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IRON SUPPLEMENTATION AND OXIDATIVE STRESS IN PATIENTS WITH UREMIA

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Abstract : The existence of oxidative stress in uremia is well proved but the relative importance of uremic status versus the role of free iron in exacerbating oxidative stress in patients with uremia is not been clarified. Serum creatinine, free iron both in ferrous and ferric state, protein thiols, lipid hydroperoxides levels were estimated by spectrophotometric methods. The study groups comprised of patients with chronic kidney disease on conservative management, on hemodialysis with and without iron supplementation, and compared with healthy controls. Free iron levels were higher in patients with chronic kidney disease on conservative management, hemodialysis patients with and without iron supplementation. Hemodialysis cases with iron supplementation had significantly higher free iron levels as compared to hemodialysis cases without iron supplementation. The levels of lipid hydroperoxides were higher and protein thiols were lower in patient groups. Creatinine correlated positively with free iron and lipid hydroperoxides, and negatively with protein thiols. In conclusion, uremia per se may be responsible for enhanced oxidative stress in patients with chronic kidney disease.

Key words : free iron hemodialysis lipid hydroperoxides
oxidative stress protein thiols

INTRODUCTION

Majority of patients with end stage renal disease are anemic and are treated with intravenous iron preparations along with recombinant human erythropoietin (1, 2). Intravenous iron supplementation in large doses may potentially exceed storage capacity and lead to the presence of free iron in plasma (3, 4). Free iron in ferric state (Fe^{3+}) can be reduced by superoxide anion or by ascorbate or any other antioxidants to ferrous state (Fe^{2+}). Ferrous iron, via Fenton reaction, can produce the extremely reactive hydroxyl radicals (2, 3, 4) which can damage biomolecules.

Although in theory, iron administered may be pro-oxidative in dialysis patients, to date there are only limited data supporting this contention. Free iron was found to be raised in conditions of iron over load (5), in

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renal failure patients with (3, 6) and without (7) iron supplementation. At present, the nature and source of free iron and the specific biochemical pathways by which patients with chronic renal failure (CRF) are subjected to increased oxidative stress are not clear (8, 9). The relative importance of the free iron and uremic toxins in exacerbating oxidative stress has not been clarified.

In the current study, free iron levels were estimated along with creatinine and other oxidative stress markers in renal failure patients on conservative management, on hemodialysis therapy with and without iron supplementation and compared with age matched healthy controls.

MATERIALS AND METHODS

Subjects

The study was carried out on renal failure patients on chronic maintenance hemodialysis with (n = 18) or without (n = 24) i.v iron supplementation and compared chronic renal failure patients on conservative management (n = 24) and age matched control (n = 20). Mean duration of hemodialysis was 16 ± 4 months. Informed consent was taken from all subjects was approved by institutional review board. The investigation confirms to the ethical guidelines for biomedical research on human subjects. None of the patients or controls received any form of antioxidant medication.

Under aseptic conditions blood samples (5 ml) were drawn into plain vacutainers, free of iron contamination, from antecubital veins of controls, chronic renal failure patients on conservative management and from arterio-venous fistula of hemodialysis patients just before starting hemodialysis. The collected blood was allowed to clot and then centrifuged at 2000 g for 15 minutes for clear separation of serum. All assays were performed immediately after serum was separated.

Special chemicals like bathophenathroline disulphonate, dithionitrobenzoic acid and xylenol orange were obtained from Sigma chemicals, St. Louis, MO, USA. All other reagents obtained were of analytical grade.

Determination of oxidative status

Serum free iron was estimated by bathophenthroline disulphonate assay (10). Briefly, sample was treated with the dye and colour developed was measured. The same procedure was followed after adding ascorbic acid to the sample. The absorbance values of sample with and without ascorbic acid were interpolated with respective standard graphs to obtain levels of free ferrous iron and free total iron. The free ferric iron concentration was calculated by subtraction of former from the latter and levels of free iron were expressed in µmol/L of serum.

Serum protein thiols were measured by a spectrophotometric method using 5′ 5′ dithio-bis (2-nitrobenzoic acid) (11) and the lipid hydroperoxide content of whole serum was determined with the FOX version II assay (12, 13). Serum creatinine was estimated by spectrophotometric method using automated analyser.

