Assessment of serum and salivary oxidative stress biomarkers with evaluation of oral health status in a sample of autistic male children

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ABSTRACT

Background: Autism is a severe neurodevelopmental disorder, presents in early childhood, characterized by severe impairments in socialization, communication and behavior. Autism is considered a multi-factorial disorder that is influenced by genetic, environmental, and immunological factors with oxidative stress as a mechanism linking these factors. Assessment of any oral manifestations; measurement of oxidative stress in saliva has to be discovered, evaluated and measured in autistics to be used as a potential diagnostic aid since saliva is an ultra-filtrate of serum and meet the demand for inexpensive, noninvasive and accessible diagnostic methodology.

Materials and methods: Oral health status: DMFT/dmft and gingival indices as well Serum and salivary Malondialdehyde (MDA) levels, glutathione (GSH), superoxide dismutase (SOD) and uric acid (UA) were estimated for 58 individuals aged (2-13) years, twenty nine of them were autistics and twenty nine were sex and age matched healthy controls.

Results: The results of this study showed that Iraqi autistic children sample was more likely to be caries-free, with significant abnormalities of the oxidative stress biomarkers.

Conclusion: Saliva can be used as adjunctive diagnostic aid for measurement of the oxidative stress in autism. Serum GSH and uric acid then serum and salivary Malonyldialdehyde followed by salivary glutathione and serum superoxide dismutase are the most powerful predictors of autism spectrum disorder respectively.

Key words: Autism spectrum disorder; Oxidative stress; Oral health status. (J Bagh Coll Dentistry 2011;23(3): 56-60).

INTRODUCTION

Autism spectrum disorders (ASDs) are prevalent neurodevelopmental disorders that affect an estimated 6 per 1,000; with male to female ratio averages 4.3:1, which means that boys are at higher risk for ASD than girls (1). Characterized by severe impairments in socialization, communicat-ion and behavior. Children diagnosed with an ASD may display a range of problem behaviors such as hyperactivity, poor attention, aggression and self-injury. In addition, to unusual responds to sensory stimuli such as hypersensitivities to light or certain sounds, colors, smells or touch and have a high threshold for pain (2). Finally, common co-morbidity conditions often associated with ASDs include gastrointestinal and autoimmune disease (3).

Investigators suggested that ASDs may result from an interaction between genetic, environmental and immunological factors, with oxidative stress as a mechanism linking these risk factors (4).

The brain is highly vulnerable to oxidative stress due to its limited antioxidant capacity, higher energy requirement, and higher amounts of lipids and iron. The brain makes up about 2% of body mass but consumes 20% of metabolic oxygen. Due to the lack of glutathione-producing capacity by neurons, the brain has a limited capacity to detoxify ROS. Therefore, neurons are the first cells to be affected by the increase in ROS and shortage of antioxidants and, as a result, are most susceptible to oxidative stress. Children are more vulnerable than adults to oxidative stress because of their naturally low glutathione levels from conception through infancy. Taken together, these studies suggest that the brain is highly vulnerable to oxidative stress, particularly during the early part of development that may result in neurodevelopmental disorders such as autism (5).

Under normal conditions, dynamic equilibrium exists between the production of reactive oxygen species (ROS) and the antioxidant capacity of the cell. ROS includes superoxide (O2−), hydroxyl, peroxy and nitric oxide (NO) free radicals (6). The two main roles of cellular antioxidant defence mechanisms are to prevent the generation of free radicals and to inactivate them after generation. This system includes enzymatic and non-enzymatic processes. Superoxide dismutase (SOD), glutathione peroxidase (GSH-Px), hydrogen peroxidase and catalase (CAT) are the antioxidant enzymes which
block the initiation of free radical chain reactions. Glutathione (GSH), alpha-tocopherol (vitamin E), ascorbic acid (vitamin C) and urate are the main non-enzymatic antioxidant molecules. 

MATERIALS AND METHODS

Sixty individuals from Central Pediatric Teaching Hospital in Al-Iskan were enrolled in this study. They were categorized into two groups:

**Autistic group:** Composed of 31 children (29 males and 2 females) who were diagnosed as autistic children, their age ranges between 2-13 years. Because the female sample very small, it was excluded from the current study.

