

Lipid peroxidation, advanced glycation end products and antioxidant status in patients on dialysis

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Abstract

Oxidative stress increases the risk of cardiovascular disease in patients on chronic dialysis. In this study, we examined markers of oxidative stress and antioxidant status in haemodialysis (HD) patients in order to find out their relationship to the dialysis treatment. Study included 25 patients on long-term haemodialysis (HD) and 30 control subjects. Levels of malonyldialdehyde+4-hydroxyalkenes (MDA+4-HNE), advanced glycation end products (AGEs) and superoxide dismutase (Cu/ZnSOD) were assayed in plasma or serum.

Mean plasma level of MDA+4-HNE was 2-fold higher and AGEs 4-fold higher in HD patients, comparing to control subjects. Also Cu/ZnSOD were significantly elevated in dialysis patients. Positive correlation between AGEs as well as Cu/ZnSOD concentration and duration of HD treatment ($r=0,68$, $p<0,007$; $r=0,53$, $p<0,006$) was found. Significantly increased oxidative stress may accelerate atherosclerotic changes and cardiovascular complications in haemodialysis patients but seems to be, at least in part, counteracted by enhanced antioxidant capacity.

Introduction

Many reports have implicated increased lipid peroxidation associated with uraemia and dialysis treatment in patients with chronic renal failure [1,2,3,4,5]; however some authors did not confirm this relation [6]. Chronic renal failure is also characterized by the accumulation of advanced glycation end products (AGEs). AGEs accumulation in uraemia is of multifactorial origin and one of them is enhanced oxidative stress [7, 8]. Increased oxidative stress contributes to long-term complications such as cardiovascular

disease in dialysis patients [7, 9]. Data on antioxidant capacity in patients on dialysis are controversial. Previous studies suggested impaired protective effect of antioxidative defence [10], but on the other hand a huge elevation of serum superoxide dismutase was noticed, that may reflect increased antioxidant function [11,12].

The aim of this study was to evaluate oxidative stress and antioxidant status in haemodialysis patients in order to find out their relationship to the dialysis treatment.

Subjects and methods

We studied 25 patients with chronic renal failure (women and men aged 50 ± 11 years) on haemodialysis, four times a week for 4.5-5.0 hours with bicarbonate using cellulose triacetate membranes. All patients were stable at least 3 months before the study started. All subjects were treated in the Department of Nephrology, Dialysis Unit. Mean duration of dialysis treatment was 72 ± 62 months.

For comparison 30 healthy subjects matched for age and sex (women and men aged 47 ± 9 years) were investigated. None of them had clinical symptoms of peripheral or coronary artery disease, diabetes mellitus, dyslipidemia or renal insufficiency.

From HD patients blood was withdrawn from punctured fistula directly before haemodialysis session. From controls fasting venous blood was taken.

Measurement of MDA+4-HNE, AGEs and Cu/ZnSOD

For MDA+4-HNE and AGEs measurement, blood was drawn into sodium-EDTA tubes and plasma was separated after centrifugation at 4°C within 15 minutes after collection. Plasma was immediately frozen at -80°C.

Plasma MDA and 4-HNE were assayed by colorimetric method (LPO-586, Oxis Research). This assay is based on the reaction of a chromogenic reagent with MDA and 4-HNE at 45°C.

Determination of AGEs is based on spectrofluorometric detection according to Munch et al. [13]. Sodium-EDTA plasma was diluted 50-fold with PBS (pH 7,4) and fluorescence 350/460 nm was measured on Fluoroscan Ascent reader.

Serum Cu/Zn SOD was measured by ELISA (Bender MedSystems, Austria). The reference value for healthy subjects was 56.5 ± 20 ng/ml, range 22.5-102.9 (according to the manufacturer).

Other measurements

Serum total cholesterol (TC), HDL-cholesterol (HDL-C), triglyceride (TG), albumin, uric acid and creatinine levels were measured on an Hitachi 912 analyzer (Roche Diagnostics,

Germany). LDL-cholesterol (LDL-C) was calculated with the Friedewald formula.

Statistics

All data are expressed as mean \pm standard deviation (SD). The Mann-Whitney U-test was used for comparison between groups. Correlations between two variables were evaluated by Spearman's rank tests. $p < 0,05$ was considered significant.

The study protocol was approved by the local Bioethical Committee of Collegium Medicum, N.C. University in Bydgoszcz.

Results

Clinical and biochemical characteristics of patients and control subjects are given in Table 1. HD patients were characterized by abnormal triglycerides, creatinine and uric acid that were significantly higher than in control subjects and significantly lower HDL-C. We found twofold higher average concentration of MDA+4HNE and fourfold higher average fluorescence level of AGEs in HD patients, compared with control subjects. Cu/ZnSOD level was significantly higher in patients than in controls (Table 2).

The longer the dialysis treatment the higher concentration of AGEs and Cu/ZnSOD were found (Table 3). We did not, however, find any association between MDA+4-HNE levels and the dialysis period (Table 3). Neither MDA nor AGEs correlated with the levels of measured biochemical parameters in HD patients. Cu/ZnSOD was positively related with creatinine and uric acid concentrations in dialysis patients ($r=0,42$, $p=0,037$; $r=0,46$, $p=0,036$).

Discussion

In this study, we examined the oxidative stress and antioxidant capacity in patients on HD treatment with the use of four selected markers.

