td>752.7 ± 205.3</td>
<td>692.1 ± 180.5</td>
<td>NS</td>
</tr>
<tr>
<td>Vitamin C (mg/dL)</td>
<td>0.69 ± 0.25</td>
<td>1.13 ± 0.38</td>
<td>&lt;0.0001</td>
</tr>
</tbody>
</table>

Values are mean ± SD.

GPx = glutathione peroxidase; Hb = hemoglobin; MDA = malondialdehyde; NS = nonsignificant; SOD = superoxide dismutase; TAC = total antioxidant capacity.
not increase the lipid peroxidation process but, when the enzymatic antioxidant systems are low, it may worsen this condition, as observed in the present study. The significantly decreased erythrocyte SOD activity in this study in comparison with control values is in agreement with data reported by Taylor et al\textsuperscript{16} and Mydlík et al\textsuperscript{20} but not with Bonnefont-Roussel et al.\textsuperscript{6} We also found a significant decline in erythrocyte GPx activity compared to controls that is in agreement with several studies,\textsuperscript{6,13,20} while Yoshimura et al\textsuperscript{21} did not find any difference in erythrocyte GPx activity. Our findings indicate that the erythrocyte GPx activity could be related to renal function, as damage of GPx activity has been observed in early stages of renal failure.\textsuperscript{6} Glutathione peroxidases are antioxidant enzymes that can detoxify hydrogen peroxide and lipid peroxides in the presence of reduced glutathione.\textsuperscript{13}

No deficiency in the two lipid-soluble antioxidants (vitamin A and E), which are not filtered through the membrane,\textsuperscript{22} was found. Even a significant increase in the levels of serum vitamin A was observed when compared with the healthy controls that is in agreement with some earlier studies.\textsuperscript{2,6} Accumulation of retinol in CRF patients can be related to the defect in retinol binding protein metabolism (vitamin A carrier).\textsuperscript{6,11} On the other hand, there was no significant difference in the serum levels of vitamin E in CAPD patients with respect to controls and were within the normal range as shown by Bonnefont-Roussel et al.\textsuperscript{6} It has been also reported that because of utilization of much more artificial materials in HD procedure, vitamin E levels were lower in hemodialysis patients than in CAPD patients.\textsuperscript{5}

Plasma vitamin C concentrations, unlike the lipophilic antioxidants were found to be markedly lower than in controls. Our findings are in accordance with the reports by Zhang\textsuperscript{23} and Finkelstein et al.,\textsuperscript{24} but not with Mydlík et al.\textsuperscript{20} Low plasma concentration of vitamin C in dialysis patients can be due to inadequate intake of fruits and vegetables, loss during the dialysis process, reduced tubular reabsorption, impaired metabolism, and/or utilization of vitamin C in regenerating or sparing vitamin E from tocopheryl radicals.\textsuperscript{11,22,23}

In the present study, plasma levels of TAC were shown to be high in CAPD patients compared with the control group, similar to earlier studies.\textsuperscript{19,25} TAC is a valuable tool for assessment of the antioxidant capacity of all antioxidants in a biological system.\textsuperscript{14,26} A possible explanation for the enhancement of TAC levels in these patients is that vitamin E concentrations remained stable and vitamin A and uric acid levels were markedly increased. It has been reported that the presence of uric acid strongly affects the TAC level,\textsuperscript{26} as, in the present study a significant correlation was observed between TAC and uric acid (r = 0.65, p < 0.001). Despite the fact that uric acid has antioxidant properties capable of scavenging oxygen free radical, but cannot be considered solely to protect against the lipid peroxidation, as there are other endogenous compounds contributing to it.\textsuperscript{14,22}

In conclusion, the findings suggest that, although elevated plasma levels of MDA (an end product of the peroxidation of polyunsaturated fatty acids) was no different from that observed in the healthy controls, the existence of oxidative stress was proved in PD patients, as evidenced by the decline in the activity of the enzymatic antioxidant defense system (SOD and GPx), and plasma vitamin C levels. Depletion of these antioxidants may contribute to accelerated atherogenesis in these patients.

Financial support

None.

Acknowledgments

We greatly appreciate the CAPD section staff in Zahedan Khatam–Al-Anbia Hospital, and the laboratory experts of the National Nutrition and Food Technology Research Institute, Tehran, Iran, for their kind cooperation and also all the individuals who willingly participated in the study.

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