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Original Article

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Oxidative stress and ferritin levels in haemodialysis patients

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Abstract

Background. Increased oxidative stress (OS) and inflammation are associated with atherosclerotic coronary artery disease in haemodialysis (HD) patients. Ferritin may have other effects in addition to its role in storing intracellular iron. This study was performed to determine any relationships between markers of OS, nutrition and inflammation in HD patients with normal and high ferritin levels.

Methods. Our cohort comprised 34 maintenance dialysis patients on erythropoietin therapy and 22 healthy controls. HD patients were divided into two groups: 17 with normal (<800 ng/ml) and 17 with high (>800 ng/ml) ferritin levels, and we measured lipid profile, albumin, highly sensitive C-reactive protein (hsCRP), anti-oxidant enzymes [whole blood glutathione peroxidase (Gpx), serum superoxide dismutase (SOD), paraoxonase, arylestherase (AE) and total anti-oxidant status (TAOC)], anti-oxidants (vitamin C) and lipid peroxidation products [red blood cell malondialdehyde (RBC MDA)].

Results. Compared with controls, the HD patients had higher serum urea, blood pressure, triglyceride, hsCRP, RBC MDA, SOD and TAOC values and lower albumin, low-density lipoprotein cholesterol, apolipoprotein AI, paraoxonase, AE and whole blood Gpx activities. Serum vitamin C, uric acid, apolipoprotein B, total- and high-density lipoprotein cholesterol, apolipoprotein B MDA, and lymphocyte levels in the HD patients with normal and high ferritin levels were similar. The OS markers of HD patients did not differ, whether or not they received intravenous iron supplementation or had transferrin saturations <50% or ≥50%.

Conclusion. HD patients are in a higher oxidative state, which results in the reduction of total anti-oxidant capacity and also have an increased inflammation status. We could not find a relationship between ferritin level and OS markers in HD patients receiving erythropoietin.

Keywords: erythropoietin; ferritin; haemodialysis; inflammation; nutrition; oxidative stress

Introduction

Certain conditions may result in the disregulation of ferritin, the main intracellular iron storage protein, which could cause this protein to act as a pro-oxidant by releasing iron [1,2]. Limited evidence shows that elevated iron stores and high-dose intravenous (IV) iron therapy may exacerbate oxidative stress (OS) in haemodialysis (HD) patients [3]. However, the lowest level of serum ferritin that might induce oxidative tissue damage is unknown [4].

OS results from an imbalance between the production of free radical and anti-oxidant activity. There is currently no consensus on an ideal marker of OS. Oxidation products of lipids, proteins, thiols and DNA are the OS parameters most commonly measured in clinical studies [5]. The anti-oxidative defense mechanisms of the body include enzymatic anti-oxidants [superoxide dismutase (SOD), gluthathione peroxidase (Gpx)] and non-enzymatic ones (vitamin C, protein sulfhydril groups, β-carotene, uric acid and vitamin E) [6]. It is well established that in HD patients OS is increased and it is associated with increased cardiovascular morbidity and mortality [7,8]. Recent data also suggest linkages between OS, inflammation, endothelial dysfunction and malnutrition in uraemic individuals [9]. These factors probably are synergistic in their effects on atherogenecity and the risk of a cardiovascular event. Studies concerning the relationship between iron storage and OS in HD patients are few [10,11]. To the best of our knowledge, however, markers of nutrition, inflammation, atherosclerosis and OS have not been studied in dialysis patients with high, normal or low ferritin levels. Therefore, the present study aims to compare these markers in regular HD patients with normal or high ferritin levels who were receiving maintenance erythropoietin (EPO) treatment.

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Subjects and methods

Patient selection

Stable subjects who were older than 18 years and on regular out-patient HD were recruited for participation in this study. We evaluated the medical data and charts of a total of 49 patients in our dialysis unit. We used the following exclusion criteria: cigarette smoking, alcohol consumption, anti-oxidant vitamin supplementations, malignancy, vasculitis, evidence of acute or chronic infection, haemoglobinopathies, anti-lipemic, non-steroidal anti-inflammatory drugs, immunosuppressive therapy, active inflammatory conditions, hepatic or respiratory diseases and evidence of significant bleeding [decrease in haemoglobin (Hb) level >2 g/dl], blood transfusion and change in transferrin saturation (TSAT) (<30 or >50%) and ferritin levels (<100 or >800 ng/ml) during the preceding 6 months. Of the 49 patients originally screened, 34 met the following inclusion criteria: on HD for at least 6 months, receiving EPO for 6 months or longer and a ferritin level greater than 100 ng/ml. The study was performed in accordance with the Declaration of Helsinki and with the approval of the local ethics committee. An informed written consent was taken from all patients before they entered the study.