**Healthy control group:** It was composed of 29 age and gender matched male children.

All individuals were evaluated by full medical history and clinical examination to exclude any other systemic disease that may affect the parameters examined in this study. Oral and periodontal examination was done for each individual and any child with symptoms and signs of any active oral inflammation and advanced periodontitis were excluded.

All parents were supplied with informed consent and the purposes of the study were explained to them. All the children subjected to extra-oral examination for any scars or trauma to the head, neck, hands and fingers; taking medical, family history and previous dental history. Intra-Oral assessment of caries experience through the application of decayed, missing and filled teeth Index (DMFT) and (dmft) for permanent and primary teeth respectively; and assessment of gingival health status through gingival index

Laboratory assessment: Blood and saliva samples were taken from autistics and control. 5 ml. of blood sample was taken from each individual, left to clot then the serum which was obtained by centrifugation at 3000 rpm for 10 minutes, transferred immediately into another tube and frozen at (-20°C) for subsequent analysis. For saliva samples each child was asked to sit down and relax as much as possible and asked to chew a piece of Arabic gum for one minute before all the saliva was removed by expectoration; chewing was then continued for ten minutes with the same piece of gum and the collection of saliva by spitting was done during this time. The collected saliva was centrifuged at 1000 rpm for 10 minutes; this done after 1 hour after collection to eliminate debris and cellular matter. The centrifuged supernatants stored frozen at (-20°C) in polyethylene tube until assayed.

Serum and salivary levels were assessed for MDA using thiobarbituric acid (TBA) method; Uric acid by using commercial kit (BioMerieux, France). GSH levels according to the method described by Burris and Ashwood; SOD activity were also assessed using the assay method involves the inhibition of autooxidation of adrenaline to adrenochrome by SOD.

All data were statistically analyzed using SPSS version 13 (Statistical Package for Social Sciences). Non-normally distributed quantitative variables (serum and salivary GSH in addition to DMFT/dmf score) are described by median and interquartile range. The remaining quantitative variables (age, serum and salivary uric acid, serum and salivary MDA, serum and salivary SOD and gingival index) were normally distributed and thus conveniently described by mean ± standard deviation. Correlation assessment was performed using the Spearman correlation analysis. The ROC analysis was used to rank the quantitative parameters from those with highest difference between Autism cases and healthy controls to lowest difference. This is done by ranking the ROC area of different parameters. Statistical significance was defined as p< 0.05.

RESULTS

The mean age for autistic children was about 5.9±3.4 years. Autistics and their controls showed homogeneity and there were no significant difference between the two groups.

**Extra-Oral Examination:** out of 29 autistics only 2 (6.9%) showed signs of trauma due to self injury habit. Parents' responses to the questionnaire regarding dental visits indicated that 28 (96.6%) of autistic children never visited dental clinic and had a negative history of treatment and follow up.

**Intra – Oral Examination:** The caries severity of children in the ASD group was statistically significant lower than that in the unaffected group for dmft (p = 0.013) but insignificant for DMFT (p = 0.73). Regarding caries prevalence, a total of 15 (51.7%) children in the ASD group had a positive caries free history (DMFT and dmf = 0), compared with 9 (31%) children in healthy control group.

According to the criteria, 96.6% of autistic children had mild gingivitis with mean value (0.55 ± 0.35) obviously lower in comparison to healthy controls (0.75 ± 0.48), but the difference failed to reach the level of statistical significance (p=0.08).

**Biochemical Findings:** It was observed that the study subjects with ASD had significantly increased serum and salivary MDA and serum UA (p< 0.001). By contrast, the study subjects with an
ASD had significantly decreased levels of serum and salivary GSH (p < 0.001) and serum SOD (p = 0.004). No overall significant differences were observed for the salivary UA and SOD among study subjects with an ASD and their controls. Tables 1 and 2 summarize an assessment of biomarkers of oxidative stress among the study subjects with ASD in comparison to the controls. Table 3 showing the tested variables ordered according to their significance in separating between autistics and healthy controls (ROC test).

