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Enhanced oxidative stress in haemodialysis patients receiving intravenous iron therapy

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Abstract

Background. Iron balance is critical for adequate erythropoiesis and there remains much debate concerning the optimal timing and dosage of iron therapy for haemodialysis patients receiving recombinant human erythropoietin therapy.

Methods. In this study, we examined the influence of baseline ferritin level and intravenous infusion of 100 mg ferric saccharate on the oxidative status of the patients on maintenance haemodialysis. The levels of antioxidant enzymes and lipid peroxides were determined in erythrocytes and plasma of 50 uraemic patients on haemodialysis. These patients were divided into groups 1, 2, and 3, based on their baseline serum ferritin levels of <300, 301–600, and >601 μg/l, respectively.

Results. We found that the mean superoxide dismutase (SOD) activities in the erythrocytes were similar in the three groups of patients and did not differ from those of the age-matched controls. On the other hand, all the haemodialysis patients showed significantly higher plasma SOD activity as compared to controls. After intravenous iron infusion, group 3 patients showed the largest decrease in plasma SOD activity. The plasma glutathione peroxidase (GSHPx) activities of the patients in all three groups and the erythrocyte GSHPx activities of the patients in the groups 2 and 3 were lower than those of the healthy controls. In all three groups of patients, no difference in GSHPx activity was found before and after intravenous iron infusion. On the other hand, we found that the average baseline levels of plasma lipid peroxides of all three groups of patients were significantly higher than that of the controls. The patients in group 3 with the highest serum ferritin levels showed the highest levels of plasma lipid peroxides. More importantly, we found that after iron infusion, the patients in all three groups, particularly those in group 3, showed significantly elevated levels of plasma lipid peroxides.

Conclusion. We demonstrated that increased oxidative stress in the blood circulation of the uraemic patients on haemodialysis is exacerbated by the elevated baseline serum ferritin levels and intravenous iron infusion. The resultant oxidative damage may contribute to the increased incidence of atherosclerosis in the patients with end-stage renal disease on long-term haemodialysis.

Key words: Key words: erythropoiesis; erythropoietin therapy; haemodialysis; intravenous iron therapy; oxidative stress; reactive oxygen species; uraemia

Introduction

Several lines of evidence have indicated that reactive oxygen species (ROS) may be involved in the uraemic toxicity of patients with end-stage renal disease (ESRD). In addition, these patients are also chronically exposed to the oxidative stress of ROS as a result of activation of neutrophils induced by their interaction with the dialysis membranes [1,2]. Increased concentration of malondialdehyde (MDA), one of the end products of the peroxidation of polyunsaturated fatty acids, has been reported in plasma and erythrocytes [3–5] of haemodialysis patients. It has been observed that plasma levels of glutathione (GSH) [6], GSH peroxidase (GSHPx) [4], vitamins C [4] and E [5] are decreased in the ESRD patients. Erythrocyte superoxide dismutase (SOD) activity was reported to be increased [7] or decreased [8] by different investigators. These studies clearly suggest that oxidative stress is enhanced, as indicated by decreased antioxidant levels and increased lipid peroxidation, exists in the haemodialysis patients.

The use of recombinant human erythropoietin (rHuEpo) to treat the anaemia of patients on maintenance dialysis has been a major advance in the care of these patients. However, this treatment is frequently blunted by the induction of iron depletion [10,11]. Recently, intravenous iron therapy has become an

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accepted mode of treatment for iron deficiency in these patients [12–14]. Several clinical studies demonstrated that intensive intravenous iron therapy not only improves erythropoiesis, but also results in a tremendous decrease (about 41%) in the amount of rHuEpo previously required [13,15]. Later, a consensus report on diagnosis and correction of iron deficiency recommended intravenous iron therapy for haemodialysis patients [16]. Nevertheless, the upper safety limit for this treatment is unclear. Eschbach *et al.* [17] suggested that iron overload may be indicated by a serum ferritin level greater than 1000 μg/l. A recent study demonstrated a profound impairment of polymorphonuclear leukocytes if haemodialysis patients had a serum ferritin level >650 μg/l even if transferrin saturation is below 20% [18]. Clearly, more studies are urgently needed to better define the potential risk of intravenous iron therapy.

