THE ROLE OF OXIDATIVE STRESS IN THE DEVELOPMENT OF DEPRESSIVE AND ANXIETY DISORDERS AFTER STROKE

SUMMARY
of dissertation work to acquire educational and scientific degree “PhD”

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The problem of the treatment and rehabilitation of patients with cerebral stroke has so far been one of the most current and difficult in modern clinical practice. Mental pathology not only comes to the forefront, but can also be crucial in restoring the motor and speech functions of the sick and their social adaptation, as well as influencing the mortality indicator. Depression is a common consequence of stroke. Approximately one-third of survivors after stroke experience significant symptoms of depression. Post-stroke depression is associated with poor quality of life, high disability and poor rehabilitation, higher healthcare use, mortality and suicidal ideation. No consensus has yet been reached on the etiology of post-stroke depression. Post-stroke depression may have multifactorial etiology including organic and reactive components.

Oxidative stress is involved in the pathogenesis of a variety of disease states and may be a common pathogenetic mechanism underlying many mental illnesses because the brain is relatively more vulnerable to oxidative damage. In the last few years, oxidative stress has been seen as one of the contributing factors in the pathogenesis of depression. Recently, it has also been discussed as a contributing factor in many chronic neurodegenerative pathologies, as well as acute cerebrovascular disorders such as stroke.

The aim of the paper is to investigate the role of oxidative stress in the etiopathogenesis of depressive and anxiety disorders in patients after stroke in order to optimize diagnostic therapeutical and medical-social approaches.

The following tasks have been placed:

1. Present the influence of the main socio-demographic characteristics (gender, age, education and marital status) and personality anxiety on the development of affective disorders of patients after stroke.

2. Analyze the dependence of the occurrence of post-stroke affective disorders on the localization of the hearth of brain damage, the severity of the stroke.

3. Present the psychopathological characterization and determine the severity of the depressive disorder in the acute phase after a stroke.

4. Investigate the correlation between depressive disorder and generalized anxiety disorder in postsynaptic patients.

5. Determine whether there is a link between the presence of depression and the presence of panic disorder in postsynaptic patients.

6. Investigate the level of reactive oxygen species (ROS) and nitrogen oxide (NO) generation in post-stroke patients depending on the presence of depressive disorder and its severity.

7. Determine the level of malondialdehyde (MDA), as a marker of oxidative damage to lipids, protein kinase (PC), oxidative damage marker of proteins, and 8-OHdG, a marker of oxidative DNA damage, in post-stroke patients depending on the presence of depressive disorder and its severity.
8. Investigate the level of the superoxide dismutase (SOD) and catalase (CAT) enzymes, as well as the non-enzymatic antioxidants (ascorbate radicals) in post-stroke patients depending on the presence of depressive disorder and its severity.

The contingent of the study was 93 stroke patients, of which 59 men (39-83 years) and 34 women (56-87 years), with average age 66.8 years. Patients treated at the Neurological Clinic to the University Hospital "Prof. Dr. Stoyan Kirkovich" in Stara Zagora for the period 2015 - 2016, meeting the criteria set out in the protocol, are being informed in advance of the objectives, tasks and methods of the study and have provided written consent for participation. All selected patients were examined for oxidative stress in the Oxidative stress and EPR spectroscopy laboratories. For comparison, a control group of 32 healthy individuals (14 men and 18 women) of the same sex and age was established.

The methodologies used are clinical, questionnaire, statistical and laboratory. The clinical methods include collecting complete information from patients, if possible and/or from close relatives, and available medical documentation on the underlying disease and concomitant illnesses, with emphasis on accompanying psychiatric history, deprivation of overall mental status, focusing on the affective sphere, somatic and neurological status and application of assessment scales (HAM-D-17, MMSE, NIHSS, STAI, PHQ-9, PHQ-15, GAD-7).

The survey method is an individual survey developed specifically for the purpose of the protocol, including socio-demographic indicators.

The statistical method includes analyzes performed using the Windows 7 statistical software package, STATISTICA version 10 (StatSoft, Inc.).

Laboratory methods include instrumental, biochemical, spectrophotometric, EPR and immuno-enzymatic methods.

