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Changes in Oxidative Stress-Related Biomarkers May Have a Role in The Pathophysiological Mechanisms Involved in Autism

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Abstract: There is evidence that oxygen free radicals play a vital part in the pathophysiology of numerous neuropsychiatric disorders. Although it has not been investigated yet, several recent studies proposed that nitric oxide (NO) and other parameters related to oxidative stress may have a pathophysiological role in autism. This study aims to evaluate the plasma levels of antioxidant enzyme, superoxide dismutase (SOD) and plasma level of Nitric oxide (NO), a marker of oxidative stress, in Egyptian autistic children. Autism is a neurodevelopmental disorder of childhood with poorly understood etiology and pathology. The present study included 40 children with autism diagnosed by DSM-V-TR criteria and Childhood Autism Rating Scale. Controls included 40 age-matched healthy children. Cases were referred to Outpatient Clinic of Children with Special Needs Department, National Research Center, Cairo, Egypt. We compared levels of SOD, and NO in children with autism and controls. Level of NO was significantly higher in autistic children compared with their controls, while SOD was significantly lower among patients than controls. These findings indicate a possible role of increased oxidative stress and altered enzymatic antioxidants, both of which may be relevant to the pathophysiology of autism.

Keywords: Autism Spectrum disorder-Nitric oxide- reactive oxygen species-Oxidative stress.

Introduction

Autism spectrum disorder (ASD) defines as a group of common, complex neurodevelopmental disorders. The Centers for Disease Control and Prevention (CDC) released the estimate of the prevalence of ASD among children aged 8 years was that 1 in 68 children in 2010 (Mandell and Lecavalier, 2014). The need to understand the causes of ASD and the underlying pathophysiology have become more acute since the number of diagnosed cases has risen markedly in recent years (Tu et al., 2013).

While the cause of autism remains elusive, autism is considered a multifactorial disorder that is influenced by genetic, environmental, and immunological factors and additionally increased vulnerability to oxidative stress (Chauhan and Chauhan, 2015, 2006).

Genetic, environmental and immunological risk factors induce the oxidative damage, promote neuronal damage, and reduce methylation activity during synthesis of myelin basic protein, which is fundamental for development of the central nervous system (Smaga et al., 2015). In fact, oxidative stress has also been implicated in the pathogenesis of other neuropsychiatric diseases, including major depressive

disorder (Nunes et al., 2013), anxiety disorders (Guney et al., 2014), and obsessive compulsive disorder (Kandemir et al., 2013).

Increasing evidence suggests a role of oxidative stress in the development and clinical manifestation of autism (Chauhan and Chauhan, 2006). Reactive Oxygen species (ROS) including superoxide anion radical ($O_2^{\bullet -}$), hydrogen peroxide (H_2O_2), hydroxyl radical ($^{\bullet}OH$), singlet oxygen ($^1O_2$) and nitric oxide ($NO^{\bullet}$) may lead to cellular injury when they are generated extremely or the antioxidant defense systems are destructed.

Nitric oxide (NO) has chemical properties that make it uniquely suitable as an intracellular and intercellular messenger. It is produced by the activity of nitric oxide synthases (NOS), which are present in peripheral tissues and in neurons. NO is known to be an oxygen radical and a neurotransmitter in the central and peripheral nervous systems. Although NO is described as an atypical neurotransmitter in the nervous system, it seems more appropriate to consider it as a second messenger. On the other hand, NO is known to affect neurodevelopmental processes in CNS (Rose et al., 2012).

It has been implicated in a number of physiological functions such as noradrenaline and dopamine release, memory and learning, and certain pathologies such as schizophrenia, bipolar disorder, and major depression (Chauhan et al., 2012).

A number of oxygenated compounds, particularly aldehydes including 4-hydroxynonenal and malondialdehyde (MDA), are produced during the attack of free radicals to membrane lipoproteins and polyunsaturated fatty acids (PUFAs). Therefore, assessment of thiobarbituric acid-reactive substances (TBARS) or 4-hydroxynonenal is probably the most commonly applied method for the measurement of lipid peroxidation (Frye et al., 2013).

Superoxide dismutase (SOD) is antioxidant protein that converts superoxide to hydrogen peroxide (Tamari et al., 2013). Excessive free radical production or oxidative stress may be involved in the pathophysiology of schizophrenia as evidenced by increased SOD activities (Wu et al., 2014). In clinical trials, the association between oxidative stress and autism has not been established and even present conflicting results. The SOD activity was either decreased in plasma (Sogut et al., 2003) and erythrocytes (Yorbik et al., 2002) or increased in plasma (Laszlo et al., 2013) and in erythrocytes (Vergani et al., 2011).

