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LIPID PEROXIDATION AND TOTAL SH GROUP IN PATIENTS WITH DIFFERENT FORMS OF GLOMERULONEPHRITIS

SUMMARY

Reactive oxygen species (ROS) are believed to be the most important intracellular signaling molecules involved in the pathogenesis of glomerulonephritis (GN). The aim of this study was to determine the concentration of lipid peroxidation products (TBARS-thiobarbituric acid reactive substances), parameters of oxidative stress in serum and urine, as well as the concentration of serum total SH groups as one of antioxidative parameters in patients with different forms of glomerulonephritis. Our study included 42 patients treated at the Institute of Nephrology and Haemodialysis, Clinical Centre Nis. All patients with various glomerular diseases were divided into four groups: I group (n=8) comprised patients with membranous glomerulonephritis (MN). II group (n=12) comprised patients with IgA nephropathy (IgA), while III group (n=10) recruited patients with lupus nephropathy (SLE). In the control group (IV group), there were 11 healthy subjects. The obtained results indicate that the level of TBARS in serum and urine patients with MN glomerulopathy was significantly decreased, while the concentration of total SH group was significantly increased compared to the control group. Linear correlation between TBARS as well as SH groups and creatinine clearance were established. Great importance of studying the role of oxidative stress for the onset of the illness or its complications in different forms of glomerulonephritis lies in the possibility of administration of antioxidants in the sense of therapeutic support and damage decrease.

Key words: **oxidative stress, glomerulonephritis**

INTRODUCTION

Reactive oxygen species (ROS) are believed to be the most important intracellular signaling molecules involved in the pathogenesis of glomerulonephritis (GN). In small doses, they are constantly produced in aerobic metabolism and play an important role in normal cell physiology during the process of signal transduction. However, in pathophysiological conditions with increased level of free radicals, these molecules become a relevant factor in initiation and spreading damage during inflammation, oncogenesis and degenerative diseases (1). Glomerulonephritis is an inflammatory disease characterized by morphological and functional changes in kidney followed by proteinuria, haematuria, azotemia, oliguria, oedema and hypertension. Increased oxidative stress is one of the most important pathogenic mechanisms involved in development of glomerulopathies. Important intracellular sources of free radicals production are cell membrane-bound, mitochondrial, and microsomal enzymes and many of these depend on present NAD(P)H as a substrate. This NAD(P)H-

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dependent enzymes are present in infiltrating leukocytes and residential mesangial cells in kidney tissue (2). In addition, xanthine oxidoreductase (XDH/XO) may be determined as very important source of ROS as demonstrated by increased xanthine oxidase activity and elevated XDH/XO mRNA and protein levels in renal tissue (3).

Membranous nephropathy glomerulonephritis (MN) is a common cause of nephrotic syndrome in adult population (50%). In this form of glomerular damage, it has been proved that free radicals are mostly produced by activities of NADPH oxidase and xanthine oxidase, followed by increase of cytochrome b_{558} in glomerular epithelial cells (4). In IgA nephropathy, intensive oxidative stress causes a great number of polymorphonuclears into kidney ti-sue, whereas in systemic lupus erythematosus (SLE), the main cause of glomerular damage is auto-antibodies formation and sedimentation of DNA-antiDNA complex in glomerulus with later complement activation. Produced inflammation in kidney is also repercuted to urinary tract, so measuring high level monocyte chemoattractant protein -1 (MCP-1) excretion and malondialdehyde in urine is better and more sensitive marker of oxidative damage (5).

AIMS

The aim of this study was to determine the parameters of oxidative stress in serum and urine of patients with different forms of glomerulonephritis. We determined the concentration of lipid peroxidation products (TBARS-thiobarbituric acid reactive substances) in serum and urine, as well as the concentration of total SH groups as one of antioxidative parameters in patients' serum and in control group of healthy subjects.

MATERIAL AND METHODS

Our study included 42 patients treated at the Institute of Nephrology and Haemodialysis, Clinical Centre Nis. All patients with various glomerular diseases were divided into four groups: I group (n=8) consisted of patients with membranous glomerulonephritis (MN). In II group (n=12) there were patients with IgA nephropathy (IgA). III group (n=10) included patients with lupus nephropathy (SLE). The control group (IV group) comprised 11 healthy subjects. All groups were matched for age and gender. Venous blood and 24-hour urine from patients and controls were collected, centrifuged and stored at -20^{0}C until assay.