DISCUSSION

Boys are at higher risk for ASD than girls and this agreed with all other studies around the world (1,3). As part of the multiple unknown developmental abnormalities, children diagnosed with autism practice self injurious behavior (SIB) at some stage in their lives. In the present study results of the extra oral assessment, types of habits, trauma and injuries revealed that out of the 29 examined children, only 2 (6.9 %) practice this behavior, and this result was in good agreement with many other studies (12,13). Heritability contributes about 90% of the risk of a child developing autism, and this support the findings in the present study in which 21 (72.4 %) of autistic children have a positive family history of neuropsychiatric illness like schizophrenia, Alzheimer’s disease, mental disorder and depression (14). In the present study 28 children (96.6%) had never visited dental clinic or received dental treatment and follow up and this could be explained by the fact that people with ASD incapable of cooperating in the dental setting owing to their impaired social interaction and communication skills. This result was in good agreement with many studies (15-18).

The current study revealed that caries severity (but failed to reach statistical significant level) in autistics were lower than in unaffected children with autism, because of their ritualistic behavior which characterized by unvarying pattern of daily activities, such as an unchanging menu so they are more regular in their behavior at meals than are unaffected children. Therefore, a lower frequency of snacking between meals and lower intake of carbohydrates could have contributed to the lower caries rate observed and this finding agreed with several studies (13,19).While disagreed with others who reported higher scores in autistic groups (20,21). Caries prevalence lower in autistic children participating in the present study and this result were in good agreement with many previous studies (13,20).

Gingival status of the autistic children in the present study showed that (96,6%) of the children had generalized mild gingivitis, which it was in good agreement with many previous studies (20,21). While Ozdemir-Ozenen and Sandalli, 2007 (22), in their study reported that the gingival index records of the children with autism was found to be significantly higher than the healthy children.

Oxidative stress and antioxidant activity is now well known to be the mechanism that links genetics, environment, and immunity as causative factors for autism (14). In the present study, aim was directed to assess and measure the oxidative stress marker (MDA) and the antioxidants (UA, GSH and SOD) in serum and saliva of autistics; and moreover any oral manifestations associated with ASD, which could be used for the early diagnosis and intervention with autism. Although there is no known cure, but early behavioral or cognitive intervention can help autistic children gain self-care, social, and communication skills.

Up to our knowledge, this study is the first of its kind that evaluate the usefulness of saliva as diagnostic/monitoring aid through measuring the oxidative stress and status of the protective antioxidant under condition of stress due to autism in a sample of Iraqi autistics.

Malondialdehyde (MDA) levels were assessed in serum and saliva of all participants. Serum MDA level was significantly higher in autistics compared to normal controls which could be due to increased generation of reactive oxygen species (ROS) due to the excessive oxidative damage generated in these children, and this agreed with many studies (23,24,25). Salivary MDA levels are directly affected by systemic oxidative stress, since they were also significantly elevated in saliva of autistics; but there was no reported previous study for compression. These levels making MDA highly significant accurate parameter in prediction of ASD as they ranked second in importance (ROC area around 0.8) for both serum and saliva (table 3).

Uric acid is the final product of purine metabolism in humans. During purine metabolism molecular oxygen was used as electron acceptor and generation of superoxide anion and other reactive oxygen products occur. Uric acid may be a marker of oxidative stress, and may have a potential therapeutic role as an antioxidant. On the other hand, like other strong reducing substances can also act as a prooxidant, particularly at elevated levels. Thus, it is unclear whether elevated levels of uric acid in diseases associated with oxidative stress are a protective response or a primary cause (26,27).

The present study found that the mean level of serum UA was significantly increased in autistics when compared with that of healthy controls and
this was in agreement with previous study of Page & Coleman, 2000 (28) which reported that urate excretion is elevated 2-3-fold in hyperuricosuric subclass of autistic patients compared to normal controls. But other studies reported that hyperuricosuric autistic subject showed improvements in speech, attention, and social interaction on a low purine diet; these findings might suggest that uric acid itself may be responsible for autistic and/or neurological symptoms (29,30).