Dialysis strategies

The dialyses were carried out using a commercially available machine (Fresenius 4008 B device, Fresenius Medical Care, Germany) and a standard bicarbonate dialysate containing (in mM) 140 Na^+, 2.0 K^+, 1.5 Ca^{2+} and 0.5 Mg^{2+}. The characteristics of the sessions were the same for all the patients. They had HD three times a week for 4–5 h with synthetically modified cellulose (Diacap SMC 1.2, B. Braun, Melsungen, Germany) (*n*: 25) or haemophan (GFS plus 11, Gambro, Hechingen, Germany) (*n*: 9) membranes. The haemodialysers were not reused. The dialysis water used was water from a reverse osmosis treatment system (Aqua RO modular, Fresenius Medical Care, Bad Hamburg, Germany) equipped with an endotoxin filter. The quality of dialysis water was regularly checked according to recommended guidelines. The patients' vascular accesses were native radial arteriovenous fistulae (*n*: 29), grafts (*n*: 3) or permanent catheters (*n*: 2). The blood flow rate was 250–300 ml/min and the dialysate flow rate was 500 ml/min. Each patient complied with fluid and diet restrictions (which consisted of 1.2 g/kg/day protein, 50 mmol sodium, restricted potassium and phosphate) and maintained a constant ultrafiltration volume. The patients were on dialysis for the following underlying causes: glomerulonephritis (*n*: 9), tubulointerstitial nephritis (*n*: 2), chronic pyelonephritis (*n*: 3), tubular necrosis (*n*: 3), polycystic kidney disease (*n*: 3), hypertension (*n*: 5), diabetic nephropathy (*n*: 1), and renal agenesis/hipogenesis (*n*: 1), or for undetermined causes (*n*: 7). Of the cohort, four were receiving β-blockers, eight were receiving angiotensin converting enzyme inhibitors (ACEI) or angiotensin receptor blockers (ARB) and/or calcium channel blockers (CCB) for hypertension, 17 received calcitriol or calcidiol and 31 received calcium-containing phosphate binders. Ten patients had anti-HCV positivity.

Iron and erythropoietin supplementation

Iron-deficient patients (serum ferritin <100 ng/ml, TSAT <20%) received IV iron sucrose (*Venofer, Abdi Ibrahim*) at a dose of 100 mg per dialysis session during initiation and then weekly (two patients), biweekly (seven patients) or monthly (six patients) based on their Hb levels, iron balance tests and their clinical status for a mean of 31 ± 16 months (range: 8–63), intermittently. Functional iron deficiency (FID) was defined as follows: ferritin >800 ng/ml, Hb <11 g/dl and TSAT <20%.

Patients whose Hb was under 10 g/dl, ferritin ≥100 ng/ml and TSAT ≥20%, received maintenance doses of recombinant human erythropoietin (rHuEPO)-α (*Eprex, Santa Farma*) or β (*Neo Recormon, Roche*) (*n*: 15 and 19, respectively) subcutaneously. The target Hb levels were 11 to 12 g/dl. The initial dose of rHuEPO was 50–150 IU/kg/week and the maintenance dose 25–75 IU/kg/week. We stopped rHuEPO treatment when a patient's Hb exceeded 12 g/dl and restarted the maintenance dose when it fell below 12 g/dl. Sufficient iron was administered to maintain serum ferritin >100 ng/ml and TSAT >20% during EPO treatment. When one or the other of ferritin and TSAT levels were >800 ng/ml or 50%, respectively, IV iron administration was stopped, unless there was a suspicion of FID.

Study design

The enrolled HD patients were divided into two groups based on serum ferritin levels: a normal ferritin group (<800 ng/ml; *n*: 17, median 525 ng/ml, range 119–790 ng/ml) and high ferritin group (>800 ng/ml; *n*: 17, median 1131 ng/ml, range 811–1650 ng/ml). The 50th percentile for ferritin levels in our cohort was 800.5 ng/ml. We used 22 healthy individuals as controls who were not on any medication and whose gender and age distributions matched those of the HD subjects. All control subjects underwent detailed examinations and they had normal findings.

