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Oxidative imbalance in adult attention deficit/hyperactivity disorder☆

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ABSTRACT

Objective: There are few studies evaluating the biochemical basis of adult attention deficit/hyperactivity disorder (A-ADHD). In the present study, we evaluated whether nitric oxide (NO), an oxidant, level and superoxide dismutase (SOD), an antioxidant, activity are associated with A-ADHD or not.
Methods: Twenty A-ADHD patients from Gaziantep University Sahinbey Research Hospital, Psychiatry Clinic, diagnosed according to The Turkish version of Adult ADD/ADHD DSM IV-Based Diagnostic Screening and Rating Scale by two psychiatrists (H.A.S. and S.S.), and twenty-one healthy volunteer controls were included. Blood samples were collected; NO levels and SOD activities were measured.
Results: The mean NO levels in patients were significantly higher than those of controls and SOD activity of patients was significantly lower than controls.
Conclusions: Remarkable high levels of oxidant NO, and low SOD activities suggest an oxidative imbalance in A-ADHD. This is the first study evaluating the oxidative metabolism in A-ADHD.

1. Introduction

Awareness against adult attention deficit/hyperactivity disorder (A-ADHD) has grown in recent years and is so common now that specific clinics in major medical centers and other outpatient clinics are devoted specifically to referrals of attention problems in adults. Attention-deficit/hyperactivity disorder usually identified in childhood persists into adulthood in about 60% of individuals with childhood onset (Elliott, 2002).

There are few studies evaluating the biochemical basis of the disorder. From syndromal aspect of view, A-ADHD may be involved with some other systems, such as oxidative metabolism. The oxidative status of other psychiatric disorders has already been studied and more evidences pointing out the possible etiological role of those molecules have been reported (Akyol et al., 2004). Oxidative stress was accused of DNA damage in some psychiatric disorders such as bipolar disorder (Andreazza et al., 2007a). The imbalance seems to be present in brain problems, but the question of which is the cause and which is the effect, or both still remains unanswered. Rare researches have evaluated the oxidative metabolism of ADHD in animals and children but not in adults (Aspide et al., 1998, Varol Tas et al., 2006). Even with very little data, antioxidant treatments were tried in ADHD. Therefore, evaluating the oxidative status of A-ADHD patients is essential for further intervention designs. Formerly, we have researched the lipid peroxidation status of A-ADHD and demonstrated the imbalance (Bulut et al., 2007), but a general oxidative status was not evaluated.

In some brain diseases some metabolic parameters are used for diagnose. Prolactin and/or creatine kinase levels may be increased just after convulsions in epilepsia, which is above the metabolic traces of brain disorders may exist in serum. Yet, there are no diagnostic blood tests for psychiatric disorders as well as A-ADHD.

Oxidant nitric oxide (NO) and antioxidant superoxide dismutase (SOD) have been implicated to play a role in the pathogenesis of some psychiatric disorders such as schizophrenia and bipolar disorder (Abdalla et al., 1986; Savas et al., 2002, 2006; Kuloglu et al., 2002, and Andreazza et al., 2007b). NO has also been implicated in a number of physiological functions such as noradrenalin and dopamine release, memory, learning, and modulation of wakefulness (Akyol et al., 2004).

SOD is a potent protective enzyme that can selectively scavenge the superoxide anion radical ($O_2^{\bullet -}$) by catalyzing its dismutation

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Table 1
Some characteristics of patients

(*N*)	Medications during blood sampling	NO levels (Median)	SOD Activity (Median)
Comorbidities		182.6 μmol/L	6.67 U/L
Anxiety disorders (8), depression (1), bipolar disorder (1), substance abuse (1)	Serotonergic (9), serotonergic and noradrenergic (1), dopamine blockers (1)		
Only ADHD (9)	None	194.4 μmol/L $Z = -1.178$ $p = 0.239$	7.3 U/L $Z = -0.575$ $p = 0.565$

to hydrogen peroxide (H_2O_2) (Fridovich, 1983). Reactive oxygen species (ROS) can be evaluated indirectly by the measurement of some antioxidant enzyme levels such as SOD. A major role of SOD may be to prevent $ONOO^-$ formation from NO (Akyol et al., 2004). When SOD activity is decreased, NO end-products damage a wide range of biological molecules including lipids, proteins, amino acids and nucleic acids (Akyol et al., 2004). Therefore, the NO–SOD levels may reflect a general oxidative status in the metabolism.

In the present study, we evaluated jointly whether NO, an oxidant, level and SOD, an antioxidant, activity are associated with A-ADHD or not. We expect to find the clues of oxidative imbalance more remarkably, since the disease nature interferes more with brain related functions.

2. Methods

20 A-ADHD patients from Gaziantep University Sahinbey Research Hospital, Psychiatry Clinic, diagnosed according to Turgay's Turkish version of Adult ADD/ADHD DSM IV-Based Diagnostic Screening and Rating Scale by two psychiatrists (H.A.S. and S.S.), and 21 healthy volunteer controls were included (Gunay et al., 2006). Case and control groups have similar distribution in age, sex and smoking status. As the researches focused on the disease entity, only attention and hyperactivity/impulsivity subscale scores were taken into consideration.

