Prooxidative-Antioxidative Balance of Cells in Different Types of Renal Replacement Therapy

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\textbf{Introduction}

The prevalence of chronic kidney disease (CKD) is on the rise all over the world. It is related to prolonged life expectancy, better medical care and access to renal replacement therapy. In Poland the number of patients who qualify for dialysis treatment is increasing by 4–6\% every year. Statistics show that in 2007, 50\% of the dialysed patients were over 65 years of age. The ageing of the society has resulted in a high morbidity due to diseases of civilization. The fastest growing group starting regular dialysis are those who suffer from diabetic kidney disease [1]. The ageing process with all its comorbidities alters body homeostasis. Impaired immunity and disturbances in repairing processes lead to intracellular damages, namely those that cause oxidation of macro- and micromolecular compounds, increased arteriosclerosis, mutations and carcinogenesis, collagen degradation, lower activity of transmembrane pumps, decreased enzyme activity, lower ATP concentration, higher platelet aggregation and changes in rheological properties of the blood. The dialysis therapy itself can exacerbate these processes by the generation of oxidative stress. However, the cells, especially erythrocytes, are equipped with an effective enzymatic and non-enzymatic antioxidant defence system.

\textbf{Key Words}

Chronic kidney disease · Dialysis · Oxidative stress · Free radicals

\textbf{Abstract}

\textbf{Background:} Patients suffering from chronic kidney disease (CKD) are exposed to increased oxidative stress and disturbances manifesting in the enzymatic and non-enzymatic antioxidant defence system. The object of the research was to assess the differences between conservative treatment, peritoneal dialysis and haemodialysis in moderating cellular antioxidant agents.

\textbf{Methods:} The group examined comprised 145 patients. The activities of superoxide dismutase, catalase, glutathione peroxidase, glutathione reductase and glucose-6-phosphate dehydrogenase were obtained using kinetic methods. The spectrophotometric method established the concentrations of reduced glutathione, albumin, uric acid, glucose, total protein and lipids.

\textbf{Results:} The type of treatment determined significant changes in antioxidative enzyme activities and concentrations of non-enzymatic antioxidative compounds.

\textbf{Conclusions:} Peritoneal dialysis provides better antioxidant protection than other types of therapy in CKD and should be considered as first-choice treatment despite more metabolic disorders.
The most important antioxidative enzymes are superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPX), glutathione reductase (GSSG-R), glutathione transferase (GST) and glucose-6-phosphate dehydrogenase (G6PDH). The non-enzymatic free radical scavengers are carotenoids, vitamin E, metal ion-binding proteins (albumin, ceruloplasmin, transferrin, ferritin) and compounds containing the SH group (glutathione, cysteine, homocysteine, coenzyme Q) [2–6].

Antioxidative enzymes cooperate in oxidative stress reduction. SOD is involved in a reaction eliminating superoxide radical ($O_2^-$) and generating hydrogen peroxide ($H_2O_2$), which is subsequently decomposed by CAT or GPX. GPX uses reduced glutathione (GSH) and transforms it into the oxidized form. Reduced GSH, necessary for that reaction, is delivered due to GSSG-R, which reduces GSSG to GSH using the nicotinamide adenine dinucleotide phosphate-reduced form (NADPH+). The source of reactive equivalents (NADPH+) in erythrocytes is the reaction catalysed by G6PDH – the main enzyme of hexose monophosphate shunt [7, 8]. The non-enzymatic antioxidant compounds in the presence of antioxidant enzymes determine a target against reactive oxygen species (ROS). The most important composites of total antioxidant capacity of blood plasma are uremic acid and albumin [9–18].

In the present article we report the influence of different types of kidney replacement therapy on the antioxidative status in patients.