The aim of the present study was to investigate the influence of the baseline serum ferritin level and intravenous iron infusion on the levels of the major antioxidant enzymes and oxidative stress in ESRD patients receiving maintenance haemodialysis.

Subjects and methods

Patients

Twenty male and 30 female patients on regular haemodialysis treatment for more than 6 months were recruited in this study after informed consent had been obtained. Their mean age was 55.8 ± 13.4 years (range 23–80 years). All of the studied patients received erythropoietin therapy. Patients underwent haemodialysis three times a week, 4 h per session. Dialysis was carried out with haemophan dialysers (Gambro AW P-15) and a dialysis bath containing 35 mmol/l bicarbonate. No patients had received blood transfusion or oral or intravenous iron therapy for 3 months preceding the study. The underlying renal diseases leading to uraemia were chronic glomerulonephritis (28 cases), diabetes mellitus (10 cases), chronic pyelonephritis (4 cases), polycystic kidney disease (3 cases), and unknown causes (5 cases). Baseline serum ferritin levels were the mean values of three measurements of ferritin levels during the 3 months preceding the study. These patients were then divided into three groups according to the baseline serum ferritin levels: group 1 serum ferritin between 100 and 300 μg/l; group 2, serum ferritin between 301 and 600 μg/l; and group 3, serum ferritin above 600 μg/l. In the present study, after obtaining the baseline samples, 100 mg of ferric saccharate was given intravenously as a slow continuous infusion for 1 h to all the patients. Samples for analysis were taken 15 min after the end of the infusion.

A control group was composed of 20 healthy volunteers, who had no history of renal or haematological diseases (10 men and 10 women) with a mean age of 57.7 ± 13.3 years. Blood samples were drawn from the antecubital veins after 12 h of fasting.

Collection and preparation of blood samples

After a 12-h fast, venous blood samples were taken from each of the patients and control subjects. Blood was collected in EDTA vacutainers and used immediately for the analysis of haematological parameters and activities of antioxidant enzymes. First, a 1-ml aliquot of blood was removed to determine haemoglobin concentration with a standard kit. Following centrifugation at 600 g for 10 min, the plasma was collected and stored in aliquots at -80°C or used immediately for enzymatic assays. Several aliquots of the same samples were transferred into other tubes to be used for the assays of other plasma or serum parameters, which were performed by routine laboratory techniques. In addition, an aliquot of 1-ml fresh blood was collected in a heparin vacutainer for biochemical analysis of the plasma level of lipid peroxides.

Measurement of antioxidant enzyme activities in erythrocytes and plasma

SOD

The SOD activities in the plasma and erythrocytes were measured by the method described by Oyanagui *et al.* [19]. Plasma or haemolysed erythrocytes, 0.2 mM hydroxylamine, and 0.2 mM hypoxanthine were mixed in 1.0 ml of 50 mM Tris–HCl buffer (pH 8.0). The reaction was initiated by adding xanthine oxidase (1.25 U/l) and 10 M EDTA to the reaction mixture and was allowed to proceed at 37°C for 40 min. Diazo dye-forming reagent was added and the rate of change of absorbance at 550 nm was then measured. The activity of SOD is expressed as nitrite U/ml or kU/g Hb.

GSHPx

Plasma or haemolysed erythrocytes, GSH reductase, and 0.15 M GSH were incubated in 1.0 ml of 50 mM phosphate buffer (pH 7.0) at 37°C for 10 min. NADPH solution (1.0 mM) and 5 mM sodium azide were then added and allowed to equilibrate for 3 min at 20°C. The enzymatic reaction was initiated by the addition of 5 mM hydroperoxide as the substrate. The conversion of NADPH to $NADPH^+$ was then followed by continuous recording of the decrease in the absorbance at 340 nm for 5 min [20]. GSHPx activity is expressed as U/l or U/g Hb.