The impact of socio-demographic factors and personality anxiety on the presence and severity of post-stroke depression were investigated. Of all the patients with depressive symptoms we studied, there were 25 (73.5%) women and 35 (59.3%) men. Of the women with depression 7 (20.6%) have mild, 7 (20.6%) have moderate, 2 (5.9%) have moderate-to-severe and 9 (26.5%) have severe depression. Of the men with depression 15 (25.4%) are mild, 11 (18.6%) have moderate, 8 (13.6%) are moderate-to-severe and 1 (1.7%) have severe depression. In women, it is more pronounced than in men (p <0.01). The incidence of generalized anxiety disorder was found in 28 (82.35%) of women and 42 (71.2%) of men. Of all women with GAD, 9 (26.5%) are mild, 10 (29.4%) are moderate and 9 (26.5%) are severe. Of all men with GAD, 17 (28.8%) are mild, 19 (32.2%) are moderate and 6 (10.2%) are severe. In contrast to depression, there was no statistically significant gender difference in GAD (p >0.05).

In the results obtained, there was no correlation between the age of the post-stroke patients and the presence of GAD, and we found no effect on the degree of depression in the family situation. On the other hand, within the framework of the results obtained, there is a
statistically significant difference between the degree of GAD in single patients (divorced, unmarried, widowed) and family (p < 0.05). In families, there is a higher degree of GAD compared to unmarried people.

When comparing Hamilton test results with the situation anxiety test, there is a strong dependence, i.e. there is a strong correlation between the degree of depression and the presence of personality anxiety (p < 0.001). With the increase in situational anxiety, the rate of depression increases. There is also a statistically significant relationship between the presence of personality anxiety and the degree of depression (p < 0.001). As the degree of personality anxiety increases, the rate of depression increases. There is a very strong relationship between the presence of situational anxiety and the degree of generalized anxiety disorder (p < 0.001). With the presence of situational anxiety, the degree of GAD is also increasing. We also found a strong relationship between the presence of personality anxiety and the degree of GAD (p < 0.001). In the presence of personality anxiety, the degree of GAD increases.

The dependence of the occurrence of post-stroke affective disorders on localization of the hearth of brain defeat and the severity of stroke was investigated. Our results show no dependence between the location of brain defect in patients with stroke and the severity of depressive disorder (p > 0.05). Of all patients studied, 73 (78.5%) with mild and 20 (21.5%) with moderate to severe stroke. There was no statistically significant relationship between the severity of the stroke and the severity of the depressive disorder (p > 0.05). Of the examined patients, 70 (75.25%) had GAD (27.95%) with mild, (31.2%) with moderate and (16.1%) with severe. Our results do not indicate relationship between the location of the stroke and the severity of the GAD (p > 0.05).

Comorbid depressive disorder in the patients we studied was diagnosed according to the ICD-10 criteria for depressive disorder: within one depressive episode (F 32) or a recurrent depressive episode (F 33). In (16.6%) of the depressed patients studied, the depressive episode was within recurrent depressive disorder and in (83.4%) depressive symptoms were newly diagnosed.

Of all post-stroke patients we studied 64.5% have depressive disorder. The severity of the diagnosed depressive states is objectively evaluated by HAM-D-17. Dominant in patients with mild depressive episode (36.6%) and moderate depressive episode (30%). Fewer are patients with moderate-to-severe depressive episode 10 (16.6%) and severe depressive episode - 10 (16.6%). We have not observed psychotic symptoms within the depressive disorder.

The levels of free radicals were examined. Free radicals consist of reactive oxygen species (ROS) and reactive nitrogen species (RNS). There was a statistically significant increase in ROS values in post-stroke patients with severe depression compared to the control group, post-stroke patients without depression and with moderate depression (p < 0.05). There is also a statistically significant increase in patients with moderate depression compared to the control group, post-stroke patients without depression and with mild depression (p < 0.05).
A statistically significant increase in post-stroke depression patients (p <0.05) was observed in nitrogen oxide levels contained in reactive nitrogen species (RNS) with regard to post-stroke patients without depression. In addition, the increase in nitrogen oxide levels in post-stroke patients and the control group (p <0.05) is also statistically significant. Statistically significant is also the increase in post-stroke patients without depression compared to the control group (p <0.05). The results show statistically significant increase in nitrogen oxide (NO) levels in patients with moderate depression compared to the control group, post-stroke patients without depression and patients with mild depression (p <0.05). There is also statistically significant increase in patients with severe depression compared to the control group and patients with mild depression (p <0.05).

The marker levels of oxidative damage to lipids, proteins and DNA were investigated. MDA (malondialdehyde) is an intermediate compound and a major indicator of the lipid peroxidation process. The results in the patients with stroke in our study showed that plasma MDA levels were significantly higher than in controls and confirmed those from other observations. Statistically significant is the increase in post-stroke patients with depression from post-stroke patients without depression (p <0.05) and the control group (p <0.05).
An increase in MDA values was observed in comorbid patients with severe depression compared to the control and follow-up group without depression, with mild and moderate depression ($p = 0.05$).