**Materials and Methods**

The study was performed at the Department of Nephrology, Transplantology and Internal Medicine of Pomeranian Medical University, Szczecin, Poland. The group examined comprised 145 patients – 54 on haemodialysis (HD), 23 on peritoneal dialysis (PD) and 68 patients on conservative treatment with CKD stage 3–5 (CKD3–5) according to the KDIGO guidelines. The characteristics of the examined group included gender, age, time since first dialysis, estimated glomerular filtration rate (eGFR), residual renal function (RRF), causes of CKD in each group, comorbidities, and medications like erythropoietin, iron, antihypertensives and statins (table 1). RRF was defined as urine output >500 ml/24 h. The duration of dialysis treatment did not differ significantly between patients on PD and HD. The selected biochemical parameters of the examined group and differences between them are shown in table 2.

Inclusion criteria for the study were as follows: diagnosis of CKD, regular HD or PD for at least 6 months before starting the study, or predialysis CKD3–5 according to KDIGO guidelines. Dialysis adequacy was defined by a Kt/V ratio ranging from 1.2 for HD patients to 1.7 for those on PD. The standard dialysis concentrate and standard PD fluid were used. All patients on PD were treated with the same dialysis fluid containing glucose of 1.5 and 2.5% in a proportion of 1:1. Thirteen patients had continuous ambulatory PD and 10 automatic PD. HD was scheduled 3 times/week (12 h weekly) using polysulphone dialysers. Treatment with antiplatelet medication, malnutrition-inflammatory-atherosclerosis syndrome, active inflammation, metastatic cancer and cigarette smoking were considered exclusion criteria from the research. Patients did not undergo any blood transfusions 1 month before the study. The study was approved by the Bioethical Committee of Pomeranian Medical University, Szczecin, Poland. All subjects gave informed consent. Blood was taken directly from the arteriovenous fistula or dialysis catheter in HD patients directly before (pre-HD) and immediately after the HD (post-HD) session. In PD and predialysis patients blood from the peripheral vein was collected during a routine visit.

The activity of erythrocyte antioxidative enzymes (SOD, CAT, GPX, GSSG-R, G6PDH) was assessed using kinetic methods. Values obtained were calculated per gram of haemoglobin (Hb). The GSH concentration was examined according to a spectrophotometric method directly after sample collection. The concentrations of albumin, uric acid, total protein and lipids were measured using spectrophotometric methods and ready-made reagents (AquaMed, Lodz, Poland).

**Statistical Analysis**

The results are presented as arithmetic mean ± SD and median (upper and lower quartile). Shapiro-Wilk’s test showed that the
distributions of the results obtained were not normal, therefore non-parametric tests were used: Wilcoxon’s pair test for dependent variables, and the ANOVA Kruskal-Wallis rank test and Mann-Whitney U test for independent variables. The qualitative differences were assessed using the χ² test. Spearman’s rank test was used to establish the correlations between measured values. The statistical significance was assumed at p < 0.05.

Results

The mean total protein concentration in patients on PD was significantly higher than in the pre-HD (p = 0.001) and post-HD (p = 0.0001) samples, but significantly lower compared to CKD3–5 patients (p = 0.0001). The HD session itself did not change the total protein concentration. The total cholesterol and HDL cholesterol levels were decreased, but triglycerides were significantly higher in the PD group compared to HD and CKD3–5 patients. The glucose concentration did not differ significantly between CKD3–5 patients and the PD group. HD patients had the lowest glucose plasma level, with a significant decrease after the HD procedure (p = 0.01) (table 2).

Enzymatic Antioxidant System

The results of enzyme activity are shown in table 3. SOD activity was significantly higher in HD patients immediately after the HD session compared to pre-HD values (p = 0.0001). SOD activity in PD patients was significantly lower than in the post-HD samples. CKD3–5 patients had a different SOD activity compared to the HD group, both before (p = 0.00001) and after the HD session (p = 0.0001).

The mean values of CAT activity were significantly different among the groups. The highest CAT activity was in CKD3–5 patients. The HD session caused a significant decrease in CAT activity (p = 0.005). In PD, CAT was significantly higher than in the post-HD samples (p = 0.04) and significantly lower compared with the CKD3–5 group (p = 0.01).