Both markers of oxidative stress, AGEs and MDA, were highly increased in HD patients in comparison to healthy subjects. Elevation of advanced glycation end products in HD patients has been known for some time [8]. Using the measurement of AGEs fluorescence, we found fourfold elevation of AGEs level in HD, comparing to controls. Recent data have demonstrated 4 to 10-fold elevations of AGEs in dialysis patients [14]. These differences however, may result from different methodologies used for AGEs determination. A variety of chemical structures of advanced glycation end products are being measured: non-fluorescent like N-carboxy-methyl-lysine (CML) or fluorescent-like pentosidines [13]. The method we used allowed only measurement of fluorescent AGEs in plasma.

Similarly, we found MDA level to be increased twofold in dialysis patients in comparison with healthy subjects, which suggests intensification of lipid peroxidation processes in the former group. Studies by others have shown that, especially before the dialysis session, there is a significant increase in plasma concentration of MDA [15,16], thiobarbituric acid reacting substances (TBARS) [17] and markedly enhanced oxidation of LDL particles [18]. Witko-Sarsat et al. also found increased level of proteins modified by peroxidation, advanced oxidation protein products (AOPP) [19] and Canestrari et al. demonstrated elevated ratio of oxidized glutathione to reduced glutathione [20]. All these findings indicate a significantly higher peroxidation process in haemodialysis patients than in healthy subjects. Pro-oxidant status in dialysis patients is mainly related with bio-incompatible

membranes and different substances from dialysate, which may cause inflammatory cell activation. In consequence vascular surface of HD patients is repeatedly exposed to the influences of cytokines, oxidative stress, coagulation products and vasoactive mediators. In addition, oxidative stress may be increased by the loss of water-soluble antioxidants during haemodialysis session.

The genesis of AGEs accumulation in uraemic patients remains to be clarified. Earlier observations have shown that AGEs levels were significantly higher in dialysis patients, comparing to diabetic subjects and appeared unrelated to elevated glucose levels in HD patients [21]. Pentosidine is mainly linked to albumin in the serum, so its accumulation cannot be attributed to a decreased removal by glomerular filtration. It was postulated that formation of AGE products such as pentosidine and CML is closely linked to oxidation process. AGEs were identified as products formed by oxidative cleavage of a glucose-derived Amadori compound. It was also demonstrated that glyoxal, which is formed after autoxidation of glucose, is an efficient precursor of AGEs. AGEs are thus products of the combined process of glycation and oxidation [22]. In addition, activity of 3-deoxyglucosone (3-DG) reductase is reduced in uraemic patients. This enzyme takes part in elimination of 3-DG, which comes from Amadori product. Accumulation of 3-DG contributes to AGE formation [23].

Significant positive correlation between the duration of haemodialysis treatment and the value of fluorescent AGEs confirms earlier findings and suggests that measurement of AGEs is a better marker of uraemic complications in haemodialysed patients than MDA concentration.

A huge elevation of serum superoxide dismutase may indicate increased antioxidative capacity in the circulation. On the other hand, high Cu/ZnSOD levels may be associated with overproduction of hydrogen peroxide, not beneficial in case of decreased activity of enzymes like glutathione peroxidase and catalase, that was mentioned earlier [11,24].

Elevated Cu/ZnSOD level was accompanied by hyperuricemia and positive correlation was observed by us between these parameters. Uric acid, a breakdown product of DNA metabolism, may be considered both as an antioxidant or a marker of cell damage [25,26,27]. Also Cu/ZnSOD can be released from the cells into circulation during late stage of apoptosis. Thus it is possible that high serum levels of Cu/ZnSOD in HD patients reflect, in part, apoptosis as well as enhanced antioxidant capacity.

In conclusion, hemodialysed patients have significantly increased levels of oxidative stress markers that seem to be, at least partly, counterbalanced by enhanced antioxidant capacity.

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Table 1 Biochemical characteristics of the subjects under study

Parameter	Control subjects n=30	HD Patients n=25	p
Age (years)	47 ± 9	50 ± 11	ns
Sex	26M, 17F	17M, 13F	-
TG [mg/dl]	95 ± 30	178 ± 101	< 0,001
TC [mg/dl]	189 ± 19	180 ± 44	ns
LDL-C [mg/dl]	108 ± 21	109 ± 35	ns
HDL-C [mg/dl]	66 ± 14	41 ± 10	< 0,001
Uric acid [mg/dl]	4,7 ± 1,0	6,7 ± 1,0	< 0,001
Albumin [g/l]	4,5 ± 0,5	4,2 ± 0,3	< 0,001
Creatinine [mg/dl]	1,0 ± 0,3	10,3 ± 2,4	< 0,001

Mean ± standard deviation (x ± SD), ns- not statistically significant

Table 2 - Markers of oxidative stress and antioxidant status in hemodialyzed patients and control subjects

Parameter	Control subjects n=30	HD patients n=25	p
MDA + 4-HNE [μ M/L]	3,9 \pm 1,4	8,4 \pm 2,4	< 0,001
AGEs [fluorescence]	7,8 \pm 1,2	30,1 \pm 6,3	< 0,001
Cu/ZnSOD [ng/ml]	99 \pm 5,1	427 \pm 82	< 0,001

Mean \pm standard deviation (x \pm SD)

Table 3 - Correlation between aemodialysis duration and concentration of MDA, AGEs and Cu/ZnSOD

Parameter	MDA+4-HNE	AGEs	Cu/ZnSOD
Duration of HD treatment	r = 0,13	r = 0,68	r = 0,53
	ns	p < 0,007	p < 0,006

- correlation coefficient