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Defining the end products of oxidation of lipids, proteins and nucleic acids in patients with post-stroke depression

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Depression is a common consequence of stroke. In the last few years, oxidative stress has been seen as one of the contributing factors in the pathogenesis of depression. Lately it has been discussed also as an accompanying factor in many chronic neurodegenerative pathologies, as well as in acute cerebrovascular disorders like stroke. The aim of the present investigation was to determine whether oxidative stress (OS) occurs during the stroke and its effect on the development of post-stroke depression. For this purpose, the levels of some important end products of oxidation of lipids, proteins and nucleic acids in post-stroke depressed patients were evaluated. The lipids we measured as the levels of malondialdehyde (MDA), the protein as protein carbonyl content (PCC) and nucleic acids were evaluated as 8-hydroxy-2'-deoxyguanosine quantity (8-OHdG) in post-stroke depressed patients.

Our study included 37 patients in the age group of 59 - 78 years old hospitalized in the Neurological Clinic of University Hospital, Stara Zagora, Bulgaria. The patients were diagnosed with a stroke, according to the criteria of International Classification of Diseases (ICD10), and separated into three groups: non depressed post-stroke (n=13), moderate depressed (n=12) and severe depressed post-stroke group (n=12). All studied parameters were compared with those of the 11 healthy controls. The observed changes in MDA levels, PCC and 8-OHdG quantity in plasma of all group patients, suggested impaired antioxidant status and presence of oxidative stress.

Keywords: Oxidative stress, Post-stroke depression, MDA, PCC, 8-OHdG.

INTRODUCTION

Oxidative stress is a biological process, which is characterized by an imbalance between reactive oxygen species (ROS) and antioxidants, with higher levels of ROS. This deviation leads to oxidative damage to proteins, lipids, nucleic acids and eventually cell death [1,2]. Oxidative stress mechanisms are implicated in the pathogenesis of mental illness. This hypothesis has a theoretical appeal because the brain is considered particularly vulnerable to oxidative damage for a number of reasons. These include its relatively high oxygen utilization and hence the generation of free radical byproducts, its modest antioxidant protections, its lipid-rich resistance that delivers ready oxidation substrates, the decreasing potential of some neurotransmitters, and the presence of redox catalytic metals such as iron and copper [3-5].

Oxidative stress results in the inactivation and modification of antioxidant enzymes and weakening of the antioxidant protection [6]. Oxidation of proteins may be responsible for their exchange in forms that are more susceptible to proteinase degradation [7, 8]. Carbonyl protein formation is an oxidative stress index as a result of modifications in amino acids. Carbonylation of proteins is a non-enzymatic addition of aldehydes or ketones to specific amino acid residues, mainly to arginine, lysine, threonine, proline, cysteine or histidine [9, 10]. Replacements in the redox equilibrium in the thiol disulfide plasma system are implicated as a key mechanism of oxidative damage and restoration of redox homeostasis in patients after acute stroke. Some authors suggest oxidative changes such as cumulative oxidative DNA damage being a common pathophysiological mechanism at the root of major depression and medical co-morbidities [11, 12].

The aim of our study is to investigate the role of oxidative stress in the etiopathogenesis of depressive disorders in post-stroke patients in order to optimize diagnostic, therapeutic and medical-social approaches. In order to achieve this, we have determined the level of malondialdehyde (MDA), marker of oxidative damage to lipids, protein-carbonyl content (PCC), oxidative damage marker for proteins, and 8-OHdG, a marker for oxidative DNA damage, as well as the enzymatic protection of the body against oxidative stress - superoxide dismutase (SOD) and catalase (CAT) in post-stroke patients according to the presence of depressive disorder and its severity.

EXPERIMENTAL

Our study included 37 patients in the age group of 59 - 78 years old hospitalized in the Neurological Clinic of University Hospital, Stara Zagora, Bulgaria. The patients were diagnosed with a stroke, according to the criteria of International

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Classification of Diseases (ICD10). The first three days after the initial diagnosis was recorded in the medical documentation of the disease and neuro-imaging study (CAT or NMR). All patients were separate into three groups: non depressed post-stroke (n=13), moderate (n=12) and severe depressed post-stroke group (n=12). The studied parameters were compared with those of the 11 healthy controls. Informed consent was obtained from all post-stroke patients and healthy volunteers enrolled in this study, according to the ethical guidelines of the Helsinki Declaration (1964). To assess the severity of depressive disorder according to criteria of ICD10 we used the Hamilton Depression Rating Scale (HAM-D-17; Hamilton, 1960).

