PROTEIN CARBONYL PRODUCTS IN BLOOD CELLS AT CHRONIC KIDNEY DISEASE

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Protein reactive carbonyl derivatives (RCD) content in erythrocytes and neutrophils in blood of patients with chronic kidney disease was investigated. The were four groups: 20 patients with nephrotic form of glomerulonephritis; 23 patients with hypertensive form of glomerulonephritis; 21 patients with pyelonephritis; 21 patients with pyelonephritis associated with arterial hypertension. Control subjects were healthy volunteers. The increasing of RCD content in neutrophils at patients of all groups were established. Two multidirectional trends of RCD content in erythrocytes were found. The probable role of RCD in erythrocytes and neutrophils in blood of patients with chronic kidney disease was discussed.

Keywords: protein reactive carbonyl derivatives, blood, chronic kidney disease

The participation of oxidative stress in the progression of kidney disorders is not doubt. Reactive oxygen intermediates and other prooxidants contribute to the development of kidney disorders by means of indirect effects on hemodynamics and adverse impact on selective permeability of glomerular membrane, provoke an acute and chronic inflammation and tissue destruction [1, 2, 3].

Besides of lipids as traditional target – prooxidants induce oxidative damage of proteins. There are different variants of modified proteins which formed depending on the types of adverse agent [4].

The increasing of the level of protein carbonyl groups, advanced oxidation protein products in blood plasma at patients with chronic renal failure and reduction of the concentration of sulfhydryl groups was described [5, 6, 7, 8]. The augmentation of protein carbonyl groups in blood of patients with chronic renal failure under regular hemodialysis was determined [9]. R. Inagi и T. Miyata [10] have created a hypothesis of participation of «carbonyl stress» in the development of complications of uremia. As has been showed later the modified proteins contribute to forming of complications of uremia by involving oxidative stress and inflammatory syndrome [11, 12]. Experimental – based results have demonstrated that possible mechanism of oxidized proteins negative effects may be connected with their participation in induction of renal fibrosis [13]. Our prior studies demonstrated an alteration of protein oxidative modifications in blood of patients at chronic pyelonephritis [14].

Great interest is studying the oxidation of proteins not only in plasma but also in blood cells, especially in neutrophils. Neutrophils are believed to play a fundamental role in mediating tissue injury with subsequent renal failure [15].

However, comparison of trends of protein carbonylation in blood cells under oxidative stress has not been performed.

The aim of our investigation was the comparative analysis of the protein carbonyl derivatives content in erythrocytes and neutrophils of patients with chronic kidney disease.

Materials and methods of research

The four groups of patients with chronic kidney disease in stage of clinical manifestations were formed. Chronic and irreversible renal structural changes proved by clinical, laboratory and instrumental studies. Anamnesis, the dynamics of clinical changes, results of laboratory testing of blood and urine were scrutinized. In difficult cases nefrobiopsiya was performed for confirmation of morphological types of nephritis. 20 patients with nephrotic form of glomerulonephritis were included in the first group. 23 patients with hypertensive form of glomerulonephritis were included in the second group. The third and fourth groups were represented by 21 patients with pyelonephritis and 21 patients with pyelonephritis associated with arterial hypertension correspondingly. Control subjects were healthy volunteers ($n = 15$) without any medication. All patients and healthy subjects were informed of any discomforts associated with the blood sampling before giving their consent to participate.

Laboratory methods

Blood sampling. Blood collected from the brachial vein (5 ml/sample) was drawn into Vacutainer tubes containing heparin in the morning after an overnight fast. Plasma was separated by low speed centrifugation at 4 °C.

For neutrophils separation we used the procedure of previously described [16]. Cells were then washed, counted, and resuspended in buffer. Purity and viability were assessed by trypan blue dye exclusion. The samples of > 85 % neutrophils with > 90 % viability were obtained. Since neutrophils are short-lived, they used within 2-4 hours of collection. Erythrocytes were washed three times in iso-osmotic saline and suspended in the physiological saline. Erythrocytes used within 2-3 hours of collection. Protein carbonyl derivatives were measured in erythrocytes by an adaptation of the method of Levine et al. [17] using the precipitates of deproteinised samples [18]. Spectrophotometric measurement of RCD values was performed and calculated using the extinction coefficient of DNPH-reactive carbonyl derivatives at 370 nm = 22,000 $mol^{-1}cm^{-1}$.

Results of research and their discussion

Data are expressed as mean ± SD (SD – standard deviation). Results compared using Mann-Whitney test for unpaired data commercial Statistica 7.0 package was used. Differences were considered significant when the P value was 0,05 or less.

The results obtained showed the increasing of RCD content in blood cells at patients of all groups in compare to control subjects (Table 1).

The most significant augmentation of RCD content was fixed in neutrophils at patients with pyelonephritis. Arterial hypertension did not provoke amplification of oxidative stress in neutrophils at patients with chronic kidney disease.

The tendency to increasing of RCD content in erythrocytes at patients of all groups in compare to control subjects was obtained. Herewith the analysis of RCD values distribution into each group demonstrated the presence of two multidirectional trends in changing of carbonyl derivates content. It did not allow us to choose the average of RCD and demanded to pool patients into two clusters (Table 2). RCD content in erythrocytes at patients of first cluster exceeded the control value ($p < 0,05$). At the same time substantial decreasing of RCD content was fixed in erythrocytes at patients of the second cluster in compare of control subjects and persons of the first cluster ($p < 0,05$).

