**Introduction**

Autism spectrum disorder (ASD) is a severe neurodevelopmental disorder. It includes five diagnostic subtypes. The current study was carried out among 2-12 years old children with ASD. Thirty seven subjected to ASD (31 males and 6 females) were selected from psychiatric research unit -Mosul University. Thirty healthy children were enrolled in this study (20 males and 10 females) with the same age as control group. ASD patients were classified into three groups according to symptoms severity grade. The results indicated a significant (p≤0.05) decrease in the serum melatonin (-81.8%), neuroglobin (-84.9%), antioxidant activity (-69.8%) and glutathione (-63.3%) in severe ASD group compared to control group. At the same time a significant (p≤0.05) increase in the serum thiobarbituric acid reactive substances (+82.6%), advanced oxidation protein products (+120.6%) and nitric oxide (+85.5%) in severe ASD patients group compared to control group were observed. Also, a positive significant (p≤0.01) correlation between antioxidant activity and melatonin, neuroglobin, glutathione was shown, while a negative significant (p≤0.01) correlation between antioxidant activity and thiobarbituric acid reactive substances, advanced oxidative protein products and nitric oxide was indicated in the current study.

**Keyword:** Autism spectrum disorder, oxidative stress, biochemical parameters.

**Abstract**

Autism spectrum disorder (ASD) is a severe neurodevelopmental disorder. It includes five diagnostic subtypes. The current study was carried out among 2-12 years old children with ASD. Thirty seven subjected to ASD (31 males and 6 females) were selected from psychiatric research unit -Mosul University. Thirty healthy children were enrolled in this study (20 males and 10 females) with the same age as control group. ASD patients were classified into three groups according to symptoms severity grade. The results indicated a significant (p≤0.05) decrease in the serum melatonin (-81.8%), neuroglobin (-84.9%), antioxidant activity (-69.8%) and glutathione (-63.3%) in severe ASD group compared to control group. At the same time a significant (p≤0.05) increase in the serum thiobarbituric acid reactive substances (+82.6%), advanced oxidation protein products (+120.6%) and nitric oxide (+85.5%) in severe ASD patients group compared to control group were observed. Also, a positive significant (p≤0.01) correlation between antioxidant activity and melatonin, neuroglobin, glutathione was shown, while a negative significant (p≤0.01) correlation between antioxidant activity and thiobarbituric acid reactive substances, advanced oxidative protein products and nitric oxide was indicated in the current study.

**Material and Methods**

**Subject:** Children with autism spectrum disorder (37 cases) were enrolled in this study (31 males and 6 females) aging 2-12 years from psychiatric research unit in College of Medicine,
University of Mosul. In addition, 30 children with the same age (20 males and 10 females) were involved as control group.

The diagnosis of ASD is applied by a specialist psychiatric doctor, and the criteria of autism spectrum disorder as defined in the diagnostic and statistical manual of disorder, fourth edition (DSM-IV) were undertaken.

Patients group were classified into three subgroup, mild, moderate and severe according to symptoms severity grade.

Blood samples: Venous blood samples were collected from both groups in antiseptic plain tubes. Serum was separated by centrifugation at 3000 rpm after blood coagulation for 15 min. Serum samples were divided in small aliquot tubes and stored at -20°C for subsequent analysis.

Biochemical analysis: Glutathione (GSH) a simple and accurate method for the determination of serum GSH was used as described by Sedlack J. and Lindsay R.H., Tietz N.V.\textsuperscript{22,23}. Ellman reagent (DTNB) was considered as a reagent for thiol group.

Thiobarbituric acid reactive substances (TBARS) were measured spectrophotometrically\textsuperscript{24}.

Advanced oxidation protein products (AOPP) were determined in serum using spectrophotometric method\textsuperscript{25}. The results were calibrated with chloramine-T as standard AOPP concentration expressed as micromoles per liter chloramines –T equivalents.

Nitric oxide (NO) a colorimetric assay was used which provided an accurate, convenient measurement of total nitrite\textsuperscript{26}.

Anti-oxidant activity (AOA) was measured spectrophotometrically\textsuperscript{27}.

Neuroglobin (NGB) was assessed based on a sandwich Enzyme-Linked Immuno Sorbent Assay (ELISA) technique using CUSABIO kit (China)\textsuperscript{28}. The analysis was performed in the immunity laboratory in Al-Salam hospital in Mosul city using BIO-TEK instruments, INC, USA.

Melatonin (MEL) Early morning blood were collected from children and used for estimation of serum melatonin. Melatonin concentration was estimated by sandwich Enzyme-Linked Immuno Sorbent Assay (ELISA) technique using CUSABIO kit (China)\textsuperscript{29}. The analysis was performed in the immunity laboratory in Al-Salam hospital in Mosul city using BIO-TEK instruments, INC, USA.

Statistical analysis: The data obtained in the current study was analyzed using Statistical Package for Social Sciences (SPSS) version 11.5. i. Standard statistical methods were used to determine the mean and standard error. ii. One way Anova (Duncan test) is used to compare between more than two parameters. iii. Pearson correlation was performed to identify the relationship between different biochemical parameters.

Results and Discussion

Statistical analysis of all used biochemical parameters on mild, moderate and severe autism spectrum disorder compared to control group are listed in table 1 as mean ± S.E for each parameter.