Glutathione S-transferase

The glutathione S-transferase (GST) activity in the plasma or erythrocytes was measured by using 1-chloro-2,4-dinitrobenzene (CDNB) as substrate according to the method described by Habig *et al.* [21]. The reaction mixture in 2 ml contained 0.2 M sodium phosphate buffer (pH 6.5), 0.95 mM CDNB, 0.95 mM GSH, and 0.5 ml plasma or lysed erythrocyte suspension. The reaction was initiated by the addition of the electrophilic substrate CDNB and the reaction was monitored at 25°C spectrophotometrically by the increase of the absorbance at 340 nm. The background rate in the absence of test samples was subtracted to correct for the non-enzymatic reaction. The GST activity is expressed as U/l or U/g Hb.

Determination of total GSH

An aliquot of 0.05 ml 10% perchloric acid (PCA) was added into 0.1 ml of whole blood to remove proteins by precipitation and centrifugation. Total free GSH (tGSH) in the whole blood was measured with an enzymatic recycling assay, which employs glutathione reductase to induce a kinetic

colorimetric reaction of 5,5'-dithiobis-(2-nitrobenzoic acid) (DTNB) [22]. The rate of change of absorbance at 412 nm was measured within 5 mim. The concentration of tGSH is expressed as μg/ml.

Determination of total free thiols

The concentration of plasma thiols was determined by a colorimetric method according to Ellman [23]. An aliquot of 0.05 ml 10% PCA was added into 0.1 ml of whole blood to remove proteins by precipitation and centrifugation. The supernatant was added with 0.05 ml of 0.1 M DTNB. After thorough mixing, the mixture was left standing at room temperature. The final absorbance at 412 nm was recorded after 30 min reaction of DTNB with all the SH-group-containing compounds. The blood concentration of total free thiols is expressed in the unit of μg/ml.

Measurement of plasma lipid peroxides

Plasma level of lipid peroxides was measured as MDA by an automated HPLC system as described previously [24]. Five hundred microlitres each of a plasma sample, reagent blank (distilled water), and 1,1,3,3-tetramethoxypropane (TMP) working standard solutions were respectively pipetted into a glass tube. After mixing, 750 μl of 0.44 M phosphoric acid and 250 μl of 0.6% thiobarbituric acid (TBA) were added. The mixture was heated for 1 h in boiling water (100°C), and then cooled at 4°C. This was followed by neutralizing 500 μl of the boiled sample with 500 μl of methanol-1N NaOH mixture (45.5:4.5, v/v) to precipitate proteins before injection into a C18 column. After centrifugation, 10–20 μl of the protein-free supernatant was fractionated by HPLC to separate the MDA–TBA adduct from interfering chromogens. The MDA–TBA adduct was quantified by a fluorescence detector using the excitation wavelength of 525 nm and emission wavelength of 550 nm. The concentration of lipid peroxides in the plasma sample is expressed as nmol MDA/ml. A calibration curve was prepared for each run by using 1,1,3,3-tetramethoxypropane as the standard.

Laboratory evaluation

Serum levels of total iron, transferrin, ferritin, total protein, albumin, haptoglobin, C-reactive protein (CRP), lactate, LDH, haematocrit, and transferrin saturation were determined before and 15 min after the infusion. Haematological parameters including haemoglobin, haematocrit, and erythrocyte index were measured on an automated counter. Biochemical assays (blood urea nitrogen (BUN), creatinine, LDH, lactate, and iron) were performed on a multiparametric autoanalyser (Paramax, Baxter, Miami). Haptoglobin and CRP levels were determined using a nephelometry method (Behring, Marburg, Germany). Serum iron and total iron binding capacity (TIBC) were measured every 3 months by standard laboratory methods. Serum ferritin concentration was determined by a microparticle enzyme immunoassay (IMX, Abbott, IL). The transferrin saturation index was calculated according the following formula: saturation (%) = serum iron/total iron binding capacity.

Statistical analysis

Data are presented as mean $\pm$ SD. ANOVA followed by Fisher's protected least significance difference test (PLSD) and Scheffe test for multiple comparisons or 2-tailed Kruskal–Wallis test were performed to assess the differences between groups. Wilcoxon's test was used for analysis of paired data. Pearson's correlation coefficient was used to determine the relationship between concentrations of antioxidants and serum ferritin levels. A P value <0.05 was considered as significant.