Laboratory measurements

Medical and demographic data were obtained for each subject from the dialysis charts. Blood pressure (BP) measurements were obtained in the seated position after 10 min of rest using a standard mercury sphygmomanometer in the morning or at noon before dialysis sessions. Body weight and height were measured in the fasting state and body mass index (BMI) was computed as weight in kilograms divided by height in metres squared. Subjective global nutritional assessment was used to evaluate the overall protein-energy nutritional status of all subjects [12]. On the basis of a subjective weighting of the data of medical history and physical examination, the patients were classified by the dietician into three groups: A, well nourished; B, mildly/moderately malnourished and C, severely malnourished.

Blood sample preparation

Pre-dialysis blood samples were collected 2 weeks before readministering iron therapy and then, HD patients were evaluated before the first dialysis session of the week. Blood was drawn from the antecubital veins of fasting patients into non-additive and EDTA-containing tubes, and was

processed in the laboratory immediately after collection. A part of the whole blood was frozen for measuring Gpx. For measuring SOD, red blood cells (RBC) were washed with saline and frozen after haemolysis. Sera and plasma were separated by centrifugation at 1500 *g* for 10 min. Plasma aliquots for measuring malondialdehyde (MDA) and serum aliquots for measuring vitamin C and total anti-oxidant status (TAOC) were kept at −70°C until the analyses were performed. To determine susceptibility of RBC and apolipoprotein (Apo) B-containing lipoproteins to oxidation, RBC and plasma samples were kept at −4°C and they were asssayed within 24 h. Other biochemical and haematological parameters were studied on the day the blood was collected.

Analysis

Serum glucose, total cholesterol (T-chol), triglyceride (TG), high-density lipoprotein cholesterol (HDL-chol), albumin, uric acid, calcium, phosphorus, iron and iron binding capacity (IBC) were measured using an autoanalyzer (Aeroset System Abbott, Abbott Laboratories, Diagnostic Division, Illinois, USA), complete blood count, including Hb, haematocrite (Hct) and lymphocyte, using an autoanalyzer (Abbott Cell-Dyn 3700SL, Abbott Laboratories, Diagnostic Division, Illinois, USA), Apo AI and Apo B by immunonephelometry (Dade Behring Marburg GmbH, Germany) and ferritin and intact parathyroid hormone (PTH) using chemiluminescent method (DPC Immulite 2000, Scientific Affairs, DPC Biermann, Germany). Highly sensitive C-reactive protein (hsCRP) was measured by a nephelometric method (Cardiophase hsCRP, BNII, Dade Behring, Marburg GmbH, Germany).

Low-density lipoprotein cholesterol (LDL-chol) concentrations were calculated according to Friedewald's formula [13]. The TSAT percentage was calculated according to the following formula: TSAT (%) = Iron/IBC × 100. True dialysis times (T), the intradialytic weight losses (UF) and patients' dry weights were obtained. Adequacy of dialysis (Kt/V) for urea was calculated using the single-compartment model of Daugirdas, standard urea removal ratio using [URR = 100 (1 − R), where R = post-dialysis urea/pre-dialysis urea] and protein catabolic rate per normalized body weight (nPCR, g/kg/day) using the formula recommended by the DOQI HD Adequacy Work Group [14].

We assayed serum paraoxonase activity in a glycine-sodium hydroxide buffer at pH 10.5 according to Eckerson *et al*. [15] and measured serum arylesterase (AE) activity using phenylacetate as substrate at pH 8.0, following the method of Haagen and Brock [16]. RBC susceptibility to lipid peroxidation was determined by RBC MDA formation, using the technique of Stocks *et al*. [17], and the results were expressed in terms of nmol MDA/g Hb; and Hb concentration was determined by the cyanmethmoglobin method [18]. In order to study lipid peroxidation in the Apo B-containing lipoprotein fraction (Apo B MDA), lipid peroxidation was assessed by measuring thiobarbituric acid-reactive substances (TBARS) after separating this fraction with the precipitation method [19]. TBARS were expressed as the MDA equivalent content per miligram of cholestrol (nmol MDA/mg chol).

TAOC was measured in serum using a commercial kit (Randox Laboratories, Antrim, UK) and the assay results are expressed as Trolox equivalent (mmol/l). RBC SOD and whole blood Gpx activities were determined using Randox kits (Antrim, UK) and the activies of the enzymes were expressed as U/ml. Serum vitamin C levels were assayed spectrophotometrically using the 2,4-dinitrophenylhydrazine method (normal range: 0.5–1.5 mg/dl) [20].

