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Original Article

Oxidative stress and haemodialysis: role of inflammation and duration of dialysis treatment

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Abstract

Background. Oxidative stress has long been demonstrated in haemodialysis patients. However, the factors influencing their oxidative status have not been characterized extensively in these patients. Therefore, the present study was designed to investigate the influence of a large number of factors known to be associated with oxidative stress.

Methods. In the present cross-sectional study, we determined the plasma levels of lipid and protein oxidation markers in 31 non-smoking haemodialysis patients and 18 non-smoking healthy subjects, together with various components of the antioxidant system at the plasma and erythrocyte level.

Results. No influence of age, diabetes or iron overload on oxidative markers and plasma and erythrocyte antioxidant systems was detected in these haemodialysis patients. The lack of an association between iron overload and oxidative status may be related to the lower level of plasma ascorbate in haemodialysis patients, since ascorbate favours the generation of free iron from ferritin-bound iron. Interestingly, plasma C reactive protein (CRP) levels measured by highly sensitive CRP assay were correlated positively with plasma levels of thiobarbituric acid reactive substances ($r = 0.38$, $P < 0.04$) and negatively with plasma α-tocopherol levels ($r = -0.46$, $P < 0.01$). Moreover, significant inverse correlations were observed between duration of dialysis treatment and plasma levels of α-tocopherol ($r = -0.49$, $P < 0.02$) and ubiquinol ($r = -0.40$, $P < 0.05$).

Conclusions. Our results suggest that inflammatory status and duration of dialysis treatment are the most important factors relating to oxidative stress in haemodialysis patients.

Keywords: advanced oxidation protein products (AOPP); glutathione; haemodialysis; highly sensitive C reactive protein assay; inflammation; oxidative stress; vitamins

Introduction

Atherosclerotic cardiovascular (CV) diseases are recognized as the major cause of morbidity and mortality in uremic patients [1,2]. Development of accelerated atherosclerosis involves multiple risk factors. Some are similar to those reported in the general population such as age, tobacco smoking, diabetes, hypertension and dyslipidemia, whereas others are more specifically associated with the consequences of nephron loss and ensuing metabolic disturbances, such as hyperfibrinogenaemia, hyperhomocysteinaemia and oxidative stress. The pro-atherogenic effects of oxidative stress, particularly by the generation of oxidized low-density lipoproteins (LDL), have been reported in haemodialysis (HD) patients [3] who combine a massive generation of reactive oxygen species (ROS) at each dialysis session [4] with a chronic deficiency in the major antioxidant systems [5,6].

The role of potential oxidative stress-inducing factors such as aging [7], inflammation [8,9], diabetes mellitus [10] and iron overload [11] has not yet been extensively evaluated in these patients. Therefore, in the present study we investigated the impact of these factors, which may contribute to exacerbation of oxidative stress in HD patients, on circulating lipid and protein oxidation markers and on plasma and erythrocyte antioxidant systems.

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Subjects and methods

Patients

Thirty-one chronic HD patients entered the present study after having given informed consent. Smokers, patients with chronic hepatitis, haematological and inflammatory disorders, cancer or immunosuppressive therapy were excluded. The main characteristics of the 31 HD patients studied are presented in Table 1. The HD patients received thrice-weekly treatments to maintain a minimum Kt/V urea index of 1.2 per session. They were dialysed using bicarbonate-buffered dialysate produced with ultrapure water (CWP 100 system, Gambro, Sweden) with high bacteriological quality (< 0.005 endotoxin units/ml). Twenty-one of the patients were regularly receiving subcutaneous recombinant human erythropoietin (rHu-Epo) (Eprex®, Janssen-Cilag, Boulogne-Billancourt, France) at a mean dose of 95 ± 8 IU/kg/week. Nine of them were receiving parenteral maltate iron supplementation of 100 mg once a week (Maltofer®; Lucien, Neuilly-sur-Seine, France), whereas the remaining 12 were not. Doses of rHu-Epo were not different between those receiving iron supplementation and those who received none. Ten patients were not treated with rHu-Epo and did not receive iron supplementation. None of the HD patients received vitamin supplementation. Eighteen non-smoking healthy subjects (eight men and ten women, mean age 45 ± 9 years) were recruited as control subjects. In HD patients, blood (10 ml) was drawn from the arteriovenous fistula just before the dialysis session and was collected in standard tubes containing 5 mM EDTA, as was the case for the venous blood drawn from controls. Following centrifugation (600 g for 10 min at 4°C), plasma was stored at −80°C. Erythrocytes were washed three times with isotonic saline solution before storage at −80°C.