Results

Tables 1 and 2 show the biochemical and haematological data of the patients in different groups that were examined in this study. There was no difference in BUN, creatinine, albumin, cholesterol, triglycerides, and uric acid among the three groups of patients. All patients had serum CRP within the normal range (0–0.50 mg/dl).

The average activities of SOD, GSHPx, and GST in the erythrocytes and plasma of the three groups of patients and controls are given in Tables 3, 4, and 5 respectively. Erythrocyte SOD activities were similar in all three groups of the haemodialysis patients and did not differ from those observed in the age-matched controls. Plasma SOD activities of the patients in all three groups were found to be higher than that of controls (106.9 ± 10.3 U/ml). Moreover, patients in group 3 showed significantly higher plasma SOD activity than the other two groups, both before and after intravenous iron infusion ($P<0.05$). After intravenous iron infusion, the patients in group 3 again showed the largest and statistically significant decrease in plasma SOD activity (from 161.4 ± 44.2 to 145.1 ± 33.3 U/ml, $P<0.05$).

The average GSHPx activity in plasma was found to be significantly lower in all the haemodialysis patients as compared with that of controls. Erythrocyte GSHPx activities in the patients of groups 2 and 3 were significantly lower than that of the controls. In addition, we found that the erythrocyte GSHPx activities of the patients in groups 1 and 2 were significantly higher than those of group 3 patients, both before and after intravenous iron infusion. However, within all three groups of patients, there was no difference in GSHPx activity before and after intravenous iron infusion (Table 4). In addition, we observed a negative correlation between GSHPx baseline activity in erythrocytes (RBC) and serum ferritin level ($r=-0.44$, $P<0.001$) (Figure 1). Similar negative correlation between erythrocytes (RBC) concentrations of GSHPx and serum ferritin level was noted after intravenous iron infusion.

Baseline activity of plasma GST was significantly decreased in all three groups of patients as compared with the controls. Plasma levels of GST did not differ significantly before and after intravenous iron infusion. As shown in Table 5, the erythrocyte GST activities of the patients in groups 1 and 3 before intravenous iron infusion were higher than those of the controls and group 2 patients. After the infusion, the erythrocyte GST activities of the patients in the group 1 were found to be significantly higher than those of patients

Table 1. Characteristis of the patients in three study groups

Parameters	Group 1 $n=16$	Group 2 $n=16$	Group 3 $n=18$	Control $n=20$
Age (years)				
mean	54.4±17.1	59.8±8.1	58.2±12.9	57.7±13.3
range	23–80	48–78	47–74	25–82
Sex (M/F)	7/9	6/10	7/11	10/10
BW (kg) mean	60.3±9.5	57.6±12.0	58.6±8.8	59.1±9.0
Duration of				
HD, months	32.6±29.4	39.9±24.8	40.9±37.9	
(range)	3–86	3–76	3–123	–
Epo(U)/month	14 002±4112	15 150±3014	15 900±4800	–

BW, body weighgt; HD, haemodialysis; Epo, erythropoeitin.

Table 2. Biochemical and haematological results of the haemodialysis patients in different groups

Parameters	Group 1 $n=16$	Group 2 $n=16$	Group 3 $n=18$
BUN	63.8±15.9	56.9±12.6	57.3±16.7
Creatinine	11.6±3.1	9.6±3.5	10.7±4.0
Ferritin	227.4±61.5	496.2±92.6	910.6±248.6
SI	71.1±13.1	72.5±23.0	68.3±21.2
TSAT before	21.8±7.7	25.4±8.9	28.8±13.6
after	68.7±30.2*	74.8±24.0*	85.6±24.6*
Hb (g%)	11.4±5.6	10.9±4.5	10.8±4.9
MCV μm^3	82.4±7.7	91.5±6.6	91.1±3.9
Albumin (g/dl)	4.1±0.4	4.0±0.4	4.0±0.3
Chol (mg/dl)	161.1±33.0	177.1±26.5	180.1±40.0
TG (mg/dl)	154.2±91.1	240.7±190.2	220.9±140.0
Uric acid (mg/dl)	6.7±0.8	6.8±1.5	6.4±1.9

SI, serum iron; TSAT, transferrin saturation; HB, haemoglobin; HCt, haematocrit; MCV, mean corpuscular volume; Chol, cholesterol; TG, triglycerides.
*Significantly different from preinfusion levels ($P<0.05$).

