SUPEROXIDE DISMUTASE AND GLUTATHIONE PEROXIDASE IN HEMODIALYZED PATIENTS AND RENAL TRANSPLANT RECIPIENTS AND THEIR RELATIONSHIP TO OSMOTIC FRAGILITY

M. NOURI*
M. RAHBANI-NOBAR*
H. ARGANI*
F. ROKHFOROOZ*

SUMMARY: Increased oxidative damage is one of the most common complications in patients suffering from chronic renal failure (CRF), undergoing hemodialysis, which leads to various abnormalities including anemia. The study was designed in order to evaluate the changes in activities of two intracellular antioxidant enzymes; superoxide dismutase (SOD) and Glutathione peroxidase (GPX); in patients with CRF under regular hemodialysis and renal transplant recipients. Relationship between the enzymes activities and altered osmotic fragility of red blood cells, one of the minor causes of anemia, was also determined in these subjects.

The activities of SOD and GPX were measured by spectrophotometric methods in red blood cells of 40 hemodialyzed patients and 26 renal transplant recipients with mean age of 42.5 ± 14 years and 38.6 ± 10 years respectively. The results were compared with those obtained from 39 age and sex matched apparently healthy individuals with mean age of 39.9 ± 15.5 years as controls. Median osmotic fragility (MOF) of red blood cells was also determined colourimeterically in each group and its relationship to the enzyme activites was evaluated.

The activity of SOD in hemodialyzed patients (1080.5 ± 700.2 U/g Hb) was markedly lower than those of renal transplant recipients (1303.5 ± 442.2 U/g Hb) and the control group (1307.8 ± 452 U/g Hb) ($p<0.05$). The activity of GPX in hemodialyzed patients (23.9 ± 9.5 U/g Hb) was significantly lower than those of renal transplant recipient (28.1 ± 16.8 U/g Hb) and control group (34.6 ± 7.5 U/g Hb) ($p<0.05$). Although the activities of the both enzymes in the renal transplant recipients were higher than that of hemodialyzed patients but they were still lower than that of the control. Calculated values for MOF in hemodialyzed patients (0.46 ± 0.03) were significantly higher than those of renal recipients (0.42 ± 0.02) and control (0.41 ± 0.01) ($p<0.05$). In patients under hemodialysis significant correlations between SOD and MOF were observed ($r = -0.3$, $p = 0.02$).

As antioxidant measurement may help to delineate susceptibility to free radical attack, decreased activities of GPX and SOD in red blood cells of patients under hemodialysis may thus contribute to increased oxidative stress in hemodialyzed patients. Elevation of MOF in hemodialyzed patients and no correlation between MOF and the activity of GPX suggests that deficiency of SOD may be one of causes of elevated MOF. It seems that measurement of total antioxidant capacity will provide useful information in these patients.

Key Words: Chronic renal failure, oxidative stress, glutathion peroxidase, supperoxide dismutase, osmotic fragility.

INTRODUCTION

Increased oxidative damage is one of the most common complications in patients suffering from chronic renal failure (CRF) who are under hemodialysis, or treated by kidney transplantation. In relation to high production of free radicals the risk of disease is high and current treatments to counteract ureamia do not decrease the risk in these subjects (1-3). Anemia is an important problem in CRF patients and it has a multifactorial origin including decrease erythropoietin production (4,5) and shortened red cell survival (6) present with hemolysis (7). Lipid peroxidation of erythrocyte membranes by toxic oxygen-free radicals plays a major role in red blood cells hemolysis (8,4). Increased sensitivity of red blood cells to oxidative damage is therefore an index of antioxidant deficiency. Prior studies have shown an imbalance between oxidants-antioxidant in CRF patients undergoing hemodialysis (1,10). The steady-state formation of oxidants is balanced by similar rate of their consumption by antioxidants (1,11).