Biochemical determinations

If not otherwise indicated, chemicals were purchased from Sigma-Aldrich Chemical (Saint-Quentin Fallavier, France).

Table 1. Main characteristics of haemodialysis patients

Mean age ± SD (years)	64 ± 18
Gender (men/women)	15/16
Residual creatinine clearance (ml/min)	1.3 ± 0.6
Renal diseases[a]	
Glomerulonephritis	2
Interstitial nephropathy	2
Vascular nephropathy	5
Diabetes mellitus	6
Polycystic kidney disease	5
Others	11
Dialysis modalities	
Duration of follow-up (years)	6.0 ± 5.8
Dialysis time per week (h)	10.1 ± 2.6
Type of dialysis membrane at time of study	
Modified cellulose	13
Synthetic	18
Dialysate buffer	Bicarbonate
Dialysis dose assessed by urea Kt/V[b]	1.2 ± 0.2

Data are expressed as mean ± SD.
[a]Number of patients.
[b]Urea Kt/V = Ln (C_0/C_T), where C_0 = pre-dialysis urea concentration and C_T = post-dialysis urea concentration.

Plasma cholesterol, triglycerides, iron and transferrin were determined using routine methods on a Hitachi 917 analyser (Roche, Meylan, France). Albumin plasma levels were determined using an immuno-nephelometric procedure (Dade Berhing, Paris, France). Plasma levels of C reactive protein (CRP) were determined by immunoturbidimetric assay (Roche, Meylan, France), and at the time of the study by highly sensitive CRP assay (Dade Berhing, Marburg, Germany). Serum ferritin levels were determined by immunoassay (IMX; Abbott, Rungis, France). Haemoglobin concentration was determined by spectrophotometry (Coulter STKS; Coultronics, Margency, France).

Plasma levels of thiobarbituric acid reactive substances (TBARS) were measured by fluorometry, and plasma levels of advanced oxidation protein products (AOPP) and protein carbonyls by spectrophotometry as described previously [12].

Ascorbate, the reduced form of vitamin C, was measured by spectrophotometry and α-tocopherol, by high performance liquid chromatography (HPLC) procedure using UV detection at 292 nm as previously described [12]. Ubiquinol (coenzyme Q_{10}) was determined by HPLC procedure [13] after plasma extraction with an ethanol/hexane mixture (2/5 v/v). Separation was performed on a Satisfaction® column (RP18AB, 250 × 4.6 mm, 5 μm; CIL Cluzeau, Puteaux, France) using methanol/hexane (85/15 v/v) containing 20 mM ammonium acetate, as the mobile phase delivered at a flow rate of 1.7 ml/min. Total ubiquinol was detected by coulometry (Coulochem Model 5100; Eurosep, Cergy-Pontoise, France) in its oxidized form using a conditioning cell set at 400 mV, and an analytical cell set at −900 mV for the first electrode potential (E_1) and 200 mV for the second electrode potential (E_2).