Protein carbonyl (PC) is a marker for oxidative damage to proteins. There is a statistically significant increase in PC levels in post-stroke patients with depression compared to the control group ($p < 0.05$), and post-stroke patients without depression compared to the control group ($p < 0.05$).

In a PC study, a statistically significant increase was seen in patients with severe, moderate, mild depressive disorder and post-stroke patients without depression compared to the control group ($p < 0.05$). As the severity of depression increases, the carbon content increases.

8-OHdG (8-hydroxy-2-deoxyguanidine) is a marker for oxidative DNA damage. There is a statistically significant increase in 8-OHdG marker levels in post-stroke patients with depression than post-stroke patients without depression ($p < 0.05$) and the control group ($p < 0.05$). Furthermore, the difference was statistically significant and between post-stroke patients and the control group ($p < 0.05$).

A statistically significant increase was observed in post-stroke patients with severe depression compared to the control group (0.81), post-stroke patients without depressive disorder and patients with mild depression ($p < 0.05$). Statistically significant is also the increase in patients with moderate depression compared to the control group, patients without
depression and patients with mild depression (p <0.05). As the severity of depression increases, levels of the 8-OHdG marker increase.

The levels of enzyme and non-enzymatic antioxidants have been investigated. The results obtained on erythrocyte SOD activity showed a statistically significant decrease in post-stroke depressive disorder compared to the control group (p <0.05) as well as in post-stroke patients without depression (p <0.05). Increased generation of ROS depletes the antioxidant enzyme SOD, which is first included in the antioxidant protection system. Therefore, superoxide anions form high amounts of hydrogen peroxide (H2O2) catalyzed by the catalase enzyme (CAT).

In the SOD study, a statistically significant decrease was observed in post-stroke patients with severe, moderate, mild depression and non-depressed patients (p <0.05). The lowest values are observed in patients with moderate depression.

Our results showed that there was a statistically significant increase in erythrocyte lysate catalase activity in post-stroke patients with depression (p <0.05) and post-stroke patients without depression (p <0.05) and compared to the control group (p <0.01). In addition, elevation was statistically significant in post-stroke patients without depression compared to the control group (p <0.05). The high activity of CAT is due to the high levels of hydrogen peroxide (H2O2), which is formed by the superoxide anion radical (O2-). The enzyme catalase significantly prevents the formation of hydroxyl radicals (OH) which are the most toxic radicals to the organism from reactive oxygen species.
Statistically significant increases in erythrocyte CAT levels in post-stroke patients with severe depression compared to the control group (p <0.05), as well as compared to patients with mild, moderate and post-stroke patients with no depression (p <0.05) have been observed. Also, the elevation of CAT in post-stroke patients without, with mild and moderate degrees of depression was statistically significant compared to the control group (p <0.05). This is due to the increase in H2O2 levels in the more severe degrees of depressive disorder.

Ascorbate radicals are non-enzymatic antioxidants that lower levels of oxidative stress. In the assay of ascorbate radicals, a statistically significant increase was observed in post-stroke patients without depression compared to the control group (p <0.05). Statistically significant is the decrease in post-stroke patients with depression compared to post-stroke patients without depression (p <0.05).

Statistically significant increases were observed in patients without depression and in patients with mild depression compared to the control group (p <0.05). On the other hand, as the severity of depression increases, the levels of ascorbate radicals decrease. A statistically significant decrease compared to the control group, patients without depression and mild depressive patients was observed in patients with severe depression (p <0.05). In moderate depressive patients, there was a statistically significant decrease compared to patients without depression and with mild depression (p <0.05).
Summarizing the results, it is found that the development of post-stroke depression is a common mood disorder affecting about a third of the patients with stroke. In our study, depressive disorder was reported in two-thirds of the patients with stroke.

When analyzing the impact of sociodemographic factors on triggering post-stroke depression, we report that female gender is associated with a statistically significant (p <0.01) higher risk of developing more severe post-stroke depression. There is also a trend that despite the lack of statistically significant difference in our study, female gender is a risk factor for the presence of GAD. We have not found a loneliness impact on post-stroke depression, whereas a negative correlation is observed with the GAD. The specific features of the person as a high level of personality anxiety are also a high risk factor for post-stroke depression. The high degree of personality anxiety also correlates with a high degree of generalized anxiety disorder.

