ORIGINAL ARTICLE

Influence of Haemodialysis on Susceptibility of Red Blood Cells to Peroxidation

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Abstract:

Background: Uremic anaemia has been the subject of several studies, since it causes serious problems. Decreased RBC production and survival seem to be due to erythropoietin deficiency combined with cell damage. *Aims and Objectives:* During haemodialysis, complement and leukocyte activation by contact with artificial surfaces promotes the formation of free radicals. However, the cell membrane is protected from this damaging effect by the presence of very efficient antioxidant enzyme defence mechanism. When this protective system is overwhelmed, it leads to cellular damage in the form of decreased RBC survival. It is postulated that antioxidant capacity in uremic patients is reduced, yet the exact mechanism remains unclear. In view of this data a study was undertaken to determine the activities of antioxidant enzymes to assess the oxidant damage to RBC in terms of TBARS (Thiobarbituric Acid Reactive Substances). *Materials and Methods:* Blood samples were collected from patients (n=40), before and after dialysis. They were compared with age and sex matched normals (n=45), who served as controls. The activities of enzymes glutathione peroxidase (GSH-Px) and Catalase (CAT) along with glutathione reduced (GSH) and malondialdehyde (MDA) expressed as TBARS in the RBC of patients on haemodialysis were determined. *Results:* The results indicated a marginal to moderate decrease in the antioxidant enzyme activities, such as CAT and GSH-Px, and increased TBARS levels before and after dialysis. *Conclusion:* Our results are suggestive of an increased susceptibility of the RBC to peroxidation on haemodialysis.

Keywords: Uremic anaemia, Decreased RBC, Thiobarbituric Acid Reactive Substances.

Introduction:

In patients on haemodialysis, RBC survival is known to decrease from normal. This decreased RBC survival is commonly attributed to chronic haemolysis due to a combination of factors. Haemodialysis, which is used to compensate for deficient renal function in uremic patients, is one of the factors which damages the RBC and the tissues [1, 2].

During haemodialysis direct contact of the RBC with insufficiently biocompatible membrane seems to contribute to cellular injury, by enhanced endogenous production of oxygen free radicals [3]. The single electron on the activated oxygen forms can be transferred to various molecular species particularly to membrane fatty acids initiating a peroxidative chain reaction with the formation of lipid peroxides, further leading to the production of MDA. The cellular injury is probably the result of an imbalance between reactive oxygen species generated and their scavengers. This change results in lipid peroxidation of red cell membranes and hence breakdown of RBC [4-6].

The present study was undertaken to determine the effects of uraemia and haemodialysis on oxidant-antioxidant levels in the RBC, in terms of TBARS, and the activities of antioxidant enzymes GSH-Px and CAT, as well as the GSH content of the RBC.

Material and Methods:

Blood sample was collected in heparinised tubes, after prior consent, from: Chronic Renal Failure

(CRF) Patients; n=40, maintained on haemodialysis twice a week and healthy age and sex matched non-smoking adults not suffering from any disease; n=45 as controls. The sample was collected from patients before and after 4 hours of dialysis. Approval was taken from the ethical clearance committee of the Institute. The study was conducted over a period of 6 months. Patients testing positive for Hepatitis B, HIV, smokers and those suffering from hepatic disease and diabetes mellitus were excluded from the study. Routine Haemoglobin (Hb), CBC and renal function tests were carried out. Whole blood was loaded on a column of α-cellulose and microcrystalline cellulose (1:1 w/w) to separate RBCs. After washing with saline, one part of the RBC suspension was taken for MDA (TBARS) estimation by the method of Stocks and Dormandy 1972 [7]. The second part of the pure RBC suspension was mixed with stabilizing solution to give RBC hemolysate (1:20), which was used for estimating the activity of GSH-Px and Catalase by the method of E. Butler 1986 [8].

Estimation of GSH was done from whole blood by the method of E. Butler 1986 [8].

Statistical analysis was carried out using the paired and unpaired students't-test. All values are reported as mean ± SD. P> 0.05 was considered as not significant (NS).

Results:

The mean haemoglobin (Hb) level in CRF patients on HD was 7.37 ± 2.42 gm% as against control value of 14.2 ± 1.28 gm%. A further fall in Hb was observed, in the post-HD samples; 6.89 ± 2.04gm%. As compared to control group the pre-HD samples showed (Table 1) a moderate fall of 27% in GSH-Px activity and a significant reduction of 57% in the Catalase activity. TBARS level showed an increase of 34%. The GSH content also fell marginally by 14%. When statistical evaluation was done between the pre and post dialysis samples a rise of 27% was seen in GSH-Px activity, while CAT exhibited a further fall of 41%.The GSH levels further decreased by 16% and the TBARS level showed a sharp increase of 92% (Fig. 1).