Glutathione peroxidase (GSH-Px) and glutathione reductase (GSSG-Red) activities were measured both in plasma and in erythrocytes as described previously [6]. Enzyme activity in erythrocytes was expressed as micromoles of NADPH oxidized per minute and per gram of haemoglobin (IU/g Hb). Erythrocyte glutathione content was simultaneously determined as its reduced (GSH) and oxidized (GSSG) forms by a modified HPLC method [14] after deproteinization by 5% metaphosphoric acid. Separation was performed on a Satisfaction® column using a 10 mM phosphate buffer saline containing 50 μM sodium octyl sulfate and 2% acetonitrile as the mobile phase delivered at a flow rate of 1 ml/min. Cell potentials were set at $E_1 = 400$ mV and $E_2 = 800$ mV, and the conditioning cell at 1000 mV. The intracellular redox potential was evaluated by the GSSG:GSH ratio, GSSG being expressed as GSH equivalent (1 mol of GSSG corresponding to two equivalents of GSH). Erythrocyte copper zinc dependent superoxide dismutase (CuZn-SOD) activity was determined by spectrometry by monitoring the auto-oxidation of pyrogallol [6], one unit of SOD representing the amount of enzyme causing 50% inhibition of the rate of pyrogallol auto-oxidation.

Statistical analysis

Data have been expressed as mean ± standard deviation (SD). The Mann–Whitney *U* test was used to compare differences between HD patients and controls. Simple regression analysis and the Spearman correlation coefficient (*r*) were used to determine the relationships between the various parameters. Independent associations between one dependent variable and more than two independant variables were assessed by multiple regression analysis.

Results

Markers of lipid and protein oxidation and antioxidant systems

As shown in Table 2, markers of lipid peroxidation, i.e. TBARS, and of protein oxidation, i.e. AOPP and protein carbonyls, were significantly increased in HD patients. Concerning enzymatic antioxidant activities in erythrocytes, a significant reduction in CuZn-SOD activity ($P<0.0001$) together with a significant increase in the GSSG:GSH ratio ($P<0.001$) was observed in HD patients (Table 2), while there was no significant difference in GSH-Px and GSSG-Red activities. In contrast, at the plasma level GSH-Px and GSSG-Red activities were markedly decreased in HD patients. Plasma α-tocopherol levels were similar in HD patients and control subjects. However, they were significantly decreased when expressed as the ratio to lipid (cholesterol and triglyceride) levels ($P<0.001$). Plasma ubiquinol levels were significantly decreased in HD patients compared with controls ($P<0.01$) and more markedly so when expressed as the ratio to lipid levels ($P<0.001$). Plasma ascorbate levels were also significantly diminished in HD patients ($P<0.0001$).

Influence of factors involved in oxidative stress

When HD patients were divided into two groups according to the median value of age, no difference could be observed for plasma levels of lipid and protein oxidation markers, or for plasma and erythrocyte levels of antioxidant systems (Table 2). It should be noted that the mean age of HD patients aged $\leqslant 70$ years was comparable to that of controls (51 ± 17 and 45 ± 9 years, respectively (non-significant)). Moreover, when HD patients were divided into two groups according to either presence ($n=9$) or absence ($n=22$) of diabetes mellitus, no difference could be detected (data not shown). Of note, no correlation was found between the residual renal function determined by residual creatinine clearance and the plasma and erythrocyte concentrations of oxidative or antioxidative markers.

When considering inflammatory status as evidenced by plasma CRP levels determined at the time of the study and using the highly sensitive CRP assay, plasma CRP levels (16 ± 33 mg/L) were correlated positively with plasma TBARS levels ($r=0.38$, $P<0.04$) and negatively with plasma lipid-normalized α-tocopherol levels ($r=-0.46$, $P<0.01$). Although plasma CRP levels can vary over time quite substantially for each HD patient, the mean of the plasma CRP levels measured over 6 months using classical assay for each HD patient, was found to be associated with plasma highly sensitive CRP levels ($r=0.51$, $P<0.005$). Of note, plasma highly sensitive CRP levels were significantly negatively correlated with plasma albumin levels ($r=-0.51$, $P<0.003$).