In PD patients GPX activity reached 14.87 ± 8.44 U/g Hb and was significantly different to that in post-HD patients (p = 0.03) and CKD3–5 patients (p = 0.04). As in the case of CAT, the highest values of GPX were assessed in the CKD3–5 group, but the lowest in post-HD patients. These activities differ significantly (p = 0.0001). The HD procedure caused a significant decrease in GPX activity (p = 0.02).

The activity of GST was lowest in the PD group compared to the other groups but the difference was significant only in comparison to post-HD patients (p = 0.05).

| Table 1. General characteristics of the groups examined |
|----------------------------------------|--------|--------|--------|
| PD (n = 23) | HD (n = 54) | CKD3–5 (n = 68) |
| Demographics | | |
| Male/female | 13/10 | 34/20 | 38/30 |
| Age, years | 46 ± 16 | 61 ± 17* | 65 ± 20* |
| Time since first HD, months | 17 ± 14 | 21 ± 13 | 0 |
| eGFR, ml/min/1.73 m² | <15 | <15 | 21.8 ± 7.8 |
| RRF (>500 ml/24 h) | 23 | 9 | 68 |
| Causes of CKD | | |
| Hypertension | 5 | 21 | 17 |
| Diabetes | 6 | 15 | 14 |
| Glomerulonephritis | 6 | 8 | 14 |
| Kidney cancer | – | 2 | 6 |
| Vesicoureteral reflux | 1 | 2 | 5 |
| ADPKD | – | 2 | 8 |
| Amyloidosis | – | 1 | – |
| Neurogenic bladder | 2 | – | – |
| Other | 3 | 3 | 4 |
| Comorbidities | | |
| Ischaemic heart disease | 11 | 39 | 35 |
| Hypertension | 10 | 24 | 30 |
| Large-vessel atherosclerosis | 3 | 20 | 17 |
| COPD | 2 | 8 | 10 |
| Liver cirrhosis | – | 4 | 6 |
| Urolithiasis | 1 | 7 | 11 |
| Gout | 2 | 9 | 12 |
| Cancer in medical history (except for kidney) | – | 3 | 3 |
| Medications | | |
| Erythropoietin | 16 | 54 | 10 |
| Iron | 23 | 54 | 37 |
| Antihypertensives | 15 | 45 | 47 |
| Statins | 11 | 19 | 31 |

ADPKD = Autosomal dominant polycystic kidney disease; COPD = chronic obstructive pulmonary disease. *p = 0.01; statistically significant value between the PD and HD or the PD and CKD groups.

The activity of G6PDH reached the lowest value in pre-HD patients and significantly increased after the HD session (p = 0.01). PD patients had the highest activity of this enzyme which differs significantly from that in the pre-HD samples.

The average activity of GSSG-R was significantly higher in the PD group compared to the HD group, both before and after the HD session. The highest value of GSSG-R was obtained in CKD3–5 patients and differs significantly from that in post-HD patients. The HD itself did not significantly impact GSSG-R activity.
Non-Enzymatic Antioxidant System

The parameters of the non-enzymatic antioxidant system are shown in Table 4. The highest concentration of GSH was observed in PD patients and differs significantly from those of the HD group, both before (p = 0.01) and after (p = 0.0001) the HD session, and the CKD3–5 group (p = 0.002). The dialysis procedure caused a significant decrease in GSH concentration.

An average albumin concentration was highest in the PD group and differs significantly from the pre-HD (p = 0.01) and post-HD (p = 0.00001) samples. The significantly lowest values were obtained in CKD3–5 patients.
The uric acid concentrations obtained in CKD3–5 patients were significantly higher than in pre-HD (p = 0.005) and post-HD (p = 0.0001) patients and in the PD group (p = 0.0001). PD patients had significantly higher concentrations compared to post-HD patients (p = 0.02).

Spearman’s rank test established the correlations between measured parameters. In PD patients some negative correlations were observed between antioxidant enzymes: SOD and CAT (R = –49, p = 0.02), GPX and GST (R = –0.46, p = 0.03), and GST and GSSG-R (R = –0.43, p = 0.04). The duration of dialysis treatment is correlated negatively with the GSH concentration in erythrocytes of PD patients (R = –0.42, p = 0.05) and in the HD group after the dialysis session (R = –0.57, p = 0.01). The activity of CAT in pre-HD patients and at the time of dialysis therapy is also negatively correlated (R = –0.45, p = 0.04). Analysis of correlations and regression models showed no dependence of age and RRF on the antioxidative system components of the patients.