The study designed to evaluate the changes in activities of two antioxidant enzymes, GPX and SOD in CRF patients undergoing hemodialysis and renal transplant recipients. Relationship between the enzyme activities and altered osmotic fragility of red blood cells, one of the minor causes of anemia was also determined in these subjects.

MATERIALS AND METHODS

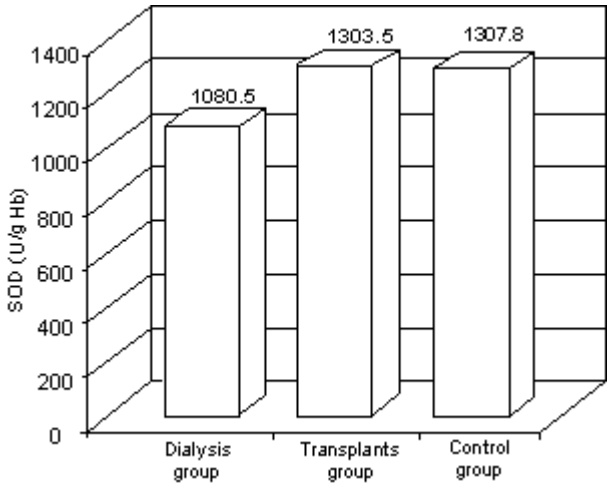
Patients

The study was carried out on 40 hemodialyzed patients (21 males and 19 females) with a mean age of 42.5 ± 14 years and 26 renal recipients (14 males and 12 females) with a mean age of 38.6 ± 10 years. A total of 39 healthy volunteers (22 males and 17 females with mean age of 39.9 ± 15.5 years without clinical or laboratory evidence of any disease were also included in the present study as control group.

Sampling

The tests were carried out on peripheral venous blood samples collected in preservative-free heparinized tubes containing EDTA. The samples were collected at fasting state and in the case of dialyzed patients before hemodialysis. The enzyme activities were measured in red blood cells of the heparinized tubes and the osmotic fragility was determined using the samples containing EDTA.

Figure 1: Comparison of activity of SOD in dialysis.



Measurements

The erythrocyte pellet was washed three times with cold isotonic saline. Lysate of erythrocytes was prepared by adding distilled water and keeping the mixture at 4°C for 15 min. The lysate was diluted by phosphate buffer PH = 7.0.

Erythrocyte SOD activity was determined with a Randox test combination. Xanthine and xanthine oxidase were used to generate superoxide radicals reacting with 2-(4-iodophenyl) 3-(4-nitrophenol) - 5-phenyl tetrazolium chloride (INT) to form a red formazan dye. Superoxide dismutase inhibits this reaction by converting the superoxide radical to oxygen. Superoxide dismutase activity was measured at 505 nm on a cessil 3000 spectrometer in hemolysates of washed erythrocytes. Results were expressed in SOD U/g hemoglobin.

Glutathione peroxidase activity was also determined with a randox test combination. Erythrocyte GPX catalyses the oxidation of glutathione by cumene hydroperoxide (12). In the presence of glutathione reductase and NADPH, the oxidized glutathione is immediately converted to the reduced form with concomitant oxidation of NADPH. The decrease in absorbance at 340 nm was measured at 37°C. The assay was performed on a hemolysate of washed erythrocytes obtained from mixing 0.05 ml of whole blood with 1 ml of cold diluting agent and 1 ml of double strength Drabkin agent. The GPX unit was defined as the enzyme activity necessary to convert 1 μmol of NADPH to $NADP^+$ in 1 min. The results were expressed as GPX U/g hemoglobin.

*From Department of Clinical Biochemistry, Faculty of Medicine, Tabriz University of Medical Sciences, Tabriz, Iran.

Table 1: Age, sex and laboratory data of subjects.

