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Real time oxidative stress markers of patients with post-stroke depression: EPR study

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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 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. To achieve evaluating the level of oxidative stress in post-stroke depression we investigated the levels of ROS products, ascorbate (Asc•) and NO• radicals as real time oxidative stress biomarkers using electron paramagnetic resonance (EPR) spectroscopy.

Keywords: Oxidative stress, Post -stroke depression, ROS, RNS, Ascorbate radicals

INTRODUCTION

Approximately one third of stroke survivors experience significant symptoms of depression [1]. Post-stroke depression may have aetiology with many factors including organic and reactive components. Oxidative stress (OS) is involved in the pathogenesis of various diseases and may be a common pathogenetic mechanism responsible for many mental illnesses as the brain is more vulnerable to oxidative damage. In the last few years, oxidative stress is considered to be one of the contributing factors in the pathogenesis of post-stroke depression.

Disrupting the balance between the processes producing ROS and antioxidant defence systems, leads to the emergence of oxidative stress causing cellular damage and direct inhibition of enzyme proteins [2]. Oxidative stress is an imbalance between the biochemical processes that generate ROS and the ability of a biological system to neutralize them. As a result, ROS are formed faster than the ability of cellular defense systems to remove them [3]. The development of oxidative stress and its consequences depend on the ability of the organism alone, or with external help, to restore the physiological balance between pro-oxidants and antioxidants [4]. Certain neurological disorders, including stroke, are associated with oxidative and nitric modification of specific proteins and the accumulation of oxidative damage. There is some evidence indicating the involvement of oxidative and nitrate stress in the pathophysiology of depression [5]. Several studies have shown increased levels of reactive oxygen and nitrogen species in depression, mainly peroxide [5] nitric oxide [6], and lowered levels of antioxidants, such as glutathione (GSH), in the brain of deceased patients with depression [7]. This is why oxidative and nitrogen mechanisms have been proposed as targets for new antidepressants [8]. The aim of the present study was to evaluate the oxidative/antioxidant status in post-stroke depression through the levels of ROS products, ascorbate (Asc•) and NO• radicals as real time oxidative stress biomarkers using electron paramagnetic resonance (EPR) spectroscopy.

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MATERIALS AND METHODS

In our study were included 93 patients hospitalized in the Neurological Clinic, diagnosed with a stroke, according to the criteria of ICD10, in 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 studied parameters were compared with those of 32 controls healthy individuals. 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. Other 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 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 10.00 a.m. Blood for determination of NO• and ROS products was collected in tubes containing 10% EDTA (ethylenediaminetetraacetic acid). All samples from each subject were split and run in triplicate.

Ex vivo electron paramagnetic resonance (EPR) study

EPR measurements were performed at a temperature of 22°C on an X-band EMX^{micro}, spectrometer Bruker, Germany. The experiments were carried out in triplicate. Spectral processing was performed using Bruker WIN-EPR and *Sinfonia* software.

Ex vivo evaluation of the levels of ROS products

The ROS levels were determined according to [9] with modification. To investigate in real time formation of ROS in the sera of PD patients and controls *ex vivo* EPR spectroscopy was used combined with PBN as a spin-trapping agent.

Ex vivo evaluation the levels of Asc•

Endogenic ascorbic acid can be oxidized by ROS to a stable ascorbate radical and the latter can be detected by direct EPR method which does not interfere with the biochemical processes. The levels of Asc• were studied according to Bailey *et al.* [10], with some modification.

Ex vivo evaluation of the levels of •NO radicals

Based on the method published by Yoshioka *et al*. [11] the EPR method for estimation of the levels of •NO radicals in serum was developed and adapted.

RESULTS AND DISCUSSION

The development of post-stroke depression is often a mood disorder affecting about a third of the patients with stroke. In our study, depressive disorder was reported by two-thirds of the patients with stroke. Previous studies have established links between post-stroke depression and severe functional impairment [12]. In our results, the depressive disorder was concomitant in 60 (64.5%) of the patients with stroke.

The brain with its high oxygen requirements and lipid-saturated environment is considered to be highly sensitive to oxidative stress or redox imbalances. The oxidative stress is prone to occur in many mental disorders, including depression. Oxidative stress is a disturbance in the balance between the biochemical processes generating ROS and the capability of a biological system to deal with them.