**Discussion**

Patients suffering from CKD are exposed to increased oxidative stress which is related to uraemia and the dialysis procedure itself. According to the stage of CKD and type of renal replacement therapy, they may present different disturbances in antioxidative mechanisms. It is known that patients undergoing regular HD manifest a high amount of oxidative stress indicators due to several factors like bioincompatible dialysis membranes, non-sterile dialysate, poor quality of dialysis water, the back-leak of contaminants across the dialysis membrane, etc. Oxidative stress causes neutrophil degranulation and the production of inflammatory mediators. The number of neutrophils drops suddenly during the first 30 min of HD and contributes to a huge formation of ROS. Other reasons for increased oxidative stress are lack of certain vitamins and microelements (C, E, selenium), advanced age of patients, high prevalence of diabetes, chronic inflammatory state, excessive parenteral iron administration, anaemia, etc. Oxidative stress links to malnutrition-inflammation-atherosclerosis syndrome and contributes to increased cardiovascular morbidity and mortality in patients with CKD. The type of renal replacement therapy can influence the outcome and possibility of subsequent kidney transplantation. In our study we compare the influence of different types of renal replacement therapy on the antioxidant defence system of patients.

SOD is considered to be the most important antioxidative enzyme. It eliminates superoxide anion which is highly reactive and may cause tissue damage due to proliferation and apoptosis of endothelial cells. The effective antioxidative system of healthy people can neutralize even 1.75 kg of these radicals daily [19]. In the present research there were no significant differences of SOD activity between patients on PD, conservatively treated patients and HD patients before the HD session. Similar results were described by Hernández de Rojas and Mateo [20] and Durak et al. [21]. Coaccioli et al. [22] showed a higher SOD activity in dialysed patients than in healthy con-
trols, but no differences between PD and HD. In this parameter Ceballos-Picot et al. [23] did not obtain differences between a control and dialysed group. Naga et al. [24] examined the patients before and 4 weeks after starting dialysis therapy. The results of this research showed a decrease in SOD activity during both HD and PD. The patients in our study were over 6 months on dialysis.

CAT and GPX prevent the accumulation of H₂O₂. In our study the highest activity of CAT was obtained in conservatively treated CKD3–5 patients. The same results were obtained by Martin-Mateo et al. [25]; in their research, CAT activity was significantly higher in nondialysed patients with CKD compared to healthy controls. Patients on PD had a similar activity of CAT compared to conservatively treated patients and HD patients before the dialysis session. The HD procedure caused a significant decrease in CAT activity, which differs significantly from the PD group. The study by Durak et al. [21] confirms our findings. Some authors described an increased activity of CAT in HD patients compared to healthy controls [20, 26]. In the present study the PD group was characterized by the negative correlation between SOD and CAT activities, but in HD patients a positive correlation occurred between these enzymes. This can indicate the involvement of many antioxidative enzymes due to the high amount and diversity of ROS produced.

GPX is involved in GSH metabolism; its role is to protect from H₂O₂ and lipid peroxides. The reduced GSH is necessary for GPX action [27]. A lower GSH concentration leads to a decreased GPX activity. In CKD excessive oxidative stress results in a reduction of GPX activity and enhancement of arteriosclerosis and cardiovascular diseases due to lipid peroxidation [28]. Reports about GPX activity in CKD are inconclusive. Some authors described a positive impact of PD, but others did not find differences compared with HD [21, 23, 24, 28–34]. Our study showed that renal replacement therapy negatively influences GPX activity. CKD3–5 patients have significantly higher values. In addition, the HD process causes a significant decrease in GPX activity. Similar results were obtained by Santangelo et al. [27] and El-Rashidy et al. [35].