The study was conducted in the form of an interview with the patient. Nine of the symptoms (low mood, feeling of guilt, suicide, retardation, activity and work, agitation, mental anxiety, somatic anxiety, hypochondriacity) are given from 0 to 4 points. Another eight symptoms (sleep disorders, disorders of sleep continuity, early awakening, gastrointestinal somatic symptoms, genital symptoms, general somatic symptoms, loss of weight, disease awareness) are given 0 to 2 points. The total score on the scale varies from 0 to 54 points, with 0 to 7 points objectively meaning lack of depression, from 8 to 13 points is meaning mild depression, 14 to 18 points - medium depression, 19 to 22 - moderate depression and 23 to 54 points - severe depression. Fasting samples of venous blood were collected in the morning between 8.00 and 10.00h. Blood for determination, MDA, PCC and 8-OHdG were collected in tubes containing 10% EDTA (ethylenediaminetetraacetic acid). All samples from each subject were split and run in triplicate.

Spectrophotometric assays

Determination of lipid-peroxidation products (MDA)

Total amount of lipid peroxidation in the plasma of healthy volunteers and patients was estimated using the thiobarbituric acid (TBA) by using a spectrophotometric assay of Plaser et al., [13] measured the malondialdehyde (MDA) reactive products at 532 nm, and all results were expressed in μmol/l.

Determination of SOD activity

The SOD activity was determined as described by Sun et al. [14], with some modifications. The hypoxanthine/xanthinoxidase system was used for superoxide anion production. This anion reduces nitro blue tetrasole (NBT) to Formosan, which was monitored at 560 nm. SOD in the samples removes the superoxide anion and suppresses the reduction. The reduction rate was used to measure of SOD activity. The end concentrations of hypoxanthine, xanthinoxidase and NBT in the measurement were 50 μmol, 10 U/ml, and 0.125 mM, respectively. One unit of SOD activity was determined as that enzyme quantity which causes 50% suppression of NBT, CAT activities reduction to Formosan. The results are presented as UI/g Hb (units per gram hemoglobin).

Determination of CAT activity

CAT activity was measured and estimated in erythrocyte lysate by the method of Beers and Sizer [15]. Hydrogen peroxide (30 mM) was used as a substrate and the decrease in its concentration at 22°C in phosphate buffer (50 mM, pH07.0) was followed at 240 nm for 1 min. One unit CAT activity was determined as that quantity of enzyme, which removes 1 μmol H_2O_2 for 1 min. The results are presented as UI/g Hb (units per gram hemoglobin).

Enzyme-linked Immunosorbent assay

Measurement of Protein Carbonyl content

PCC was measured by using a commercial ELISA kit followed manufacturer's instructions. Protein carbon content in the samples was determined by a standard curve prepared from the absorbance obtained on the basis of the oxidized/reduced BSA standards and the protein carbonyl content in the samples were calculated in nmol/mg.

Measurement of 8-OHdG Quantity

The quantitative measurement of 8-OHdG were carried out using commercial ELISA kit, followed manufacturer's instructions. The kit has an 8-OHdG detection sensitivity range of 100 pg/mL – 20 ng/mL.

Statistical analysis

Statistical analysis was performed using Statistica 8.0, Stasoft, Inc., one-way ANOVA, Student t-test to determine significant difference among data groups. The results were expressed as means ± standard error (SE). A value of $p > 0.05$ was considered statistically. To define which groups are different from each other we have used LSD post hoc test.

RESULTS AND DISCUSSION

The depressive disorder was concomitant in 60 (64.5%) of the patients with stroke. There is some evidence, pointing out that stroke is associated by oxidative stress. There is little understanding, however, on the subject of oxidative stress and depression in post-stroke patients.

