

The Determination of Oxidized Proteins and Albumin in Plasma of Chronic Kidney Disease Patients

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Abstract

Background: The study of oxidized proteins in blood of chronic kidney disease patients has been made without taking an initial clinical form of disease into account. The purpose of the research is to study oxidized proteins and albumin in blood plasma of patients with chronic glomerulonephritis and chronic pyelonephritis as an initial clinical form of chronic kidney disease. **Methods:** Blood plasma taken from 132 patients with various stages of chronic kidney disease and degree of chronic renal failure has been used for investigation. Blood of 32 healthy donors has been used for control testing. In blood plasma, modified proteins (protein reactive carbonyl products, advanced oxidative protein products), and albumin have been estimated. **Results:** In blood plasma of patients with chronic glomerulonephritis, as an initial clinical form of the chronic kidney disease, the significant increase in all types of oxidized proteins and significant decrease in albumin have been observed, comparing to the control samples. In blood plasma of patients with chronic pyelonephritis, as an initial clinical form of the chronic kidney disease, the significant increase in protein reactive carbonyl products has been observed comparing to the control samples only. **Conclusions:** the obtained results may unravel new targets for pharmacological intervention at an early stage of chronic kidney disease based on the initial clinical form of the disease.

Keywords: Chronic kidney disease; Plasma; Protein reactive carbonyl products; Advanced oxidative protein products; Albumin

Introduction

From year to year chronic damage of kidney parenchyma and interstitial tissue has become a very serious problem, eventually starting to get out of control. According to the large population-based registers, the prevalence of chronic kidney disease is at least 10%, reaching 20% or more in certain categories of people (the elderly, people with diabetes or secondary renal disease, etc.) [1]. Some kidney diseases have acquired continuously progressive character with the development of chronic renal failure (CRF) and its outcome in the terminal stage. That is why, fundamental studies of the chronic renal failure formation and progression mechanisms, especially at the predialysis stage, are so crucial.

Analysis of the previous results has shown that the study of oxidative stress is one of the most important directions of the development and progression of chronic kidney disease mechanism research (CKD) [2].

An increase in the protein reactive carbonyl products concentration in blood of the CKD patients associated with arterial hypertension has been noted [3]. An increase in the advanced oxidative protein products (AOPP) has been detected in blood of the CKD patients under dialysis [4]. A large proportion of total plasma antioxidant properties may be attributed to albumin. The previous results have shown the alteration of albumin concentration and imbalance of its oxidized and reduced forms in blood plasma of the CKD patients [5].

Unfortunately, the investigation of pathophysiological mechanisms CKD progression has been made without taking an initial clinical form

of disease into account. We have hypothesized that generation of oxidized proteins in blood plasma of the CKD patients might depend on the initial clinical form of disease.

The main purpose of the research is to study the concentration of oxidized proteins and albumin level in plasma of the CKD patients with different initial clinical forms of the disease.

Materials and Methods

Patients

132 patients with various stages of CKD and degree of chronic renal failure (CRF) participated in the study. There were 47 males and 85 females, age 53, 7 ± 5 , 7 years. The patients have been split into 4 groups consisting of 38 patients with chronic pyelonephritis and CKD of 1-2 stages (CRF 0), 21 patients with chronic glomerulonephritis and CKD of 1-2 stages (CRF 0), 44 patients with chronic pyelonephritis and 3 stage of CKD (CRF 1), 29 patients with chronic glomerulonephritis and 3 stages of CKD (CRF 1). Clinical examination of the patients has been conducted by Professor V. Molotov-Luchanskiy. 32 healthy volunteers, age- and sex-matched, formed a test control group. These volunteers were healthy, as determined by a medical history questionnaire, physical examination, and normal results of clinical laboratory tests and blood pressure.

Ethics

The medical ethics committee of the Medical University (Karaganda) has approved the study. All patients and volunteers have received the full information on possible inconveniences and complications at the blood sampling stage before giving their consent to participate

Methods

Peripheral venous blood from fasted healthy volunteers and fasted patients was collected into the tubes, containing the anticoagulant heparin. To obtain plasma samples, the blood was centrifuged immediately at $2000 \times g$ for 15 min. The plasma has been used within 1 hour of collection.