Statistical

The results were expressed as mean ± standard error of mean (SEM). A P value of <0.05 was considered statistically significant. Analysis of variance (ANOVA) was used to compare mean values in the four groups, followed by multiple comparisons by post-hoc test. Pearson correlation test was applied to correlate between the parameters.

RESULTS

The mean urea and creatinine levels in renal failure cases on conservative management were 80.2 ± 10.3, 5.9 ± 1.2; in hemodialysis with iron supplementation were 40.34 ± 4.2, 3.8 ± 1.3; and in hemodialysis without iron supplementation were 41.31 ± 3.8, 3.1 ± 1.1. As depicted in Table I, there was a significant increase in free Fe^{2+}, free Fe^{3+} and total free iron in hemodialysis patients with and without iron supplementation, and CRF patients on conservative management compared to controls (P<0.01). Free iron levels were significantly higher in hemodialysis cases who were receiving intravenous iron as compared to patients who were not receiving any kind of iron supplementation (P<0.01).

Serum creatinine levels were significantly higher in CRF patients on conservative management compared to hemodialysis cases with and without iron supplementation, and controls (P<0.01). Serum lipid hydroperoxides were significantly higher and protein thiols were lower in hemodialysis cases with and without iron supplementation, and CRF patients on conservative management compared to controls (P<0.01). However, there was no significant difference between the patient groups.

On applying the Pearson correlation test, serum creatinine was correlated positively with Fe^{2+} (P<0.01), Fe^{3+} (P<0.01), total free iron (P<0.01), lipid hydroperoxides (P<0.01) and negatively with protein thiols (P<0.01).

DISCUSSION

Presence of oxidative stress in renal failure is well established (14). The specific biochemical pathway that leads to the generation of reactive oxygen species is still not known (9). One of the proposed pathways is generation of hydroxyl radicals via Fenton reaction. In the current study, free iron levels were higher in all the three patient groups but the levels were found significantly higher in hemodialysis cases receiving intravenous iron supplementation.

TABLE I: Status of serum non-transferrin bound iron, creatinine, protein thiols and lipid hydroperoxides in controls, chronic renal failure and hemodialysis patients. The data are expressed in mean±SEM.

	Control (n = 20)	*Chronic renal failure cases* (n = 24)	*Group I hemodialysis cases* (n = 18)	*Group II hemodialysis cases* (n = 24)
Total free Iron (µmol/L)	2.23±0.17	12.65±2.32	69.49±6.52♦*	43.32±4.09*
Free Fe^{+3} Iron (µmol/L)	1.74±0.41	10.12±3.54	54.23±5.43♦*	36.47±5.15*
Free Fe^{+2} Iron (µmol/L)	0.80±0.52	2.43±0.17	14.65±2.71♦*	8.21±2.23*
Protein thiols (µmol/L)	342.34±43.43	191.26±16.75*	164.12±13.54*	173.43±10.24*
Lipid hydroperoxides (µmol/L)	0.18±0.09	1.78±0.46*	2.17±0.45*	1.79±0.32*

Data is expressed in mean±SEM, *P<0.01 compared to controls, ♦P<0.01 compared to hemodialysis cases without iron supplementation. Group I – Received i.v. iron supplementation; Group II – Received no iron supplementation.

The positive correlation between free iron and creatinine indicates some unknown mechanism which could lead to the presence of increased levels of free iron in these patients. In the present study, serum creatinine correlated positively with lipid hydroperoxides and negatively with protein thiols, indicating there may be a direct relationship between the extent of renal damage and the increased presence of free radicals and decreased antioxidant status in these patients. Although, free iron levels were higher in hemodialysis cases receiving supplemental iron but, there was no significant difference in free radical and antioxidant levels between these patients and those not receiving intravenous iron. This may possibly indicates catalytically inactive nature of free iron as observed earlier (8).

We found increased lipid hydroperoxides and decreased protein thiols in proportion to serum creatinine indicating the role of uremia in exacerbating the oxidative stress in patients with chronic kidney disease. This fact is also supported by the recently published work by Witko-Sarat et al who reported positive correlation between serum creatinine and advanced oxidation protein products (15).

In conclusion, this study suggests that uremia results in retention of reactive aldehydes, hydroperoxides and depletion of reduced thiol antioxidants. These changes exacerbate oxidative stress. Free iron although present at higher levels in uremia may not be a contributing factor to the oxidative stress.

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