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Original Article

Purine metabolism and oxidative stress in children with autistic spectrum disorders

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Abstract

Objectives: Autism and related autism spectrum disorders (ASD) are heterogeneous neurodevelopmental disorders behaviorally defined by significant deficits in social interaction and communication and by the presence of restricted interests and repetitive behaviors. It has been suggested that oxidative stress and abnormal purine metabolism may play a role in the pathogenesis of ASD, but the literature reports somewhat contradictory results. The aim of this study is to assess the status of purine metabolism - expressed as serum adenosine diaminase (ADA) - and oxidative stress - expressed as serum malondialdehyde (MDA) and serum superoxide dismutase (SOD) - in male children with autism.

Methods: The present study is a cross-sectional study performed at Al-Kadhimiya Teaching Hospital, Baghdad, Iraq including measurement of serum ADA in boys with ASD. A total of 35 patients (age range 14-19 years) with autism were involved in this study together with a matching group of 40 apparently healthy boys (age range 14-16 years) who were included as controls.

Results: Serum ADA and SOD were significantly lower in boys with autism accompanied by significant higher serum MDA levels when compared with controls.

Conclusion: Patients with ASD have impaired purine metabolism and increased oxidative stress which was supported by low levels of ADA and SOD, and high level of MDA. Further biochemical or genetic studies are required to explore the nature of autism.

INTRODUCTION

Autism and related autism spectrum disorders (ASDs) are heterogeneous neurodevelopmental disorders behaviorally defined by significant deficits in social interaction and communication and by the presence of restricted interests and repetitive behaviors. Despite intense research, the etiology of autism remains largely unknown but is likely multifactorial, including biologic, genetic, and environmental factors [1].

It has been suggested that oxidative stress may play a role in the etiopathogenesis of ASD [2]. Oxidative stress is defined as the disruption of the normal intracellular balance between reactive oxygen species (ROS), produced either during aerobic metabolism or as a consequence of pathologic processes, and antioxidant defence mechanisms [3]. Oxidative stress, in turn, induces the secretion of numerous vasoactive and pro-inflammatory molecules [4] leading to neuro-inflammation [5]. Oxidative stress has been suggested to underlie several other mental disorders, including schizophrenia and bipolar disorder [6], and neurodegenerative pathologies such as Alzheimer disease [7]. Oxidative stress is the result of increased production of pro-oxidant species or decreased antioxidant defences; glutathione redox status has indeed been found to be decreased in autistic patients, also in the post-mortem analysis of Autistic brain tissues [8].

Oxidative stress can be detected by studying a panel of different markers [9], some of which such as DNA, proteins and polyunsaturated fatty acids (PUFAs) residues are pathognomonic for oxidative damage of biomolecules. It is worth mentioning that lipid peroxidation was found to be elevated in autism and that PUFA are important for neurodevelopment [10]. Several markers of oxidative stress are available such

as malondialdehyde (MDA), a marker of lipid peroxidation [11].

Inborn errors of purine metabolism have been implicated as a cause for some cases of autism [12]. Adenosine deaminase (ADA) is an enzyme involved in purine metabolism. It is needed for the breakdown of adenosine from food and for the turnover of nucleic acids in tissues. It has also been proposed that ADA, in addition to adenosine breakdown, stimulates release of excitatory amino acids and is necessary to the coupling of adenosine receptors and heterotrimeric G proteins, which may be altered in some autistic patients [13]. However, the full physiological role of ADA is not yet completely understood [12].

Given together, this study was conducted to assess the status of some biological markers related to purine metabolism and oxidative stress in male children with ASD.

MATERIALS AND METHODS

Subjects

The study was a cross-sectional study carried out in the Psychiatry Department at Al-Kadhimiya Teaching Hospital, Baghdad, Iraq. The diagnosis of autism was confirmed in all subjects by a consultant psychiatrist using the Autism Diagnostic Interview-Revised (ADI-R) [14], the Autism Diagnostic Observation Schedule-Generic (ADOS-G) [15], and the Developmental, Dimensional and Diagnostic Interview (3di) [16] criteria. All patients had simplex autism, and all were negative for Fragile X. None of the patients were on special diets or alternative treatments.

