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Original Article

Oxidative State of Glutathione: Its Possible Role in the Anemia of Childhood Chronic Renal Failure

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Abstract

Objectives: This work was carried out to evaluate the relation between the anemia of chronic renal failure (CRF) and glutathione system through measurement of the reduced and oxidized forms of glutathione (GSH and GSSG respectively).

Methods: RBCs and plasma of 30 children suffering from CRF: 20 on hemodialysis (HD) and 10 treated conservatively were compared to 10 normal controls.

Results: CRF patients had their mean RBCs GSH significantly lower and plasma GSSG significantly higher than controls. This could be the result of reduced activity of glutathione reductase enzyme or its overexhaustion in detoxifying oxidants present in uremic blood. GSSG was significantly lower in uremic RBCs as it can readily pass through the RBCs membrane. No differences were observed among patients under HD and those treated conservatively. Erythropoietin therapy had a beneficial effect on glutathione system through raising RBCs GSH. Vitamin E supplementation induced significant decrease in frequency of blood transfusions.

Conclusions: Uremic children are exposed to oxidative stress that could affect the survival of RBCs. HD does not pose an extra-burden on glutathione system. Every attempt must be made to keep this system in an optimal state possibly through vitamin E and selenium supplementation.

INTRODUCTION

Anemia occurs with relatively high incidence in patients affected by chronic renal failure (CRF). It has been described as normocytic, normochromic anemia. Erythropoietin (EPO) deficiency seems to the main cause of anemia in these patients[1]. The red blood cells (RBCs) of uremic patients, although reduced in number, show a shortened life span. The biochemical alterations involved in this event have yet to be completely clarified[2].

Different mechanisms have been implicated in aging and lysis of RBCs; lipid peroxidation of the RBC membrane induced by free radicals could be the most important[3]. In vitro studies showed that plasma from CRF patients contains various oxidants[2].

RBCs have several lines of defense against oxidative stresses. Reduced form of glutathione (GSH), together with its related enzymes is one of the major scavengers of activated oxygen radicals in RBCs[4]. Red cells release oxidized glutathione (GSSG) when exposed to oxidative stresses, most probably as a consequence of an increased glutathione peroxidase activity[5]. Hence,

measurement of RBCs and plasma reduced and oxidized glutathione of children with CRF was carried out to test the presence, in plasma, of oxidants which might be affecting the integrity of the glutathione system and the role of hemodialysis.

SUBJECTS AND METHODS

Thirty children with CRF - followed up in the Pediatric Nephrology Clinic and Hemodialysis unit of Ain-Shams University - were enrolled in this study as a stratified random sample. The first group comprised 20 children with CRF on regular HD. They were 12 males and 8 females. Their ages ranged from 6 to 17 years with a mean of 12.7 ± 3.2 years. Seven patients (35%) were on erythropoietin (EPO) therapy. All patients needed blood transfusions at a frequency of 1 to 4 per 3 months. The mean duration on HD was 20.4 ± 15.3 months with a mean of 6.9 ± 2.2 hours of dialysis / week.

The second group included 10 children with CRF treated conservatively (not on HD). They were 7 males and 3 females. Their ages ranged from 1 to 16 years with a mean of 11.6 ± 4.7 years. Only one patient was on EPO. Fifty % needed blood transfusions at a frequency of 1 to 2 per 3 months.

Ten healthy children were studied as a control group. They were 6 males and 4 females with an age range of 8 to 15 years and a mean age of 11.7 ± 2.2 years.

All children were evaluated clinically with accurate recording of the cause of CRF, duration under HD therapy, frequency of blood transfusions, erythropoietin therapy, drugs with oxidant effect as sulpha drugs, salicylates, antimalarials, chloramphenicol and others.

- Complete blood counts were done on Coulter Counter (Model T890) and reticulocytic count using the method of Dacie and Lewis (1985)[6].
- Measurement of BUN, serum creatinine, creatinine clearance, serum sodium and potassium was done using Bechman Synchron CX automated system.
- Quantitative measurement of plasma and red cell reduced and oxidized glutathione was done employing the enzymatic method described by Tietze (1969)[7] The reagents and enzymes used were of analytical grade and were purchased from Sigma chemical company.