The current study of post-stroke depression is related to its high prevalence and its negative impact on the rehabilitation process. Its research is

very important for solving a number of practical tasks such as post-stroke depressive disorder diagnosis, choosing optimal therapy methods depending on the clinical picture and the presence of concomitant psychiatric disorders as well as the possibility of prophylaxis of the post-stroke depression. Brain ischemia unlocks a complex cascade of metabolic events, most of which involve the formation of modified protein products that have been observed under various conditions such as aging, cell differentiation and apoptosis [16].

We investigated the levels of lipid peroxidation products in post-depressive patients with varying degrees of depression. Our study showed a statistically significant increase in MDA values in comorbid patients with severe depression (mean 5.74 ± 0.11 μmol/ml vs mean1.76 ± 0.2 μmol/ml, p> 0.00, t-test). On Figure 1 is seen statistically significant increase in other two groups: non-depressed post-stroke (mean 2.9 ± 0.2μmol/ml p> 0.00, t-test), and moderate post-stroke depressed (mean 3.38 ± 0.9 μmol/ml p> 0.00, t-test), compare with controls (see Fig. 1). Moreover, there is statistically significant increase between the depressed groups' p>0.05.

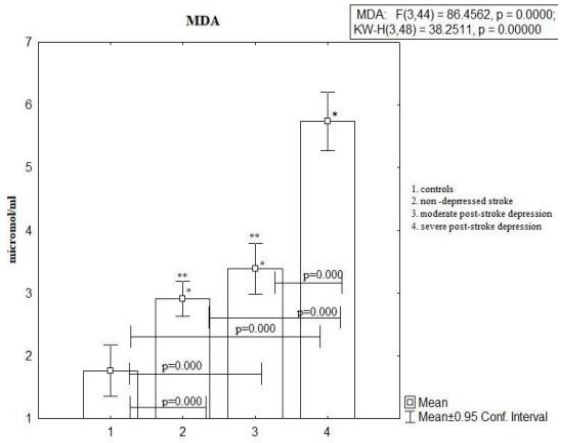

Fig. 1. Levels of MDA in post-stroke patients and healthy controls; (*) p>0.05 vs controls; (**) p>0.05 vs severe post-stroke depressed group

The presented results showed that plasma MDA levels were significantly higher than in controls and confirmed those from other observations. A study of Iida *et al*., [17] reports increased MDA levels in patients with affective disorders. There are reports that the major depressive disorder was associated with elevated MDA levels [18]. Another study demonstrated that an elevated serum MDA level at admission was positively associated with an increased risk of developing depression after acute stroke, especially minor stroke [19].

The present study is designed to investigate the possible relationship between the level of typical markers of oxidative and nitrative protein modifications (protein-carbonyl groups) and the degree of post-stroke depression. Oxidation of amino acid residues leads to the formation of relatively stable protein-carbonyl groups, which may be qualitative and quantitative markers allowing the evaluation of oxidative damage to proteins [20, 21].

The PCC in post-stroke patients showed a statistically significant increase in patients with severe depression (mean 6.67±0.3 nmol/ml, p> 0.00, t-test), moderate groups (5.71 ± 0.3 nmol/ml, p> 0.00, t-test) compare to control group (mean 3.24±0.4 nmol/ml), (Figure 2). The results in non-depressed post-stroke group (mean 4.65±0.6 nmol/ml) are increased but not statistically compare to controls. Non-depressed group showed statistically significant increase versus severe depressed group (mean 4.65 ± 0.6 nmol/ml vs mean 6.67±0.3 nmol/ml, p> 0.00, t-test). As the severity of depression increases, the protein carbonyl content increases.

The oxidative damage to proteins has been documented as an increased concentration of protein-carbonyl groups in plasma proteins in patients after ischemic stroke. In addition, there is a significant positive correlation between the level of carbonylation and the severity of depression.

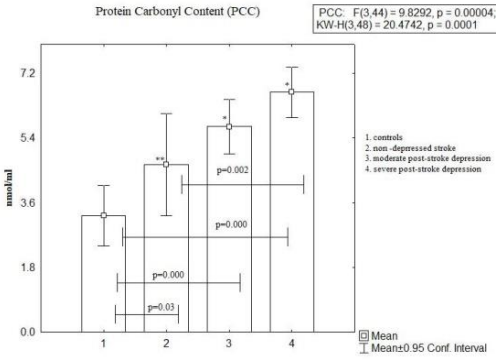

Fig. 2. PCC in plasma of healthy controls, and post – stroke depressed patients; (*) p>0.05 vs controls; (**) p> 0.05 vs severe post-stroke.