In the majority of cases, the comorbid depressive disorder in the patients studied is mild and moderate. Against the backdrop of low mood (81.6%) and sadness (73.3%), increased mental (86.6%) and somatic (78.4%) anxiety, insomnia, predominantly early morning wake, the low degree of expressiveness of endogenous depression, the supernatural thoughts of inferiority, guilt and hopelessness. Suicidal thoughts shared 1.7% of depressed patients, with only one experiencing suicidal experiences in the past. Most patients, 52 (86.7%), with post-stroke depression, are involved in somatic complaints (weight in the extremities, back or head, back pain, head or muscle, loss of energy and easy tiredness) and their extraordinarily hypochondriac thoughts (78.3%). Using Factor Analysis, Savina MA (2016) outlines the limits of post-stroke depressive syndrome that has a mosaic structure and includes the following depressive symptoms: grief (87.3%), anhedonia (55.1%), depressive apathy (25.4%), hopelessness (25.4%) and dysphoria (50.8%), as well as the presence of vegetative disorders. The syndrome it describes does not fully comply with the criteria of international classifications: it often involves dysphoria, as well as asthenopoietic disorders such as weakness and tiredness, which add to the organic spectrum disorders.

Arnaudova M. 2015 discusses concerns about own body health, late-depression with frequent "masking" behind somatic symptoms, making it difficult to diagnose and treat these conditions in a timely manner. However, there is no doubt that somatic symptoms in the elderly may not be associated with depression, which would underestimate actual somatic problems. Depressions in the elderly are to a large extent "somaticated", i.e. the somatic symptoms prevail over the others. Insofar as the clinical manifestations of the depressive disorder of the stroke are predominantly somaticated, with predominance of the anxiety disorders (Surname et al., 2005) it is necessary to take attention to the high sensitivity of patients, even to a slight increase in body discomfort in post-stroke depression. The sociocultural traditions of the Bulgarian people also contribute to the fact that patients more often and willingly communicate to the treating physicians somatic complaints, considering the emotional discomfort as an unimportant and unreasonable discussion.

The brain with its high oxygen consumption and lipid-rich environment is considered highly susceptible to oxidative stress or redox imbalances. Oxidative stress is an imbalance between the biochemical processes generating ROS and the ability of a biological system to
neutralize them. As a result, ROS are formed faster than cellular defense systems can remove them (Gadjeva 2007). It has been shown that depression in somatically healthy patients is associated with increased blood cytokine concentrations (Dowlati et al., 2010), increased levels of ROS and RNS (Maes et al 2011a, Maes 2011b, Suzuki et al 2001, Dhir et al 2011). In addition, significantly lower plasma concentrations of several key antioxidants such as vitamin E, zinc and coenzyme Q10, and antioxidant-reduced enzyme activity, as well as reduced brain glutathione (GSH) levels, have been reported in a major depressive episode (Maes et al., Gawryluk et al., 2011). Free radicals are the result of the oxidative-reductional reactions occurring in the body. They are paramagnetic particles, molecules, atoms or ions that can accept or deliver electrons and create a chain reaction that can cause significant biological damage (Gadjeva 2007a, 2007b, 2007c). The existence of a possible link between depression and inflammation of the central nervous system is suggested in several recent articles on neuroimmunology of stroke (Vogelgesang et al., 2011, Pachner et al., 2012). 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 weaken cognitive sensitivity. Recent studies have shown that oxidative and nitrate stress can contribute to the pathogenesis of depression by participating in neurogenesis, neuroplasticity, nerve inflammation and monoamine reuptake (Pae et al., 2004, Pae et al., 2008). The latter trend is that antidepressants have a therapeutic effect by suppressing the production of inflammatory cytokines, ROS and RNS, or even increasing antioxidant protection (Lee et al., 2013). In our study, a statistically significant increase in ROS values was observed in patients with severe depression compared to the control group, patients without depression and with moderate depression (p <0.05).

Brain ischemia unlocks a complex cascade of metabolic events, most of which involve the formation of nitrogen and oxygen free radicals. Oxidative stress during stroke has a significant impact on irreversible damage to neurons (Safwen et al., 2014, Chen et al., 2014, Abd-Elsameea et al., 2014, Lee et al., 2014). As a result of hypoxia, oxygen and glucose are depleted in the brain tissue, which are the main products for ATP synthesis. This inhibits the activity of the sodium-potassium pump and increases the flow of calcium ions into the cell. Increased Ca2+ concentration in the cell results in the activation of Ca2+ dependent enzymes, including proteases and nucleases responsible for neuronal degradation processes (Broughton et al., 2009).

