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Oxidative Stress Evaluation in Uremic Patients Undergoing Continuous Ambulatory Peritoneal Dialysis

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Introduction

Oxidative stress - primarily due to the uremic state per se, but also to certain aspects of dialysis procedure - have been documented in chronic renal failure (CRF) patients. Markers of oxidative damage like increased lipid peroxidation products, increased plasma protein carbonyl groups and decreased plasma protein thiol groups, as well as reduced antioxidant defence parameters were reported before the initiation of dialysis therapy and seem to gradually worsen as the renal function declines [1].

Dialysis-related factors could add deletorious effects on the oxidant status of CRF patients with regard to both reactive species generation and antioxidant losses. The bioincompatibility of the dialysis materials such as dialyzer membranes (especially cellulose) and bacterial contaminants from dialysate were involved in the augmented production of reactive oxygen species in hemodialysed patients. However, their roles are still debated [1].

Also, peritoneal dialysis might enhance oxidative stress by the presence of high concentration of glucose in dialysis fluids [2]. Moreover, the glucose degradation products formed mainly during the heat sterilization process of the solutions, as well as the low pH and the high osmolality contribute to the bioincompatibility of the conventional peritoneal dialysis fluids that could account for the increased local and systemic oxidative burden [3]. In addition, impairment of the antioxidant status, expressed by lower levels of plasma ascorbate and whole-blood reduced glutathione was found in chronic peritoneal dialysis patients [3].

Considering that the great bulk of evidences about uremia-associated oxidative stress came from haemodialysis patients and only scant data is available concerning peritoneal dialysis, we aimed to evaluate the oxidative status in CRF patients on continuous ambulatory peritoneal dialysis (CAPD).

Materials and Methods

Subjects

Eighteen clinically stable CAPD patients (10 female and 8 male, mean age 55.6±16.9 years), on renal replacement therapy for at least 6 months, none of them treated with erythropoietin or intravenous iron were included.

Sixteen age- and gender-matched non-dialysed CRF patients (6 female and 10 male, mean age 55±15.7 years, mean serum creatinine 4.5±2.7mg/dl) were randomly selected from those attending the Nephrology Clinic for routine periodical examination and composed the control patients group.

Exclusion criteria for the patients were active inflammatory and infectious conditions (including exit or tunnel infections and/or peritonitis two months before enrollment), malignancies, diabetes mellitus, active liver diseases, severe anaemia, and medications with antioxidative potentialities.

Normal control group consisted of twelve matched healthy volunteers (7 female and 5 male, mean age 45.5±12.1 years) with normal routine biochemistry and urine analyses. They did not take any medication or nutritive supplements.

An informed consent was obtained from all participants.

Methods

Blood samples were drawn after overnight fasting into standard vacuum tubes with lithium heparin, were transported on ice, in the dark, and were processed within 30 minutes of collection by centrifugation (3000 *g* for 10 minutes at room temperature). The plasma aliquots were stored at -70°C until analysed for oxidative stress determinations or used immediately for routine laboratory parameters. Erythrocytes were washed three times with isotonic saline solution and lysed in cold ultrapure water. The red cell stroma was removed by centrifugation (10000 *g* for 15 minutes at 4°C) and the clear supernatant was stored at -70°C until erythrocytes antioxidant parameters were assayed.

All the oxidative stress parameters were determined by spectrophotometry.

Plasma thiobarbituric acid reactive substances (TBARS; nmol/g protein), measured according to the method described by Esterbauer [4], were used to assess plasma lipid peroxidation.

Plasma total free thiols (Pt-SH; mcmol/g protein), assayed by the Ellman's method [5], served as marker of extracellular antioxidant status.

Erythrocyte superoxide dismutase activity (SOD; U/g haemoglobin) was assessed by analysing the rate of inhibition of pyrogallol auto-oxidation, according to Marklund and Marklund [6].

Erythrocyte glutathione peroxidase activity (EGPx; U/g haemoglobin) was determined indirectly by measuring the rate of consumption of NADPH during the regeneration of reduced glutathione from the oxidised glutathione produced by EGPx in the presence of oxidising substrate [7].

Erythrocyte non-protein thiols (E-SH; mcmol/g haemoglobin), which consist mainly of reduced glutathione

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(GSH) were measured according to the method of Beutler *et al* [8].

