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Protein oxidation in chronic kidney disease

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Abstract. An imbalance between oxidative processes and antioxidant systems has been widely demonstrated in chronic kidney diseases (CKD). In this study we enrolled 26 healthy subjects, 27 patients with CKD on conservative treatment (CT-CKD) with various degrees of renal failure, and 31 CKD subjects in haemodialysis treatment (HD-CKD), evaluated before and after a standard haemodialysis session. In each group we measured protein carbonyl groups (PC) as an index of protein oxidation, lipid peroxidation (TBARS) and two plasma markers of leukocyte activation, elastase and myeloperoxidase (MPO). In CT-CKD subjects the PC level was significantly higher than in normal controls, and it was negatively correlated with creatinine clearance. In HD-CKD patients the PC concentration was significantly increased also in comparison with CT-CKD. An increase in TBARS was present both in CT-CKD and in HD-CKD patients, but in HD-CKD patients TBARS were lower than in CT-CKD. Elastase was increased in both CKD groups, while MPO was not different among control and patient groups. In HD-CKD patients the HD session was followed by a further increase in PC, as well as by an increase in elastase and MPO, whereas TBARS did not change. Protein oxidation accelerates the glycation processes and seems to be connected with the chronic inflammatory state detectable in renal failure, although we did not observe any significant correlation between PC level and leukocyte activation markers.

1. Introduction

Chronic kidney disease (CKD) is associated with enhanced oxidative stress, which has been widely investigated in its effects on lipids [9, 13].

When the reactive oxygen/nitrogen species attack the protein molecules protein carbonyl groups are produced (PC). PC measurement is a marker of protein oxidation [6], and it has been explored in CKD [2, 7, 9–11, 13–15]. Another indicator examined in CKD is the advanced oxidation protein products [3, 8].

We have previously [4, 5] examined elastase, myeloperoxidase and lipid peroxidation (thiobarbituric acid reactive substances, TBARS) in CKD subjects on conservative and haemodialysis treatment, observing an increase in elastase and in TBARS.

This research was aimed to evaluate PC in two groups of CKD patients, on conservative therapy (CT-CKD) or haemodialysis treatment (HD-CKD); in the latter the evaluation was made before and after a haemodialysis session; we also evaluated TBARS, elastase and myeloperoxidase, and their correlation with PC.

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2. Subjects and methods

2.1. Subjects

We enrolled 27 subjects (15 men and 12 women, mean age 58.2 ± 7.6 years) with clinically stable CT-CKD at stages 2–5 according to the K/DOQI classification. The cause of CKD was diabetic nephropathy in 6 patients, nephroangiosclerosis in 5, chronic glomerulonephritis in 3, polycystic kidney disease in 2. The cause was unknown in 11 patients. In this group creatinine was 3.15 ± 1.70 mg/dl, creatinine clearance was 26.63 ± 17.05 ml/min, leukocyte was 7721 ± 3092 μl, haemoglobin was 12.60 ± 2.15 g/dl. We also enrolled 31 HD-CKD patients (16 men and 15 women, mean age 61.5 ± 12.8 years). In this group the cause of CKD was nephroangiosclerosis in 9 patients, diabetic nephropathy in 6, chronic glomerulonephritis in 5. The cause was unknown in 11 subjects. Dialysis duration was 48.5 ± 35.7 months. We also studied, as control group, 26 healthy subjects (17 men and 9 women, mean age 43.54 ± 6.92).

2.2. Protein oxidation

PC were measured by an enzyme-linked immunosorbent assay (ELISA) kit (BioCell PC test kit, Enzo Life Sciences AG, Switzerland). It uses the reagent 2,4-dinitrophenyl-hydrazine (DNP), which reacts with the PC forming a stable hydrazone product. Plasma samples were incubated with DNP, and then plasma proteins were non-specifically adsorbed to the wells of an ELISA plate. Unconjugated DNP and non-protein constituents were washed away. The adsorbed proteins were probed with a biotinylated anti-DNP antibody, followed by streptavidin-linked horseradish peroxidase. A chromatin reagent was added, and the reaction was stopped by adding an acid solution. Absorbance for each well was measured at 450 nm and related to a standard curve prepared for serum albumin, containing increasing proportions of hypochlorous acid-oxidized protein, calibrated colorimetrically.

2.3. Lipid peroxidation

The oxidation of polyunsaturated fatty acids was evaluated in plasma by detection of the TBARS generated by peroxidative processes. The evaluation of TBARS was made by fluorimetry, using 1,1,3,3-tetramethoxypropane as standard.

2.4. Plasma elastase

Elastase was determined as elastase/α1-proteinase inhibitor complex (elastase/α1-PI) using the *Elastase ELISA kit* (Oncogene Research Products, San Diego, USA).

2.5. Plasma MPO

MPO was evaluated employing the *Myeloperoxidase ELISA kit* (Calbiochem, San Diego, USA).