Statistical analysis

Clinical and laboratory data were expressed as mean ± SD. The numerical variables were compared with the Wilcoxon signed rank test in intragroup comparisons and with the Mann–Whitney U-test in intergroup comparisons. Kruskal–Wallis non-parametric analysis of variance (ANOVA) was used for the comparison of HD patients according to the tertiles. If significant, two-group comparisons were performed using the Mann–Whitney U-test. To compare the ratios of categorical variables, we used the chi-squared test. The relationship between serum ferritin and other parameters was estimated by Pearson's correlation analysis. The variables were studied with binary logistic regression analysis to determine whether they directly influenced hyperferritinaemia or not. All statistical analyses were done using the SPSS 13.0 statistical software (SPSS Inc., Chicago, IL, USA). The P-values ≤ 0.05 was considered significant.

Results

Age and gender distribution of the HD and control groups were similar. Compared with controls, the HD patients had higher serum urea, systolic and diastolic BPs, but lower Hb and Hct levels (Table 1). Various markers, such as ferritin, TSAT, Hb, albumin and Kt/V, were followed in 34 HD patients (Figure 1). At the initiation of the study, only serum ferritin levels of 34 patients (median 800, range 119–1650 ng/ml) were lower than the values in 2 (median 792, range 186–1840 ng/ml) and 4 months (median 806, range 142–1650 ng/ml) before the study ($P < 0.01$, Figure 1C). The patients with high ferritin levels had higher serum calcium, ferritin and TSAT and lower phosphorus levels than patients with normal ferritin

Table 1. Characteristics and biochemical and haematological data of the subjects in HD and control groups

	Healthy control (*n*: 22)	HD patients (*n*: 34)
Age (year)	35.8 ± 7.2	41.3 ± 12.4
Gender (M/F)	7/15	18/16
BMI (kg/m^2)	26.8 ± 5.2	23.5 ± 5.1*
Systolic BP (mmHg)	110 ± 17	126 ± 19**
Diastolic BP (mmHg)	67 ± 12	80 ± 13**
Hb (g/dl)	13.6 ± 1.3	10.8 ± 1.1**
Hct (%)	40.4 ± 3.6	32.6 ± 3.6**
Urea (mg/dl)	25 ± 6	165 ± 38**
Albumin (g/dl)	4.7 ± 0.2	4.1 ± 0.2**

HD, haemodialysis; M, male; F, female; BMI, body mass index; BP, blood pressure; Hb, haemoglobin; Hct, haematocrite.
*$P < 0.05$; **$P < 0.001$, compared with controls.

