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Content of Carbonyl Compounds and Parameters of Glutathione Metabolism in Men with Type 1 Diabetes Mellitus at Preclinical Stages of Diabetic Nephropathy

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The content of carbonyl compounds (methylglyoxal and TBA-reactive substances) and components of the glutathione system (activities of glutathione-dependent enzymes, content of oxidized and reduced glutathione) and their interrelationships were studied in men of young reproductive age with type 1 diabetes mellitus at the stages of normo- and microalbuminuria. In patients with normoalbuminuria, the level of methylglyoxal, reduced and oxidized glutathione, and glutathione reductase activity were increased and the content of TBA-reactive substances was decreased. In the group with microalbuminuria, an increase in content of methylglyoxal and activity of glutathione-dependent enzymes relative to the control values were observed; the content of TBA-reactive substances was increased and glutathione reductase activity was decreased relative to the group with normoalbuminuria. In patients with microalbuminuria, a strong correlation between the mean glomerular filtration rate and the blood level of methylglyoxal was revealed.

Key Words: *carbonyl compounds; glutathione-dependent enzymes; type 1 diabetes mellitus; microalbuminuria; men*

Diabetes mellitus (DM) remains one of the main socially significant diseases. The number of patients suffering from DM has increased by 2.8 times over the past 17 years and continues to increase every year [14]. In the Russian Federation, more than 250,000 people live with type 1 DM (DM1). Kidney damage in this form of DM and further progression of diabetic nephropathy is a serious problem that impairs quality of life and leads to early disability and premature death of patients [11]. Despite available algorithms for screening this complication among patients with DM1, the identification of the mechanisms of the development of diabetic nephropathy remains an urgent problem. According to the modern classification, there are three stages of diabetic nephropathy: albuminuria, proteinuria, and renal failure [9]. The last two stages are irreversible, because by the time proteinuria occurs, 50-70% of the renal mass is already undergoing sclerosis. According to current data, 29.5% of patients with DM1 have diabetic nephropathy in the stage of albuminuria [11]. With timely prescribed therapy, this stage of diabetic nephropathy can regress. However, the efficiency of currently available therapeutic options remains low and cannot stop disease progression [9,11].

An important factor in the development of diabetic complications is oxidative glycosylation that includes glucose autooxidation, formation of dicarbonyl intermediates, and binding of oxidized carbohydrates to proteins [10]. The accumulation of natural dicarbonyls (methylglyoxal and TBA-reactive substances), secondary reaction products of free radical oxidation of lipids and carbohydrates can contribute to the modification and disruption of normal metabolism of vari-

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ous cell biomolecules [1,13]. Glutathione-dependent enzymes, the main component of the antioxidant defense (AOD), are evolutionarily programmed to optimize the balance between oxidative processes and the activity of various AOD systems [4,8]. Activity of these compounds at the preclinical stages of diabetic nephropathy and their relationship with the parameters of renal damage remain little studied.

The aim of this study was to analyze the parameters of carbonyl stress and glutathione metabolism in men with DM1 depending on the level of albuminuria.

MATERIALS AND METHODS

The study included 40 men of young reproductive age (mean age 30.25±8.51 years) with DM1 and unsatisfactory glycemic profile, patients of the Endocrinology Department of the Irkutsk Regional Clinical Hospital. The patients were grouped depending on the level of albuminuria: 20 men with normoalbuminuria (NAU) (mean age 29.38±9.78 years) and 20 men with microalbuminuria (MAU) (mean age 30.88±7.54 years). The control group included 28 healthy men (mean age 29.71±4.59). The inclusion criteria for the main group were signed informed consent of the patient to participate in the study, age 18-40 years, male sex, verified diagnosis of DM1, glomerular filtration rate (GFR) ≥60 ml/min/1.73 m^2. The exclusion criteria for the main group were T2DM/other types of DM, female sex, severe complications of DM (proteinuria, renal failure, and macrovascular complications), presence of other endocrine diseases, severe concomitant somatic pathologies, or primary kidney damage (infectious, vascular, toxic, immuno-inflammatory, and tumor). The inclusion criteria for the control group were the absence of acute or exacerbation of chronic diseases at the time of examination, normal glucose tolerance, and absence of hereditary predisposition to DM.

All participants signed informed consent to participate in the study in accordance with the World Medical Association Declaration of Helsinki (2013). The Committee on Biomedical Ethics at the Research Centre for Family Health and Human Reproduction Problems approved the study (No. 8.2 dated November 2, 2018).

During examination, a comprehensive assessment of clinical and laboratory data was carried out, methods for assessing early kidney damage were used (calculation of GFR, determination of albumin content, ratio of creatinine and microalbumin in urine). The content of albumin and the ratio of albumin to creatinine in the urine were determined on the Synchron CX9 Pro biochemical analyzer (Beckman Coulter) by immunoturbidimetric method. The GFR was calculated using the CKD-EPI formula (ml/min/1.73 m^2).

