Antioxidant activity in children with ADHD – a comparison in untreated and treated subjects with normal children

Ruchi K[a], Anil Kumar S[b], Sunil G[c], Bashir A[d], Prabhat S[e]

[a] Family Medicine Resident, St. Francis Hospital, Wilmington, USA,
[b] Dept of Basic Medical Sciences, Faculty of Medicine, International Islamic University Malaysia,
[c] Monash University, Sunway Campus, JC School of Medicine & Health Sciences, Malaysia,
[d] JLN Institute of Molecular Biology, CSM Medical University, Lucknow, India,
[e] Department of Psychiatry, CSM Medical University, Lucknow, India

ABSTRACT

Introduction: Attention deficit hyperactivity disorder (ADHD) is a frequently encountered clinical condition in children. Based on DSM IV-TR criteria it can be sub-classified into three distinct types namely hyperactive-impulsive, inattentive and combined. **Materials and Methods**: In the present study, salivary antioxidant activity (AOA) in children with ADHD was compared with age-matched normal control subjects, both as a whole and also with regard to the three subtypes. Additionally, the effect of therapy on the altered AOA levels was investigated following short term (<3 months) and long term (1–3 years) treatments. AOA and catalase activities in the saliva were estimated employing previously reported biochemical procedures. **Results**: While AOA is decreased in ADHD patients as compared to normal subjects, statistically significant decrease is seen only in the combined and the hyperactive-impulsive subtypes. Restoration of AOA and catalase activities is seen only after sustained therapy and not in the short term. **Conclusion**: It is concluded that ADHD is associated with decrease in AOA and this should possibly also be addressed for limiting the long term outcomes of this condition.

KEYWORDS: Attention deficit hyperactivity disorder, oxidation-reduction, free radicals, catalase, antioxidants

INTRODUCTION

Attention deficit hyperactivity disorder (ADHD) is one of the most common neuro-behavioural disorders in children characterized by hyperactivity, impulsiveness and inattention beyond the norm for a child's age. The children with ADHD have pronounced impairments and can experience long term adverse effects on academic performance, vocational success and social-emotional development. The impact of ADHD on society is enormous in terms of stress to families, financial cost, disruption in schooling and potential for development of criminality and substance abuse.

Despite many clinical studies, our knowledge about the aetiology and pathogenesis of ADHD remains largely speculative. Indirect evidences however, have been variously put forth to suggest the possible involvement of multiple factors such as neurochemical deficits, cerebral circulatory impairments and subtle foetal and perinatal brain damage due to toxic, metabolic or physical insult.[1] Stress and anxiety have also been found to complicate clinical manifestations of ADHD[2,3] and may be relevant for its initiation and/or perpetuation.

Oxidative stress is widely recognized nowadays as an important feature of many neurological disorders because of the high susceptibility of the brain to oxidative damage caused by free radicals. However, not much is known about the possible role of oxidative stress in the pathogenesis of ADHD.

Total antioxidant activity (AOA) is a parameter, characterizing the sum of activities of anti-oxidants present in the material studied which may be considered as a marker of the antioxidant status of the body. Body fluids, especially blood, have been used as a tool for assessment of antioxidant activity in patients in many diseases.[6] In the last decade, several methods have been developed for assaying the antioxidant activity in saliva.[7,8] The advantage of using saliva is that it can be easily obtained by simple, non-invasive means. This becomes especially important where repeated sampling is required or in study groups which may not be suitable for invasive methods such as hyperactive children with ADHD.

The present study was, therefore, designed to evaluate the AOA of children with ADHD as compared to age-matched healthy children by monitoring the antioxidant potential of saliva as a biomarker of ox

Corresponding author:

Dr. Anil Kumar Saxena
13, Jalan IM 13/1
Astana Golf Resort
25200 Kuantan, Pahang, Malaysia
Email: drak.saxena@gmail.com

idative stress. An attempt was also made to assess the effect of standard drug therapy on salivary AOA and its correlation with the clinical outcome.