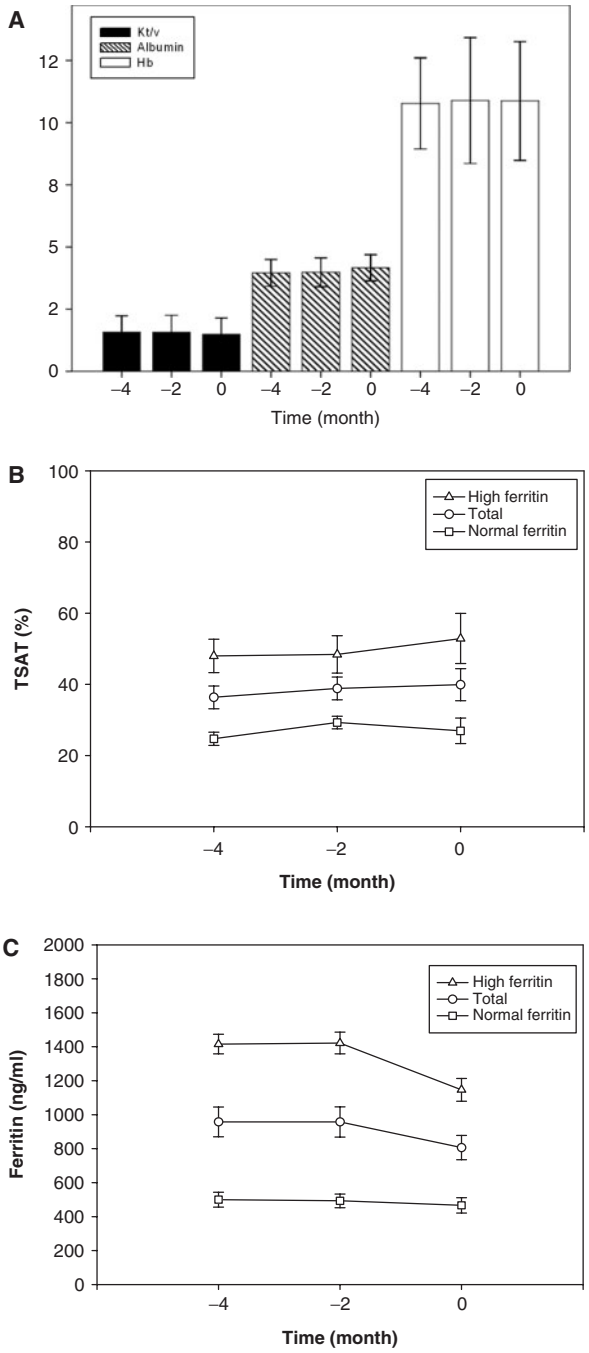

Fig. 1. The trends over time of the haemoglobin (Hb), albumin, Kt/V (mean ± SD) (**A**), serum transferrin saturation (TSAT) (**B**) and ferritin (mean ± SE) (**C**) in haemodialysis patients four and 2 months before and at the initiation of the study.

(Table 2). There were two patients with FID in the high ferritin group.

Serum LDL-chol and Apo AI levels, paraoxonase, AE and whole blood Gpx activities were significantly lower in HD patients than in controls; however, serum triglyceride, hsCRP, RBC MDA and TAOC and RBC SOD activities were higher. There was no difference in serum Apo B, T-chol and HDL-chol and Apo B MDA levels between the two groups. There was no difference

Table 2. Characteristics of HD patients with normal and high ferritin levels

	Normal ferritin (*n*: 17)	High ferritin (*n*: 17)
Age (year)	41.5 ± 14.8	41.1 ± 9.8
Gender (M/F)	11/6	7/10
BMI (kg/m^2)	23.3 ± 6.6	23.7 ± 3.2
Dialysis duration (month)	48.1 ± 26.9	89.1 ± 68.1
Interdialytic weight gain (kg)	2.7 ± 0.9	2.8 ± 0.7
Hb (g/dl)	10.6 ± 1.2	11.0 ± 1.1
Ferritin (ng/ml)	466 ± 185	1146 ± 275*
TSAT (%)	26.9 ± 14.8	52.8 ± 29.0*
EPO dosage (U/kg/week)	117 ± 60	127 ± 62
Iron dosage (mg/week) (*n*)	48 ± 25 (13)	37 ± 17 (2)
nPCR (g/kg/day)	1.24 ± 0.1	1.15 ± 0.2
Albumin (g/dl)	4.1 ± 0.3	4.2 ± 0.1
Ca (mg/dl)	8.5 ± 0.5	9.3 ± 0.6*
P (mg/dl)	5.7 ± 1.2	4.9 ± 1.0**
CaxP	48 ± 11	46 ± 10
PTH (pg/ml)	453 ± 291	387 ± 280
Kt/V	1.49 ± 0.2	1.49 ± 0.3
URR	71.8 ± 6.8	72.2 ± 5.4

HD, haemodialysis; M, male; F, female; BMI, body mass index; Hb, haemoglobin; TSAT, transferrin saturation; EPO, erythropoietin; nPCR, normalized protein catabolic rate; Ca, calcium; P, phosporus; PTH, parathyroid hormone; URR, urea removal ratio. *$P < 0.01$; **$P < 0.05$, compared with the other group.

between these parameters in the HD patients with normal and high ferritin levels (Table 3). Serum vitamin C and uric acid levels in HD groups with normal and high ferritin level were similar (0.38 ± 0.4 *vs* 0.27 ± 0.1 mg/dl and 7.3 ± 1.1 *vs* 6.6 ± 1.2 mg/dl, respectively, $P > 0.05$).

The BMI and serum albumin values of HD patients were lower than those of the control group but T-chol levels were similar (Tables 1 and 3). Between the HD patients with normal and high ferritin levels, there were no differences between BMI (23.3 ± 6.6 *vs* 23.7 ± 3.2 kg/m^2, respectively), serum T-chol (165 ± 32 *vs* 161 ± 47 mg/dl), albumin (4.1 ± 0.3 *vs* 4.2 ± 0.1 g/dl) and lymphocyte count (1.54 ± 0.4 *vs* 1.47 ± 0.5 × 10^3/mm^3) ($P > 0.05$). Subjective global nutritional assessment scores were A (well nourished) in all HD patients.