Table 3. SOD activities in the controls and haemodialysis patients of different groups

	SOD Plasma (U/ml)	SOD RBC (kU/g Hb)
Controls ($n=20$)	106.9±10.3	111.8±14.0
Group 1 before	125.9±23.3[a,b]	113.3±18.6
($n=16$) after	118.5±21.1[c]	117.3±12.2
Group 2 before	131.5±26.3[a,b]	112.8±16.8
($n=16$) after	122.3±22.4[c]	110.1±15.4
Group 3 before	161.4±44.2[a]	111.6±17.6
($n=18$) after	145.1±33.3	110.6±17.5

[a]Significantly different from control (group 1 and 2; $P<0.05$; group 3; $P<0.001$).
[b]Significantly different from group 3 before infusion ($P<0.05$).
[c]Significantly different from group 3 after infusion ($P<0.05$).
[d]Significantly different from group 3 before infusion ($P<0.05$).
ANOVA with least significant difference was used to compare the various groups of patients.

in groups 2 and 3. We found that group 1 patients with lower baseline level of ferritin exhibited more drastic increase in the GST activity than did the controls. It is noteworthy that there was no significant

Table 4. Activities of GSHPx in the controls and haemodialysis patients of different groups

	GSHPx Plasma (U/ml)	GSHPx RBC (U/g Hb)
Controls ($n=20$)	636.6±77.4	62.4±2.0[b]
Group 1 before	248.2±37.9[a]	59.1±11.1[b]
($n=16$) after	265.6±36.5	58.2±6.9[c]
Group 2 before	248.1±50.8[a]	54.5±10.7[b]
($n=16$) after	261.2±57.6	53.1±7.8[d]
Group 3 before	254.9±65.0[a]	47.1±7.7
($n=18$) after	292.8±69.9	45.0±8.7

[a]Significantly different from control before infusion ($P<0.001$).
[b]Significantly different from group 3 before infusion ($P<0.05$).
[c]Significantly different from group 3 after infusion ($P<0.001$).
[d]Significantly different from group 3 after infusion ($P<0.05$).
ANOVA with least significant difference was used to compare the various groups of patients.

Table 5. Activities of GST in the controls and haemodialysis patients of different groups

		GST Plasma (U/l)	GST RBC (U/g Hb)
Controls ($n=20$)	Before	7.5±0.2	5.7±0.3
Group 1	Before	4.3±0.7[a]	8.5±2.7[b]
($n=16$)	After	4.6±0.9	8.3±2.6
Group 2	Before	4.0±1.1[a]	6.7±1.5[c]
($n=16$)	After	4.5±1.2	6.0±1.6[d]
Group 3	Before	4.4±1.5[a]	7.7±3.2[b]
($n=18$)	After	5.4±2.1	6.6±3.3[d]

[a]Significantly different from control before infusion ($P<0.001$).
[b]Significantly different from control before infusion ($P<0.05$).
[c]Significantly different from group 1 before infusion ($P<0.05$).
[d]Significantly different from group 1 after infusion ($P<0.05$).
ANOVA with least significant difference was used to compare the various groups of patients.

difference in the GST activity after iron infusion. Table 6 shows free thiols and total GSH levels in plasma of all the studied patients before and after iron infusion. The plasma levels of free thiols in all haemodialysis patients were significantly lower than those of the controls. On further analysis, the plasma levels of