Exclusion criteria included presence of organic aciduria, dysmorphic features, diagnosis of Fragile X or other serious neurological (*e.g.* seizures) or psychiatric (*e.g.* bipolar disorder) conditions, known medical conditions including endocrine, cardiovascular, pulmonary, liver, kidney or other diseases. The protocol for the study was approved by the Ethical Committee of Al-Nahrain Medical College, and informed consent was signed by each subject.

Blood samples

Five milliliters of random venous blood were withdrawn from each patient, in supine position, without application of tourniquet. Samples were transferred into clean new plane tube, left at room temperature for 15 min for clotting, centrifuged at 1,800g for 10 min at 4°C, and the separated serum was transferred into eppendorf tubes that were stored at -20°C until analysis.

Biochemical analyses

Serum adenosine deaminase was measured by an ELISA kit (USCN Life Sciences; Wuhan, PR China) which relays on a sandwich enzyme immunoassay for the in vitro quantitative measurement of ADA in human serum [17]. The microtiter plate provided in this kit has been pre-coated with a monoclonal antibody specific to ADA. Standards or samples are then added to the appropriate microtiter plate wells with a biotin-conjugated polyclonal antibody preparation specific for ADA. Next, avidin conjugated to horseradish peroxidase (HRP) is added to each microplate well and incubated. Then a tetramethylbenzidine (TMB) substrate solution is added to each well. Only those wells that contain ADA, biotin-conjugated antibody and enzyme-conjugated avidin will exhibit a change in color. The enzyme-substrate reaction is terminated by the addition of a sulphuric acid solution and the color change is measured spectrophotometrically at a wavelength of 450 ± 10 nm. The concentration of ADA in the samples is then determined by comparing the optical density (OD) of the samples to the standard curve. The detection range was 0.312-20 ng/ml; the standard curve concentrations used for the ELISA's were 20 ng/ml, 10 ng/ml, 5 ng/ml, 2.5 ng/ml, 1.25 ng/ml, 0.625 ng/ml, and 0.312 ng/ml.

Serum superoxide dismutase (SOD) was measured using the inhibition rate of 2-(4-indophenyl)-(4-nitrophenol)-5-phenyltetrazolium chloride reduction method (modified method of Sun *et al* [18]) via a commercial kit (Randox; Antrim, Northern Ireland, UK). One unit of SOD activity was defined as the amount of protein that inhibits 2-(4-indophenyl)-(4-nitrophenol)-5-phenyltetrazolium chloride reduction rate by 50%.

Serum malondialdehyde was determined by the method of Draper and Hadley [19] based on the reaction of MDA with thiobarbituric acid (TBA) at 95°C. In the TBA test reaction, MDA and TBA react to form a pink pigment with an absorption maximum at 532 nm. The MDA concentrations were calculated using the molar extinction coefficient of 1.5×10^5. The results were expressed as nmol/ml serum.

Statistical analysis

Statistical analysis was done using descriptive (mean and standard deviation) and inferential statistics (t-test) to test the significancy of mean difference. When P value was less than 0.05, the difference was considered statistically significant.

RESULTS

Subjects

A total of 35 male patients with ASD were enrolled in this study: age range 14-19 years (mean age 15.3 ± 2.8 years). Another 40 apparently healthy male subjects were used as healthy controls: age range 14-16 years (mean age 14.23 ± 2.2 years) (Table 1).

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All participants were screened via parental interview for current and past physical illness.

Biochemical outcome

Serum Adenosine deaminase was found to be highly significant lower in ASD group compared to healthy controls ($P < 0.001$, Table 2).

Serum superoxide dismutase was significantly less in ASD group compared to healthy controls ($P < 0.05$, Table 2).

Serum malondialdehyde was significantly higher in ASD group than healthy controls ($P < 0.05$, Table 2).