MATERIALS AND METHODS

The study was conducted in children with ADHD attending the child and adolescent psychiatry out-patient department of Gandhi Memorial and Associated Hospitals, Lucknow, India after obtaining institutional ethical approval. Normal, healthy age-matched children from local schools were included in the study as control subjects after ensuring their satisfactory and average performance in school and at home. The purpose of the study was explained to the parents/ guardians and an informed consent for participation was obtained from them.

The patients of ADHD were selected for this study according to the following inclusion and exclusion criteria. The inclusion criteria were diagnosis of ADHD according to the criteria laid down in DSM IV-TR,[9] age below 16 years and availability of a reliable informant. The exclusion criteria were the presence of any uncontrolled co-existing medical illness and severe mental retardation.

The initial rating of behaviour and mental status was done by the Parent Symptom Questionnaire Hyperactivity Impulsivity Factor (PSQ) and the Kiddie Schedule for Affective Disorder and Schizophrenia Present and Lifetime.[10] A score of two or more on PSQ is considered screen positive. Assessment of intelligence was done by a clinical psychologist. Following the initial assessment a consensus diagnosis was made using DSM IV-TR criteria. According to their symptoms, ADHD patients were categorized into Hyperactive-Impulsive (ADHD-HI), Inattentive (ADHD-I) and Combined (ADHD-C) subtypes.

Participants of the study were divided into the control group (normal subjects) and the ADHD group. The ADHD group was further classified into the Pre-treatment group and Post-treatment group. The Pre-treatment group was categorized into 3 subtypes, the ADHD-HI, ADHD-I and ADHD-C. The Post-treatment group was analysed according to the length of follow-up; short-term follow-up group of less than three months and long-term follow-up group of one to three years. Short-term follow-up post-treatment group included only those children from the pre-treatment group who could be followed up to a period of three months. A long term follow-up post-treatment group (1-3 years) was included in the study for the purpose of comparison. For this group, subjects were recruited based on the same inclusion and exclusion criteria from those patients who had been registered with the out-patient department for varying lengths of time prior to the commencement of this study and were clinically well controlled on drug treatment.

Samples of saliva from ADHD patients and control subjects were collected in sterile sample vials. The subjects were asked to rinse the mouth 20 minutes prior to sample collection and no oral intake was allowed after this till collection of the sample. The samples were stored at 4-8ºC and examined for AOA and catalase activity on the same day.

Antioxidant capacity of saliva was estimated using the method described by Ziobro and Bartosz.[11] The reaction mixture containing 0.5 ml of phosphate buffer (100 mmole/L, pH7.4), 0.5 ml of sodium benzoate (10 mmole/L), 0.2 ml of Fe-EDTA (2 mmole/L EDTA + 2 mmole/L of $Fe[NH_4]_2SO_4$), 0.2 ml 0f H_2O_2 (10 mmole/L) and 0.01 ml of saliva was incubated for 60 minutes at 37ºC. The reaction was stopped by the addition of 1 ml of 20% acetic acid. One ml of thiobarbituric acid (TBA) solution (0.8% in 50 mmole/l Na OH) was added and the solution was heated for 10 minutes at 100ºC. The absorbance of the pink colour thus formed was estimated spectrophotometrically at 532 nm. 0.01 ml of uric acid (1mmole/L in 5 mmole/l NaOH) was used as standard antioxidant for determining the AOA of unknown samples. Proper blanks (K_0, A_0 and UA_0) were run under similar experimental conditions.

The AOA of each sample was calculated as follows:

$$\text{AOA (mmole/L)} = \frac{(CU)\,(K\text{-}A)}{(K\text{-}UA)}$$

Where K= absorbance of control (K_1- K_0); A= absorbance of sample (A_1-A_0) ; UA= absorbance of uric acid solution (UA_1 – UA_0) ; CU= concentration of uric acid (mmole/L)

The catalase activity in saliva was determined spectrophotometrically by monitoring the decomposition of H_2O_2 at 240 nm.[12] The reaction mixture (1.5 ml) consisted of phosphate buffer (1.2 ml; 0.05 M; pH 7.0), H_2O_2 (200 µl; 0.88 M) and 0.1 ml of saliva. The reaction was followed for five minutes at 30ºC. The enzyme activity was expressed as µmole H_2O_2 decomposed min-1 ml-1 saliva using extinction coefficient 43.6 M^{-1} cm^{-1} of H_2O_2.

