A Comparative Study of Antioxidant System and Intensity of Lipid Peroxidation in Type 2 Diabetes Mellitus and Ischemic Stroke Aggravated and Not Aggravated by Type 2 Diabetes Mellitus

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The survey was conducted on patients with type 2 diabetes mellitus and on patients with ischemic stroke aggravated and not aggravated by type 2 diabetes mellitus. A comparative study of the function of antioxidant system and the intensity of oxidative stress induced by lipid peroxidation (LPO) in the blood was carried out. Stroke aggravated by diabetes was characterized by higher intensity of LPO than stroke not aggravated by diabetes, which, apparently, determines the more severe course of stroke in patients with diabetes. The mechanisms of compensatory response to oxidative stress at the level of antioxidants in stroke aggravated by diabetes also differed from those in stroke not aggravated by diabetes. These data indicate the need of using water-soluble low-molecular-weight antioxidants in the treatment of stroke aggravated by diabetes.

Key Words: *ischemic stroke; diabetes; antioxidants; lipid hydroperoxides*

Damage to cerebral structures during cerebral ischemia occurs as a result of progressive development of a complex of pathological disorders at the molecular and cellular levels. They are caused by reduced oxygen content in the arterial blood, on the one hand, and toxic effects of oxygen intermediates (oxidative stress), on the other. Oxidative stress plays an important role in the pathogenesis of acute ischemic stroke (IS). The generation of free radicals and damage to tissue are among the major factors determining the severity of IS and its outcome [1,7,10]. Brain tissue is characterized by high content of polyunsaturated fatty acids most prone to free radical-induced peroxidation and iron ions catalyzing generation of free radicals, on the one hand, and relatively low content of enzymes neutralizing free radicals, which makes the organ highly vulnerable to oxidative stress [4,6,8]. Due to the violation of structural and functional integrity of the blood-brain barrier in patients with IS, destructive processes induced by ischemia become systemic and are recorded in both the cerebrospinal fluid and blood [2,11].

Oxidative stress and production of free radicals are the main causes leading to the development of insulin resistance, β-cell dysfunction, decrease in glucose tolerance, and type 2 diabetes mellitus (DM2). They are also involved in the development of diabetic complications, micro- and macroangiopathy [13]. On the other hand, it is known that diabetic angiopathies of cerebral arteries lead to microcirculation disturbances and contribute to the development of cerebral ischemia. DM is a risk factor for IS, and the prevalence of IS, its severity and frequency of deaths among patients with DM is much higher [3,12]. Comparative analysis

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of the molecular mechanisms responsible for the development of ischemia-induced destructive processes in patients with IS aggravated and not aggravated by DM can greatly improve the efficiency of prediction, prevention, and treatment of IS in patients with DM.

The aim of this work was comparative analysis of the functional status of antioxidant system and intensity of LPO induced by oxidative stress in the blood of patients with DM2 and with IS aggravated and not aggravated by DM2.

MATERIALS AND METHODS

Characteristics of the groups are presented in Table 1. Among patients with IS 7 had cardioembolic stroke and 32 patients had aterothromboembolic stroke. The infarction zone was localized in the brain stem in 5 patients and in the cortico-subcortical areas of brain hemispheres in 34 patients. Atherosclerosis was diagnosed in 27 patients, hypertension in 17 patients, CHD in 9 patients, congestive heart failure in 4 patients, and atrial fibrillation in 8 patients. Seven patients with IS+DM2 had cardioembolic stroke and 38 patients had aterothromboembolic stroke. In five patients, the infarction area was located in the brain stem and in 40 patients in the cortico-subcortical areas of brain hemispheres. Atherosclerosis was diagnosed in 33 patients, hypertension in 16 patients, CHD in 6 patients, congestive heart failure in 12 patients, and atrial fibrillation in 7 patients. The mean disease duration in patients with DM2 and IS was 11±7 years (M±σ) and in patients with DM2 10±6 years (M±σ). In all DM2 and IS+DM2 patients, angio- and neuropathies typical of late stages of DM were found.

All patients were treated in St. Gregory the Illuminator Scientific Medical Center and Armenia Medical Center. Diagnosis of patients was conducted in accordance with International Classification of Diseases (ICD-10). The control group (no family history of IS, myocardial infarction, and DM) consisted of employees of the National Academy of Sciences of the Republic of Armenia. None of the examined subjects suffered from cancer or autoimmune diseases, myocardial infarction, acute infectious diseases or underwent surgery at least 12 months before blood sampling. All subjects gave their consent to give blood for the research, permission of the Ethics Committee of the Institute of Molecular Biology was also obtained.

The blood was taken at 9:00 h after overnight fast from the cubital vein, in patients with IS and IS+DM2 the blood was taken on the first day after stroke. The samples were immediately placed on ice and then centrifuged at 3000g for 10 min. The serum was collected and used in further experiments. Serum samples were stored at -30°C.