Creatinine (mol/l) levels were measured with original kits using autoanalizer Bio Systems A25 in serum and urine and creatinine clearance rate was calculated. Serum TBARS levels (6) and urine TBARS (7) were determined by spectrophotometry at 532 nm after boiling the sample and condensing it with thiobarbituric acid (TBA). Results were expressed as mol/l for serum and mol/gr creatinine for urine. Concentration of total SH group in serum was determined by using 5-5'-dithiobis (2-nitrobenzoic acid) (DTNB)(8). Absorbance was measured at 412 nm against blank samples without DTNB and expresed as mol/l. All results were expressed as mean ± S.D. A Student's t-test and Mann-Whitney Rank Sum test were used to estimate differences between the groups. The criterion for significance was $p<0.05$. In addition, all patients were divided in three groups compared to creatinine clearance rate (CCr):

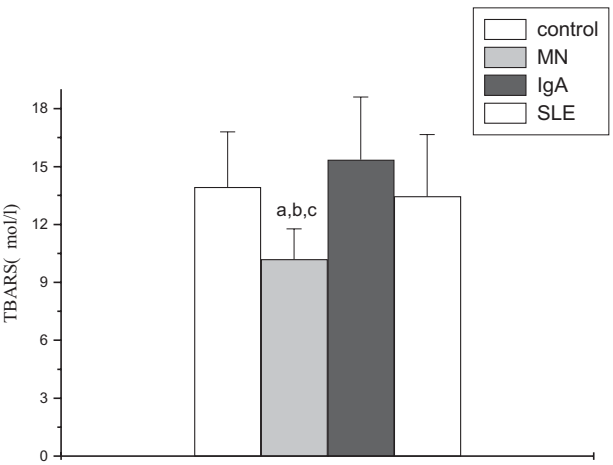
With CCr > 78 ml/min and normal kidney function

CCr- 48-78 ml/min and

CCr-< 48 ml/min.

RESULTS

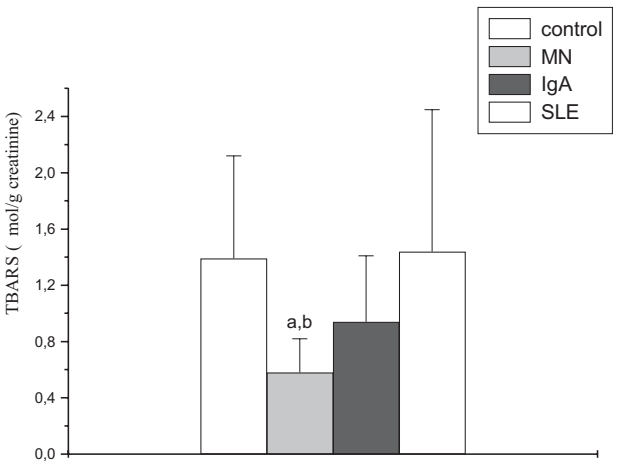
Serum TBARS level was 10,19±1,58 mol/l in patients with MN glomerulonephritis which was significantly lower than that of the control group (13,94±2,86; $p<0,005$), SLE group (13,46±3,19; $p<0,05$) and IgA group (15,36±3,23; $p<0,001$).



a-p<0.005 vs control; b-p<0.05 vs SLE group; c-p<0.001 vs IgAgroup

Figure 1. Concentration of TBARS in serum of patients with glomerulonephritis

As shown in Figure 2, concentration of TBARS in urine patients with MN glomerulonephritis was significantly lower, too, (0.58±0.24 mol/g creatinine) when compared to the control group (1.39±0.73; $p<0.01$) and SLE group (1.44±1.01; $p<0.01$).



a-p<0.01 vs control; b-p<0.01 vs SLE group
Figure 2. Concentration of TBARS in urine of patients with glomerulonephritis

In urine patients with IgA glomerulopathy we did not determine statistically significant difference (0.94±0.47).

Concentration of total SH group in serum was significantly decreased in MN group compared to all other groups (Table 1).

Table 1. Concentration of total SH group in serum of patients

	Number of patients	Total SH group (mol/l)
control	11	250.68±25.42
MN	8	181.38±36.42[a,b,c]
IgA	12	253.28±56.21
SLE	10	229.68±25.42

a-p<0.00 1 vs control; b-p<0.05 vs SLE group; c-p<0.05 vs IgA group

Table 2. Concentration of TBARS in urine, serum and concentration of total SH group in serum of patients compared to creatinine clearance

GROUP	Number of patients	TBARS (urine) mol/g creatinine	TBARS (serum) mol/l	Total SH (serum) mol/l
I (>90ml/min)	12	0.582±0.151[a,b]	11.24±1.94 [c,d]	257.57±47.85 [e,b]
II (48-90ml/min)	10	1.487±0.984	15.38±4.06	189.40±34.40
III (<48ml/min)	8	1.42±0.70	14.92±3.34	200.67±35.94

a-p<0.001 vs II group; b-p<0,01 vs III group; c-p<0.05 vs II group; d-p<0,005 vs III group ; e-p<0.005 vs II group

Table 2 presents the linear correlation between concentration of serum and urine TBARS as well as total SH groups and creatinine clearance rate. In serum and urine patients with normal kidney function, concentration of serum and urine TBARS were significantly decreased compared to patients with renal failure and diminished creatinine clearance (48-90ml/min and <48ml/min).

The concentration of total SH group was statistically significantly higher in patients with good creatinine clearance compared to the two other groups.

