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The Change of Glutathione Dependent Anti-Oxidant Mechanism in Patients with Chronic Renal Disease by Hemodialysis

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Abstract: In order to test the for existence of a possible effect during hemodialysis we investigated glutathione and related enzymes before and after dialysis of 36 patients (20 M/16 F) with chronic renal failure (CRF). 25 healthy person (15 M/10F) served as controls. Erythrocyte reduced glutathione (GSH) concentrations, glucose –6- phosphate dehydrogenase (G-6-PD), glutathione peroxidase (GPX) activities and plasma GPX activities were significantly decreased in patients ($p<0.01$). These levels were significantly increased with dialysis ($p<0.01$) but were still lower than in controls. Predialysis plasma GSH levels of patients were not significantly different from the control group. But postdialysis plasma GSH levels increased from predialysis levels ($p<0.01$). Plasma Selenyum (Se) concentrations were lower in patients than in controls ($p<0.01$) and not significantly changed with dialysis.

These results suggest that the anti-oxidant system related to GSH is insufficient in CRF patients and this system improves only partly after hemodialysis.

Key Words: Glutathione, Glutathione peroxidase, Glucose 6-Phosphate dehydrogenase, Hemodialysis

Introduction

Glutathione is the most abundant low molecular weight thiol-containing containing comopund in living cells. Its reduced form (GSH) contributes to the viability of erythrocytes by stabilizing thiol groups of membrane enzymes and hemoglobin, and by acting as a reducing agent for hydroperoxides and free radicals, thus protecting the red cells against oxidative damage (1). Intracellular GSH is converted into oxidized glutathione (GSSG) by glutathione peroxidase (GPX), which catalyzes the reduction of peroxides (2). Selenium is an essential component of the GPX and GPX activity is related to the blood selenium (Se) level (3). Glucose-6-phosphate dehydrogenase (G-6-PD) as a source of NADPH guarantees the reduction of GSSG to GSH. Disorders in GSH related metabolism are known to produce hemolysis (2). Anaemia in chronic renal failure (CRF) is a common manifestation and it has been described as normocytic and normochromic (4). Deficiency of erythropoietin (5), and a hypoporoliferative bone marrow appear to be the principal factors of the anaemia (6).

The biochemical alterations involved in this event have not yet been completely clarified. Different mechanisms have been implicated in lysis of erythrocytes. It has been claimed that CRF leads to a lipoperoxidative process. The evidence consistly mostly of the detection of breakdown products of lipid peroxides such as malonaldehyde (7). While data regarding the antioxidant enzyme superoxides dismutase, catalase, and GPX are contradictory (8-11). A changed erythrocyte membrane fluidity (12) impairment of the Na-K pump in the erythrocytes (13) could contribute to hemolytic anaemia in CRF.

Our previous study showed a decrease of glutathione related scavenger systems of oxygen radicals in CRF patients (14). This study was therefore designed to examine the effect of hemodialysis on glutathione levels, GPX activities in plasma and erythrocytes, and the G-6-PD activities in erythrocytes of patients with CRF.

Material and Methods

Thirty six patients with CRF (20 men, 16 women aged 37±11 years) and 25 (15 men, 10 women) age-matched healty colunteers were studied. The patients had been on maintenance hemodialysis three times a week for 2-5 years. The dialysate had the following composition: Sodium 142 mEq/L, potassium 2mEq/L,

calcium 3.5 mEq/L, magneisum 1.5 mEq/L, chloride 117 mEq/L, bicarbonate 30 mEq/L, acetic acid 2 mEq/L. The dializers were equipped with a cuprophane fiber dialyzer membrane. The patients were not on any drug therapies (vitamin E, vitamin D, erythropoietin, Se, etc.) The patients had low Hb and Htc levels and high urea nitrogen and creatinine levels (Table 1) Pre and post hemodialysis blood samples were collected into two tubes one of with EDTA for GPX assay and the other with ACD for G-6-PD, glutathione assays. Informed consent for entry into the study was obtained from all patients and volunteers.