Discussion:

In response to different stimuli, leading to tissue injury, the glomerular cells experience a burst of oxidative metabolism with a marked increase in

Table 1: GSH-Px, CAT, GSH and TBARS in CRF patients before (Pre-HD) and after (Post-HD) Haemodialysis and in Controls

Parameters	Controls Mean ± S.D	Pre-HD Mean ± S.D	Post-HD Mean ± S.D
GSH-Px (I.U./gm Hb)	27.81±7.22	20.33±3.72 (27%) **	25.79±3.78 (27%) **
Catalase (I.U./gm Hb)	07.9±2.68	03.41 ±1.55 (57%) **	02.03±1.07 (41%) **
GSH (µmoles/gm Hb)	06.35±1.5	05.48±2.49 (14%) *	04.6 ±2.23 (16%) *
TBARS (nmoles/gm Hb)	28.22±12.59	37.86±18.11 (34%) **	72.61±30.13 (92%) **

*p<0.01 *, p<0.001 ** Figure in parenthesis indicates % rise/fall when Pre-HD samples compared with controls while post – HD samples compared against pre-HD.*

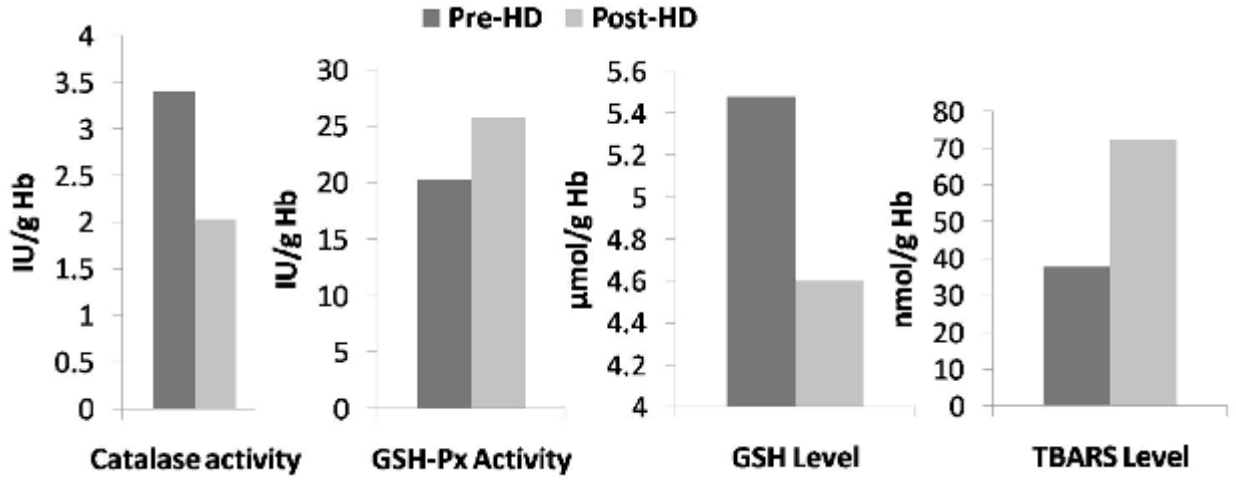

Fig. 1: Comparison of GSH-Px & CAT Activities, GSH and TBARS Level Before (pre-HD) and After Haemodialysis (post-HD)

oxygen uptake. This in turn leads to increased generation of the superoxide anion and its related species [6].

The circulating RBC is exposed to this oxidative damage on the outside. Haemodialysis on the other hand evokes injurious effects on the RBC by enhanced endogenous production of oxygen free radicals. It causes mechanical stress to the RBC and also increases generation of free radicals [9]. The RBC tries to combat this two-way attack by gearing up its defence system. The overall oxidative damage has been observed to accelerate RBC aging and haemolysis through lipid peroxidation [3].

Low GSH levels have been observed by earlier workers [4, 10] in the RBC of CRF patients before dialysis. The activities of the enzymes catalase and GSH-Px have been shown to be lower than those of controls by Zachara B A *et al* [11] and Durak I *et al* [12], in keeping with our observations. Higher TBARS levels in pre-HD samples as against controls, reported by us, supports the observations made by Evelyne P *et al* [1], and Zwolinska D *et al* [13]. Whether or not dialysis alleviates oxidative stress in CRF remains debatable. Several studies demonstrate partial restoration of the metabolic abnormalities in the RBC after dialysis [4, 14], while many others fail to observe such an effect [15-17]. Different reasons have been put forth for the fall in GSH level in CRF patients on HD. Markus D *et al* [18] have observed low GSH after dialysis, and explained it on the basis of dialytic removal of GSH precursors. Canestrari F *et al* [3] have observed a rise in the GSSG level and have attributed this to the utilisation of GSH against the effects of ROS. However, both the studies have reported low GSH levels which are supported by the present study. A moderate rise in GSH-Px activity and a fall in GSH level after HD suggest that it is probably inadequate to reduce Hydrogen Peroxide (H_2O_2) formed as reflected by the higher TBARS levels after dialysis, observed in this study and supported by others [19, 20]. Catalase seems to be of little help in this mechanism. A decrease in its activity [12, 21] could probably be on account of inhibitory effect of supraconcentrations of H_2O_2. It has also been proposed that under the influence of reactive oxygen species, the enzyme binds to the cytosolic face of the RBC membrane [12].

Our results indicate that the RBC, in CRF patients on HD, have greater susceptibility to Reactive Oxygen Species (ROS) as reflected by the significant rise in TBARS levels which are also supported by others [19, 20] combined with a fall in antioxidant enzyme and GSH activity.

Conclusion:

The data helps to generate a greater insight into the understanding of mechanisms regulating ROS metabolism and identification of processes that promote oxidative excess. Such studies would help to target antioxidant therapies more effectively and help to reduce the detrimental actions of vascular oxygen free radicals.

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