The chemicals used in this study were Sodium dihydrogen orthophosphate dehydrate and sodium benzoate (Thomas Baker, India); Disodium hydrogen phosphate (Sarabhai Chemicals, India) ; Ferrous ammonium sulphate (BDH, Glaxo Laboratories, India); Thiobarbituric acid (Sigma, USA); Acetic acid, 100% and ethylene dinitrilotetracetic acid disodium (Merck, India) and Sodium hydroxide (Qualigens Fine Chemicals, Glaxo, India). All the chemicals were of analytical grade and were prepared in double distilled water.

Statistical analysis was performed with 'GraphPad Prism' software. All values were expressed as mean ± S.E. The data were subjected to one-way analysis of variance followed by Dunnet's multiple comparison test and Student's t-test for judging the statistical significance of the differences between means. A difference at $p<0.05$ was considered statistically significant.

Table I: Age and sex distribution of ADHD patients and normal controls

Group	n	Age (yrs) Mean ± S.E.	Males (%)	Females (%)
Control	35	9.01 ± 0.41	66	34
ADHD patients	47	8.71 ± 0.43		
Pre-treatment (total)	32	8.27 ± 0.49	88	12
Subtypes				
ADHD-HI	10	7.81 ± 0.91	100	0
ADHD-I	6	10.87 ± 1.08	83	17
ADHD-C	16	7.58 ± 0.56	81	19
Post-treatment (total)	23	8.91 ± 0.65	87	13
Short-term follow-up # (3 months)	8	7.53 ± 0.91	88	12
Long-term follow-up (1-3 years)	15	9.64 ± 0.83	87	13

#These eight patients were re-evaluated during follow up after treatment.

Table II: Salivary AOA in normal controls and ADHD patients.

GROUP	N	AOA ± S.E. (mmol/L)
Control	35	1.24 ± 0.08
ADHD Patients	32	0.86 ± 0.07**
Subtypes		
ADHD-HI	10	0.83 ± 0.07*
ADHD-I	6	± 0.17
ADHD-C	16	0.82 ± 0.11**

*** p < 0.05; ** p < 0.01 as compared to control**

Table III: Comparison of AOA values in the short and long term post-treatment groups with normal controls.

GROUP	N	AOA ± S.E. (mmol/L)
Control	35	1.24 ± 0.08
Post-treatment		
Short term	8	0.79 ± 0.18 *
Long term	15	1.30 ± 0.11#

*** p < 0.01 as compared to control ; # p > 0.05 as compared to control.**

RESULTS

Saliva was used as a biomarker of redox status in the present study. Total antioxidant potential and catalase activity of saliva were estimated and compared between ADHD subjects and normal age-matched healthy controls. Additional comparisons of these parameters were also made between the three identified subtypes of ADHD, as also between the pre-treatment and post-treatment values of these markers.

The age and sex distribution of the 82 subjects in the present study is depicted in Table I. There was no significant difference in the mean ages of the subjects in different groups. However, sex distribution exhibits a predominant male preponderance in all the groups.

The mean level of AOA of saliva in the control group was 1.24 ± 0.08 mmol/L (Table II). AOA in the pre-treatment group was found to be significantly lower (0.86 ± 0.07 mmol/L; p< 0.01) as compared to the control group. The mean AOA in ADHD-HI and ADHD-C were significantly lower as compared to control. ADHD-I group also showed a lower mean value for AOA which however, was not statistically significant as compared to controls.

In the post-treatment groups less than three months (short term follow-up), eight ADHD patients who exhibited good control of their clinical signs and symptoms, as judged by their parents/guardians and the consultant psychiatrist in-charge, were subjected to a second estimation of salivary AOA. The mean AOA value in this group was found to be significantly lower as compared to control (Table III).The post-treatment long term follow-up group consisted of a different set of patients who were clinically well controlled on different drugs for the disease for varying periods of time ranging from 1 to 3 years. The mean AOA value in this set did not show any significant difference as compared to the controls (Table III).