Salivary UA on the other hand, showed insignificant increase in autistics when compared with that of healthy controls. But there were no reported previous studies on level of UA in saliva of autistics to compare our results with.

In consistence with the above findings, Pearson correlation coefficient was applied in this study between parameters revealed that serum UA had a highly significant moderately strong positive (direct) correlation with serum MDA (r = 0.483) which is widely utilized as a marker of lipid peroxidation in states of elevated oxidative stress. And according to results of ROC test serum UA was significantly accurate, (parameter number one) in this study in prediction of Autism spectrum disorder as shown in table 3.

Glutathione (GSH) high concentration and its central role in maintaining the cell's redox state, therefore it is one of the most important cellular antioxidants. In the present study, serum GSH level of autistics was significantly depleted in comparison to that of healthy controls, and this could be correlated to the impaired defense mechanism against damage by ROS in autism which supported through considering the previous reports (3,4,23). Increased lipid peroxidation together with the observed depletion of GSH supports the oxidative stress hypothesis in ASD. Salivary GSH level in this study was shown to be significantly decreased in comparison to healthy controls but there was no previous reported studies to compare with. The ROC test results of this study revealed the diagnostic value of serum GSH, as the most accurate parameter in prediction of ASD since it ranked number one (ROC area around 0.9). Salivary GSH was also highly significant accurate in prediction of ASD as ranked third in order of importance (ROC area around 0.7) in this study as shown in table 3.

Superoxide dismutase (SOD) is a potent protective enzyme that can selectively scavenge superoxide anion by catalyzing its dismutation to H2O2 and oxygen (O2). Several studies proposed that altered activities of antioxidant system might have a pathophysiologlal role in autism (31).

Results obtained from current study demonstrate a significant reduced level of SOD in serum of autistic children, which confirmed the remarkable depletion SOD in autistic samples, since the brain contains high levels of oxidizable lipids that must be protected by antioxidants. This could find a support through considering previous works (7), but disagreed with many others (32,33).Salivary SOD activity was insignificantly lower in autistics compared to control, but there was no previous study on SOD level in saliva of autistics to compare with. The results of ROC test in this study revealed that serum SOD was significantly accurate in prediction of ASD, since ranked as parameter number three in order of importance in this study (table 3).

REFERENCES


Table 1: Mean±SD for tested parameters

<table>
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<th>Markers</th>
<th>Cases</th>
<th>Controls</th>
<th>p</th>
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</thead>
<tbody>
<tr>
<td>Serum UA (µmol/L)</td>
<td>296.2 ± 28.2</td>
<td>164.3 ±74.3</td>
<td>&lt;0.001</td>
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<td>Salivary MDA (µmol/L)</td>
<td>89.2 ± 29.5</td>
<td>83.9 ±88.1</td>
<td>0.78 [NS]</td>
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<td>Serum MDA (µmol/L)</td>
<td>13.6 ± 4.5</td>
<td>6.6 ±4.8</td>
<td>&lt;0.001</td>
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<td>Salivary MDA (µmol/L)</td>
<td>11.6 ± 3.3</td>
<td>5.7 ±3.9</td>
<td>&lt;0.001</td>
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<td>Serum SOD (unit/L)</td>
<td>0.059 ±0.085</td>
<td>0.123 ±0.032</td>
<td>0.004</td>
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<td>Salivary SOD (unit/L)</td>
<td>1.392 ±0.21</td>
<td>1.561±0.358</td>
<td>0.05 [NS]</td>
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Table 2: Median level for selected parameters

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<th>Markers</th>
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<tbody>
<tr>
<td>Serum GSH (µmol/L)</td>
<td>147.2</td>
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<td>Salivary GSH (µmol/L)</td>
<td>36.8</td>
<td>588.8</td>
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Table 3: ROC analysis for tested parameters

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<th>parameters</th>
<th>Area under the curve</th>
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<td>Serum MDA</td>
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<td>Salivary GSH</td>
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<td>Serum SOD</td>
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<td>Dmf</td>
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<td>Salivary UA</td>
<td>0.652</td>
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<td>Gingival index</td>
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<td>0.11 [NS]</td>
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<td>DMF</td>
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<td>0.79 [NS]</td>
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