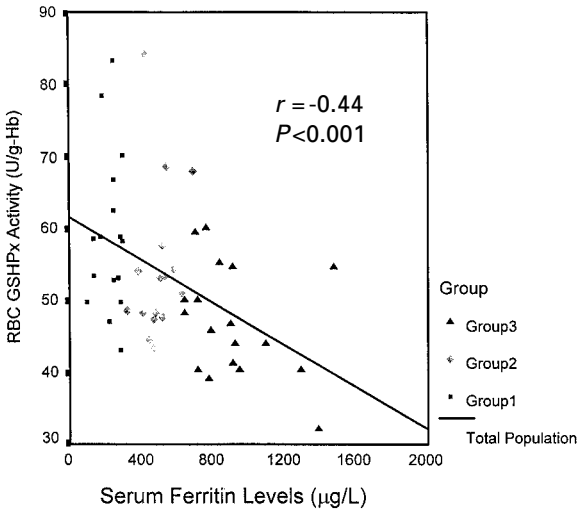

Fig. 1. Correlation between serum ferritin levels and RBC GSHPx activity in 50 haemodialysis patients. ($r = -0.44$, $P < 0.001$).

Table 6. Activities of GSH in the controls and haemodialysis patients of different groups

	f-thiols (μg/ml)	*t*-GSH (μg/ml)
Controls ($n=20$)	339.8 ± 11.1	201.0 ± 10.0
Group 1 before	312.2 ± 35.8[a]	206.2 ± 29.8
($n=16$) after	325.3 ± 40.1	220.0 ± 39.9
Group 2 before	233.7 ± 39.5[a,b,c]	167.7 ± 31.1[f]
($n=16$) after	230.3 ± 46.3[d,e]	179.3 ± 30.8[f]
Group 3 before	263.8 ± 62.4[a,b]	190.2 ± 58.5
($n=18$) after	286.6 ± 62.9[d]	206.9 ± 55.9

[a]Significantly different from control before infusion (group 1, ($P < 0.05$; groups 2 and 3, $P < 0.001$).
[b]Significantly different from group 1 before infusion ($P < 0.05$).
[c]Significantly different from group 3 before infusion ($P < 0.05$).
[d]Significantly different from group 1 after infusion ($P < 0.05$).
[e]Significantly different from group 3 after infusion ($P < 0.05$).
[f]Significantly different from group 1 ($P < 0.05$).
ANOVA with least significant difference was used to compare the various groups of patients.

free thiols of group 2 patients were significantly lower than those of patients in the other two groups. On the other hand, we observed significantly lower levels of GSH in the patients of group 2 as compared to controls and group 1 patients. After iron infusion, GSH concentration was significantly increased in patients of all three groups.

Baseline plasma MDA values in all haemodialysis patients were significantly higher than those of the controls. In addition, the patients in group 3 showed significantly higher baseline MDA levels than group 1 patients. Most importantly, we found that after infusion of iron, the patients in all three groups showed significantly elevated plasma levels of MDA. The group 3 patients, who had the highest baseline of serum ferritin, exhibited significantly higher mean post-infusion MDA values when compared with patients in group 2 (Table 7). Furthermore, we found a positive correlation between MDA and serum ferritin level in the patients on haemodialysis ($r = 0.39$, $P < 0.05$) (Figure 2).

Haemoglobin, haematocrit, albumin, cholesterol, triglycerides, haptoglobin, and uric acid did not change significantly during iron infusion. LDH was found to rise in all the patients but lactate increased only in the patients of group 3 after iron infusion (2.7 ± 0.7 *vs* 3.8 ± 1.4 IU/l; $P < 0.05$). As expected, the rise in transferrin saturation 15 min after iron infusion was statistically significant ($P < 0.001$) as shown in Table 2.

Discussion

The treatment of anaemia in ESRD patients on haemodialysis is frequently hindered by the presence of

Table 7. Plasma MDA levels in the controls and studied groups of haemodialysis patients

	Before	After
Controls ($n=20$)	1.4 ± 0.1	–
Group 1 ($n=16$)	1.7 ± 0.6[a]	4.5 ± 1.7[d]
Group 2 ($n=16$)	2.1 ± 0.5[a]	3.6 ± 1.6[d]
Group 3 ($n=18$)	2.3 ± 0.7[a,b]	5.4 ± 2.3[c,d]

[a]Significantly different from control (group 1, $P < 0.05$; group 2 and 3, $P < 0.001$).
[b]Significantly different from group 1 ($P < 0.05$).
[c]Significantly different from group 2 ($P < 0.05$).
[d]Significantly different from after iron infusion (group 2, $P < 0.05$; groups 1 and 3, $P < 0.001$).
ANOVA with least significant difference was used to compare the various groups of patients while Wilcoxon test was used to compare paired data.