Catalase activity in saliva was estimated in control (n=18) and ADHD patients (pre-treatment group, n=13; long-term post-treatment group, n=7). It was found to be reduced in the pre-treatment ADHD group when compared with the normal controls (Table IV). However its mean level in the long term post-treatment group was higher than the control group although the finding was not statistically significant (p>0.05).

Table IV: Salivary catalase activity in control, pre-treatment and post-treatment groups.

GROUP	N	Catalase activity (H_2O_2 decomposed min^{-1} ml^{-1})
Control	18	172 ± 16
Pre-treatment	13	120 ± 12*
Post-treatment *(Long term)*	7	210 ± 32

***p< 0.05**

DISCUSSION

An increasing amount of evidence suggests that oxidative stress is important in either the primary or the secondary patho-physiological mechanisms underlying many neurological disorders. The damage can become widespread due to weakened cellular antioxidant defence system.[13] In the present study, total antioxidant activity in the saliva of ADHD children was evaluated and compared with that of healthy age-matched controls. The suitability of saliva for the evaluation of total antioxidant activity of the body has been well established.[7,11]

The male predominance of ADHD observed in our study has also been reported earlier.[14] The ratio of boys to girls in ADHD patients generally varies from 3:1 to 9:1.

Our study shows an attenuation of total antioxidant capacity in children with ADHD as compared to healthy, age-matched children (Table 1). The salivary AOA in the pre-treatment group showed significantly lower AOA in children suffering from combined ($p<0.01$) and hyperactive-impulsive ($p<0.05$) subtypes of ADHD as compared to the control group. In the ADHD-inattentive subtype also the mean AOA value was somewhat lower as compared to controls but this was not statistically significant. Excessive exercise or physical activity has been reported to increase oxidative stress due to enhanced generation of free radicals in the body and/or a compromised antioxidant defence system.[15,16] This may be one of the possible reasons for the reduced AOA in the children with combined and hyperactive-impulsive subtypes of ADHD since these are associated with hyperactivity.
Some earlier studies have reported reduction in antioxidant capacity in children with ADHD but ours is the first study which has investigated the antioxidant status in the three subtypes of ADHD patients wherein a significant decrease in AOA was observed only in the HI and C subtypes and not in the I subtype.

There was no statistically significant difference between the pre-treatment and post-treatment values of AOA at the short term follow-up. The post-treatment values still remained significantly lower ($p<0.05$) as compared to control during this period (Table III). Most patients in the short term follow up group showed variable or no marked clinical improvement as judged by their parents/guardians and the consultant psychiatrist in-charge.

The post-treatment long-term follow-up group of ADHD patients had been receiving different drugs for varying periods of time (1–3 years). The baseline AOA values prior to treatment of these patients were not available since these patients had registered prior to the commencement of this study. However, the mean AOA value of this group was found to be significantly higher ($p<0.01$) as compared to the pre-treatment AOA values in the newly diagnosed group and comparable with the AOA levels of the normal controls ($p > 0.05$; Table III). Most patients in this group had improved clinically as judged by the psychiatrist in-charge and their parents/guardians both at home and at school. Although the findings in this group do not give a direct comparison for the AOA values with their previous values (since these were not available), it nevertheless shows that the AOA levels in treated, clinically improved ADHD patients do not differ significantly from those of normal children while those with recently diagnosed and as yet, uncontrolled ADHD are significantly altered.The catalase activity in saliva showed a pattern similar to that of total AOA in the pre-treatment and long-term follow up groups indicating that a decrease in catalase activity might be one of the factors responsible for the attenuation of AOA in ADHD patients.

From the above it can be surmised that there is a decrease in the antioxidant capacity of the body in patients of ADHD and it is likely that antioxidant capacity recovers towards control values after sustained drug therapy and in parallel with clinical improvement. The absence of a similar beneficial effect in the short term follow-up group indicates that the normalization of the AOA is not because of the drugs used in the treatment of ADHD since the AOA values remained low in subjects who were followed up for up to 3 months after commencement of drug therapy. The subjects in the short-term follow-up group were yet to show significant clinical improvement during this short period. Hence, it would appear that the improvement in antioxidant status follows the clinical improvement. Whether this also means that the increase in oxidative stress present in patients of ADHD is a manifestation of the disease process and not a factor in its causation requires further exploration.