An inverse correlation was also observed between duration of dialysis treatment and plasma α-tocopherol ($r=-0.49$, $P<0.02$) or ubiquinol ($r=-0.40$, $P<0.05$) levels (Figure 1), but not with other antioxidants.

Table 2. Plasma and erythrocyte levels of oxidative stress markers and antioxidant systems in 31 haemodialysis (HD) patients and 18 control subjects

	Controls ($n=18$)	HD patients ($n=31$)	HD patients $\leqslant 70$ years ($n=16$)	HD patients >70 years ($n=15$)
Mean age (years)	45 ± 9	64 ± 18^{b}	51 ± 17	77 ± 6^{d}
Plasma levels of oxidative stress markers				
TBARS (μmol/l)	2.27 ± 0.23	2.80 ± 0.41^{c}	2.69 ± 0.29	2.92 ± 0.49
AOPP (μmol/l)	45 ± 18	151 ± 45^{c}	152 ± 52	150 ± 43
Carbonyls (nmol/mg protein)	0.37 ± 0.09	0.55 ± 0.25^{a}	0.63 ± 0.29	0.57 ± 0.17
Erythrocyte antioxidant systems				
CuZn-SOD (IU/g Hb)	1.49 ± 0.23	1.05 ± 0.24^{c}	1.03 ± 0.20	1.07 ± 0.29
GSH (μmol/g Hb)	4.45 ± 1.62	3.27 ± 1.44^{a}	3.31 ± 1.32	3.23 ± 1.60
GSSG (μmol/g Hb)	0.66 ± 0.34	1.17 ± 0.41^{c}	1.21 ± 0.43	1.12 ± 0.39
GSSG:GSH ratio[e]	0.36 ± 0.25	0.89 ± 0.55^{b}	0.92 ± 0.66	0.86 ± 0.43
GSH-Px (IU/g Hb)	8.9 ± 2.2	7.8 ± 2.1	8.5 ± 1.7	7.0 ± 2.3
GSSG-Red (IU/g Hb)	5.6 ± 0.8	6.3 ± 1.4	6.2 ± 1.1	6.5 ± 1.7
Plasma antioxidant systems				
α-Tocopherol (μmol/l)	31.6 ± 5.7	28.6 ± 8.2	26.5 ± 5.6	31.0 ± 9.9
(μmol/mmol lipids)	5.0 ± 0.7	4.1 ± 0.8^{b}	4.1 ± 0.6	4.1 ± 1.0
Ubiquinol (μmol/l)	1.39 ± 0.31	1.11 ± 0.37^{a}	1.12 ± 0.37	1.10 ± 0.37
(μmol/mmol lipids)	0.22 ± 0.05	0.16 ± 0.05^{a}	0.18 ± 0.06	0.15 ± 0.04
Ascorbate (μmol/l)	78 ± 27	28 ± 24^{c}	29 ± 28	21 ± 14
GSH-Px (IU/l)	82 ± 29	24 ± 10^{c}	28 ± 10	19 ± 8
GSSG-Red (IU/l)	50 ± 19	37 ± 13^{a}	35 ± 10	32 ± 6

P values, by Mann–Whitney U-test for patients *vs* controls: [a]$P<0.01$, [b]$P<0.001$, [c]$P<0001$, and for HD patients $\leqslant 70$ years *vs* >70 years: [d]$P<0.0001$.

[e]where GSSG was expressed as GSH equivalent.

The increase in plasma ferritin levels observed in HD patients under erythropoietin (Epo) treatment compared with untreated patients (447 ± 209 µg/l *vs* 245 ± 181 µg/l, $P < 0.05$) was not associated with an increase in the level of lipid and protein oxidative stress markers (Table 3). A significant decrease in erythrocyte CuZn-SOD activity in rHu-Epo-treated HD patients was observed ($P < 0.05$).

