Erythrocyte glutathione peroxidase activity, plasma malondialdehyde and erythrocyte glutathione levels in hemodialysis and CAPD patients

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Abstract

Objectives: Cardiovascular disease is the major cause of mortality in patients receiving hemodialysis (HD) and continuous ambulatory peritoneal dialysis (CAPD) due to chronic renal failure. Increased lipid peroxidation and depletion of antioxidants may contribute to increased risk of atherosclerosis. We have therefore assessed the effect of hemodialysis and CAPD on oxidant and antioxidant status.

Design and methods: Plasma malondialdehyde (MDA), Glutathione (GSH) levels and glutathione peroxidase (Gpx) activities were determined in 20 healthy persons (control), 20 patients on HD, 16 patients on CAPD.

Results: MDA was elevated in posthemodialysis and CAPD patients in comparison to prehemodialysis and control groups (posthemodialysis 1.39 ± 0.38 nmol/mL, CAPD 1.26 ± 0.27 nmol/mL, prehemodialysis 0.83 ± 0.22 nmol/mL, controls 0.72 ± 0.21 nmol/mL, p < 0.0001). With respect to antioxidants, glutathione levels were significantly lower in prehemodialysis, posthemodialysis and CAPD groups than those in control group (prehemodialysis 16.82 ± 6.73 mg/dL RBC, posthemodialysis 31.43 ± 11.88 mg/dL RBC, CAPD 40 ± 12.72 mg/dL RBC, controls 62.26 ± 24.01 mg/dL RBC, p < 0.0001). While erythrocyte GSH levels were significantly lower in the prehemodialysis patients than those in posthemodialysis and CAPD patients (p < 0.0001), it was significantly lower in posthemodialysis patients than those in CAPD patients (p < 0.05). There were no significant differences with respect to erythrocyte Gpx levels among the groups (p > 0.05).

Conclusions: These findings indicate oxidative stress in patients with chronic renal failure which is further exacerbated by hemodialysis and CAPD, as evidenced by increased lipid peroxidation and low antioxidant levels. © 2002 The Canadian Society of Clinical Chemists. All rights reserved.

Keywords: Continuous ambulatory peritoneal dialysis; Hemodialysis; Glutathione; Glutathione peroxidase; Malondialdehyde

1. Introduction

Polyunsaturated fatty acids are oxidized in vivo by free radicals and other reactive species. Subsequent degradation of oxidized lipid molecules lead to the formation of several specific metabolites that include aldehydes of variable chain length [such as malondialdehyde (MDA) and Hexanal] [1]. MDA, a water soluble lipid peroxidation product, is partially excreted via the urine under normal conditions [2, 3, 4, 5]. However, it is not known what amount of MDA formed in the body is eliminated by the kidney. It is therefore unknown whether elevated MDA plasma concentrations in uremia reflect increased lipid peroxidation or reduced metabolic clearance [6]. Free radical mediated lipid peroxidation is reported to play a pivotal role in atherogenesis, initiating a series of events that lead to enhanced uptake of low-density lipoprotein (LDL), formation of foam cells and finally to the production of an atherosclerotic plaque [7]. Recent studies have reported increased susceptibility to oxidation of LDL isolated from patients with chronic renal failure treated by hemodialysis or continuous ambulatory peritoneal dialysis (CAPD) [8]. Protection from oxidant injury involves complex pathways at both the blood and intracellular levels. These mechanisms are thought to prevent free radicals from causing irreparable damage by reactions with lipids, proteins and nucleic acids. It has been proposed that patients with chronic renal failure have antioxidant deficiencies and that this impaired redox status...
increases their risk for cumulative injury to multiple end organs [9]. The extent of the oxidative stress could be exacerbated by a decreased efficiency in the natural antioxidant systems [10,11]. Reduced glutathione (GSH), together with its related enzymes, is one of the major scavengers of activated oxygen species in red blood cells (RBC) [12]. GSH is easily oxidized to disulfide (GSSG); high levels of GSH are maintained in the cells by the NADPH dependent enzyme glutathione-reductase. Glutathione peroxidases (Gpx) are antioxidant enzymes that can detoxify hydrogen peroxide and lipid hydroperoxides in the presence of reduced glutathione [13]. Several types of Gpx have been identified in the blood, two of which are cellular Gpx (cGpx), found in red blood cells, and extracellular Gpx (eGpx), found in plasma [14].