By bivariate correlation analysis, serum ferritin had a significant relation with serum calcium ($r = 0.442$, $P = 0.009$) and uric acid ($r = -0.401$, $P = 0.019$; Figure 2), but not with other studied parameters. When a logistic regression model was applied for all factors related to ferritin, each of age, gender, BMI, Hb (<11 or ≥11 g/dl), serum albumin (<4 or ≥4 g/dl), CRP levels, anti-HCV positivity and Kt/V (<1.2 or ≥1.2) were found to be unrelated to hyperferritinaemia (>800 ng/ml), but not to receiving IV iron supplementation [odds ratio (OR): 37, 95% confidence interval (CI): 2.1–675, $P < 0.01$].

Subgroup analysis

The HD patients were divided into three groups based on tertiles of serum ferritin levels as

Table 3. Lipid profile and parameters of the oxidative-antioxidative system of the control and patient groups

	Healthy control (*n*: 22)	HD patients (*n*: 34)	HD groups	
			Normal ferritin (*n*: 17)	High ferritin (*n*: 17)
TG (mg/dl)	113 ± 57	174 ± 81*	178 ± 89	171 ± 75
T-chol (mg/dl)	173 ± 33	163 ± 40	165 ± 32	161 ± 47
LDL-chol (mg/dl)	106 ± 29	87 ± 30**	89 ± 26	85 ± 35
HDL-chol (mg/dl)	44 ± 8	41 ± 7	40 ± 8	42 ± 6
Apo AI (mg/dl)	159 ± 21	110 ± 18***	108 ± 15	112 ± 21
Apo B (mg/dl)	85 ± 23	81 ± 24	82 ± 22	80 ± 27
Paraoxonase (U/l)	215 ± 108	113 ± 51***	100 ± 40	126 ± 58
AE (kU/l)	75 ± 34	48 ± 15**	48 ± 16	48 ± 15
Apo B MDA (nmol MDA/mg chol)	6.8 ± 0.7	6.8 ± 0.4	6.7 ± 0.4	6.9 ± 0.4
RBC MDA (nmol MDA/g Hb)	86 ± 15	102 ± 22**	107 ± 23	98 ± 22
Gpx (U/ml)	5196 ± 1501	2118 ± 720***	2009 ± 659	2214 ± 776
SOD (U/ml)	1243 ± 510	10609 ± 4905***	10198 ± 4652	11020 ± 5256
TAOC (mmol/l)	1.1 ± 0.2	2.1 ± 0.2***	2.0 ± 0.2	2.1 ± 0.2
hsCRP (mg/dl)	0.19 ± 0.1	0.66 ± 0.8**	0.72 ± 1.0	0.61 ± 0.5

HD, haemodialysis; TG, triglyceride; T-chol, total cholesterol; LDL-chol, low-density lipoprotein cholesterol; HDL-chol, high-density lipoprotein cholesterol; Apo AI, apolipoprotein AI; Apo B, apolipoprotein B; AE, arylesterase; RBC MDA, red blood cell malondialdehyde; Gpx, glutathione peroxidase; SOD, superoxide dismutase; TAOC, total anti-oxidant status; hsCRP, highly sensitive C-reactive protein.
*$P < 0.01$; **$P < 0.05$; ***$P < 0.001$, compared with controls.

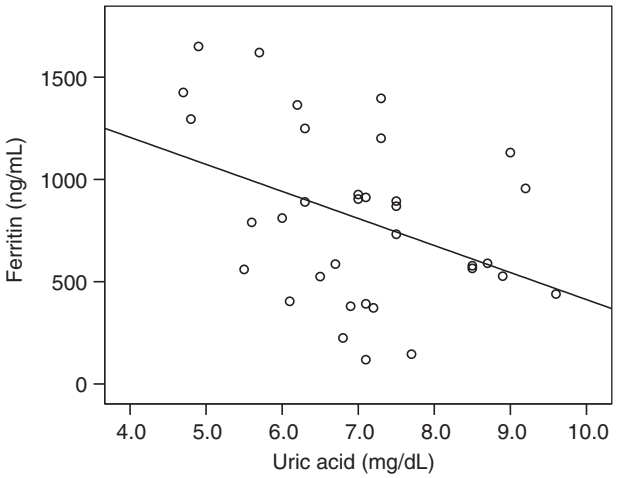

Fig. 2. Correlation between ferritin and uric acid in haemodialysis patients.

group I (median 392, range 119–560 ng/ml, *n*: 11), group II (median 800, range 565–912 ng/ml, *n*: 12) and group III (median 1295, range 926–1650 ng/ml, *n*: 11). When the parameters of OS were compared between these groups, only the AE activities of patients with the lowest ferritin tertile were higher than those of the other two groups (55 ± 15 *vs* 41 ± 15 and 49 ± 13 kU/l). The difference between group I and group II was significant ($P < 0.05$).

