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Oxidants and antioxidants in long-term haemodialysis patients☆

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Abstract

Survival for decades is now possible in end-stage renal disease patients (ESRD) treated with haemodialysis (HD). Long-term survivors may present dialysis-related pathology (DRP). Alterations in lipid metabolism and oxidative stress are recognized as important risk factors that could be prevented or reduced by optimal therapy.

We have studied markers of oxidative stress in patients receiving HD treatment for more than 20 years. In order to evaluate a preventive intervention against oxidative damage we measured the factors implied for the prooxidative and antioxidative mechanisms in haemodialysis patients. Ten long-term HD survivors (HD duration: 274.2 months) and ten patients with recent onset of HD (HD duration: 17.8 months), had blood drawn for plasma vitamins A and E, malondialdehyde (MDA), plasma and RBC glutathione peroxidase (GPx), RBC superoxide dismutase (SOD), plasma and erythrocyte glutathione reductase (GSSG-R), oxidized and reduced glutathione (GSH) assessment.

Despite normal levels of antioxidant vitamins, an usual finding in this setting, increased MDA, and oxidized GSH, and decreased plasma GPx and reduced GSH show that oxidant stress is markedly present in both recent onset and long-term HD patients.

It would appear highly advantageous to reduce complications of long-term dialysis patients with preventing modalities. © 2001 Éditions scientifiques et médicales Elsevier SAS

Keywords: Oxidative stress; Antioxidants; Glutathione; Malondialdehyde; Haemodialysis

Survival for decades is now possible in end-stage renal disease patients (ESRD) treated with haemodialysis (HD). Long-term survivors may present dialysis-related pathology (DRP) with joints and bone amyloidosis, progressive wasting, skin fragility (the 'shrinking patient'). Among DRP, cardiovascular disease is the most frequent, being the major cause of mortality in haemodialysis patients. Alterations in lipid metabolism and oxidative stress are recognized as important risk factors that could be prevented or reduced by optimal therapy. Reactive oxygen species (ROS) have been implicated in various forms of cellular injury. ROS may cause cell damage and are involved in pathophysiology of several diseases, including atherosclerosis and chronic inflammation. Uremic patients have a decreased ability to withstand oxidative stress. It is postulated that antioxidant capacity is reduced, yet the mechanism remains unclear.

Oxidative stress and alterations in lipid metabolism are caused by haemodialysis mainly due to bioincompatibility type of reactions such as production of ROS by inflammatory cells due to complement-mediated or independent pathways, and the imbalance between oxidants and antioxidants due to the diffusive loss of hydrophilic vitamins such as ascorbic acid.

We have studied markers of oxidative stress in 20 patients receiving HD treatment for more than 20 years. Primary renal diseases included: chronic glomerulonephritis (four cases), nephroangiosclerosis (three cases), renal insufficiency (five cases), amyloidosis (one case), Alport syndrome (one case), Pyelonephritis (two cases), Polykystosis (two cases). The haemodialysis schedule was 4 h, 3 times/week, using cellulosique

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hollow fiber dialyzers and acetate (15 mM)-bicarbonate (20 mM) dialysate.

In order to evaluate a preventive intervention against oxidative damage we measured the factors implied for the prooxidative and antioxidative mechanisms in haemodialysis patients.

Ten long-term HD survivors (5F/5M; 68.0 years; HD duration: 274.2 months) and ten patients with recent onset of HD (5F/5M; 66.6 years; HD duration: 17.8 months) had blood drawn for plasma vitamins A and E, malondialdehyde (MDA), plasma and RBC glutathione peroxidase (GPx), RBC superoxyde dismutase (SOD), plasma and RBC glutathione reductase (GSSG-R), oxidized (GSSG) and reduced glutathione (GSH) assessment. Lipid determinations were obtained before dialysis after an overnight fast of at least 12 h. Blood was drawn on heparinate as anticoagulant; samples were immediately centrifuged at 1500 g for 5 min at 4°C. The plasma was subdivided and frozen (-80°C) until analysis.

Serum cholesterol and triglycerides were determined by enzymatic colorimetric assay, serum apoproteines A1 and B were measured by immunoturbidimetry.

Vitamins A and E, oxidized and reduced glutathione were determined using liquid chromatography. TBA reactants-MDA were determined using spectrofluorimetry method (SOBIODA-Grenoble. France), plasma antioxidant status (TAS), SOD, GPx and GSG-R activities were assayed by spectrophotometric methods, using available commercial kits (Randox Labs. Ltd, Grumlin, UK) with a ABX Cobas Mira Plus analyzer (Roche, F. Hoffman. La Roche and Co, Basle, Switzerland).