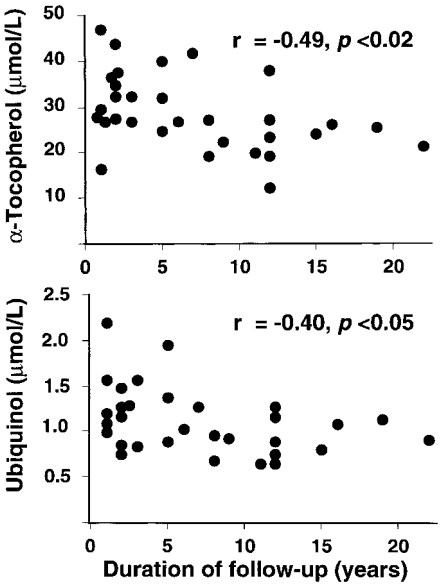

Fig. 1. Relationship between duration of dialysis treatment and plasma levels of α-tocopherol and ubiquinol for 31 HD patients. *r* indicates Spearman correlation coefficient.

Discussion

Our data confirm the presence of an increase in oxidative stress markers and decreased antioxidant defense in HD patients. Moreover, they point to a significant role of inflammatory processes and duration of dialysis treatment in HD-associated oxidative stress. In contrast, age, diabetes and iron overload were not found to be important contributive factors in these patients.

In our study, both lipid and protein oxidative markers were significantly increased in HD patients compared with controls. The more marked increase in plasma levels of AOPP, which are advanced oxidation protein products characterized by a high dityrosine content, compared with protein carbonyl levels could be explained by the lower susceptibility to proteolysis of the latter cross-linked proteins and their subsequent accumulation in plasma [15].

Concerning the antioxidant system, the significant decrease in erythrocyte CuZn-SOD activity observed in HD patients compared with control subjects ($P < 0.0001$) is in accord with previous reports [5]. While total erythrocyte glutathione concentrations were similar in HD patients and control subjects, interestingly the ratio between oxidized and reduced glutathione was significantly increased. This ratio is a reliable indicator of the cellular redox potential, and its increase could be due to oxidative stress at the cellular level. This occurs in spite of unchanged *in vitro* erythrocyte GSSG-Red and GSH-Px activities. The explanation for this discrepancy could be an impairment of the *in vivo* activity of these enzymes. Interestingly,

Table 3. Iron and oxidative status in HD patients treated with Epo receiving iron supplementation (Epo + Fe+) or not (Epo + Fe−) and non-Epo-treated (Epo−) HD patients

	(Epo−) ($n = 10$)	(Epo + Fe−) ($n = 12$)	(Epo + Fe+) ($n = 9$)
Iron status			
Hb (g/dl)	9.8 ± 1.0	10.0 ± 1.3	10.3 ± 1.4
Iron (mg/l)	12.5 ± 3.5	10.6 ± 4.0	11.4 ± 4.3
Transferrin (g/l)	1.8 ± 0.3	1.5 ± 0.3	2.0 ± 1.0
Saturation coefficient (%)	42.6 ± 7.7	32.6 ± 4.9^{b}	38.7 ± 6.4^{d}
Ferritin (µg/l)	245 ± 180	416 ± 205^{a}	487 ± 218^{f}
Oxidative stress markers			
TBARS (µmol/l)	2.73 ± 0.35	2.81 ± 0.55	2.87 ± 0.26
AOPP (µmol/l)	157 ± 56	135 ± 32	167 ± 52
Carbonyls (nmol/mg protein)	0.54 ± 0.39	0.51 ± 0.13	0.61 ± 0.16
Plasma antioxidant systems			
α-Tocopherol (µmol/mmol lipids)	4.03 ± 0.84	4.13 ± 1.06	4.18 ± 0.49
Ubiquinol (µmol/mmol lipids)	0.19 ± 0.05	0.15 ± 0.05	0.16 ± 0.06
Ascorbate (µmol/l)	25 ± 23	28 ± 23	18 ± 8
GSH-Px (IU/l)	28 ± 12	19 ± 10^{a}	25 ± 5
Erythrocyte antioxidant systems			
CuZn-SOD (IU/g Hb)	1.19 ± 0.20	0.84 ± 0.17^{c}	1.15 ± 0.16^{e}
GSH-Px (IU/g Hb)	7.4 ± 1.8	7.2 ± 1.9	9.0 ± 2.4
GSH (µmol/g Hb)	3.29 ± 1.25	3.27 ± 1.62	3.26 ± 1.50
GSSG:GSH ratio	0.82 ± 0.40	0.86 ± 0.42	1.02 ± 0.82