Galili et al. [33] described a higher expression of genes coding GST during oxidative stress. In CKD a shift of the prooxidative-antioxidative equilibrium towards the oxidation reactions is observed, so we expected higher activity of GST [25]. The present results confirm this premise. In our study there were no significant differences between GST activities among the whole examined groups; however, it was above the normal range. Some authors showed a higher activity of GST in HD than in PD patients [33–35]. The HD procedure did not influence this enzyme activity. The negative correlation between GST and GPX activity in PD patients may indicate a competition for a common substrate. Some authors claim that GST activity depends on the stage of kidney disease and may become a marker of uraemic toxicity and kidney graft function [33, 34, 36, 37].

GSSG-R is an enzyme dependent on the availability of NADPH+H⁺. The increase of GSSG (the oxidized form of GSH) concentration or lower synthesis of NADPH+H⁺ due to decreased G6PDH activity may inhibit the GSSG-R action. It results in a reduced regeneration of GSH and an inadequate function of the antioxidative system [38]. A decreased activity of GSSG-R is frequently observed in both conservatively treated patients and HD patients. In our study the activity of GSSG-R of HD patients was significantly lower compared to that of the PD and CKD3–5 groups. Similar results were obtained by Santangelo et al. [27] and Ahmadpoor et al. [28]. Ceballos-Picot et al. [23] showed increased activities of GSSG-R without significant differences between HD and PD patients.

G6PDH is one of the enzymes responsible for the maintenance of an adequate NADPH+H⁺ concentration in the cell. The lower activity of G6PDH results in an increased susceptibility to haemolysis during contact with the dialysis membrane. There is a proven connection between oxidative damage of erythrocytes and a lack of NADPH+H⁺ [7, 39]. In our study no significant differences in G6PDH activity were observed between PD group, pre-HD samples and CKD3–5 patients. HD treatment caused a significant increase in this enzyme activity. Similar results were described by Paşaoğlu et al. [32] and Alhamdani et al. [40]. Other authors also showed that the activity of G6PDH depends on the glucose content in the dialysate. The activity was the highest in the presence of glucose [41–43].

The GSH thiol group (SH) is oxidized to GSSG by a reaction with xenobiotics, H₂O₂, organic peroxides and free radicals. Uraemic toxins and highly reactive GPX are also oxidizing substances utilizing GSH [27]. Its concentration in healthy people is significantly higher compared to CKD patients [23–25, 32, 41, 44, 45]. In the present study PD patients had significantly higher concentrations of GSH than the HD and CKD3–5 groups. Similar results were obtained by Tarn et al. [46]. During the HD session a significant decrease in GSH concentration was observed [47]. This may be caused by excessive oxidizing of GSH and a lack of its reduced form or low activity of γ-glutamylcysteine, which is necessary for regaining GSH [47].
The longer the time since the first dialysis, the lower the GSH concentrations obtained.

The albumin concentration in PD patients is significantly higher than in the HD and CKD3–5 groups. Zachara et al. [48] estimated a linear decrease in total protein and albumin along with progression in kidney insufficiency. A higher urinary albumin loss was noted in diabetic patients [49]. The concentration of albumin and total protein in PD patients with diabetes is significantly lower than in patients without this comorbidity [50]. The authors suspect that it is caused by a greater permeability of the diabetic peritoneum.

The uric acid concentration was significantly lower in PD patients compared to the CKD3–5 group, but significantly higher than in patients examined after the HD session. HD treatment is very effective in removing this compound from the blood plasma. Uric acid represents the total plasma antioxidative capacity. Its increased synthesis in CKD is connected with the production of superoxide anion and H₂O₂, which may play an important role in the pathogenesis of the disease [13].

In conclusion, PD provides better antioxidant protection than HD and should be considered the first-choice treatment of CKD as often as possible. Unfortunately, it is connected with some metabolic disorders like hypertriglyceridaemia and higher levels of uric acid. In order to avoid such abnormalities we should use fluids with low glucose concentrations or without glucose. PD gives better candidates for kidney transplantation and, in long-term treatment, can minimalize the complications resulting from excessive oxidative stress, especially cardiovascular diseases.

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References


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