The OS markers of HD patients who either received IV iron or did not, and who had TSAT levels <50% or ≥50% were similar (Table 4). Also, the OS markers of HD patients who either received or did not receive ACEI, ARB or CCB as anti-hypertensive treatment did not differ from each other ($P > 0.05$).

Discussion

Uraemic patients have an increased cardiovascular risk that cannot be explained completely by traditional cardiovascular risk factors. Non-traditional cardiovascular risk factors, such as OS, abnormal calcium and phosphate metabolism, hyperhomocysteinaemia, malnutrition and inflammation syndrome, represent novel therapeutic targets for clinical interventions in this patient population [9]. Several reports have found in uraemic patients increased markers of protein oxidation (advanced oxidation protein products; AOPP) and lipid peroxidation (TBARS, MDA, 4-hydroxynonenal, oxidated LDL and esterified F2 isoprostanes) as well as of oxidation of carbohydrates and nucleic acids. A decreased free oxygen radical scavenger status has been suggested by measurements of low levels of vitamin C, selenium, RBC Gpx and SOD [6,21]. Human serum paraoxonase (PON1) is an HDL-associated esterase enzyme that is capable of hydrolysing lipid peroxides and exerts paraoxonase and AE activities [22]. PON1 is known to retard the oxidation of LDL by preventing and inhibiting the generation of lipid peroxides [23]. There is evidence that atherogenesis is accelerated in conditions accompanied with low serum paraoxonase activity [24]. In patients on HD maintenance, serum paraoxonase or AE activities are abnormally low [25]. We found an increase in serum TG, hsCRP, RBC MDA, TAOC and SOD activities, and a decrease in serum Apo AI, paraoxonase, AE and the whole-blood Gpx activities of HD patients compared with controls. These findings are in accordance with previous reports of increased OS and inflammation in HD patients [3,25–27].

Increased body iron stores may increase the oxidative consumption of anti-oxidants and may contribute

Table 4. Parameters of the oxidative–antioxidative system and of inflammation of HD patients according to iron supplementation status and TSAT levels

	Supplemantal iron		TSAT	
	No (*n*: 19)	Yes (*n*: 15)	<50% (*n*: 23)	≥50% (*n*: 11)
Paraoxonase (U/l)	128 ± 59	95 ± 31	104 ± 43	132 ± 61
AE (kU/l)	48 ± 16	48 ± 15	46 ± 13	52 ± 19
Apo B MDA (nmol MDA/mg chol)	6.9 ± 0.4	6.6 ± 0.5	6.8 ± 0.4	6.8 ± 0.5
RBC MDA (nmol MDA/g Hb)	103 ± 28	101 ± 12	106 ± 25	95 ± 12
Gpx (U/ml)	2280 ± 756	1910 ± 635	1945 ± 656	2449 ± 749
SOD (U/ml)	10423 ± 5407	10844 ± 4360	9921 ± 4338	12046 ± 5885
TAOC (mmol/l)	2.11 ± 0.3	2.11 ± 0.1	2.11 ± 0.2	2.13 ± 0.2
Vitamin C (mg/dl)	0.27 ± 0.1	0.4 ± 0.4	0.35 ± 0.4	0.28 ± 0.11
Uric acid (mg/dl)	7.1 ± 1.2	6.9 ± 1.3	7.0 ± 1.1	7.0 ± 1.5
hsCRP (mg/dl)	0.64 ± 0.6	0.7 ± 1.0	0.8 ± 0.9	0.39 ± 0.4

HD, haemodialysis; TSAT, transferrin saturation; AE, arylesterase; Apo B, apolipoprotein B; RBC MDA, red blood cell malondialdehyde; Gpx, glutathione peroxidase; SOD, superoxide dismutase; TAOC, total anti-oxidant status; hsCRP, highly sensitive C-reactive protein.