Groups / Variables	Hemodialyzed patients	Kidney transplant recipients	Controls
Number	40	26	39
Age (year)	42.50* ± 14**	38.6 ± 10	39.90 ± 15
Sex (male-female)	21:19	14:12	22:17
SOD (U/g Hb)	1080 ± 477	1303 ± 320	1378 ± 452
GPX (U/g Hb)	23.90 ± 9.50	28.1 ± 16.8	34.60 ± 7.5
MOF (NaCl %)	0.46 ± 0.03	0.42 ± 0.02	0.41 ± 0.01
Hb (g/dl)	10.03 ± 1.78	13.50 ± 2.50	14.70 ± 1.30
Hct (%)	32.80 ± 5.90	42.90 ± 7.90	45.60 ± 4.10

* Mean, **SD

To evaluate the osmotic fragility of Red Blood cells the concentrations of NaCl in the test tubes were divided into 12 different grades, ranging from 0.0 - 0.6%. 20 microliters of Red Blood cells of each individual was then incubated in solutions containing different concentrations of NaCl. The absorbance of the supernatant versus water was determined at 575 nm using cessil 3000 spectrometer. An osmotic fragility curve was determined for each individual using this instrument. The percentage of NaCl at which 50% of RBC were lysed was considered the median osmotic fragility (MOF). Hemoglobin and Hematocrit and other hematologic parameters were measured by sysmex one-1000.

Figure 2: Comparison of mean activity of GPX in dialysis.

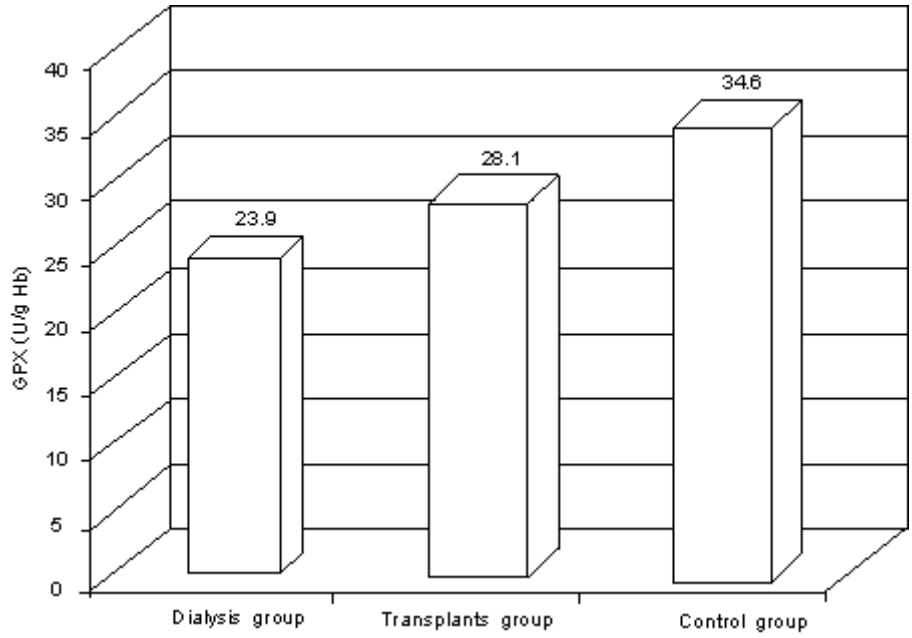
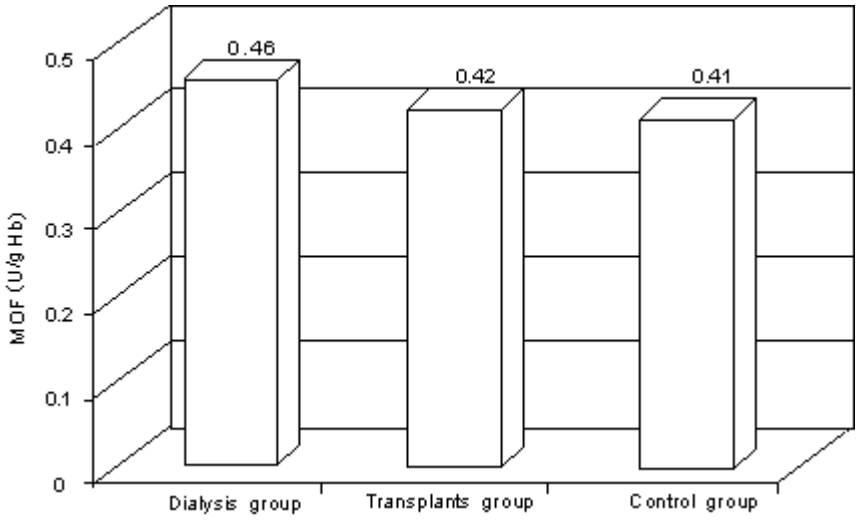