Our goals were to compare plasma MDA, erythrocyte GSH levels, and Gpx activities in groups of hemodialysis and peritoneal dialysis patients and healthy control subjects.

2. Patients and methods

2.1. Patients and controls

Twenty patients (15 males and 5 females, 20–65 yr old, mean ± SD 44.5 ± 15.65) receiving regular hemodialysis and 16 patients (9 males and 7 females, 18–65 yr old, mean ± SD 46.56 ± 15.39) receiving continuous ambulatory peritoneal dialysis for end stage renal failure were recruited and compared with 20 healthy controls (10 males and 10 females, 20–65 yr old, mean ± SD 43.45 ± 14.31). Exclusion criteria were patients with diabetes, intercurrent infection, chronic inflammatory conditions and who were alcoholics and smokers. All patients receiving hemodialysis (HD) had been on regular hemodialysis for at least 6 months (mean ± SD 14.5 ± 8.1 months) and were dialysed three times a week each for 4 h, using bicarbonate dialysate and hemophan dialyzing membranes. The use of duration of peritoneal dialysis was seven to 32 months (mean ± SD 18.4 ± 9.7 months) (Table 1). The diet of the dialysis patients was not modified from that already prescribed for their end stage renal disease. All patients were treated with, antihypertensive (nifedipine, prazosin), active vitamine D (calcitriol), 20 healthy subjects were recruited from the staff of the Medical Faculty of the Kocaeli University. None took medicine or antioxidant supplements.

2.2. Methods

Blood samples were collected from hemodialysis patients immediately before a dialysis session and at the end of the dialysis and also from CAPD patients between 9 and 11 AM during regular outpatient visits. Venous blood samples were taken after an overnight fast and were collected in vacutainer plain tubes and containing disodium EDTA (1.5 mg/mL) in the case of GSH, Gpx and MDA measurement. Two milliliters aliquot was removed to determine hemoglobin (Hb), hematocrit (Hct) and erthrocytes. Samples were immediately centrifuged (3000 rpm for 10 min. at 4°C) and serum samples were used for urea, creatinine, iron, albumin, uric acid assays. Whole blood samples, RBCs, and plasma samples were used for GSH, erythrocyte Gpx and MDA measurements, respectively.

Lipid peroxidation level was monitored by determining the end product of lipid peroxidation, MDA by the thiobarbituric acid method [15]. Plasma MDA values were calculated using the extinction coefficient of MDA-thiobarbituric acid complex at 532 nm = 1.56 × 10^5 M^-1 cm^-1 and expressed as nmol/mL. We measured Gpx activity by the modified method of Paglia and Valentine [16] as described by Jacobson et al. [17], using tert-butyl hydroperoxide as substrate on Bayer Opera autoanalyzer. Gpx catalyzed the oxidation of reduced glutathione in the presence of cumene hydroperoxide. The rate of glutathione oxidation was measured by monitoring the disappearance of NADPH + H⁺ in the reaction medium (decrease of absorbance at 340 nm), since NADPH + H⁺ was consumed for the reduction of oxidized glutathione by glutathione reductase. Gpx activi-

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Table 1

<table>
<thead>
<tr>
<th>Biochemical parameters of subjects</th>
<th>Control (n = 20)</th>
<th>Hemodialysis (n = 20)</th>
<th>CAPD (n = 16)</th>
<th>Laboratory reference range</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>43.45 ± 14.31</td>
<td>44.5 ± 15.5</td>
<td>46.56 ± 15.39</td>
<td>18–45</td>
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<tr>
<td>Duration of dialysis (months)</td>
<td>14.5 ± 8.1</td>
<td>18.4 ± 9.7</td>
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<tr>
<td>Urea (mg/dL)</td>
<td>30.35 ± 9.43</td>
<td>185.05 ± 55.72</td>
<td>84.40 ± 32.74</td>
<td>18–45</td>
</tr>
<tr>
<td>Creatinine (mg/dL)</td>
<td>0.99 ± 0.15</td>
<td>10.7 ± 3.81</td>
<td>8.31 ± 3.29</td>
<td>0.7–1.5</td>
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<tr>
<td>Albumin (g/dL)</td>
<td>4.39 ± 0.37</td>
<td>3.69 ± 0.38</td>
<td>3.56 ± 0.48</td>
<td>3.0–5.5</td>
</tr>
<tr>
<td>Iron (µg/dL)</td>
<td>75.75 ± 33.5</td>
<td>57.45 ± 32.29</td>
<td>53.56 ± 18.65</td>
<td>35–140</td>
</tr>
<tr>
<td>Urate (mg/dL)</td>
<td>3.2 ± 1.11</td>
<td>5.76 ± 1.59</td>
<td>4.56 ± 0.87</td>
<td>2.3–8.6</td>
</tr>
</tbody>
</table>