Vitamin E supplementation was given to all patients at a daily dose of 5 mg and reevaluation of the frequency of blood transfusion and hemoglobin (Hb) concentration was done 3 months later.

Statistical analysis of the results was done using a standard computer program (Statview) employing Student's t-test for paired and unpaired data and correlation coefficient.

RESULTS

The mean Hb concentration and RBCs count were significantly lower in patients than in controls. The reticulocytic count was normal in all studied patients (mean value was 0.7 ± 0.4 % in HD patients and 0.6 ± 0.3 in non-dialyzed patients). The mean RBC GSH and GSSG were significantly lower in CRF patients of both groups than in controls. The RBC GSSG/GSH ratio was

Table 1: Laboratory data of CRF patients versus those of the control group

Variable	Patients on HD n = 20	t	p	Patients on conservative n = 10	t	p	Control group n = 10
	Mean ± SD			Mean ± SD			Mean ± SD
Hb (g/dL)	6.00 ± 0.97	10.42	≤ 0.001	8.80 ± 2.10	3.93	≤ 0.001	12.20 ± 1.75
RBCs count (xI0^{12}/L)	2.17 ± 0.38	15.56	≤ 0.001	3.36 ± 0.78	3.94	≤ 0.01	4.45 ± 0.38
Plasma GSSG (µg/mL)	8.01 ± 1.90	14.29	≤ 0.001	5.96 ± 3.61	3.72	≤ 0.01	1.68 ± 0.41
Plasma GSH (µg/mL)	2.46 ± 0.47	1.05	> 0.05	2.27 ± 0.40	0.08	> 0.05	2.29 ± 0.33
Plasma GSSG/GSH	3.391 ± 0.988	11.55	≤ 0.001	2.800 ± 1.735	3.72	≤ 0.01	0.744 ± 0.193
RBCs GSSG (µg/mL)	10.18 ± 1.07	2.66	≤ 0.05	9.58 ± 0.86	3.83	≤ 0.01	11.31 ± 1.14
RBCs GSH (µg/mL)	461.17 ± 41.73	11.22	≤ 0.001	445.37 ± 47.24	9.67	≤ 0.001	652.85 ± 48.74
RBCs GSSG/GSH	0.022 ± 0.003	5.22	≤ 0.001	0.022 ± 0.004	3.45	≤ 0.01	0.018 ± 0.002

significantly higher in CRF patients. There was no significant difference in plasma GSH however. The plasma GSSG of patients was significantly higher than in controls. This is shown in Table (1).

Although the mean Hb and RBC count in patients on HD were significantly lower than in those treated conservatively, yet there were no significant differences in their RBCs and plasma GSH and GSSG. Furthermore, the levels of glutathione did not differ with the underlying cause of CRF whether SLE, polycystic kidneys, obstructive uropathy or others.

Concerning the effect of EPO therapy, there was a significantly higher red cell GSH in patients receiving EPO than in those not receiving EPO ($P < 0.01$), although their Hb concentration and RBC count were comparable (6.2 ± 2.1 g/dL and 2.2 ± 0.6 x 10^{12}/L versus 6.0 ± 0.9 g/dL and 2.2 ± 0.3 x 10^{12}/L).

A significantly positive correlation was found between the age of CRF patients and RBCs GSH ($r = 0.46$, $P < 0.01$). Another significantly positive correlation was found between RBCs GSH and Hb ($r = 0.95$, $P < 0.001$).

Vitamin E supplementation caused a significant decrease in the frequency of blood transfusions in HD patients from a mean of 2.6 ± 1.1/3 months to a mean of 1.3 ± 0.6/3 months ($P < 0.01$) although the rise in Hb concentration was insignificant.

DISCUSSION

RBCs-GSH was significantly lower in CRF patients, whether on HD or on conservative treatment as compared to controls. This is in agreement with the

results of Costagliola et al. (1989)[8] in their study on adult CRF patients. On the contrary, the earlier reports by Kocak-Toker et al. (1986)[3] failed to demonstrate such a decrease.