Figure 3: Comparison of mean of MOF in dialysis.



Statistical analysis

SPSS-9.1 was employed to evaluate statistical significance. Regression analysis was calculated for correlations between parameters. The level of significance was set at $p<0.05$. All data are expressed as the mean ± SD.

RESULTS

The results in control group and the study groups, hemodialyzed patients and kidney transplant recipients are presented in Table 1.

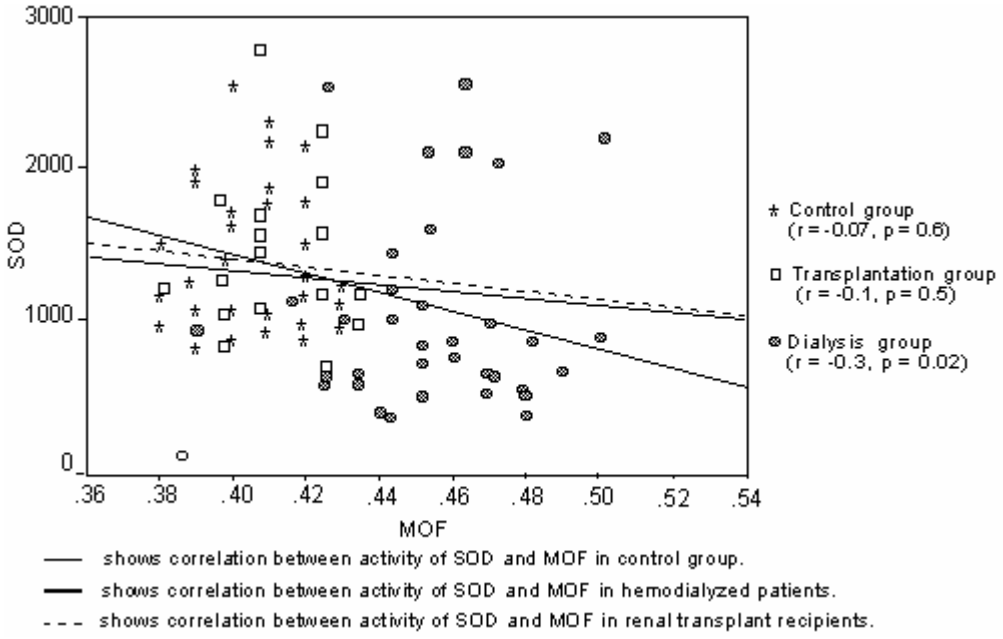
As shown in Table 1 and Figure 1 comparing with controls and kidney transplant recipients significant reduction in the SOD activity of RBC in hemodialyzed patients was noticed ($p<0.05$). No significant differences between kidney transplant recipient and the controls were noticed ($p>0.05$). The activity of GPX in RBC of hemodialyzed patients (Figure 2) was also markedly lower than those of other groups ($p<0.05$). Although the activity of GPX in the RBC of kidney transplant recipients was lower than that of control but it was not meaningful ($p>0.05$). The values for Hb and Hct in hemodialyzed patients were significantly lower than those of other two groups ($p<0.05$), but MOF (NaCl%) of erythrocytes in this group (Figure 3) was markedly higher than those of kidney transplant recipients and control groups ($p<0.05$). Studying correlation between MOF and activities of the antioxidant enzymes in erythrocyte a negative and significant relationship between SOD and MOF ($r = -0.3$, $p<0.02$) was noticed (Figure 4).

