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Original article

Evaluation of ADMA, carbonyl groups, CAT and NKA in depressed patients with and without posttraumatic stress disorder

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ABSTRACT

Background: It has been shown that asymmetric dimethylarginine (ADMA), carbonyl groups, catalase (CAT) and neurokinin A (NKA) are actively involved in neuronal processes such as depression and posttraumatic stress disorder (PTSD). One of their roles is to protect the body from oxidative damage. This is done by affecting neuronal growth, development and plasticity. The study aimed at assessing the concentrations of ADMA, carbonyl groups, CAT and NKA in patients with varying levels of depression severity, PTSD, and depression concurrent with PTSD.

Methods: The study covered 460 people. Out of them, 120 suffered from different types of depression. The study groups comprised: 60 subjects with mild depression (MD), 60 subjects with moderate depression (MOD), 60 subjects with severe depression (SeD), 60 subjects with MD and PTSD (MD + PTSD), 60 subjects with MOD and PTSD (MOD + PTSD), 60 subjects with SeD and PTSD (SeD + PTSD), and 60 subjects with PTSD alone. Each group of 60 participants included 30 males and 30 females. The concentrations of all blood parameters were determined at 7 a.m. using the ELISA method.

Results: Depressive episodes became more severe as the concentration levels of studied markers increased.

Conclusions: ADMA, carbonyl groups, CAT and NKA can be useful markers of chronic stress in both males and females with depression, PTSD, and depression concurrent with PTSD. They can be utilized when making an initial diagnosis and evaluating the severity of disease. Changes in their concentration levels may show a biological response to oxidative stress characteristic of depression.

Introduction

Psychiatric disorders such as depression and posttraumatic stress disorder (PTSD) are characterized by biochemical imbalances in the central nervous system (CNS) and the activation of inflammatory as well as oxidative and nitrosative (O&NS) pathways [1]. As a consequence of being exposed to chronic stress, these biochemical imbalances, also called an allostatic load, may occur in depressed patients [2]. Reactive oxygen species (ROS) have also been shown to play a significant role in the pathogenesis of depression and PTSD by inducing protein oxidation. They include radical species such as superoxide ($O_2^{\bullet -}$), hydroxyl ($OH^{\bullet}$), peroxyl ($RO_2^{\bullet}$), alkoxyl ($RO^{\bullet}$), hydroperoxyl ($HO_2^{\bullet}$), and nonradical species such as hydrogen peroxide (H_2O_2), hypochlorous acid (HOCl), ozone (O_3), singlet oxygen ($^1O_2$), and peroxynitrite ($ONOO^-$), [3]. Major depressive disorder (MDD) is related to the overproduction of ROS. This may result in lipid peroxidation and damage to proteins, DNA and mitochondria [4]. Also, it may induce an autoimmune response against neoepitopes of fatty acids and proteins. The main reason for disease neuroprogression is damage caused by oxygen-nitrogen stress (e.g. an overproduction of nitrogen oxide or impaired oxidative metabolism in mitochondria) and a subsequent autoimmune response [5]. This damage then leads to disturbed cell signaling, mitochondrial dysfunction, impaired axonal regeneration and increased apoptosis. Oxygen-nitrogen stress activates inflammatory processes in the central nervous system and contributes to elevated levels of proinflammatory cytokines, decreased neurogenesis, disease neuroprogression, as well as mitochondrial and hypothalamic-pituitary-adrenal (HPA) axis dysfunction. Moreover, antioxidant concentrations are reported to be decreased and oxidative stress increased [6]. Both inflammatory and mitochondrial oxidative processes generate free radicals that are highly reactive species. Oxidative stress inactivates and modifies antioxidant enzymes and impairs antioxidant defense [7]. Catalase is one of the major intracellular antioxidant enzymes that forms the antioxidant defense system against radical-mediated damage [8].

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Asymmetric dimethylarginine (ADMA) is a natural product of asymmetric methylation of proteins. It is also an endogenous inhibitor of endothelial nitric oxide synthase (eNOS). When circulating ADMA competes with L-arginine (the substrate of nitric oxide synthases, NOSs), it is considered to be a crucial risk factor for chronic stress in depressed individuals. As a consequence, the L-arginine-nitric oxide pathway becomes impaired and oxidative stress increases, leading to endothelial dysfunction [9,10].

Increased steady-state ROS levels may cause protein oxidation. This can result in their conversion into forms that are more prone toward proteinase degradation [11]. Carbonyl groups are markers of oxidative damage to proteins. Protein carbonyl formation is an index of oxidative stress as a result of amino acid modifications. Protein carbonylation is the non-enzymatic addition of aldehydes or ketones to particular amino acid residues, such as arginine, lysine, threonine, proline, cysteine, and histidine. Also, carbonyl groups may be introduced into proteins *via* secondary reactions of the nucleophilic side chains of Cys, His, and Lys residues with aldehydes that are produced during lipid peroxidation, or with reactive carbonyl derivatives that are created from the reduction of sugars or their oxidation products. Protein carbonyl content is the most general indicator and by far the most commonly used marker of protein oxidation. The accumulation of protein carbonyls has been reported in several human diseases, such as depression and posttraumatic stress disorder, among other conditions [12–14].