It is not possible to establish a cause-effect relationship with the present results. However, decreased antioxidant capacity found in patients of ADHD, specially the ADHD-HI and combined types, is likely to result in further oxidative organ damage. Sustained, long-term drug therapy, in addition to providing symptomatic control might also tend to limit damage due to the disturbed redox status. However, since improvement in AOA appears to occur after a longer term of drug treatment, supplementation with anti-oxidants, at least during the early stages of treatment may be in-

dicated with a view to provide additional benefits in these patients. Further studies in this direction are required.

CONCLUSION

From the results of the present study we can conclude that ADHD is associated with a concomitant decrease in the antioxidant capacity in the children suffering from this disorder. This decrease in AOA is especially pronounced in the hyperactive-impulsive and the combined types of ADHD. ADHD subjects who are clinically improved after sustained long term drug treatment on the other hand display AOA levels similar to normal children. The recovery of AOA levels towards control values does not appear after short term treatment of up to three months. Antioxidant supplementation at least during the early phase of treatment might be considered with a view to limit the long term deleterious consequences of impaired antioxidant mechanisms.

REFERENCES

1. Farone SV, Biederman J. Pathophysiology of attention deficit/hyperactivity disorder in Neuropsychopharmacology: The fifth genera tion of progress eds; Davis KL, Charney D, Coyle J T, Nemeroff C. American college of Neuropsychopharmacology, 2002, pp 577 – 96.
2. Meehan KB, Ueng-McHale JY, Reynoso JS, et al. Self-regulation and internal resources in school-aged children with ADHD symptoma tology: an investigation using the Rorschach inkblot method. Bull Menninger Clin 2008; 72(4):259-82.
3. Hastings PD, Fortier I, Utendale WT, Simard LR, Robaey P. Adrenocortical functioning in boys with attention-deficit/hyperactivity dis order: examining subtypes of ADHD and asso ciated comorbid conditions. J Abnorm Child Psychol 2009; 37(4):565-78.
4. Rice-Evans C, Miller NJ. Total antioxidant status in plasma and body fluids. Meth Enzym 1994; 234:279-93.
5. Battino M, Ferreiro MS, Gallardo I, Newman HN, Bullon P. The antioxidant capacity of sa liva. Review. J Clin Periodontol 2002; 29(3):189-94.
6. Stefanescu A, Purice M, Iancu C. A non-inva sive strategy for the assessment of oxida tive stress by salivary indices, in healthy stu dents. Romanian J Endocrinol 2002; 40:21-30.
7. Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition, Text Revision. 2000; American Psychiatric Association
8. Kauffmann J, Birmaher B, Brent D. Schedule for affective disorder and schizophrenia for school-age children present and lifetime ver sion (K-SADS-PL): initial reliability and valid ity data. J Am Acad Child Adolsc Psychia try 1997; 36:980-88.
9. Ziobro A, Bartosz G. A comparison of the to tal antioxidant capacity of some human body fluids. Cellular and Molecular Biology Letters 2003; 8:415-19.
10. Aebi H. Catalase in vitro. Methods Enzymol 1984; 105:121–6.
11. Gilgun-Sherki Y, Rosenbaum Z, Melamed E, Of fen D. Antioxidant therapy in acute central nervous system injury: Current State. Phar macological Reviews 2002; 54:271-84.
12. Swanson H, Sergeant JA, Taylor E, et al. At tention-deficit hyperactivity disorder and hy perkinetic disorder. Lancet 1998; 351:429-33.
13. Ji LL. Antioxidants and oxidative stress in ex ercise. Proc Soc Exp Biol Med 1999; 222: 283-92.
14. Chevion S, Moran CS, Heled Y, et al. Plasma antioxidant status and cell injury after se vere exercise. Proc Natl Acad Sci (USA) 2003; 100:5119-23.
15. Ross BM, McKenzie I, Glen I, Bennet CPW. Increased level of ethane, a non-invasive marker of n-3 fatty acid oxidation, in breath of children with attention deficit hyperactiv ity disorder. Nutr Neurosci 2003; 6:277-81.
16. Dvoráková M, Sivonová M, Trebatická J, et al. Redox Rep 2006; 11(4):163-72.