Assessment of the activities of these free radical scavenging enzymes in plasma may help to understand better the changes in antioxidative status in autism. The hypothesis is that the imbalance between oxidant and antioxidant systems might be involved in the pathophysiology of autism like other psychiatric diseases such as schizophrenia, bipolar disorders, etc. It was also demonstrated that mice lacking the fragile X

mental retardation protein showed a reduced SOD expression and these mice were more sensitive to oxidative stress and demonstrated behavioral characteristics of autism (Bechara et al., 2009).

Therefore, the purpose of this study was to investigate the plasma level of NO and the potential role of SOD in Egyptian children with ASD by measuring plasma circulating levels of SOD and comparing them with age and gender-matched typically-developing children.

Subject and Method

- This study was carried out on forty children with autism, their ages range between 3 to 5 years, at the Out-patient clinic for "children with autism" of the Department of Children with Special Needs at the Centre of excellence of Medical Research Centre, National Research Centre and another forty age –matched normal children as controls.
- This study was included newly diagnosed cases (before receiving any treatment). The diagnosis of autism was made by using 3 psychometric assessments; Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition, Text Revision (DSM-V) (American Psychiatric Association, 2013), Childhood Autism Rating Scale (CARS) (Schopler et al., 1993) and Autism Diagnostic Interview- Revised (ADI-R) (Lord et al., 1994).
- **Blood Sampling**: venous blood samples from both the autistic and control children were collected in EDTA coated tubes, plasma was separated by centrifugation 20-minutes at the speed of 3000 r.p.m and used for biochemical analysis including:
    - Plasma Superoxide Dismutase was determined using ELISA system using an ELISA commercial kit according to the manufacturer's protocol (eBioscience company, USA)
    - The Nitric oxide level in human plasma was assessed based on the modified Griess method according to Tatsch et al., (2011).

Results

The results showed that mean concentration of Nitric oxide was significantly high, while SOD plasma level was deceased in autistic children compared to control children, table 1.

Table 1: Plasma levels of NO and SOD in autistic children compared to age-matching controls.

Parameter / Groups	Nitric Oxide(umol/L) (Mean ± S.D.)	SOD (U/ml) (Mean ± S.D.)
Autistic	27.21 ± 6.92	1.02 ± 1.40
Control	13.73 ± 3.45	1.67 ± 0.90
P value	≤ 0.01	≤ 0.01

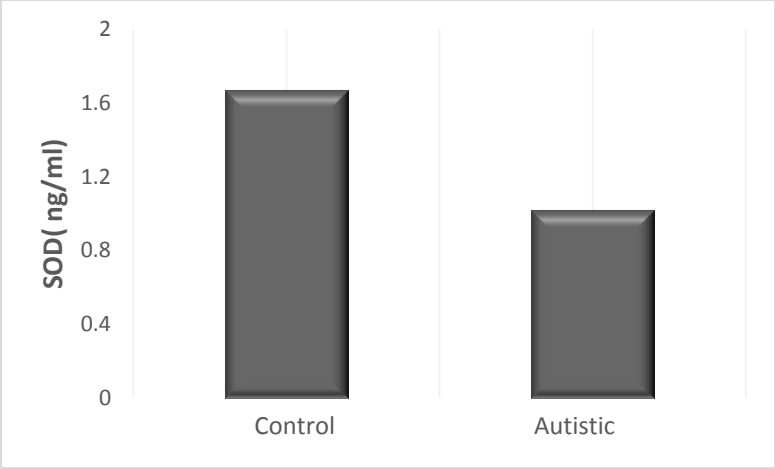

Figure1: plasma concentration of SOD in autistic group in comparison to control group

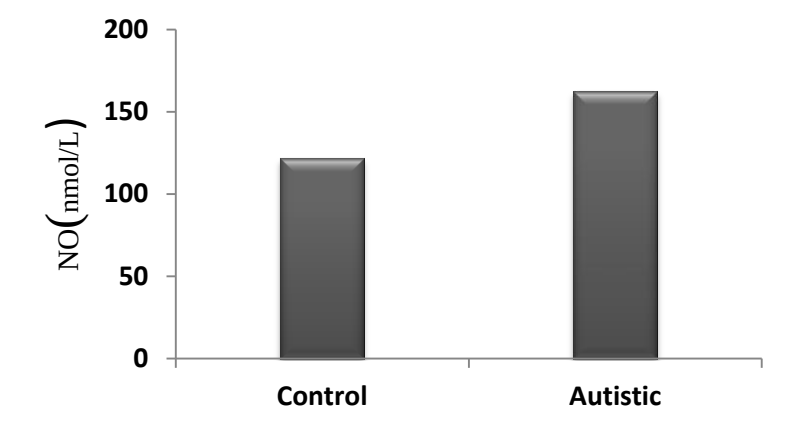

Figure 2: plasma concentration of NO in autistic group in comparison to control group

Discussion

Oxidative stress happens when cellular antioxidant defense mechanisms fail to balance and control reactive oxygen species (ROS) generation. Oxidative stress in autism has been studied at the membrane level and also by measuring products of lipidperoxidation, detoxifying agents (for example, glutathione), and antioxidants required in the defense system against ROS (Smaga et al., 2015).