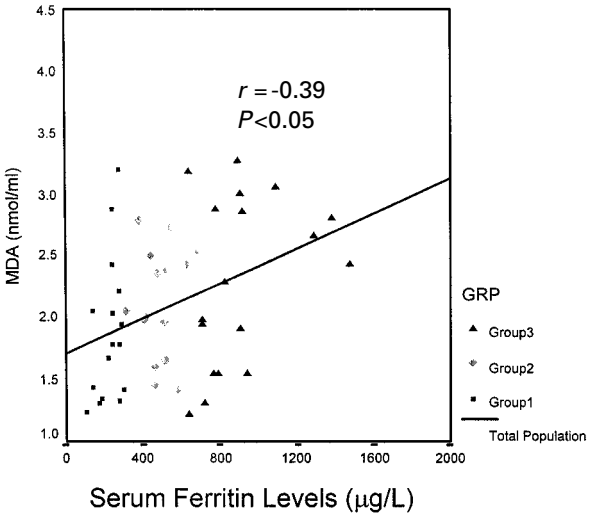

Fig. 2. Correlation between serum ferritin levels on plasma MDA levels in 50 haemodialysis patients. ($r = 0.39$, $P < 0.05$).

suboptimal iron stores. Intravenous iron therapy is mandatory to replenish the diminished iron stores. However, little information is available concerning the influence of iron therapy on the oxidative status of uraemic patients.

Intracellular ROS bear low specificity in terms of the biological molecules that they attack. Lipid peroxidation, protein oxidation, and oxidative damage to DNA are the major consequences of the oxidative stress. Fenton reaction, which leads to the formation of hydroxyl radicals or putative organic radicals, has been proposed to mediate most of these oxidative modifications and this reaction requires the participation of metal ions such as Fe and Cu [25,26]. Nevertheless, protective mechanisms that inhibit free-radical reactions as well as non-enzymatic free radical scavengers exist in the animal and human cells to protect them against free-radical damage. Oxidative stress develops when the levels of these antioxidants are deprived or when production of free radicals exceeds the capacity of the cell to dispose of them.

In several previous studies [1,2], uraemic patients on maintenance haemodialysis were found to be exposed to higher levels of ROS. Owing to such increase in ROS production, lipid peroxidation has been invariably found to be enhanced in the plasma and erythrocytes of uraemic patients [4,9,27]. Consistent with these observations, the haemodialysis patients examined in this study displayed a significant increase of lipid peroxidation and aberration of antioxidant enzymes in the plasma and erythrocytes. An imbalance between the generation and the removal of ROS and free radicals may be a contributory factor of haemodialysis-related complications.

The SOD activities in the plasma or erythrocytes were found to be either decreased [8,28], increased [7,9,29] or unchanged [30] in uraemic patients. In this study, the erythrocyte SOD activities were similar in all three groups of haemodialysis patients and no difference was found as compared with those of the controls. Since the erythrocytes lack the ability of *de novo* protein synthesis, no induction of antioxidant enzymes occurs [31]. Correction of anaemia by increased erythropoiesis results in an increase of precursor cells of erythrocytes. These young cells are known to contain elevated levels of SOD activity [32]. Hence during accelerated erythropoiesis, an increase in the number of young haematopoietic cells may replenish the erythrocyte SOD activity. Our data showed that plasma SOD activities were significantly increased in all the haemodialysis patients as compared to the controls. Meanwhile, those patients in group 3 showed significantly higher plasma SOD activity than the patients in the other two groups both before and after intravenous iron infusion. After intravenous iron infusion, group 3 patients showed the largest decrease in the plasma SOD activity. This augmented oxidative stress probably reflects the deleterious effect of high iron stores.