Statistical analysis

Results were expressed as mean ± standard deviation and were compared using Student's *t* test. A *p* value <0.05 was considered significant.

Results

There were no significant differences among groups regarding age, gender, smoking habit, aetiology of CRF, serum iron or lipids status (data not shown). All subjects had serum C-reactive protein within the normal range (0-5 mg/dl).

TBARS levels were increased only in CAPD patients versus controls. These patients, also, had markedly lower activity of erythrocyte SOD as compared to the other studied groups. As concerns the glutathione-dependent antioxidants, a significant decrease of plasma thiols was noticed in both uremic groups, while the EGPx and E-SH showed gradually elevated values in patients, with the bigger increment in CAPD group (Table 1).

Table 1. Values of the oxidative stress parameters in studied groups

Parameter	CAPD group	CRF non-D group	Controls	*p* CAPD vs. non-D	*p* CAPD vs. Controls	*p* non-D vs. Controls
TBARS	†58.3±19.8	51.8±20.5	42.7±12.4	0.36	0.01	0.15
Pt-SH	†6.3±0.9	†6.1±1.0	7.2±1.1	0.51	0.04	0.02
SOD	†‡164.2±47.5	463.2±77.5	409.8±58.8	<0.001	<0.001	0.06
EGPx	†‡7.9±0.9	†4.5±1.3	3.3±0.9	<0.001	<0.001	0.01
E-SH	†‡11.9±1.8	†4.8±2.5	3.2±0.9	<0.001	<0.001	0.04

p<0.05: † vs. Controls; ‡ vs. CRF non-D group.

Discussion and Conclusions

In accordance with other studies [9,10], we found the lipid peroxidation marker higher in CAPD patients than in normal controls, indirectly suggesting an enhancement of reactive oxygen species generation in uremic patients on peritoneal dialysis. A tendency toward higher TBARS levels was also seen in CRF non-dialysed group, but without reaching statistical significance. This might argue against significant oxidative processes accounting for lipid peroxidation in CRF before renal replacement therapy, but more probably it is related to the low sensibility and specificity of the used assay. Alternatively, the serum levels of TBARS might be kept in the normal range by some protective mechanisms like hepatic microsomes that are capable of both TBARS generation and degradation, as previously supported by *Gotoh* et al [11].

In fact, the decrease of Pt-SH levels observed in all CRF patients as compared to healthy individuals, which reflects a deficiency of the extracellular antioxidant defence, could be speculated in favour of an increased consumption for counteracting the potential oxidative stress. The presumable contribution of hypoalbuminaemia to the lower plasma thiols appears implausible since no significant correlations were found between serum albumin and Pt-SH in either CRF groups (data not shown).

The deficiency of antioxidant systems in peritoneal dialysis patients is further sustained in our results by the marked decline of erythrocyte SOD. Therefore, one can assume that both extracellular and enzymatic intracellular defence against oxidative stress are defective in uremic patients undergoing CAPD.

Concerning the other two studied erythrocyte antioxidant parameters, contrary to previous published data [10], significantly higher values were noticed in CRF groups. We hypothesised that an enzymatic induction occurs in response to oxidative stress and accounts for the increased EGPx activity and the regeneration of the intracellular pool of reduced glutathione (GSH). Indeed, the ability of glutathione-peroxidase to adapt its activity according to oxidative stress was demonstrated in chronic haemodialysed children [12]. Also, it was stated that the oxidants-induced up-regulation of glucose-6-phosphate dehydrogenase might play a role in the increase of intracellular GSH [13].

We concluded that peritoneal dialysis patients are exposed to oxidative stress, evidenced by both an increase in plasma lipid peroxidation (TBARS) and a suppressed activity of antioxidants (erythrocyte SOD). Also, the impairment of extracellular antioxidant system expressed by the lower levels of plasma free thiols, probably accounted for by the enhanced consumption in order to offset the oxidative burden, plead for an imbalance between reactive species production and antioxidant defence mechanisms in CAPD patients. The enhancement of erythrocyte glutathione-dependent antioxidants might reflect rather an adaptive response to oxidative processes and, therefore, indirectly argues in favour of oxidative stress occurring before initiation of dialysis and augmented thereafter.

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