Plasma and erythrocyte hemolysate were used as the study material. Activities of glutathione peroxidase (GPx), glutathione reductase (GR), and glutathione-S-transferase (GST) were measured by enzyme immunoassay using commercial kits from Randox. The content of carbonyl compounds (methylglyoxal) was determined using commercial Human Methylglyoxal (MGO) ELISA Kit. The level of reduced and oxidized glutathione (GSH and GSSG) was measured using the method proposed by P. J. Hisin and R. Hilf (1976). The concentration of TBA-reactive substances (TBARS) was determined fluorometrically by the method proposed by V. B. Gavrilov (1987) [3]. The measurements were carried out on a Shimadzu RF-1501 spectrofluorophotometer and a Shimadzu RF-1650 spectrophotometer.

The data were analyzed using Statistica 6.1 software (StatSoft, Inc.). To determine the proximity to normal distribution law of quantitative features, we used the visual-graphical method and the Kolmogorov—Smirnov agreement criteria with the Lilliefors and Shapiro—Wilk tests. The equality of the general variances was checked using the Fischer test (F test). To present quantitative data, descriptive statistics were given: mean (M) and variance (σ). The parametric Student's test and nonparametric Mann—Whitney test were used for the analysis of intergroup differences for independent samples. The Spearman's method was used for the correlation analysis. The critical significance level was set at 5% (0.05).

RESULTS

The analysis of the results showed the presence of significant differences in the content of carbonyl compounds and in some parameters of the glutathione status in patients with DM1 irrespectively of the microalbuminuria level. In men with DM1 and NAU, we observed an increase in the blood level of methylglyoxal (by 1.41 times; p=0.014), GR activity (by 2.78 times; p=0.011), content of GSH (by 1.33 times; p=0.004) and GSSG (by 1.32 times; p=0.007), and the level of TBARS (by 1.73 times; p=0.002) in comparison with the control (Fig. 1).

In the group with DM1 and MAU, an increase in methylglyoxal content (by 1.52 times; p=0.009), activities of GR (by 1.52 times; p<0.0001) and GPx (by 1.28 times; p<0.0001), and content of GSH (by 1.51 times; p<0.001) and GSSG (by 1.4 times; p<0.0001) relative to the control values (Fig. 2).

The inter-group comparison showed higher values of TBARS (by 1.4 times; p=0.0173) and reduced GR activity (by 1.4 times; p=0.0183) in the group with DM1 and MAU in comparison with NAU.

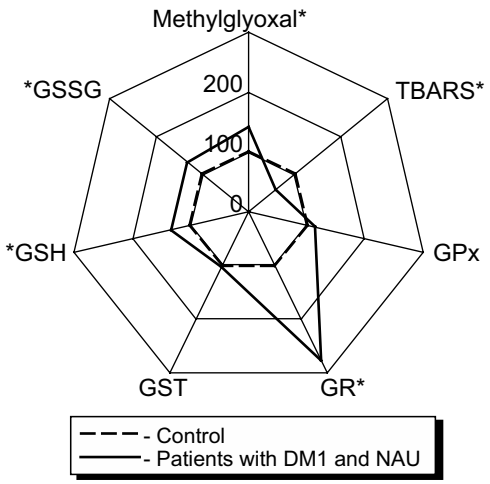

Fig. 1. The level of carbonyl compounds and components of glutathione system in men with DM1 and NAU. *Significant differences from the control (taken as 0%).

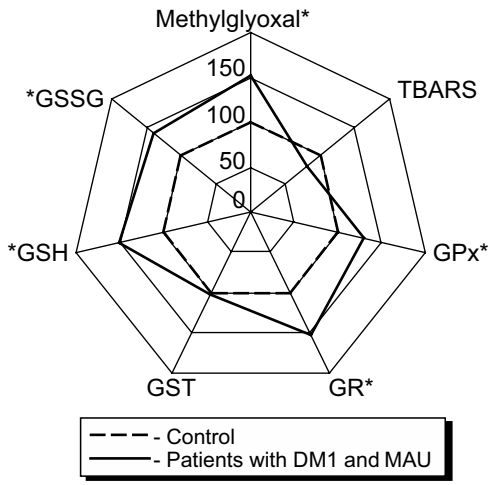

Fig. 2. The level of carbonyl compounds and components of glutathione system in men with DM1 and MAU. *Significant differences from the control (taken as 0%).