We showed more than two fold increases in protein-carbonyl groups in post-stroke patients compared to healthy subjects, and our results are consistent with previous reports [22]. The obtained results show a significant correlation between the degree of oxidation of amino acid residues in plasma proteins and the severity of depression in patients after a stroke.

A marker for oxidative DNA damage is an 8-OHdG (8-hydroxy-2-deoxyguanidine). Still, there are no studies on its levels in post-depressive patients with depression. Still a number of authors confirm the high levels of 8-OHdG in patients with

depression only [23-25]. The 8-OHdG marker showed a statistically significant increase in post-stroke patients with severe depression compare to healthy controls (mean 3.45 ±0.3 ng/ml vs mean 0.77±0.3 ng/ml, p=0.000, t-test). Moreover the same statistically significant increase was observed in other two post-stroke groups: non-depressed (mean 1.35±0.04 ng/ml vs mean 0.77±0.3 ng/ml, p=0.000, t-test), and moderate (mean 1.88 ±0.1 ng/ml vs mean 0.77±0.3 ng/ml, p=0.000, t-test), (Fig. 3). Statistically significant increase was also seen between post –stroke depressed group (p<0.05). As the severity of depression increases, levels of the 8-OHdG marker increase.

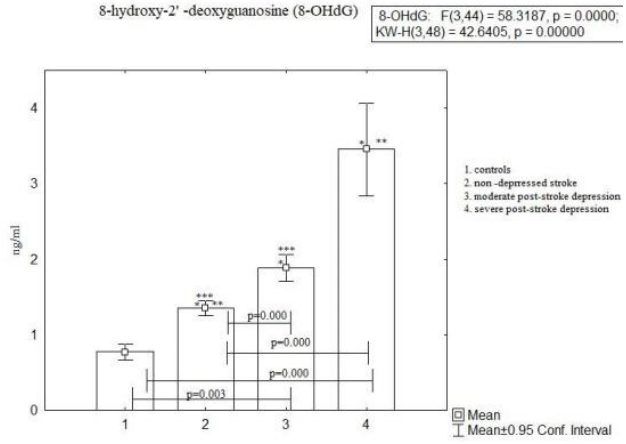

Fig. 3. 8-OHdG in plasma of healthy controls, and post –stroke depressed patients; (*) p>0.05 vs controls; (**) p>0.05 vs moderate post -stroke; (***) p>0.05 vs severe post-stroke.

Study of SOD activity, it has seen a statistically significant decrease in patients with severe (mean 540.3 ± 55 UI/gHb, p >0.00, t-test), moderate (mean 983.1 ± 167.5 UI/gHb, p>0.00, t-test), and non-depressed groups (mead 927.66 ± 163.1 UI/gHb, p> 0.00, t-test) compared to the controls (14364 ± 1989.5 UI/gHb), (p>0.05) (Fig. 4). The lowest levels are seen in patients with moderate depression.

There was a statistically significant increase in erythrocyte CAT activity in all post-stroke depressed groups: severe depressed group (mean 28454.7 ± 1280 UI/gHb, p> 0.00, t-test), moderate (mean 23249.7 ± 1329 UI/gHb, p> 0.00, t-test) and non-depressed (13257.8 ± 1003 UI/gHb, p> 0.00, t-test) compared to the controls (mean 1483.45 ± 78.6 UI/gHb) (Fig.5). A statistically significant increase in CAT activity were observed between post-stroke depressed groups and non-depressed patients (p >0.05).