Table 1. Uremic and hematological parameters of the patients

	Reference range	Hemodialysis patients (mean±SD)
Creatinine (mg/dl)	0.5 - 1.6	12.9 ± 0.4
Urea nitrogen (mg/dl)	8 - 22	96 ± 5
Hb (g/dl)	12 - 16	7.6 ± 1.9
Hct (%)	37 - 54	24 ± 6

Blood samples were centrifuged at 2000 g. for 5 min. at 4ºC and the plasma were removed from the packed RBC. The remaining red cells were washed three times with 0.9 % NaCl and suspended in the same solution to yield hemotocrit values of 70 %.

Plasma and erythrocytes GSH were measured using Tietze method (15), GPX activities were determined by Pleban et al. Method (16). The principle of GPX activity is based on the decrease in NADPH absorbance at 340 nm. By measuring the absorbance change per min of NADPH, GPX activity of erythrocytes were expressed in U/g Hb of hemolysate. Erythrocytes G-6-PD activities were measured using Sigma UV kinetic assay (Cat No: 345-A).

The reaction catalyzed by G-6-PD was as follows

$$\text{G-6-P} + \text{NADP}^{+} \xrightarrow{\text{G-6-PD}} \text{6-phosphogluconate} + \text{NADPH} + \text{H}^{+}$$

The enzyme activity is determined by measurement of the rate of increase in NADPH concentration. Plasma Se concentrations were determined by Jacopson and Lockitch method (17) using Hitachi Z-8000 atomic absorption spectrophotometer graphite furnace. The reagents and enzymes used were of analytical grade and were purchased from the Sigma chemical company (USA).

All data were expressed as the mean values SD. Data were analysed using student's test.

Results

The activity of GPX, G-6-PD and the GSH content of controls and patients are shown in Table 2 and 3.

Erythrocytes GSH concentrations were significantly decreased in the pre-and postdialysis samples. Predialysis plasma GSH levels were not different from controls. After dialysis, plasma and erythrocytes GSH levels significantly increased compared with predialysis levels. Erythrocytes and plasma GPX activities were significantly lower in patients (pre and postdialysis) than in controls. Also erythrocytes G-6-PD activities were low. G-6-PD and GPx activities were significantly increased by dialysis. Plasma Se concentrations were significantly lower in pre and postdialysis samples than controls ($p<0.01$) and not changed with dialysis.

Table 2. Erythrocyte GPX (U/g Hb), G-6-PD (U/g Hb) activities and GSH (µg/ml) levels of patients and controls

	n	G-6-PD	GPX	GSH
Control	25	5.4±0.2	26.1±4.9	363.6±23.2
Predialysis	36	3.3±0.2[a]	20.2±4.2[a]	231.4±19.1
Postdialysis	36	4.1±0.2[a,b]	21.2±4.5[a,b]	294.6±22.3[a,b]

a: $p<0.01$ from controls

b: $p<0.01$ from predialysis

Table 3. Plasma GPX (U/dL) activity and GSH (µg/ml), Se (µg/L) concentrations of patients and controls

	n	GPX	GSH	Se
Control	25	32.6±4.3	2.35±0.13	101.8±8.6
Predialysis	36	19.7±4.4[a]	2.29±0.15	77.4±8.8[a]
Postdialysis	36	21.1±4.2[ab]	2.38±0.12[b]	76.6±9.5[a]

a: $p<0.01$ from controls

b: $p<0.01$ from predialysis

Discussion

Glutathione is involved in a wide variety of biological reactions such as the maintenance of protein thiol groups (-SH) in the reduced state, removal of hydrogen peroxide, amino acid transport, detoxification of xenobiotics (18,19). It is likely that GSH, a cofactor for GPX, could also be involveld in the reduction of oxygen radicals as a free radical scavenger (9). Intracellular GSH is converted to oxidized glutathione (GSSG) by GPX, which catalyzed the reduction lof the peroxides whose substrate is NADPH and G-6-PD as a source of NADPH quarantee the reduction of GSSG for the maintenance pool of GSH (2,19).