P values, by Mann–Whitney U-test for HD patients from (Epo−) *vs* (Epo + Fe−) groups: $^{a}P < 0.05$, $^{b}P < 0.001$, $^{c}P < 0.0001$, for (Epo + Fe−) *vs* (Epo + Fe+) groups: $^{d}P < 0.05$, $^{e}P < 0.01$; and for (Epo−) *vs* (Epo + Fe+) groups: $^{f}P < 0.01$.

an alteration in the pentose pathway has been reported in uremic patients [16] and thus a disturbed generation of NADPH, which is an essential cofactor of the glutathione-related enzymatic system.

In plasma, GSSG-Red and GSH-Px activities were effectively decreased. This diminution could be attributed to decreased enzyme synthesis in the kidney [6] or to an enzymatic protein alteration leading to their inactivation. Several modifications of protein structure and function occur in uremia, including oxidation, glycation and carbamylation. Thus, for instance, GSH-Px was shown to be particularly susceptible to inactivation by myeloperoxidase-derived hypochlorous acid [17]. High plasma levels of HOCl-modified proteins in HD patients, as reflected by AOPP, support a possible inactivation of enzymatic antioxidant systems. Moreover, such modifications could easily occur because of the marked reduction of non-enzymatic antioxidant systems in plasma, as observed in our HD patients.

It has been suggested previously that inflammation is associated with increased cardiovascular risk and mortality in uremic patients [9,18]. Elevated plasma CRP levels are associated with acute phase response; however, more recent epidemiological studies suggest that even small changes in CRP levels, up to 3 mg/l, are associated with increased cardiovascular risk, at least in the general population [19]. In our study, plasma CRP levels of HD patients were regularly determined every 3 months. For each patient, the mean value of plasma CRP levels was closely related to plasma highly sensitive CRP level ($r=0.51$, $P<0.006$). Those presenting chronically high plasma CRP levels were also found to be elevated with the highly sensitive CRP assay. The reason for the elevated plasma CRP levels in these HD patients was not clear. It is possible that an increase in production of cytokines such as tumor necrosis factor α, interleukin 1 (IL-1) and IL-6 during dialysis sessions was one of the underlying mechanisms [20]. On the other hand, we found that plasma highly sensitive CRP levels were correlated positively with plasma levels of lipid peroxidation products, TBARS ($r=0.38$, $P<0.04$) and negatively with those of lipid-normalized α-tocopherol ($r=-0.46$, $P<0.01$). Our results suggest that inflammatory processes inducing an increased production of ROS lead to the consumption of fat-soluble antioxidants and subsequently to the generation of lipid peroxidation products and oxidized LDL. Thus, inflammation and oxidative stress may act in synergy in a vicious cycle, leading eventually to accelerated atherosclerosis.

Interestingly, we observed an inverse relationship between duration of dialysis and liposoluble antioxidants, i.e. α-tocopherol and ubiquinol. Decreased plasma levels of α-tocopherol and ubiquinol have been previously reported in uremic patients on intermittent HD [21]. Since dialytic loss or malnutrition were unlikely in the patients of the present study (plasma albumin concentration was 36.2 ± 3.8 g/l), the decrease in lipid-normalized α-tocopherol and ubiquinol could be due to elevated consumption of these liposoluble molecules as antioxidants. Insufficient protection of increased amounts of lipids could lead to an increased susceptibility of plasma lipoproteins to oxidation. Thus it has been reported that very low-density lipoproteins (VLDL), which are generally increased in dyslipidemic uremic patients, showed an increased susceptibility to oxidation [22].