Neurokinin A (NKA; NK-2) is one of the two most thoroughly described members of the tachykinin family of neuropeptides. The other one is neurokinin B (NKB; NK-1). They are both putative neurotransmitters that exert crucial physiological functions in both the central nervous system and peripheral tissues. NKA influences target cells by interacting with specific receptors, which have been cloned. Also, it is said to have seven transmembrane spanning sequences and to be coupled to G-proteins and the phosphoinositide-signaling pathway. Three distinct receptors have been identified so far. One of them is called neurokinin-2 receptor (NK-2R). As a receptor for Substance K (SK) in the human brain, NK-2R may offer a treatment target in depressed patients with comorbid PTSD. NK2 receptors are expressed in the amygdala, hippocampus and other components of neural stress-response circuitry. Based on the studies on both animals and humans, exposure to stressors causes a release of SK in the amygdala, while selective antagonism of NK2 receptors inhibits behavioral stress responses [15–17].

In order to understand the biochemical mechanisms of depression with and without PTSD, it is crucial to understand the degree of oxidative stress and the status of the antioxidant defense system in affected individuals.

Material and methods

The study covered 460 individuals, 120 of whom were female and 120 male. The mean age was 45.2/4.5 years (range: 19–47 years). The groups comprised: 60 subjects with mild depression (MD), 60 subjects with moderate depression (MOD), 60 subjects with severe depression (SeD), 60 subjects with MD and PTSD (MD + PTSD), 60 subjects with MOD and PTSD (MOD + PTSD), 60 subjects with SeD and PTSD (SeD + PTSD), and 60 subjects with PTSD alone. Each group of 60 participants included 30 females and 30 males. In order to diagnose PTSD, the authors used the fifth edition of the Diagnostic and Statistical Manual of Mental Disorders (DSM-5). The DSM-5 criteria are divided into four diagnostic clusters: intrusion, avoidance, negative cognitions and mood, and alterations in arousal and reactivity. Having been exposed to traumatic events, PTSD patients are reported to persistently re-experience them thereafter. This eventually cause them to avoid any distressing trauma-related stimuli. In addition, such patients are also reported to have negative alterations in cognitions and mood. They are manifested as an inability to recall significant details of the traumatic event, negative trauma-related beliefs and emotions, as well as reduced affect. Moreover, these affected individuals find it impossible to experience positive emotions from that moment. Trauma-related alterations in arousal and reactivity are also observed, and they manifest as aggressive and self-destructive behaviors. Patients display aggressive and self-destructive behaviors. The symptoms are observed for at least one month. Personality disorders are mostly characterized by impaired self and interpersonal functioning accompanied by pathological personality traits. Depressive disorders were diagnosed by means of the fifth edition of the Diagnostic and Statistical Manual of Mental Disorders. The psychometric properties of the Beck Depression Inventory (BDI-II) by Aaron T. Beck were used to measure depression severity. The BDI-II consists of twenty one items. Each item corresponds to various depressive symptoms. The respondents were asked to fill out a multiple choice questionnaire by selecting one of four responses that best described their emotions over the past 30 days. They could receive 0–3 points for each answer. The total score that could be earned was between 0 and 63. In order to measure depression severity, the following cut-off points were used: 0–11 = no depression, 12–19 = mild depression, 20–25 = moderate depression, over 26 = severe depression.

Depressed subjects underwent treatment at the Department of Psychiatry between 2012 and 2016. The control group comprised forty sex- and age-matched healthy individuals. The mean age of the controls was 42.4 +/− 4.1 years (range: 23–48 years). Exclusion criteria for both groups included: organic damage to the central nervous system, diagnosed mental illnesses other than specified, diagnosed alcohol or other substance abuse, treatment for infectious and chronic systemic diseases. Individuals who were using tobacco or taking medication were also excluded from the study. In addition, none of the female subjects or controls were going through menopause, and blood was taken for lab tests during the follicular phase of the menstrual cycle. Prior to pharmacological treatment, all subjects had fifteen millimeters of blood collected from the median cubital vein on admission. Neither participant had been taking any medication for two weeks prior to blood draw. Only healthy individuals comprised the control group.

Determination methods

Blood samples were collected between 7 and 9 a.m. from all subjects and sent for lab tests. Then, they were centrifuged at 3500 rpm for 10 min. Following centrifugation, the samples were pipetted into sterile 2 ml microcentrifuge tubes and stored at −70 °C. For laboratory analysis, the enzyme-linked immunosorbent assay (ELISA) was used. The following markers were determined: asymmetric dimethylarginine (ADMA), (Immunodiagnostik, Bensheim-Germany); protein carbonyl (CO) groups, (Immunodiagnostik, Bensheim-Germany); catalase (CAT), (Cloud-Clone Corp., USA); neurokinin-A (NKA), (Ray Biotech., USA). The tests were performed in duplicate using properly diluted plasma samples. Standard solutions were added to the plate to verify reliability of the results. Having measured the concentrations, a calibration curve was prepared. The plate was coated with a monoclonal antibody specific for a particular antigen. Then, the plasma samples were applied to the plate and incubated for 60 min. Having appeared in the plasma, a specific antigen bound to a specific antibody. All unbound antigens were washed away and an enzyme-linked antibody specific for a particular marker was added. Following this, the samples were incubated for 30 min and the plates were rinsed. The next stage involved adding a substrate solution to produce a color reaction. Color intensity differed