The results from the levels of ROS in patients with stroke are given in Figure 1. We have discovered a statistically significant increase of ROS values in post-stroke patients with severe post-stroke depression (3.75±0.19 arb. units) compared to controls (0,62±0.11 arb. units), compared to non-depressed patients (2.44±0.2 arb. units) and compared to patients with moderate level of depression (2.56±0.2 arb. units), ($p<0.05$). There is also statistically significant increase in patients with moderate level of depression (3.19±0.2 arb. units) compared to the control group, post-stroke non-depressed patients and patients with mild depression ($p<0.05$). As a result, ROS products formed faster than their removal by the cellular defense systems [3]. Although some studies have established a link between oxidative stress and mental disorders, the causal relationship between them is not fully defined. Several mechanisms have been proposed, including genetic predisposition, disturbances in traditional signal transduction pathways, and oxidative stress participating in the pathogenesis of depression theory [13]. It has been proven that depression in somatically healthy patients is associated with elevated concentrations of blood cytokine [14] and increased levels of ROS and RNS [6, 7, 15]. Mechanisms associated with post-stroke depression may include an imbalance between pro-inflammatory and anti-inflammatory activity that is responsible for increasing oxidative stress and may in turn weaken cognitive sensitivity. Wei *et al*. [16] have reported an increase in ROS levels in a major depressive episode, causing immune dysregulation by suppressing T-cell responses.

The latest trend is that antidepressants have a therapeutic effect by suppressing the production of inflammatory cytokines, ROS and RNS, or even increasing antioxidant protection [9].

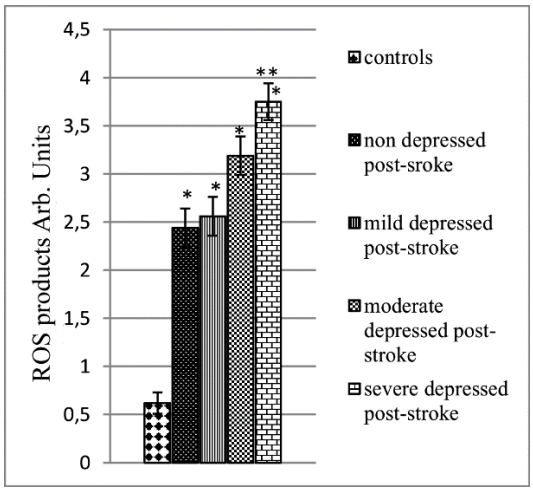

Fig. 1. Levels of reactive oxygen species in patients with stroke

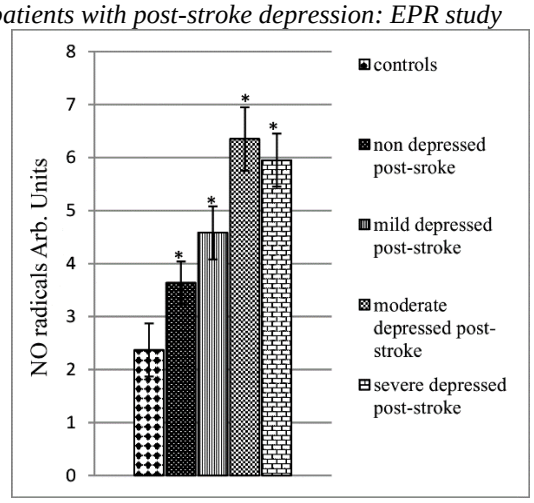

Fig. 2. Nitric oxide levels in patients with stroke

Accordingly, these studies place particular emphasis on the consideration of biochemical parameters that may have a direct impact on improving the functionality of patients in compliance with the severity of their depression.

Brain ischemia unlocks a complex cascade of metabolic events, most of which involve the formation of nitrogen and oxygen free radicals. Oxidative stress associated with inflammation or post-cerebral reperfusion may lead to oxidative nitrate modifications of the protein structure, resulting in changes in their functional properties. It can also lead to the accumulation of modified protein products that have been observed under various conditions such as aging, cell differentiation and apoptosis [17].

Nitric oxide levels in patients with stroke are given in Figure 2. The results show a statistically significant increase of levels of NO in patients with moderate depression (6.35±0.6 arb. units) compared to the control group (2.37±0.9 arb. units), non-depressed post-stroke patients (3.64±0.5 arb. units) and patients with mild depression (4.58±0.48 arb. units), ($p<0.05$), (Fig. 2). Statistically significant increase was detected also in patients with severe depression (5.95±0.5 arb. units) compared to the control group and patients with mild level of depression. ($p<0.05$).

In our study, we observed increases in NO levels in patients with post-stroke depressive disorder, with the highest levels in moderate and severe depressive disorders. According to research of Kudlow *et al*. [18] low concentrations of nitric oxide are neuroprotective and mediate physiological stimulation, whereas high concentrations mediate neuroinflammatory reactions and are neurotoxic.