DISCUSSION

Glomerulopathies are characterized by glomerulosclerosis, tubulointerstitial sclerosis and tubular dilatation, periglomerular infiltration of macrophages/monocytes and myofibroblasts (9). Early cell proliferation followed by subsequent fibrosis is a prominent hallmark of proliferative glomerulonephritis, and it may ultimately lead to the end stage renal diseases. Oxidative stress can cause glomerular injury, not only by inflicting direct damage on glomerular structures, but also by inducing ERK (mitogen-activated protein kinase) activation and cell proliferation (10).

Therefore, produced free radicals destroy the basal membrane of glomerulus, disturb tubular function, degrading collagen type IV and other components of matrix, disturb electrostatic barrier, which leads to proteinuria (11). After H_2O_2 infusion in left renal artery, dose-dependent urinary increase of protein excretion occurs, due to the damage of capillary wall, without visible ultrastructural abnormalities (12). Obtained results in this study show that TBARS concentration in urine and serum patients with IgA and SLE nephropathy does not significantly change in comparison to the control group. A decrease proved in patients with membranous glomerulonephritis could be the consequence of intensive inclusion of antioxidative protection, since a significant decrease of total SH group in serum was proved in the same group. Data from literature show that proved oxidative stress in active Heymann nephritis (membranous nephropathy in humans) starts with increase of TBARS in the 9th week, with maximum peak in the 23rd week, having reduction of total antioxidative capacity (13). During acute experimentally caused glomerulonephritis, concentration of lipid peroxide products significantly rises on the 2nd, 3rd and 12th day in plasma and kidney tissues, with decrease of total antioxidative plasma capacity (14). Literature data also show that patients with lupus glomerulonephritis have increased concentration of lipid peroxides which have positive correlation with the level of proteinuria.

Contradiction obtained in this study is the consequence of personality of each patient, duration of illness and generally good reaction to given therapy. In order to clarify the obtained results, all

patients, regardless of the form of glomerulonephritis, were divided into three experimental groups in respect to creatinine clearance. Data worked out in this way show that the level of oxidative damage greatly depends on the level of kidney damage, since the concentration of TBARS is significantly increased in serum and urine in patients with diminished creatinine clearance. In I group comprising 12 patients with normal creatinine clearance (>78ml/min), there were eight patients having MN glomerulonephritis (of 11 patients in total), which partially explains decreased oxidative stress in this group of patients. Proved oxidative stress in patients with chronic glomerulonephritis followed by increase of lipid peroxides and NO in plasma and erythrocytes, having decrease of antioxidative enzymes, depends on creatinine increase of concentration and illness duration (15). At the same time, concentration of SH groups is greatly decreased and is directly dependant on the level of renal function degradation. Follow-up of the patients with different forms of glomerulonephritis showed increase of oxidized glutathione in erythrocytes, having diminished possibility of GSH regeneration and GSH-GSSG relation normalization in period of recurrence (16).

The oxidant-antioxidant imbalance may contribute to pathogenic changes in different types of glomerulonephritis. Great importance of studying the role of oxidative stress for the onset of the illness or its complications in different forms of glomerulonephritis lies in the possibility of administration of antioxidants in the sense of therapeutic support and damage decrease.

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LIPIDNA PEROKSIDACIJA I UKUPNA SH GRUPA KOD BOLESNIKA SA RAZLIČITIM OBLICIMA GLOMERULONEFRITISA

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SAŽETAK

Reaktivne kiseonične vrste (ROS) su veoma važni signalni molekuli uključeni u patogenezu glomerulonefritisa (GN). Cilj ovog rada bio je da se kod bolesnika sa različitim formama glomerulonefritisa odrede parametri oksidativnog oštećenja lipida (TBARS) u serumu i urinu, kao i koncentracija ukupnih SH grupa u serumu bolesnika i kontrolnoj grupi zdravih ispitanika. U radu je ukupno obrađeno 42 ispitanika lečenih na Institutu za nefrologiju i hemodijalizu Kliničkog centra u Nišu koji su podeljeni u 4 eksperimentalne grupe:

I grupa obolelih od membranoznog glomerulonefritisa (MN)- 8 bolesnika;

II grupa obolelih od IgA nefropatije (IgA)-12 bolesnika;

III grupa obolelih od Sistemskog Lupusa Eritematodesa sa istoimenom glomerulopatijom (SLE)-10 bolesnika i

IV grupa zdravih ispitanika (kontrola)- 11.

Dobijeni rezultati pokazuju da je koncentracija TBARS statistički značajno smanjena u urinu i serumu bolesnika sa membranoznim glomerulonefritom uz porast koncentracije SH grupa. U odnosu na klirens kreatinina, sa smanjenjem klirensa i propadanjem bubrežne funkcije raste koncentracija TBARS i smanjuje se koncentracija SH grupa.

Ovo može biti posledica multifaktorijalnog karaktera same bolesti kao i metaboličkog odgovora specifičnog za svakog bolesnika pojedinačno. Primena antioksidanasa imala bi za cilj dalje poboljšanje osnovne bolesti i nastalih komplikacija.

Ključne reči: **oksidativni stres, glomerulonefritis**