In general, red cells with decreased GSH levels show shortened survival time in vivo and an increased susceptibility to hemolysis[9]. Therefore, the finding of decreased GSH levels in CRF patients may ***partly*** account for the shortened red cell survival time in uremia previously noticed by Hofti et al. (1984)[10] and for the anemia frequently encountered in these patients. This is supported by the finding of a significantly positive correlation between RBCs-GSH and Hb concentration in CRF patients of this study.

Hemolysis of RBCs is expected to produce reticulocytosis as a compensation by a normal erythroid marrow. In this work however, CRF patients had a normal mean reticulocytic count. In view of the well-known suppressed erythropoiesis in uremia[11], the finding of normal reticulocytic count does not exclude hemolysis.

High levels of RBCs-GSH are maintained normally by the NADPH-dependent enzyme glutathione reductase. There is controversy concerning the level of this enzyme in CRF. However, the alterations in the hexose-monophosphate shunt resulting from insulin resistance, fails to produce adequate amounts of NADPH which is a key element in the glutathione system[12].

The mean RBCs GSSG was significantly lower than that of controls. Costagliola et al. (1989)[8] also reported reduced levels of GSSG. One might speculate that such low levels are due to its continuous reduction by glutathione reductase enzyme. However, this is not the case as the levels of RBCs GSH were also low. A more likely explanation for the low RBC-GSSG is that it can readily pass through the red cell membrane. This is supported by the finding of a significantly higher mean plasma GSSG and GSSG/GSH ratio than in controls.

High levels of GSSG in plasma could exert two important effects on RBCs. First is the inhibition of G6PD activity, with consequent alteration of the glutathione system. The second effect is that GSSG easily reacts with hemoglobin to produce hemoglobin-glutathione mixed disulfides with a consequent protein aggregation, precipitation and hemolysis[8].

These alterations in the oxidative state of glutathione are probably the result of accumulation of oxidant substances in the plasma of patients with CRF with overexhaustion of the glutathione reductase enzyme system. The end result is accumulation of reactive species which initiate the peroxidation of membrane polyunsaturated fatty acids[13,14]. Although there are several reports indicating involvement of distorted free radical metabolism in the pathogenesis of anemia in CRF, yet the exact mechanism has not been completely clarified. It has been suggested that GSH is needed to protect the erythrocytes against peroxides continuously generated endogenously[15].

Although it has been suggested that the

loss of plasma antioxidant or addition of oxidative substances during HD may contribute to an oxidation reduction imbalance[2], yet there were no differences in the levels of GSH and GSSG in plasma and RBCs of patients on regular HD and those on conservative treatment, nor did their levels correlate with the duration of HD therapy in patients on HD. This indicated that HD does not pose an extra burden of free radicals on the glutathione system.

In the present study, the mean RBCs GSH level was significantly higher in CRF receiving erythropoietin therapy (EPO) as compared to those who were not. This occurred in spite of the lack of significant difference in hemoglobin concentration and RBCs count among them, supporting the concept of a direct effect of EPO on the glutathione system rather than through a simple increase in RBCs count. On the contrary, Bozfakiogla et al. (1992)[16] reported that GSH level and glutathione peroxidase remained unchanged after EPO therapy. However, in a study by Chakraborty et al. (1988)[17], EPO deficiency due to starvation has been reported to increase red cell lipid peroxidation and depress antioxidant system.

Vitamin E has been shown to have an antioxidant activity and to stimulate GSH synthetase[18]. Supplementation with vitamin E was carried out for patients of this study. A significant decline in the frequency of blood transfusions was noticed.

The RBCs GSH in CRF patients correlated significantly with age. This could be related to the hormonal changes occurring with growth and the possible role of estrogen as an antioxidant which has been described by Halliwell (1991)[19].

The results thus far obtained suggest that children with CRF have disturbances in the oxidative state of glutathione that is probably the result of the oxidative stress imposed by the uremic state. Such alterations might affect RBC survival and contribute to the anemia of CRF. Supplementation with vitamin E and probably vitamins A and C and selenium might help improve the anemia of CRF.

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