Based on the facts above, the present study is designed to investigate the possible relationship between the level of typical markers of oxidative and nitrile protein modifications (protein-carbonyl groups, CAT) and the degree of post-stroke depression. Oxidation of amino acid residues leads to the formation of relatively stable protein-carbonyl groups, which can be qualitative and quantitative markers allowing the evaluation of oxidative damage to proteins (Dalle-Donne et al 2003a, Dalle-Donne et al., 2003b). In our study, 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. We showed more than double increases in the protein-carbonyl groups in the post-stroke patients
compared to healthy subjects. Our results are consistent with previous reports (Cichon et al., 2015). The results show for the first time 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.

We reported an increase in NO levels in patients with post-stroke depression, with the highest levels in moderate and severe depressive disorders. Although the clinical benefit of NO synthase polymorphisms has not yet been determined, Kudlow et al. 2016 highlight the 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 (eg, microglial activity) (Liu et al., 2011, Lu et al. 2013, Tynan et al. 2012) or phosphodiesterase enzyme activity (Reierson et al., 2011). Perhaps the approach complementary to three strands where traditional antidepressants are used in combination with pharmacological agents aimed at normalizing NO and inflammatory signaling pathways, would be most appropriate.

The 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 in hydrogen peroxide (H2O2) and oxygen (O2). Hydrogen peroxide is then reduced to water and molecular oxygen by the enzymes peroxidase, glutathione and catalase (Venarucci et al., 1999). 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. 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 dependence being almost double higher 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. Of importance for the decrease of SOD is the presence of depressive disorder after a stroke. In our study in depressive post-stroke patients, CAT levels are high, which decomposes hydrogen peroxide as a second level of protection against oxidative stress, and the potential for dealing with the body's oxidative stress is still high in the early days after the stroke. Severe depressive disorder correlates statistically significant with the high CAT levels. In post-stroke patients with depressive disorders, non-enzymatic ascorbate radicals are rapidly depleted as a biomarker of oxidative stress. We observed that in the severe degrees of depression the lowest level of ascorbate radicals compared to the milder degrees of depression is therefore the lowest opportunity to compensate for oxidative stress. For this reason, complex therapy of depression is a recommended intake of vitamin C.

From this study we can draw the following conclusions:
1. The role of socio-demographic factors in the development of affective disorders of patients after a stroke is relatively weak.
1.1. Self-importance is only gender for the development of depression. In lonely patients, GAD occurs at a lower level than in family. The remaining socio-demographic factors studied (age and education) have no effect on the occurrence of affective disorders.

1.2. The specific features of the personality as a high level of personality anxiety are also a high risk factor for post-stroke depression and generalized anxiety disorder.

2. There was no statistically significant dependence of the occurrence of post-stroke affective disorders on localization of the hearth of brain damage and the severity of the stroke in post-stroke patients. There is no statistically significant correlation between the severity of depression and localization and the severity of the stroke.

3. Depressive disorder in the acute phase after stroke was found in approximately 2/3 (64.5%) of patients with stroke, prevail those with mild and moderate-to-severe depressive disorders with mood swings, sadness, increased mental and somatic anxiety, insomnia, somatic complaints, with extravagant hypochondriac thoughts.

4. Generalized anxiety disorder in post-stroke patients was found in 70 (75.25%). There is a statistically significant positive correlation between depressive disorders and generalized anxiety disorders - increasing of severity of depressive disorder increases the severity of generalized anxiety disorder as well.

5. There is no statistically significant correlation between the presence of depression and the presence of panic disorder in post-stroke patients.

6. The levels of reactive oxygen species (ROS) and nitric oxide (NO) generation in post-stroke patients are dependent on the presence of depressive disorder and its severity.

6.1. In post-stroke patients with depression, there is increased generation of reactive oxygen species (ROS). The highest are the ROS levels in the severe compared to the lower degrees of depression.

6.2. Nitrogen Oxide (NO) is significantly increased in patients with post-stroke depression. Severe degrees of depression correlate statistically significantly with higher levels of nitric oxide.

7. Malondialdehyde (MDA), a product of lipid peroxidation during the oxidative stress, protein-carbonyl (PC), as a marker for oxidative damage to proteins and the level of 8-OHdG, a marker of oxidative DNA damage, are increased in depressive post-stroke patients. The worst degrees of depression correlate statistically significant with the highest levels of all three markers.

8. The superoxide dismutase enzyme (SOD) decreases in the presence of depressive disorder after a stroke, but there is no significant difference in SOD levels at different degrees of depression. In depressive post-stroke patients, catalase enzyme (CAT) levels are high and severe depressive disorder correlates statistically significant with higher CAT levels. The smallest opportunity to deal with oxidative stress is found in severe degrees of depression due to low levels of ascorbate radicals. Because of these results, antioxidants such as vitamin C,
coenzyme Q, omega 3 and others are also appropriate for the complex treatment of depression with antidepressants.