Finally, the potential role of rHu-Epo treatment and iron status in oxidative stress of HD patients must be addressed. Haemoglobin levels were close to 10 g/dl in the rHu-Epo-treated HD patients (Table 3). In these patients, we solely observed a significant decrease in erythrocyte CuZn-SOD activity compared with untreated patients ($P<0.05$). This decrease was observed even in patients treated with rHu-Epo alone, in the absence of concomitent iron supplementation, suggesting a direct effect of rHu-Epo or anaemia correction on CuZn-SOD activity, as reported in an other study [11]. Approximatively half of the rHu-Epo-treated patients received intravenous iron supplementation. This might promote the generation of ROS by metal ion-dependent oxidative process [23]. In our study, however, serum ferritin concentration was not related to the plasma or erythrocyte concentrations of oxidative or antioxidative markers. One possible explanation could be the lower plasma levels of ascorbate, i.e. the reduced active form of vitamin C, related to its ROS scavenging capacity and its transformation into dehydroascorbic acid. The decrease of plasma ascorbate levels is in favour of a mainly non-functioning vitamin C in HD patients [24]. Since ascorbate is a strong reducing agent, it can favour the generation of free iron from ferritin-bound iron and its release into plasma, and thereby induce lipid and protein oxidation [25]. Although ascorbate supplementation may be beneficial in increasing iron bioavailability and decreasing rHu-Epo resistance, it could also be harmful by increasing oxidative stress [26]. Therefore, controlled trials are needed to assess the safety and efficacy of ascorbate supplementation associated with an intravenous iron supplementation in rHu-Epo-treated HD patients.

In conclusion, inflammatory processes and the duration of dialysis treatment appear to be the main factors involved in the oxidative stress of HD patients. Conversely, oxidative stress may also induce inflammatory processes.

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References

1. Brown JH, Hunt LP, Vites NP, Short CD, Gokal R, Mallick NP. Comparative mortality from cardiovascular disease in patients with chronic renal failure. *Nephrol Dial Transplant* 1994; 9: 1136–1142
2. London GM, Drüeke TB. Atherosclerosis and arteriosclerosis in chronic renal failure. *Kidney Int* 1997; 51: 1678–1695