2.6. Statistical analysis

Results were expressed as means ± S.D. The comparison among control subjects, CT-CKD and HD-CKD patients, was effected according to the ANOVA model, followed by the Bonferroni's test. The

Table 1
Means ± S.D. of oxidative parameters and leukocyte enzymes in control subjects and in CKD patients

	Control subjects	CT-CKD	HD-CKD	F
PC (nmol/mg prot)	0.440 ± 0.134	0.709 ± 0.107*	1.230 ± 0.192*§	203.2[b]
TBARS (nmol/ml)	6.35 ± 1.26	10.59 ± 2.0*	8.91 ± 1.48*§	35.7[b]
MPO (ng/ml)	48.6 ± 14.8	57.9 ± 27.1	51.0 ± 35.0	0.78
Elastase (ng/ml)	61.2 ± 19.0	92.2 ± 34.5 #	83.2 ± 24.6[¶]	6.68[a]

[a]$p < 0.01$; [b]$p < 0.001$ (ANOVA); [¶]$p < 0.05$; # $p < 0.01$; *$p < 0.001$ vs control subjects (Bonferroni's test); §$p < 0.001$ vs CT-CKD (Bonferroni's test). CT-CKD = chronic kidney disease on conservative treatment; HD-CKD = chronic kidney disease on haemodialysis treatment; PC = protein carbonyl groups; TBARS = thiobarbituric acid-reactive substances; MPO = myeloperoxidase.

Table 2
Means ± S.D. of oxidative parameters and leukocyte enzymes in HD-CKD patients before and after a dialytic session

	HD-CKD before dialysis	HD-CKD after dialysis
PC (nmol/mg prot)	1.230 ± 0.192	1.394 ± 0.352[#]
TBARS (nmol/ml)	8.91 ± 1.48	9.21 ± 1.71
MPO (ng/ml)	51.0 ± 35.0	118.1 ± 62.1*
Elastase (ng/ml)	83.2 ± 24.6	164.5 ± 86.6*

[#]$p < 0.01$; *$p < 0.001$ vs before dialysis (Student's t test for paired data).

Student's "t" test was used to compare HD-CKD patients before and after a haemodialysis session; the correlation was performed using the linear regression.

3. Results

In CT-CKD subjects we observed a PC increase; HD-CKD subjects had higher values of PC not only in comparison with control, but also in comparison with CT-CKD patients (Table 1).

An increase in TBARS was present both in CT-CKD and in HD-CKD patients, but in HD-CKD patients TBARS were lower than in CT-CKD (Table 1).

Elastase was increased in both CKD groups, while MPO was not different among control and patient groups (Table 1).

In CT-CKD patients there was a negative correlation between PC and creatinine clearance ($r = -0.46$, $p < 0.02$), while the correlation was not significant between TBARS and creatinine clearance. Neither PC nor TBARS were correlated with creatinine, leukocyte or haemoglobin. There was no correlation between PC and TBARS, nor was either of them related to elastase or MPO in any group.

In HD-CKD patients the HD session was followed by a further increase in PC, as well as by an increase in elastase and MPO, whereas TBARS did not change (Table 2).

4. Discussion

PC increase in CT-CKD suggests the presence of oxidative stress at the initial stages of the disease, but the literature data are controversial. In the paper by Mimić-Oka [12], the PC were increased in

CKD patients with creatinine clearance >50 ml/min, while in a recent study [15] PC were normal in two groups of CKD subjects, with mean creatinine clearance of 22 ml/min (IV CKD) and 12 ml/min (V CKD) respectively.

In CT-CKD we found a negative correlation between PC and creatinine clearance. This relation was also described by Aveles et al. [1], but not by others [13].

We had previously evaluated [4, 5], in different groups of CT-CKD and HD-CKD patients, TBARS, elastase and MPO and the results obtained now confirm the enhanced TBARS and elastase levels in CKD patients, without changes of MPO. Differently from PC, lipid peroxidation seemed to take advantage of HD treatment, being the pre-dialytic TBARS in HD-CKD lower than in CT-CKD.

As regards the effect of a HD session on the oxidation markers, once again PC behaved differently from TBARS. The latter, in accordance with previous data [4], did not change significantly after the HD session. However, the PC behaviour observed by us is not a constant finding in CKD. Other authors did not find any difference of PC before and after a standard HD session, as well as between CT-CKD and HD-CKD subjects [7].

We did not observe any difference in PC subdividing HD-CKD subjects according to dialysis vintage or to the different filters used in HD (data not shown). The literature data are contrasting about the influence of dialysis vintage: some authors [9] found an increase in PC when HD was continued for more than 12 months, while others [14] did not observe any variation. As regards the filters, Bordoni described a reduction in PC only when a high flux membrane was used, but did not note any difference between polysulfone and cellulose membranes [2].

Peritoneal dialysis might have a favourable impact on protein oxidation [3, 10]. After kidney transplantation, AOPP and PC levels decreased significantly [1].

In conclusion, protein oxidation is increased in CKD on conservative and on haemodialysis treatment and then it is useful to pursue therapeutic strategies able to oppose protein oxidation.

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