DISCUSSION

Since the entire range of toxic metabolites in the body is excreted mainly from the kidney, this organ is endowed with significant antioxidant defence system next only to liver. This is understandable because reactive oxygen species (ROS) play a key intermediary role in the pathophysiologic processes of a wide variety of clinical and experimental renal diseases (11,13,14).

In this study marked decrease in the activities of SOD and GPX in erythrocyte of hemodialyzed patients was noticed. Reduction in erythrocyte of SOD activity could be explained by increased H_2O_2 concentration (15). Because of the decreased GPX activity in erythrocytes the accumulation of H_2O_2 may cause inhibition of SOD activity (16). The increase in H_2O_2 may originate from either excess releasing of H_2O_2 from activated neutrophils or excess production of H_2O_2 in erythrocytes in patients under regular hemodialysis. Blood 'Se' levels are frequently reported to be lower in hemodialyzed patients (17). The integrity of GPX requires ade-

Figure 4: Correlation between activity of SOD (u/g Hb) and MOF (NaCl %) in dialysis and transplants and controls (Tabriz 78-79).



quate intake of 'Se' (18) and its deficiency causes low activity of GPX and reduction in GPX-protein (apoenzyme) synthesis (19). During oxidative stress, inactivation of GPX may occur, and on the other hand superoxide anion itself can inhibit peroxidase function (20). Simultaneous reduction of the antioxidant enzymes in erythrocytes of hemodialyzed patients has been reported by other investigators (13). So GPX must be considered to be complementary to SOD.The main mechanisms of anemia in chronic renal failure are relative erythropoietin deficiency, shortened erythrocyte survival, toxic metabolites that inhibits erythropoiesis and bleeding due to qualitative platelet defect (21). In our study comparing with control and kidney transplant recipient groups significant decrease in hemoglobin concentration and hematocrit percentage was noticed ($p<0.01$). After successful kidney transplantation marked improvement was observed. In anemia of chronic renal failure the most important factor in the shortened erythrocyte survival may be lipid peroxidation of the cell membrane. Defective antioxidant activity may increase this damage (22). In normal subjects free radicals are removed by enzymatic and nonenzymatic antioxidant defence systems. Most important of the former are SOD and GPX. Comparing with control and kidney transplant recipient groups significant elevation of MOF was found in hemodialyzed group. Relationship between the enzymes activities and MOF was also studied in the three groups. A significant and negative correlation was only observed between erythrocyte SOD and MOF in hemodialyzed patients ($r = -0.3$, $p<0.02$) but the relationship between GPX and MOF was not meaningful. As mentioned previously reductions in the activity of SOD causes oxidative damage by accumulation of superoxide anions. The causes of increased MOF in hemodialyzed patients have been reported (23). It seems reasonable to assume that imbalance in the activity of antioxidant enzymes may also involve in altered MOF of patients suffering from chronic renal failure.

Based on these findings, it may be concluded that decreased activities of SOD and GPX in erythrocytes of patients under hemodialysis may present increased oxidative stress in tissues. Elevation of enzymes activ-

ities in erythrocytes of renal transplant recipients indicate that transplantation of kidney may provide an improvement of oxidative stress in patients under hemodialysis. Elevation of MOF in hemodialyzed patients and no correlation between MOF and activity of GPX suggest that deficiency of SOD and other antioxidants may cause elevation of MOF. It seems that measurement of total antioxidants capacity will provide useful information in these patients.

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Correspondence:
M. Nouri
Department of Clinical Biochemistry,
Faculty of Medicine,
Tabriz University of Medical Sciences,
Tabriz, IRAN.
e-mail: nourim@tbzmed.ac.ir