TABLE 1. Characteristics of Groups

Group	N	Men/women	Age (M±σ), years
IS	39	13/26	69±9
IS+DM2	35	11/24	69±9
DM2	33	26/7	58±9
Healthy volunteers (control)	38	21/17	55±9

Total serum activity of non-enzymatic water-soluble low-molecular-weight antioxidants was determined by photochemiluminescent analysis on a Photochem device (Analytik Jena AG) using commercial kit (ACW; Analytik Jena AG), including luminol as the photosensitizer and ascorbic acid as the standard. Antioxidant activity was calculated automatically with Photochem unit software in equivalent units of ascorbic acid concentration (mmol/liter).

Ferroxidase activity of ceruloplasmin in blood serum was determined as described previously by using double ammonium sulfate and iron sulfate (Mohr's salt) as the substrate [5] and expressed in μmol ferrous ammonium sulfate per 1 liter serum per 1 min (μmol/liter/min).

The content of lipid hydroperoxides in the serum as a measure of LPO intensity was determined as described previously [9] and expressed in optical density units at λ=480 nm (A480).

Statistical processing of the data including nonparametric Mann–Whitney U test and Spearman correlation analysis was performed using SPSS-13 software. The differences were significant at $p<0.05$.

RESULTS

Mean values of the studied parameters for all groups are presented in Table 2.

No significant correlations were found between the studied parameters, on the one hand, and age and sex of patients, on the other.

The content of lipid hydroperoxide in the serum in patients was significantly higher than in healthy individuals. Thus, the content of lipid hydroperoxides in patients with IS and DM2 was by 3 times higher and in patients with IS+DM2 by 4 times higher ($p<0.0001$) than in the control. High blood content of lipid hydroperoxides in patients with IS and IS+DM2 was a marker of LPO intensification induced by oxidative stress characteristic of pathogenesis of stroke [1,10]. This was also true for patients with DM2, although the mechanisms and factors that contribute to the development of oxidative stress in this pathology are

TABLE 2. Parameters of Oxidative Stress and Antioxidant Activity ($M \pm \sigma$) of the Serum of Patients and Healthy Volunteers

Group	*N*	Content of lipid hydroperoxide, A480	Total activity of low-molecular-weight water-soluble antioxidants, mmol/liter	Ceruloplasmin ferroxidase activity, μmol/liter/min
IS	39	0.09±0.03*	3.4±1.1*ˣ	475±107
IS+DM2	35	0.12±0.04*⁺	2.8±0.9	574±129*ᵒ
DM2	33	0.09±0.03*	2.6±0.8	483±111
Healthy volunteers (control)	38	0.03±0.01	2.5±0.8	429±94

Note. $p<0.0001$ compared to: *control, ˣpatients with IS+DM2; ᵒpatients with IS and DM2; ⁺$p<0.05$ compared to patients with IS.

different from those observed in IS [13]. This fact explains higher levels of lipid hydroperoxide in the blood of patients with IS+DM2 compared to patients with IS (1.3 times, $p<0.05$). Apparently, it is a factor determining more severe course of stroke complicated by DM2 [3,12].

Total serum activity of water-soluble low-molecular-weight antioxidants in patients with IS was approximately by 1.4 times higher than that in healthy individuals; in patients with IS+DM2 and DM2 it was higher by 1.3 and 1.2 times respectively ($p<0.0001$). In patients with IS+DM2 and DM2, this parameter did not differ significantly from the values characteristic of healthy individuals.

Significant changes in ceruloplasmin ferroxidase activity were observed only in patients with IS+DM2: it by 1.3 times surpassed the control values and the parameters in patients with IS and DM2 ($p<0.0001$). In other groups, ferroxidase ceruloplasmin activity did not differ from that in healthy individuals.

Thus, we showed that the increase in functional activity of the antioxidant system is a compensatory mechanism triggered by oxidative stress in stroke. Moreover, IS is associated with activation of low-molecular-weight non-enzymatic components of the system, while IS+DM2 is accompanied by activation of the enzyme antioxidant ceruloplasmin. In other words, the mechanisms of compensatory response to oxidative stress at the level of antioxidants in IS+DM2 differ from those observed in IS. It is obvious that metabolic, molecular, and cellular shifts typical of pathogenesis of DM2 impair compensatory mechanisms protecting the body from oxidative stress, with participation of water-soluble nonenzymatic low-molecular-weight antioxidants. Despite the fact that our data do not answer the question of how changes specific for the pathogenesis of DM2 lead to disturbances in the specified compensatory mechanisms, our results clearly demonstrate the necessity of using water-soluble low-molecular-weight antioxidants for improving the effectiveness of treatment IS aggravated by DM2.

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