In the present study, we exhibited that plasma NO levels were significantly increased while SOD levels were significantly diminished in children with ASD compared with typically-developing children, indicating a conceivable part of expanded oxidative stress and altered enzymatic antioxidants, may be relevant to the pathophysiology of autism. Consistent with our findings, Gonzalez-Fraguela et al. (2012) suggested the notion that oxidative stress was associated with autism. Similarly, Sogut et al. (2003) found that increased oxidative stress and altered enzymatic antioxidants may be relevant to the pathophysiology of autism. Several studies have reported increased oxidative damage as evident by increased lipid peroxidation,

protein and DNA oxidation as well as decreased antioxidant status(enzymatic and non-enzymatic) indicated by glutathione—redox imbalance and decreased activities of antioxidant enzymes in subjects with autism (Chauhan and Chauhan, 2006).

Nitric oxide has been recognized as a biological neural messenger molecule although it is best known as a toxic reactive free radical in the CNS. NO or NO derived nitrogen oxides must interact with neuromodulators in order to modify these modulators, especially monoamines, and thereby change their regulatory action on synaptic transmission (Ankarali et al., 2009).

NO is synthesized on demand by the enzyme NO synthase (NOS) from L-arginine. A critical reaction that NO undergoes in oxygenated biological media is a direct bimolecular reaction with O2 yielding peroxynitrite (ONOO_). Peroxynitrite and its further products have been linked to several interactions which may contribute to cellular injury, including lipid peroxidation, nitrosylation of some molecules, and inactivation of sodium channels. Liposomes exposed to XO-derived reactive species in the presence of NO exhibited both stimulation and inhibition of lipid peroxidation, depending on the ratio of the rates of ROS production and NO introduction into reaction system. Therefore, ONOO_ has been shown to oxidize a variety of biological molecules and may be responsible for certain types of NO-mediated toxicity (Valko et al., 2007).

Taken together, NO or closely related molecules have been reported to be neurodestructive. NO can induce neurological toxicity under conditions of excessive formation (Talarowska et al., 2014).Previous studies suggest that peripheral NO metabolites can be used as a marker of CNS dependent NO changes. The changes in NO levels may be meaningful in autism owing to the aforementioned functions of NO in the nervous system. There was a remarkable increase in total nitrite levels Fig. 2.

Increased oxidant end-products by the reactions of NO with other free radicals may probably contribute to the neuropathophysiology of autism because of the preferential vulnerability of the brain to oxidative injury. In this study, we found that plasma levels of SOD are associated with risk of ASD. These findings, if replicated, have numerous implications for the progression and treatment of ASD.

Rose et al. (2012) indicated that increased oxidative stress in the autism brain may have functional consequence in terms of achronic inflammatory response, increased mitochondrial superoxide production, and oxidative protein and DNA damage. It is important to discuss whether reduced plasma levels of SOD in children with ASD have physiological or pathological roles.SOD may play pathophysiological role in autism through several paths. First, oxidative stress-induced production of lipid peroxides and their by-products is known to lead to the loss of membrane functions and integrity (Chauhan and Chauhan, 2006). Second, several studies have suggested a link between oxidative stress and the immune response (Sutti et al., 2014).

The connections between the immune system and the nervous system, including its possible role in the development of ASD had been suggested. Third, the connection between inflammatory response and oxidative stress had been suggested. A number of studies have implicated oxidative stress as a major upstream component in the signaling cascade involved in activation of redox-sensitive transcription factors and pro-inflammatory gene expression leading to inflammatory response (Rossignol and Frye, 2012a).

Fourth, excitotoxicity has been suggested as a result of oxidative stress (Basso and Ratan, 2013). Substantial evidence suggests that excitotoxicity, oxidative stress and impaired mitochondrial function are the leading cause of neuronal dysfunction in autistic patients (Essa et al., 2013). We inferred that children with autism are more susceptible to oxidative stress and lacking antioxidant defense mechanism. We highlight that autistic children might profit from antioxidants supplementation. in addition, early assessment of antioxidant status would have better prognosis as it might diminish the oxidative stress before inducing more irreversible brain damage.

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