3. Maggi E, Bellazzi R, Falaschi F *et al*. Enhanced LDL oxidation in uremic patients: an additional mechanism for accelerated atherosclerosis? *Kidney Int* 1994; 45: 876–883
4. Nguyen AT, Lethias C, Zingraff J, Herbelin A, Naret C, Descamps-Latscha B. Hemodialysis membrane induced activation of phagocyte oxidative metabolism detected *in vivo* and *in vitro* within microamounts of whole blood. *Kidney Int* 1985; 28: 158–167
5. Paul JL, Sall ND, Soni T *et al*. Lipid peroxidation abnormalities in hemodialyzed patients. *Nephron* 1993; 64: 106–109
6. Céballos-Picot I, Witko-Sarsat V, Merad-Boudia M *et al*. Glutathione antioxidant system as a marker of oxidative stress in chronic renal failure. *Free Radical Biol Med* 1996; 21: 845–853
7. Olivieri O, Stanzial AM, Girelli D, Trevisan MT, Guarini P, Terzi M. Selenium status, fatty acids, vitamins A and E, and aging: the Nove study. *Am J Clin Nutr* 1994; 60: 510–517
8. Witko-Sarsat V, Friedlander M, Nguyen-Khoa T *et al*. Advanced oxidation protein products as novel mediators of inflammation and monocyte activation in chronic renal failure. *J Immunol* 1998; 161: 2524–2532
9. Zimmermann J, Herrlinger S, Pruy A, Metzger T, Wanner C. Inflammation enhances cardiovascular risk and mortality in hemodialysis patients. *Kidney Int* 1999; 55: 648–658
10. Miyata T, Maeda K, Kurokawa K, van Ypersele de Strihou C. Oxidation conspires with glycation to generate noxious advanced glycation end products in renal failure. *Nephrol Dial Transplant* 1997; 12: 255–258
11. Delmas-Beauvieux MC, Combe C, Peuchant E *et al*. Evaluation of red blood cell lipoperoxidation in hemodialysed patients during erythropoietin therapy supplemented or not with iron. *Nephron* 1995; 69: 404–410
12. Nguyen-Khoa T, Massy ZA, Witko-Sarsat V *et al*. Critical evaluation of plasma and LDL oxidant-trapping potential in hemodialysis patients. *Kidney Int* 1999; 56: 747–753
13. Kontush A, Reich A, Baum K *et al*. Plasma ubiquinol-10 is decreased in patients with hyperlipidaemia. *Atherosclerosis* 1997; 129: 119–126
14. Acworth IN, Bailey B. Protection against oxidants. In: Acworth IN, Bailey B, eds. *The Handbook of Oxidative Metabolism*. ESA Inc, Chelmsford, 1995, 1–13
15. Witko-Sarsat V, Friedlander M, Capeillère-Blandin C *et al*. Advanced oxidation protein products as a novel marker of oxidative stress in uremia. *Kidney Int* 1996; 49: 1304–1313
16. Yawata Y, Jacob HS. Abnormal red cell metabolism in patients with chronic uremia: Nature of the defect and its persistence despite adequate hemodialysis. *Blood* 1975; 45: 231–239
17. Aruoma OI, Halliwell B. Action of hypochlorous acid on the antioxidant protective enzymes superoxide dismutase, catalase and glutathione peroxidase. *Biochem J* 1987; 248: 973–976
18. Stenvinkel P, Heimbürger O, Paultre F *et al*. Strong association between malnutrition, inflammation, and atherosclerosis in chronic renal failure. *Kidney Int* 1999; 55: 1899–1911
19. Ridker PM, Cushman M, Stampfer MJ, Tracy RP, Hennekens CH. Plasma concentration of C-reactive protein and risk of developing peripheral vascular disease. *Circulation* 1998; 97: 425–428
20. Descamps-Latscha B, Goldfarb B, Nguyen AT *et al*. Establishing the relationship between complement activation and stimulation of phagocyte oxidative metabolism in hemodialyzed patients: a randomized prospective study. *Nephron* 1991; 59: 279–285
21. Triolo L, Lippa S, Oradei A, De Sole P, Mori R. Serum coenzyme Q10 in uremic patients on chronic hemodialysis. *Nephron* 1994; 66: 153–156
22. McEneny J, Loughrey CM, McNamee PT, Trimble ER, Young IS. Susceptibility of VLDL to oxidation in patients on regular haemodialysis. *Atherosclerosis* 1997; 129: 215–220
23. Cristol JP, Bosc JY, Badiou S *et al*. Erythropoietin and oxidative stress in haemodialysis: beneficial effects of vitamin E supplementation. *Nephrol Dial Transplant* 1997; 12: 2312–2317
24. Miyata T, Wada Y, Cai Z *et al*. Implication of an increased oxidative stress in the formation of advanced glycation end products in patients with end-stage renal failure. *Kidney Int* 1997; 51: 1170–1181
25. Dai LI, Winyard PG, Zhang Z, Blake DR, Morris CJ. Ascorbate promotes low density lipoprotein oxidation in the presence of ferritin. *Biochim Biophys Acta* 1996; 1304: 223–228
26. Tarng DC, Wei YH, Huang TP, Kuo BIT, Yang WC. Intravenous ascorbic acid as an adjuvant therapy for recombinant erythropoietin in hemodialysis patients with hyperferritinemia. *Kidney Int* 1999; 55: 2477–2486

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