Determination of protein reactive carbonyl products

We measured the concentration of protein reactive carbonyl products using the protocol of Levine R et al. [6]. Triplicate aliquots of of plasma (0.8 ml) have been added with 0.2 ml of 10% trichloroacetic acid. The samples have been centrifuged and 1 ml of either 2M HCl or 10 mM 2, 4 - dinitrophenylhydrazine (DNPH) in 2M HCl have been added to the precipitates and incubated at 37°C for 90 min. After the samples have been centrifuged (8,000g, 10 min), the DNPH excess has been removed with ethanol-ethyl acetate 1:1 (v/v). The samples then have been resuspended in 6M of guanidine hydrochloride. Quantification has been performed using a spectrophotometer PD - 303 UV APEL (Japan) at an absorbance of 366 nm. Concentration of carbonyl derivatives has been calculated using the molar absorption coefficient of 22,000 mol/cm. Protein reactive carbonyl products values have been given in nmol/ml.

AOPPs determination

Determination of AOPPs has been based on spectrophotometric detection according to Witko-Sarsat et al. [7]. 200 μ l of plasma (diluted 1:5 with phosphate-buffered saline (PBS), 200 μ l of chloramin T (0-100 μ mol/L) for calibration, and 200 μ l of PBS have been mixed. 10 μ l of 1.16 M potassium iodide and 20 μ l of acetic acid have been added to each sample, and absorbance at 340 nm has been measured immediately against a blank containing 200 μ l of PBS, 10 μ l of KI and 20 μ l of 10% acetic acid. Concentration of AOPPs has been given in chloramine units (μ mol/ml). Albumin concentration was measured

using Albumin-Vital kit. Albumin concentration has been given in g/L.

Statistical Analysis

Data have been expressed as mean \pm SEM. Comparisons between the patients and the control groups have been performed using non-parametric Mann-Whitney U-test (for independent variables).

Results

The albumin concentration significantly decreased only in plasma of the CKD patients with chronic glomerulonephritis as an initial clinical form of the disease (Table 1). In plasma of the patients in all four groups, the concentration of protein reactive carbonyl products was significantly lower than in controls samples and did not depend on the initial form of CKD. AOPPs significantly increased only in plasma of the CKD patients with chronic glomerulonephritis as an initial clinical form of the disease to comparison with the control group (Table 1). We have observed that the beginning of CRF did not induce significant alteration of oxidized proteins in plasma of the patients of the 3rd and 4th groups.

Discussion

The obtained results demonstrated that at the early stages of CKD the accumulation of AOPPs and decreasing of protein reactive carbonyl products took place in plasma of the patients with chronic glomerulonephritis, as an initial form of the disease. The oxidative damage of proteins was accompanied by the albumin concentration decrease.

At the early stages of CKD, the decreasing of protein reactive carbonyl products took place only in plasma of the patients with chronic pyelonephritis, as an initial form of the disease.

Groups	AOPPs, μ mol/ml	Albumin, g/L	Protein reactive carbonyl products, nmol/ml
Group 1: Patients with CKD 1,2 (CRF0), (chronic glomerulonephritis as an initial clinical form of the disease)	0.80 \pm 0.19*	35.13 \pm 4.67*	0.41 \pm 0.03*
Group 2: Patients with CKD 1,2 (CRF0) (chronic pyelonephritis as an initial clinical form of the disease)	0.45 \pm 0.06	45.60 \pm 2.1	0.46 \pm 0.03*
Group 3: Patients with CKD 3 (CRF1), (chronic pyelonephritis as an initial clinical form of the disease)	0.65 \pm 0.23	46.25 \pm 3.17	0.49 \pm 0.053*
Group 4: Patients with CKD 3 (CRF1), (chronic glomerulonephritis as an initial clinical form of the disease)	0.59 \pm 0.09*	30 \pm 1,4*	0.40 \pm 0.06*
Control subjects	0.30 \pm 0.12	47 \pm 3.5	1.26 \pm 0.11

* in comparison with control subjects ($p \leq 0,05$)

Table 1: Oxidized proteins and albumin concentration in plasma of the CKD patients with chronic glomerulonephritis / chronic pyelonephritis as an initial clinical form of the disease (M \pm m)

Oxidative damage of proteins can have a wide range of metabolic and functional consequences, including increased susceptibility to aggregation, and to binding with blood cells. Those processes induce an impairment of biochemical and reological properties of blood. Decrease in albumin supports high level of oxidative stress. We

hypothesized that all those metabolic and functional disorders contribute to CKD progression more significantly in case of chronic glomerulonephritis as an initial form of the disease.

Conclusion

The obtained results have broadened our understanding of the pathways crucial to the CKD development depending on the initial form of the disease. It would be interesting to discuss new markers of CRF progression in CKD patients.

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