Table 1. Demographic criteria of patients and control subjects (range and mean ± SD)

Group	ASD group	Healthy controls
Number (subjects)	35	40
Mean age (years)	15.3 ± 2.8	14.2 ± 2.2
Age range (years)	14-19	14-16

Table 2. Serum adenosine deaminase (ADA), superoxide dismutase (SOD) and malondialdehyde (MDA) levels (mean ± SD)

Variable	ASD	Control	P
ADA (ng/ml)	9.2 ± 1.9	16.5 ± 3.4	< 0.001
SOD (U/ml)	0.61±0.21	0.66±0.41	< 0.05
MDA (nmol/ml)	0.75±0.17	0.69±0.12	< 0.05

DISCUSSION

Autism is a complex neurodevelopmental disorder that is thought to involve an interaction between multiple, variable susceptibility genes [20], epigenetic effects [21], and environmental factors [22]. The apparent increase in the diagnosis of ASD from 4-5 per 10,000 children in the 1980s to 30-60 per 10,000 children in the 1990s has raised great concern [23]. This increased prevalence of autism has enormous future public health implications and has stimulated intense research into potential etiologic factors and candidate genes.

Because abnormal purine metabolism and low activity of adenosine deaminase have been reported in other neurologic disorders, including Alzheimer disease, Parkinson disease, schizophrenia, and Down syndrome [24], the activity of ADA was measured in a group of autistic children with respect to the control children [25]; in the present study the results were within the range of values previously found in several other studies.

The observed imbalance in ADA activity in the autistic children is complex and not easily explained by perturbation of a single pathway or isolated genetic or nutritional deficiency. Moreover, a possible relationship between the losses of adenosine homeostasis due to the currently reported reduced activity of ADA, and the impairment of neurotransmitter profile is suggested in patients with autism. This is also supported by the findings of a recent work by El-Ansary [26] who showed a low plasma and high brain serotonin and dopamine concentration in patients with autism compared with control subjects. Based on these results, it seems likely that tight control of adenosine levels could play an important role in brain development and neural plasticity [27], and that any dysfunction in homeostatic control of adenosine, an important modulator of the brain immune system, could upset the balance between pro-inflammatory and anti-inflammatory cytokines, which is crucial for normal brain development [28].

It is possibly relevant that, in autistic children, decreased activity of ADA has been shown to be associated with increased frequency of ADA polymorphisms [29]. The observed increase in adenosine could be due to either an inhibition of adenosine kinase or an increase in 5-nucleotidase, both of which have been shown to occur with oxidative stress [30]. Elevated intracellular adenosine has been shown to increase oxidative stress [30].

In the present work, among the oxidative stress parameters that were evaluated in autistic group compared to healthy controls, a significant increase in MDA, a marker of lipid peroxidation, accompanied by significant reduction in SOD, an enzyme reflecting free radical scavenging activity, was observed. Similar findings were reported by other groups [31, 32]. This finding suggests that oxidative stress was involved in the pathogenesis of conditions related to the neuronal tissues. The fatty acid composition of the brain and neural tissues is characterized by high PUFA concentrations which play a very important role in signal transduction [33], neuro-inflammation [34] and cellular repair and survival [35]; this tissue appears to be involved by peroxidation process.

Alternatively, a genetic predisposition to environmental agents or conditions that promote oxidative stress could contribute to the abnormal metabolic profile observed in the autistic children. The contribution of MDA-related oxidative injury to autism was explored by autoimmunity as an etiological factor of autism [36]. Autoimmunity is an abnormal immune reaction in which the immune system becomes primed to react against body organs. It can result from an immune response against altered self proteins, *e.g.* modified by adduction of lipid-derived electrophiles generated by oxidative injury. Immunoglobulin (Ig)G, IgA, IgM and myelin basic protein anti-brain auto antibodies were reported to be present in high percentage of sera from children with autism compared to healthy children [37].

In conclusion, the present study indicates that abnormal purine metabolism and oxidative stress might contribute to the pathogenesis of ASD. This suggestion is supported by the finding of low serum ADA and SOD activities and high levels of serum MDA in autistic boys with respect to healthy boys. Further studies on other markers of purine metabolism and oxidative stress, in addition to genetic study in a larger number of children (boys and girls) with ASD are required to explore the nature of ASD.

COMPETING INTERESTS

The authors declare that they have no conflict of interest.

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