The major defence mechanisms against ROS-induced cellular damage include GSH and enzymes associated with its metabolism. Two GSH-dependent enzymes that are essential for the disposal of free radicals are GSHPx and GST. GSHPx is a selenium-dependent enzyme [33] and it has been suggested that GSHPx activity is related to the blood selenium level [34]. GSHPx catalyses the reduction of hydrogen peroxide to water at the expense of GSH. On the other hand, GST catalyses the reaction of GSH with electrophilic reagents [35] such as those generated by microsomal metabolism of drugs and foreign chemicals such as acetaminophen and halogenated aromatic hydrocarbons or those generated in the blood of uraemic patients. In this study, the GSHPx activities of both erythrocytes and plasma of haemodialysis patients were lower than those of the controls. These findings are consistent with the results of previous studies [31,36]. It is noteworthy that neither baseline ferritin level nor the intravenous iron infusion directly affected the enzyme activity of GSHPx in the erythrocytes.

GSH is usually the most important and abundant physiological thiol compound in tissue cells. It is an antioxidant that protects the cell against the deleterious effects of ROS and toxic compounds. In uraemic patients on regular haemodialysis, blood GSH level was reported to be increased [37] or decreased [6]. In addition, Ceballot-Picot *et al*. [6] found that GSH concentration is closely correlated with the degree of renal failure. Its depletion could be due to a decrease of GSH synthesis and/or to an increase of GSH consumption. The results obtained in this study demonstrated that patients with higher baseline level of ferritin have lower concentrations of total GSH and free thiols in plasma. Such negative correlation may be attributed to the increase of oxidative stress in the patients with higher iron stores. Deficiency of GSH and free thiols may lead to elevation of free radicals and oxidative stress in the tissue cells. In this study, we observed that after iron infusion, the plasma GSH and free thiols were increased in the patients of all 3 groups. This suggests that acute iron infusion may impose a greater need of GSH to dispose of ROS and free radicals, which play an important role in eliciting oxidative stress and oxidative damage in haemodialysis patients.

Moreover, our study showed that the GST activity was increased in the erythrocytes of uraemic patients and was further increased in the uraemic patients with the lowest level of serum ferritin. Interestingly, infusion of iron did not significantly change GST activity. This finding probably indicates that the uraemic milieu with its toxic metabolites may have caused maximal induction of this GSH-related defence system [36,38]. On the other hand, the observed decrease in plasma GST activity in all three groups of uraemic patients may be attributed to the ongoing uraemia-induced imbalance between oxidants and antioxidants.

Some previous studies demonstrated that uraemic patients undergoing chronic haemodialysis exhibited augmented lipid peroxidation in the erythrocytes. In plasma, a significant correlation between plasma MDA

and platelet MDA was observed [39] as thrombocytes are one of the principal sources of lipid peroxides. All three groups of patients showed significant higher levels of plasma MDA as compared to the controls ($P<0.05$ for group 1; $P<0.001$ for groups 2 and 3). However, patients with higher baseline ferritin levels had significantly and progressive higher levels of plasma MDA. In the patients of all three groups, the MDA levels were significantly increased after intravenous iron infusion. An interesting finding of this study was that the increment of plasma MDA level in group 2 patients was lower than those of the patients in the other groups under enhanced oxidative stress. An increased number of young cells such as reticulocytes in blood circulation of group 2 patients may play an important role.

Finally, with the dosage and infusion rate of intravenous iron used in this study, we found that the transferrin saturation was over 50% in many patients. We found a mild increase in LDH after the intravenous infusion in all patients, but only patients in group 3 showed significant increase in the blood lactate level after iron infusion. The increase in lactate may serve to protect the vascular components against ROS-mediated oxidative damage [40]. We believe that in patients with lower iron stores (e.g. <600–800 µg/l), the possibility of inducing free-radical toxicity will be significantly reduced using regular low-dose intravenous iron therapy.

Taken together, our findings suggest that the increased oxidative stress in uraemic patients on haemodialysis is exacerbated by the elevated baseline serum ferritin levels and intravenous iron infusion. It warrants further investigation as to whether the resultant oxidative damage is a contributory factor for the accelerated atherosclerosis frequently seen in haemodialysis patients.

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Editor's note

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