Our study showed that the patients with CRF had low erythrocyte GSH levels but normal plasma GSH levels. Also our previous results showed that the GSSG/GSH ratio in erythrocytes and plasma were higher in CRF patients than in controls (14). There are conflicting results on GSH levels of hemodialysis patients. Some authors reported high (1,20) and some reported low (8-10,21,22) GSH levels of erytrocytes in CRF patients. In agreement with our studies Vanella et al (8) found low GSH content in erythrocytes of uremic patients before dialysis and GSH levels were significantly increased after hemodialysis. But Chaunhan et al (10) suggest that there was a decrease in red cell GSH after hemodialysis. They did could not explain this result since their G-6-PD levels of patients were low and after adequate dialysis or renal transplantation the G-6-PD values returned to normal.

We determined low G-6-PD and GPx activity in erythrocytes of our patients and after dialysis. These values were more elevated than predialysis. These results supported our increased GSH levels after dialysis.

It has been reported that plasma of uremic patients shows an inhibitory effects towards G-6-PD of normal erythrocytes. Also the increase of G-6-PD values after hemodialysis may suggest that the G-6-PD deficiency in uremic subjects may be reversible by appropriate treatment (23).

This condition and our results suggest that decreased GSH level in erythrocyte is due to the decreased availability of NADPH by decreased G-6-PD activities. We observed erythrocyte and plasma low GPX activities. These alterations may be related to Se deficiency because we demonstrated low plasma Se levels in patients. Se deficiency decreases GPX activity (3,11,24). In contrast to our results Turi et al. (22) found a decreased GPX activity with hemoldialysis (6 CRP patients). They suggest that decreased antioxidant enzyme activity and GSH content may be the result of the accumulation of RBC metabolites and uraemic toxins. This was not improved by dialysis, which may be related to the non-dialysable character of these toxic substances. Also they stated that there was an increased oxidatic stress in uraemic patients treated with chronic hemodialysis. However, our findings are in accordance with some other (24,26). Saint Georges et al (26) reported that plasma Se, GPX and erythrocyte GPX activity were lower in hemodialyzed patients than controls. After Se treatment of patients plasma GPX activity increased to a plateau but remained lower than in controls. Erythrocyte GPX activity rose progressively and then stabilized at the control levels. In our study plasma Se did not significantly change during dialysis but GPX activity significantly elevated with dialysis. These result may suggest that the mdecrease of GPX activity in erythrocyte and plasma not only related to Se deficiency but could also be related to the accumulation of metabolites and toxins in the blood inhibiting the GPX activity and enzymatic system such as G-6-PD. These differences may be related to the hemodialysis years of the patients or the type of hemodialysis membrane and/or dialysate, or major minor contributinos to numerous clinical manifestations associated with CRF and dialysis. The decrease of G-6-PD, GPx activity and GSH content observed in erythrocytes of uremic patients may be related to the accumulation in metabolites and toxins inhibiting the enzymatic system of GSH metabolism. The deplation of the scavenger system for oxygen free radicals makes the uremic erythrocytes more vulnerable to oxidative damage and may contribute, in part, to anaemia in the uremic patients. Further studies will be necessary on antioxidant mechanism in hemodialysis patients.

Oxidative haemolysis seems to be a mutifactorial abnormality caused by a reduced level of GSH regeneration due to a defect (G-6-PD) of hexose monophosphate and a decreased antioxidant enzyme activity.

In conclusion the anti-oxidant system related to GSH are insufficient in CRP patients who are on hemodialysis treatment and this system improves only partly after hemodialysis.

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