to the acceleration of the process of atherosclerosis in HD patients. The recent report of Drueke *et al.* [28] indicates that the carotid artery intima-media thickness is associated with plasma AOPP, serum ferritin and annual IV iron dose administered, and it supports the concept of a role of OS, iron and ferritin in the early stages of atherosclerosis in end-stage renal disease patients. Serum ferritin levels were found to be an independent determinant of the serum 8-hydroxy-2′-deoxyguanosine (8-OHdG) level, a marker of DNA oxidative injury, in HD patients [29]. In the same study, the serum ferritin and 8-OHdG levels of nine patients with serum ferritin levels >1000 ng/ml at the inception of the study decreased significantly during 6 months of follow-up without iron supplementation. We hypothesize that OS could be associated with ferritin levels in stable HD patients. Reddi *et al.* [10] did not find a difference either in anti-oxidant enzymes, anti-oxidants or lipid peroxidation products (plasma and RBC MDA) between dialysis patients who receive EPO treatment, most of whom also received vitamin C supplementation (20 of 27), with normal (<325 ng/ml) or higher than normal (>325 ng/ml) serum ferritin levels after 4 weeks of iron dextran treatment. In another study, Lim *et al.* [3] divided HD patients into three groups based on baseline serum ferritin levels of <300, 301–600, and >601 ng/ml, and found that OS was exacerbated by the elevated baseline serum ferritin levels and IV iron infusion. The patients with the highest serum ferritin levels showed the highest levels of plasma lipid peroxides and SOD activity and the lowest levels of RBC Gpx compared with the patients in the other two groups. These patients also showed the largest decrease in plasma SOD activity and increase in MDA after IV iron infusion. The same authors reported that the MDA levels of dialysis patients with the highest ferritin levels (657–1251 ng/ml) were significantly higher than those of the other two groups with lower ferritin levels (296–556 and 559–804 ng/ml, respectively) in another study [11]. Also, elevated serum ferritin levels affected the levels of the lipophilic anti-oxidants, such as lycopene, β- and α-carotene. The ferritin cut-off values in these studies were different from our study. Therefore, we also performed percentile subgroup analysis. But the analysis performed by splitting HD patients according to ferritin levels in two groups (< or >800 ng/ml) as well as tertiles (<563, 563–913 and >913 ng/ml) revealed no relationship between ferritin levels and OS and inflammation parameters, with the exception of serum uric acid. Furthermore, the TSAT level did not relate to OS markers. For the first time, we observed that PON and AE enzymes were similar in HD patients with normal and high ferritin levels. Although AE activity in HD patients with lower ferritins was higher in the tertile analysis, this finding needs to be confirmed by further studies.

As an acute-phase reactant, serum ferritin increases during an inflammation [4]. Albumin is the predominant oxidatively modified plasma protein in patients on HD. Oxidation of albumin will decrease plasma anti-oxidant defenses and increase the likelihood of OS-induced tissue injury and cardiovascular disease in these patients [30]. Although there were no malnourished patients or patients with inflammation in our cohort, our patients had higher hsCRP and lower albumin values than the controls. A recent study found significantly higher serum ferritin levels in HD patients who had one or both of malnourishment and inflammation, and it showed that serum CRP was significantly higher in those with a serum ferritin >800 ng/ml than in those whose serum ferritin was <800 ng/ml [31]. On the contrary, in HD patients with normal and high ferritin levels, we did not find differences in markers of inflammation (hsCRP) and malnutrition (BMI, lymphocyte, albumin and T-chol).

A limitation of the present study is its cross-sectional, observational nature, as well as the relatively small sample size. IV iron supplementation, iron formulation, prescribed anti-hypertensive agents and grade of anaemia could have influenced the oxidative status of our HD patients. Sodium ferric gluconate and

iron sucrose seem to produce more lipid peroxidation (increase in MDA) compared to iron dextran after a single IV dose, which was statistically significant for the first of these iron formulation [32]. A recent study showed that equipotent anti-hypertensive therapy with amlodipine and valsartan led to significant reductions in many parameters of OS [5]. It has been reported that the administration of EPO might have an anti-oxidant or pro-oxidant effect in HD patients [33,34]. Our findings did not parallel these observations. Furthermore, 1-year rHuEPO therapy without concomitant iron supplementation seems to exert no additional influence on coagulation, endothelial cell damage/activation markers or on OS in HD patients [35].

Our data do not support the presence of a relationship between ferritin level and OS markers in HD patients who receive EPO treatment. However, prospective long-term follow-up studies are needed to clarify the influence of iron stores and iron therapy on overall and cardiovascular morbidity and mortality in HD patients.

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Conflict of interest statement. None declared.

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