The correlation analysis of the data revealed the presence of significant dependencies of TBARS with GSH (r=0.88; p=0.021) and GSSG (r=–0.95; p=0.004) in the group with DM1 and NAU. In the group with DM1 and MAU, the correlation of TBARS with methylglyoxal (r=0.72; p=0.003) and GSSG (r=-0.55; p=0.033) and GST with GSH (r=0.72; p=0.002) were revealed. Analysis of interrelationships of the parameters of renal damage with the studied parameters revealed a direct dependence of the GFR level on the content of methylglyoxal (r=0.64; p=0.0431).

The obtained results indicated increased levels of methylglyoxal in patients with DM1 regardless of the albuminuria level. Chronic hyperglycemia associated with DM is usually accompanied by elevation in intracellular glucose content, activation of pathological pathways of its metabolism, and insufficient utilization during implementation of the pentosophosphate pathway [15]. Activation of the polyol and hexosoamine pathways stimulates intensive accumulation of dihydroxyacetone phosphate, which leads to the production of glyceraldehyde-3-phosphate, dihydroxyacetone-3-phosphate, and some other metabolites that stimulate the protein kinase C (PKC) pathway [5]. These compounds are the precursors of a natural dicarbonyl methylglyoxal, a precursor of advanced glycation end-products (AGE) [10]. Methylglyoxal, along with glyoxal, bind to different AGE receptors or interact with biomolecules, thus causing oxidative stress directly or indirectly through the activation of PKC [1,7].

Further development of events can include an increase in vascular permeability, neovascularization, violation of the integrity of the tissue—blood barrier in nephrons, *etc*. [2,6]. Methylglyoxal is now considered as an important biomarker of diabetic complications due to its close association with glycation reactions, β-cell dysfunction, and insulin resistance [13]. This compound is considered the most reactive among AGE-products due to its direct involvement in modulation of the function of insulin molecules, as well as impaired signal transmission, which leads to a decrease in insulin secretion [12].

Methylglyoxal is also considered an independent risk factor for changes in metabolic parameters, including BP, GFR, *etc*. [13]. Experiments showed that the kidneys are a direct target for AGE-mediated damage, because they are the main site of their excretion [6]. Moreover, increased level of AGE is closely associated with various structural and functional changes typical of diabetic nephropathy, including thickening of the glomerular basement membrane, mesangial expansion, glomerulosclerosis, and tubulointerstitial fibrosis [5,6].

The most convincing evidence of the role of AGE in the development of diabetic nephropathy was obtained in studies of inhibitors of AGE formation, such as aminoguanidine and pyridoxamine [5]. In addition, AGE receptor knockout mice showed less pronounced functional and structural damage to the kidneys [12].

Thus, increased concentration of methylglyoxal in the blood of patients with NAU can serve as an unfavorable sign of DM1 development; under conditions of MAU, this indicator can reflect the potential role of AGE in the development of diabetic nephropathy. Moreover, in patients with MAU, we found a positive relationship of methylglyoxal with the GFR parameter, which can confirm the pathogenetic role of AGE in diabetic nephropathy. TBARS level decreased in the group with NAU relative to both the control and MAU group, which can be a result of compensatory

processes and activity of the glutathione system components GR and GSH.

Increased activities of antioxidant enzymes GR and GPx were also found in the group with MAU. In the literature, there are conflicting data on activity of antioxidant enzymes in patients with DM1 depending on the level of glycemic control, duration of the disease, and concomitant complications [4]. Some researchers observed no differences in activity of GPx and GR in comparison with the control. It is known that GR, as a homodimeric enzyme, restores the disulfide bond of oxidized glutathione to its sulfhydryl form GSH. GR is also important in the formation of innate immunity [1,4]. GPx, in addition to hydroperoxides, recycles most of the phospholipid peroxides and fatty acids using glutathione. The most plausible explanation of increased activity of the glutathione system in MAU is participation of components of the glutathione system in the mechanisms of the methylglyoxal detoxification by the glyoxylase system. The enzymes of this system are usually significantly reduced in DM, and reduced glutathione acts as a catalyst that binds methylglyoxal to form hemithioacetal for reaction with glyoxylase 1 [12]. In addition, GSH is a regulator of various cellular processes, such as gene expression, DNA and protein synthesis, proteolysis, cell proliferation and apoptosis, cytokine production and immune defense, regulation of mitochondrial function, cellular and oxidative status, *etc*. [7,15]. However, progressive growth of methylglyoxal in MAU can indicate insufficient activity of the glutathione system components in this case.

Thus, the results of our study indicate an increase in the concentration of methylglyoxal and a compensatory increase in activity of the glutathione system components in men with DM1 irrespective of the albuminuria level in the presence of a close relationship between the glycation index and markers of renal damage. Monitoring of these indicators in male patients with DM1 can be an important component of the pathogenetic treatment and prevention of a severe DM complication, diabetic nephropathy.

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