Data are the mean ± SD
ties of erythrocytes were expressed in U/g Hb of hemolysate. GSH levels were assayed by using methods of Beutler et al. [18]. Method principle is; nonprotein sulfhydryl groups of RBCs are in the form of reduced GSH. 5,5'-Dithiobis (2-nitrobenzoic acid) (DTNB) is a disulfide chromogen to an intensely yellow compound. The absorbance of the reduced chromogen is measured at 412 nm and is directly proportional to the GSH concentration. GSH concentration were expressed in mg/dL of RBCs. Hb, Hct, erythrocytes, urea, creatinine, iron, albumin, uric acid concentrations were determined using routine laboratory methods.

The study was approved by the Ethics Commitee of the Medical Faculty of the Kocaeli University and all participants gave informed consent.

2.3. Statistical analysis

All data were expressed as the mean ± SD and median. Data were analyzed using the Kruskall Wallis and Mann-Whitney-U test. Prehemodialysis and posthemodialysis groups were compared with Wilcoxon test. Differences were considered significant when the probability was p < 0.05.

3. Results

Plasma MDA levels were elevated in both posthemodialysis (1.39 ± 0.38 nmol/mL) and CAPD (1.26 ± 0.27 nmol/mL) patients than those in prehemodialysis (0.83 ± 0.22 nmol/mL) and in control group (0.72 ± 0.21 nmol/mL) (p < 0.0001, Table 2, Fig. 1). Also plasma MDA levels were elevated in posthemodialysis patients compared to CAPD patients (p < 0.05, Table 2, Fig. 1). Compared to controls (62.26 ± 24.01 mg/dL RBC) erythrocyte GSH levels were significantly lower in prehemodialysis (16.82 ± 6.73 mg/dL RBC), posthemodialysis (31.43 ± 11.88 mg/dL RBC) and CAPD patients (40.0 ± 12.72 mg/dL RBC) (p < 0.0001 Table 2, Fig. 2). While erythrocyte GSH levels were significantly lower in the prehemodialysis patients than those in posthemodialysis and CAPD patients (p < 0.0001), it was significantly lower in posthemodialysis patients than those in CAPD patients (p < 0.05, Table 2, Fig. 2).

No statistical differences were found for the erythrocyte Gpx activities among the groups (Table 2, Fig. 3).

4. Discussion

This study has shown plasma MDA levels to be increased in posthemodialysis and CAPD patients and confirms reports published by a number of other groups [6,19, 20,21].

Table 2
Levels of malondialdehyde (MDA), glutathione (GSH) and glutathione peroxidase (Gpx)

<table>
<thead>
<tr>
<th></th>
<th>Control (n = 20) mean±SD (median)</th>
<th>Hemodialysis (n = 20) mean±SD (median)</th>
<th>CAPD (n = 16) mean±SD (median)</th>
</tr>
</thead>
<tbody>
<tr>
<td>MDA (nmol/mL)</td>
<td>0.72 ± 0.21 (0.79)</td>
<td>0.83 ± 0.22 (0.81)</td>
<td>1.26 ± 0.27 (1.23)</td>
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<tr>
<td>Prehemodialysis</td>
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<tr>
<td>Posthemodialysis</td>
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<tr>
<td>GSH (mg/dL RBC)</td>
<td>62.26 ± 24.01 (55.2)</td>
<td>16.82 ± 6.73 (17.05)</td>
<td>40.0 ± 12.72 (40.65)</td>
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<tr>
<td>Prehemodialysis</td>
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<tr>
<td>Posthemodialysis</td>
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<tr>
<td>Gpx (U/g Hb)</td>
<td>20.27 ± 5.8 (18.26)</td>
<td>16.31 ± 3.79 (16.35)</td>
<td>21.75 ± 9.69 (16.64)</td>
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</tbody>
</table>