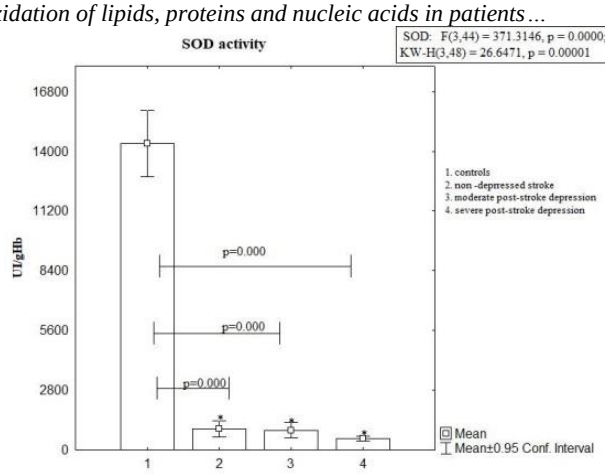

Fig. 4. SOD activity in post –stroke depressed patients and controls, (*) p>0.05 vs controls

Production of radicals in the brain is due to catecholamine metabolism, such as dopamine and norepinephrine, and is increased by the presence of transition metals and antioxidant deficiency. Due to the high toxicity of free radicals, the body has developed a number of specialized and effective methods for deactivating them. The superoxide dismutase enzyme (SOD) catalyzes dissociation of the superoxide radical into hydrogen peroxide (H_2O_2) and oxygen (O_2) [26]. Hydrogen peroxide is then reduced to water and molecular oxygen by the enzymes peroxidase glutathione and catalase [26].

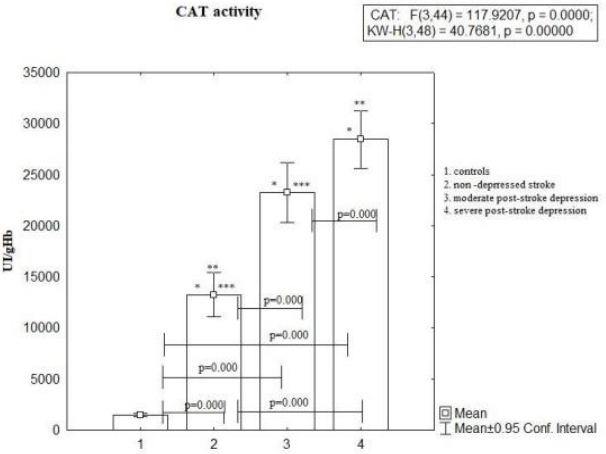

Fig. 5. CAT activity in post –stroke depressed patients and controls, (*) p>0.05 vs contrls; (**) p>0.05 vs moderate; (***) p>0.05 vs severe post-stroke.

Catalase is not directly involved in the elimination of ROS, but does not allow Fenton's reaction in which hydrogen peroxide is converted to highly toxic hydroxyl radicals [26]. According to literature, the SOD does not change in the first 24 hours after the lesion, whereas CAT activity levels decrease [26]. Reduced catalase activity in patients with stroke reduces the efficiency of deactivation of H_2O_2 [26], which increase oxidative stress. In our study, we noticed that the catalase activity in erythrocytes of patients with stroke was about five times higher than that of a healthy control group,

this relationship being almost doubled in patients with severe post-stroke depression. The superoxide dismutase enzyme (SOD) is inhibited by ROS in depressive patients. There is no significant difference in SOD levels at different degrees of depression. The presence of depressive disorder after a stroke is important for the decline of SOD activity. In our study, CAT levels are high, and the potential for dealing with the oxidative stress of the body is still high in the early days after the stroke. The severe degree of depressive disorder has a statistically significant correlation with high CAT levels. Our results are confirmed by a study of Miller *et al* [28], which shows a positive correlation between the concentration of carbonyl groups and the results of the Geriatric Depression Scale. Moreover Miller *et al*., [28, 29] found significantly lower levels of protein carbonyl in the acute phase of a major depressive episode compared to healthy subjects.

Non-enzymatic Ascorbate radicals are rapidly exhausted in post-stroke patients with depressive disorder, as a biomarker of oxidative stress. We observed that in the worse degrees of depression the level of ascorbate radicals is the lowest compared to the lighter degrees of depression, therefore the lowest possibility of compensating for oxidative stress. For this reason in the complex therapy of depression is recommended intake of vitamin C.

CONCLUSIONS

The finding presents a certain advantage when using ACE and renin inhibitors to ARB with respect to the real time oxidative stress indicators. This fact alone is not a basis for the benefit of ACE inhibitor therapy to ARB, even with accompanying diseases in which etiopathogenesis is discussed increased oxidative stress. The choice of antihypertensive agents should be made individually according to the relevant European guidelines.

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