They also point out that the increased concentrations of nitric oxide increase the synthesis of reactive nitrogen species (RNS) and reactive oxygen species (ROS). Although the clinical benefit of NO-synthase polymorphisms has not yet been determined, there is a possibility of treating a major depressive disorder with pharmacological agents by reducing NO levels. Evidence suggests that many current antidepressants, initially thought to primarily act on neurotransmitters, can actually be mediated by normalizing NO levels by affecting several interconnected pathways (e.g., microglial activity [19] or phosphodiesterase enzyme activity [20]. Perhaps the three-pronged approach by which traditional antidepressants are used in combination with pharmacological agents aimed at normalizing NO and inflammatory signaling pathways will turn out to be the most appropriate one. It should be emphasized that despite polymorphisms in the enzyme NO synthase can identify significant subpopulation; these variants are unlikely to be a sufficient or necessary condition for the induction of a major depressive disorder. Rather, some polymorphisms in NO synthase enzymes may be involved in a larger model of vulnerability. Vulnerability may include multiple factors (e.g., genetic, epigenetic, environmental factors) that contribute to a comprehensive patho-etiological model explaining why some people are more susceptible and others less prone to depressive symptoms [21].

Statistically significant increase of ascorbate radicals can be seen in non-depressed patients (0.44±0.09 arb. units) and patients with mild depression (0.46±0.2 arb. units) compared to the control group (0.31±0.04 arb. units), ($p<0.05$), (Figure 3). On the other hand, with the increase in the severity of depression, levels of ascorbate radicals decline. Statistically significant decrease compared to the control group, non-depressed

patients and patients with mild depression, can be seen in patients with severe depression (0.21±0.09 arb. units), ($p<0.05$). Patients with moderate depression (0.28±0.05 arb units) display a statistically significant decrease compared to non-depressed and mildly depressed patients ($p<0.05$).

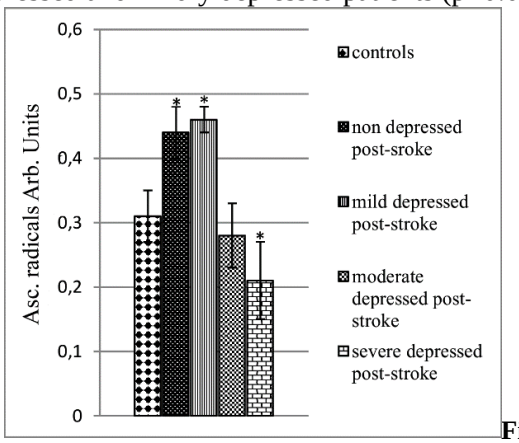

Fig. 3. Levels of ascorbate radicals in patients with stroke

There are reports that the major depressive disorder is associated with decreased levels of ascorbate radicals [22]. This is confirmed by a study of vitamin C concentration and symptoms of depression in adult patients (over 65 years of age) [28]. We observed that in heavier degrees of depression the level of ascorbate radicals is lower than in lower degrees of depression, therefore is the lowest possible level of oxidative stress compensation. For this reason, the complex therapy of depression recommends an intake of vitamin C. Ascorbate radicals and ROS products have been studied in patients with ischemic stroke using EPR [23]. The use of vitamin C as a substitution therapy has been studied by many authors, but the results are contradictory. Sahranian *et al.* [24], in a double-blind study found that adding vitamin C to citalopram did not increase its effectiveness in a major depressive episode. Conversely, a study of [25] shows positive results from the addition of vitamin C to fluoxetine during a major depressive episode in infant ages. More extensive studies are needed to expand the evidence of oxidative stress in post-stroke depression and to establish a potential therapeutic approach.

CONCLUSION

The levels of reactive oxygen species (ROS) and nitric oxide (NO) generation in post-stroke patients are dependent on the presence of a depressive disorder and its severity. The smallest opportunity to cope with oxidative stress is found in heavier degrees of depression due to low levels of ascorbate radicals. Because of these results, anti-depressant antioxidants such as vitamin C, coenzyme Q, omega 3, etc., should also be involved in the complex treatment of depression with antidepressants.

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МАРКЕРИ В РЕАЛНО ВРЕМЕ ЗА ОКСИДАТИВЕН СТРЕС ПРИ ПАЦИЕНТИ С ПОСТ-ИНСУЛТНА ДЕПРЕСИЯ

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(Резюме)

Депресията е обичайно следствие при инсулт. През последните години оксидативният стрес се счита за един от факторите, допринасящи към патогенезата на депресията. Напоследък се дискутира, че той е придружаващ фактор при много навродегенеративни патологии, както и при акутни мозъчно-съдови нарушения, например инсулт. Целта на нашето изследване е да се изучи ролята на оксидативния стрес в етиопатогенезата на депресивни нарушения при пациенти след инсулт с оглед да се оптимизират диагностичните, терапевтичните и медико-социалните подходи. За оценка на нивото на оксидативния стрес при след-инсултна депресия са изследвани нивата на продуктите на реактивни кислородни форми (ROS), аскорбатни (Asc•) и NO• радикали като биомаркери на оксидативния стрес в реално време с използване на EPR спектроскопия.