Data are the mean±SD (median)

a Control and posthemodialysis, peritoneal dialysis, p < 0.0001
b Prehemodialysis and posthemodialysis, peritoneal dialysis, p < 0.0001
c Posthemodialysis and peritoneal dialysis, p < 0.05
d Control and prehemodialysis, posthemodialysis, peritoneal dialysis, p < 0.0001
e Prehemodialysis and posthemodialysis, peritoneal dialysis, p < 0.0001
f Posthemodialysis and peritoneal dialysis, p < 0.05
CAPD patients have raised MDA levels, but a lower level than hemodialysis patients; this suggests that hemodialysis exacerbate an endogenous tendency to increased lipid peroxidation in uremia [22]. Hemodialysis patients are at particular oxidative risk in that the bioincompatibility of dialyzer membranes can lead to the release of free radicals from activated neutrophils [23]. Multiple mechanisms are responsible for protection against oxidant stress, which is thought to be exacerbated by chronic renal failure. Maher et al. noted elevated lipid peroxidation products 30 min. after beginning of hemodialysis and suggested that complement activation or heparin dependent increase in free fatty acids might be responsible [24]. The lipid peroxidation products, in comparison to controls, were not to be elevated between dialysis sessions; this is the same as in our findings. HD patients were dialyzed per 48 h. MDA levels tend to decrease during this hemodialysis intervals in these patients. The low glutathione levels in prehemodialysis patients explain this situation because antioxidants are used for suppressing oxidant stress.

An imbalance between antioxidant and oxidant-generating systems leading to an oxidative stress has already been proposed in the pathogenesis of uremia-associated inflammation disorders and could be an initial event triggering the atherogenic mechanism in uremia [25]. Our study has shown erythrocyte GSH levels to be decreased in prehemodialysis, posthemodialysis and CAPD patients. Yawata et al. have demonstrated a metabolic blockage of the pentose phosphate shunt producing NADPH in patients with treatment hemodialysis [26]. This could impair the reduction of oxidized glutathione via glutathione reductase. GSH depletion could be rather attributed to a decreased GSH synthesis and/or to an increased degradation because its precursors (glycine, glutamate and cystein) are elevated or in the normal range in the plasma of chronic renal failure patients [9,27] demonstrated that there were significantly elevated plasma levels of cystein in hemodialysis patients compared with normal control subjects. They also demonstrated peritoneal dialysis patients had plasma cystein levels higher than those of normals [9].

In our study, erythrocyte GSH levels significantly decreased in the prehemodialysis group when compared the other groups. These data are influenced by the lower hematocrits of the dialysis patients and dialysis induced hemoconcentration explains the higher values after the treatments [9]. Furthermore, as we mentioned above, GSH were used for suppressing the oxidant stress.

Gpx is a selenium dependent enzyme and its activity is related to the blood selenium level [28]. NADPH is involved in the generation of reduced glutathione which is essential for detoxifying hydrogen peroxide through the reaction catalyzed by Gpx. The hexose monophosphate pathway is the principal source of NADPH in erythrocytes, and it has been suggested that an abnormality in this pathway exist in chronic renal failure patients [29]. We found no statistical differences among the groups in the erythrocyte Gpx activity. While Yoshimura et al. found that plasma Gpx activity was lower in peritoneal and hemodialysis patients than control subjects, they observed that there wasn’t any differences in erythrocyte Gpx activity [30].

In conclusion that patients with hemodialysis and peritoneal dialysis are subject to oxidative stress, as indicated by increased lipid peroxidation and reduced antioxidant levels. It appears that the patients with peritoneal dialysis are affected less than patients with hemodialysis. Further studies will be necessary to be found of causes of the differences between hemodialysis and peritoneal dialysis patients.

Acknowledgements

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References