Table 1

Comparison of the mean of RCD content in erythrocytes and neutrophils at patients with chronic kidney disease and normal controls

Patients with chronic kidney disease:	RCD content in	
	Erythrocytes nmol/ml	Neutrophils nmol/ 10^{-6}
Nephrotic form of glomerulonephritis, $N = 20$	10,77 ± 3,62	0,33 ± 0,06*
Hypertensive form of glomerulonephritis, $N = 23$	9,23 ± 4,11	0,35 ± 0,1*
Pyelonephritis, $N = 21$	12,44±4,18	0,51 ± 0,23*
Pyelonephritis associated with arterial hypertension, $N = 21$	11,89 ± 5,18	0,38 ± 0,14*
Control subjects, $n = 15$	8,24 ± 1,67	0,02 ± 0,009

The notes:
*Significant difference between control subjects and patients with chronic kidney disease, $P < 0,05$.

Table 2

Different pools of RCD content in erythrocytes at chronic kidney disease

Patients with chronic kidney disease:	RCD content (nmol/ml)
Nephrotic form of glomerulonephritis, $N = 20$	10,77 ± 3,62
Cluster 1 $N = 11$	14,39 ± 1,97*
Cluster 2 $N = 9$	6,25 ± 1,06#
Hypertensive form of glomerulonephritis, $N = 23$	9,23 ± 4,11
Cluster 1, $N = 12$	13,46 ± 4,09*
Cluster 2, $N = 11$	4,41 ± 0,91*#
Pyelonephritis, $N = 21$	12,44 ± 4,18*
Cluster 1 $N = 13$	15,26 ± 1,65*
Cluster 2 $N = 8$	5,04 ± 0,98*#
Pyelonephritis associated with arterial hypertension, $N = 21$	11,89 ± 5,18
Cluster 1 $N = 17$	15,60 ± 2,58*
Cluster 2 $N = 9$	4,89 ± 1,48*#
Control subjects, $N = 15$	8.24 ± 1,67

The notes:
*Significant difference between control subjects and patients, $P < 0,05$;
Significant difference between patients of cluster 1and patients of cluster 2, $P < 0,05$.

It is interesting to discuss increasing of intracellular concentration of oxidized proteins in neutrophils of all groups of patients. Accumulation of RCD in neutrophils suggests the development of intracellular oxidative stress. We suppose that the surplus content of carbonyl derivatives of proteins may be prerequisite for formation of scaffold for neutrophil extracellular traps (NETs).

According to accepted model [19, 20], the stages of NET formation include the destruction of nuclear envelope, disorganization of chromatin with following accession of neutrophil's granule proteins to its components, rupture of cell membrane and releasing complex including nuclear acids from neutrophil. The final formation of NETs occurs in blood. Reactive oxygen spe-

cies are involved in this process at the stages of the initiation and regulation of NETs formation. In this case we assume that the action of reactive oxygen species may be connected with carbonylation of histones and other chromatin proteins.

Earlier we described the changing of the ratio of H1, H2A, H2B, H3 and H4 histones in neutrophils at patients with chronic kidney disease [21]. This data may be useful for substantiation of our conjecture. The RCD affect on spatial structure of proteins and induce the formation the new binding sites. This position also corresponds to the adopted model of NETs.

We also suppose that the surplus intracellular carbonylation of proteins can promote the incorrect forming of NETs. The appearance in blood free components of NETs (free nuclear acids, histones, other proteins, RCD) or NETs with reduced efficiency exacerbates the disorders of endothelium and disturbance of hemostasis. Fuchs TA et al [22] have proposed the model of NETs participation in red thrombus formation.

The coexistence of erythrocytes with increased and decreased content of RCD is very interesting. We have offered special term for indicating this phenomenon – «Delta carbonyl derivatives». Such striking difference of clusters based on «Delta carbonyl derivatives» draws attention and demands explore the reasons of it. The phenomenon «Delta carbonyl derivatives» may be connected with imbalance of young and old erythrocytes in bloodstream at patients with chronic kidney disease. The erythrocytes demonstrate different age-related sensitivity to pro – oxidant action [23].

RCD formation is connected with oxidative damage of wide range of erythrocyte proteins. The proteins of cytoskeleton and membranes may be one of main targets for pro – oxidants action. Oxidative damage of cytoskeleton and membranes proteins may induce alterations of stability, deformability and ability of erythrocytes to reversible aggregation. Such red cells are subject to hemolysis into renal glomerular apparatus. Free hemoglobin has a strong toxic effect and aggravates of renal damage [24].

On the other hand, the presence in bloodstream of erythrocytes with decreased content of RCD makes it possible to surmise augmentation of rigidity of red cell membranes, leading to lower efficiency of gas-transport function. Arterial hypertension may be independent factor of influence; its role in maintaining of carbonyl stress must be clarified. In any case further research must be continued.

Thus, RCD are not only involved in presentation of the oxidative modification of proteins. RCD appear to be the active components of the second echelon of pro – oxidant attack, which impact on metabolism, functions and demeanor of erythrocytes and neutrophils.

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