Statistical analysis: All data were expressed as the mean value $\pm$ SD. Data were analyzed using the Mann–Whitney test and also subjected to a multivariate analysis of variance (ANOVA). Differences were considered significant when the probability was $P < 0.05$.

Patients on haemodialysis, in comparison with control subjects had no significantly differences for TAS, for erythrocyte GPx, GSSG-R, SOD and total glutathione. We found significant differences for plasma GSSG-R and reduced glutathione when we compared the recent and long-term HD patients (Table 1).

Despite normal levels of antioxidant vitamins E and A, an usual finding in this setting, increased MDA and oxidized GSSG, and decreased plasma GPx and reduced GSH show that oxidant stress is markedly present in both recent onset and long-term HD patients compared with healthy control subjects. Such prooxidant status has deleterious consequences since it has been demonstrated that antioxidant status could modulate cell functions. These data indicate that HD patients have an impaired antioxidant response, which may be attributed in part, to plasma GSH deficiency. We also found that triglycerides were significantly higher in haemodialysis patients than in the control group [1].

These results suggest that patients on chronic haemodialysis are particularly prone to oxidative stress and that dialysis itself may worsen this condition. The study supports the view that the antioxidant system is largely inadequate.

Rather than a weakening of antioxidant defenses, the susceptibility of chronic renal failure patients to oxidative stress might be ascribed to free radical and reactive oxygen metabolite production and to increased levels of oxidizable substrates notably triglycerides with their unsaturated fatty acids.

Further studies will be necessary to establish the relationships between measures of oxidative stress and cardiovascular complications in chronic renal failure patients under haemodialysis and whether treatment with antioxidants may reduce oxidative stress or reverse adverse effects associated with haemodialysis.

Oxidative stress, which occurs when there is excessive free-radical production or low antioxidants levels, has been implicated as a causative factor in atherogenosis.

Table 1
Markers of oxidative results

		Haemodialysis patients		Healthy subjects
		Long-term	Recent	
Vitamin A	(μM/l)	5.10 ± 1.72	6.01 ± 1.42	1.75 ± 3.5
Vitamin E	(μM/l)	43 ± 12	43 ± 17	18.6 ± 34.9
TAS	(mM/l)	1.56 ± 0.14	1.63 ± 0.27	1.50 ± 0.20
TBARS-MDA	(μM/l)	3.15 ± 0.36	3.20 ± 0.52	2.0 ± 0.6
Plasma GPx	(U/l)	272 ± 69	253 ± 73	719 ± 100
RBC GPx	(U/g Hb)	35.4 ± 15.6	32.9 ± 10.5	40 ± 16
Plasma GSS-G-réductase	(U/g Hb)	68.4 ± 13.2	57.1 ± 9.0	53 ± 20
RBC GSS-G-réductase	(U/g Hb)	10.9 ± 4.0	9.3 ± 2.1	9.0 ± 4.3
RBC-SOD	(U/g Hb)	1166 ± 567	1206 ± 257	1450 ± 350
Oxidized glutathione	(mM/l)	0.56 ± 0.37	0.82 ± 0.32	0.15 ± 0.05
Reduced glutathione	(mM/l)	1.18 ± 0.4	0.78 ± 0.21	1.75 ± 0.15
Total glutathione	(mM/l)	1.74 ± 0.21	1.55 ± 0.24	1.90 ± 0.2

It would appear highly advantageous to reduce complications of long-term dialysis patients with preventing modalities. Preventive modalities, including use of biocompatible membrane, ultrapure dialysate, exogenous supplementation of antioxidant vitamins, extracorporeal removal of ROS and oxidatively modified substances, would appear highly desirable to reduce complications of long-term dialysis patients [2].

Vitamin E, in vivo and in vitro conditions, has been proposed to partially correct the lipid peroxidation in plasma and blood cell membranes, and apoptosis in peripheral blood leukocytes. New dialysis strategies [3] with an antioxidant approach to the protection against the oxidant stress could be used. Their rationale is based on the emerging role of vitamin E and ascorbic acid in counteracting some biological effects associated with oxidative stress.

Prolonged exposure of long-term HD survivors to this condition may favor the emergence of long-term complications of HD treatment and may require prolonged antioxidant therapy.

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