The medical records of the patients were reviewed by the researcher (S.S.) and patients with a history of chronic systemic diseases such as diabetes mellitus, hypertension and severe head injury were excluded. After complete description of the study to the subjects, a written informed consent was obtained from all subjects. Ethics committee of the Gaziantep University Medical School approved the trial. Also a semi-structured form was used to detect several socio-demographic and clinical variables of the patients. Comorbid patients were included only when the other psychiatric conditions were in remission according to their Clinical Global Impression Scale Scores below 2 points at least 2 months therefore, the patients were allowed to take their medications. The comorbids were having antidepressants, benzodiazepines and atypical antipsychotics. Case and control groups had similar distribution in age, sex and smoking status.

The subjects strictly refrained from alcohol or food intake and physical exercise after 08:00 p.m. on the day before collection. Mentioned metabolites were measured in plasma samples of study groups. Blood samples were collected during routine laboratory evaluation at 08.00 a.m. Only one sample was collected. After immediate centrifugation (1000 × *g*, 10 min), serum sample were stored frozen at −70 °C.

Since NO is a very labile molecule, its direct measurement in the biological samples is very difficult (Moncada et al., 1991). Therefore, the stable oxidation end products of NO, nitrite (NO_2^-) and nitrate (NO_3^-), can be readily measured in biological fluids and have been used in vitro and in vivo as indicators of NO production. Quantization of nitrate and nitrite was based on the Griess reaction, in which a chromophore with a strong absorbance at 545 nm is formed by reaction of nitrite with a mixture of *N*-naphthlethylenediamine and sulphanilamide (Cortas and Wakid, 1990). For nitrite plus nitrate (total nitrite) detection, an aliquote of the sample was treated with copporized cadmium (Cd) in glycine buffer at pH 9.7 (2.5–3 g of Cd granules for a 4 mL reaction mixture) to reduce nitrate to nitrite and then mixed with fresh reagent and the absorbance was measured in a spectrophotometer (UV-1601 Shimadzu, Japan). Linear regression was done using the peak areas from the nitrite standards. The resulting equation was then used to calculate the unknown sample concentrations. All chemicals used in this assay were obtained from Sigma except cadmium granules (Fluka). The results were expressed as micromoles per liter (μmol/L).

The principle of the total SOD (EC 1.15.1.1) activity method is based, briefly, on the inhibition of nitroblue tetrazolium (NBT) reduction by $O_2^{\bullet -}$ generated by xanthine/xanthine oxidase system (Durak et al., 1993). One unit of SOD was defined as the enzyme amount causing 50% inhibition in the NBT reduction rate. Activity was expressed as Units per milliliter serum (U/mL).

SPSS® for Windows 13.0 statistical program was used for the statistical analysis of data. The normality of the serum measures was tested with drawn *Q*–*Q* plots. The significance of differences between groups was estimated by two-tailed *T*-test. In case of nonparametric hypothesis, Mann–Whitney *U*-test was used when comparing two-independent samples. Differences were accepted as significant when $p < 0.01$. Bonferroni's correction was applied for multiple comparisons. Bivariate comparisons were examined via Spearman correlation coefficients and values were corrected for ties.

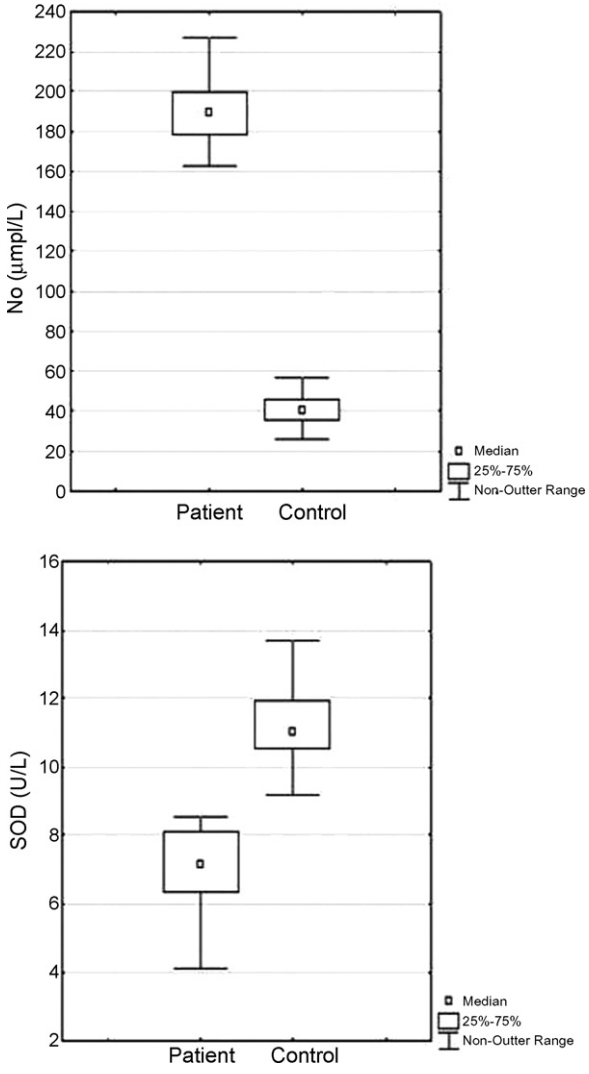

Fig. 1. NO levels and SOD activites of groups.