Table 1 summarizes a significant (P \(\leq 0.05\)) decrease in the serum melatonin, neuroglobin, antioxidant activity and glutathione in ASD patients group compared to control group while, there was a significant (P\(\leq0.05\)) increase levels of thiobarbituric acid reactive substances, advanced oxidation protein products and nitric oxide in moderate and severe ASD patients as compared to control and mild groups. Also table 1 demonstrates the change percentage of each parameter.

The data in table 2 indicate the correlation between antioxidant activity with the other biochemical parameters in patients and control groups. Pearson correlation analysis was performed to study the association. A positive significant correlation between AOA with glutathione, melatonin and neuroglobin of ASD patients (to the left) compared to control group (to the right) as illustrated in figure 1 and negative significant correlation with thiobarbituric acid reactive substances, advanced oxidation protein products and nitric oxide of ASD patients group (to the left) compared to control group (to the right) as indicated in figure 2.

The present study is an attempt to examine the oxidative stress and status of the protective antioxidant under condition of stress due to ASD. Oxidative stress is the deregulation of oxidant – antioxidant system which causes tissue damage through the production of reactive oxygen species, lipid peroxidation and protein oxidation\textsuperscript{30}.

Reactive oxygen species have been suggested as important mediators in traumatic brain injury, strokes, neurodegenerative diseases\textsuperscript{31} and psychiatric disorder \textsuperscript{32} and in some individual with ASD\textsuperscript{33}.

Purkinje cells are especially sensitive to ischemia and hypoxia\textsuperscript{9}. The decrease in Purkinje cells number is likely to affect the neuronal communication and could contribute to autistic behavior, learning, cognitive and emotional processes\textsuperscript{34}. Several studies showed that oxidative stress, lipid peroxidation, DNA and protein oxidation are increased in autism spectrum disorder\textsuperscript{35}. Multiple oxidative stress biomarkers have been identified in ASD children\textsuperscript{36}. Glutathione is required to maintain the normal reduced state of cells and to counteract all the deleterious effects of oxidative stress. GSH is said to be involved in many cellular processes including the detoxification of endogenous and exogenous compounds\textsuperscript{37}. 

\textsuperscript{8} International Science Congress Association
There is evidence that GSH, itself is a neurotransmitter or neuromodulator and any change in either GSH levels turnover rates or oxidation state would adversely affect central nervous system activity\(^3^8\). The results in table 1 showed a significant (p≤0.05) decrease in serum GSH levels in ASD patients groups (-36.0%, -52.4%, -63.3%) which indicated oxidative stress. Glutathione contains thiol group and considered the strongest antioxidant like melatonin and glutathione might be representative of oxidative stress, have been reported in the ASD patients\(^4^1\). Increased lipid peroxidation is well reported in neurodegenerative diseases\(^4^2\). Similarity, a significant (p≤0.05) increase in the levels of serum TBARS in ASD patients, especially moderate and severe groups compared to control (+26.1%, +82.6%) respectively were observed in table 1. It was suggested that ROS attacks double bonds producing free radicals. This will increase lysosomal enzymes causing tissue and membrane damage with lipid peroxidation leading to loss of membrane function and integrity\(^4^3\).

**Table-1**

Concentration of some biochemical parameters as mean ± S.E control group, mild, moderate and severe autism spectrum disorder patients

<table>
<thead>
<tr>
<th>Biochemical parameters</th>
<th>Control Mean ±S.E</th>
<th>Mild % change</th>
<th>Moderate % change</th>
<th>Severe % change</th>
</tr>
</thead>
<tbody>
<tr>
<td>Melatonin pg/ml d</td>
<td>36.34±2.50</td>
<td>27.06±1.18</td>
<td>b</td>
<td>a</td>
</tr>
<tr>
<td>Neuroglobin ng/ml c</td>
<td>51.97±4.88</td>
<td>29.68±3.39</td>
<td>a</td>
<td>a</td>
</tr>
<tr>
<td>Antioxidant activity μmol/L c</td>
<td>38.52±2.1</td>
<td>38.83±1.66</td>
<td>a</td>
<td>a</td>
</tr>
<tr>
<td>Glutathione μmol/L b</td>
<td>4.58±0.2</td>
<td>2.93±0.11</td>
<td>a</td>
<td>a</td>
</tr>
<tr>
<td>Thiobarbituric acid reactive substances μmol/L a</td>
<td>0.69±0.04</td>
<td>0.66±0.02</td>
<td>a</td>
<td>a</td>
</tr>
<tr>
<td>Advanced oxidation protein products μmol/L a</td>
<td>38.82±1.32</td>
<td>39.39±2.0</td>
<td>a</td>
<td>a</td>
</tr>
<tr>
<td>Nitric oxide μmol/L a</td>
<td>10.33±0.23</td>
<td>9.93±0.2</td>
<td>a</td>
<td>a</td>
</tr>
</tbody>
</table>

S.E = Standard Error .. Different letters horizontally a, b, c, d indicate that the mean is different significantly at p≤0.05.

**Table-2**

Correlation between antioxidant activity and other measured biochemical parameters of control and ASD patients groups

<table>
<thead>
<tr>
<th></th>
<th>AOA</th>
<th>GSH</th>
<th>MEL</th>
<th>NGB</th>
<th>AOPP</th>
<th>TBARS</th>
<th>NO</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>r</td>
<td>1.0</td>
<td>0.308</td>
<td>0.069</td>
<td>0.005</td>
<td>-0.447</td>
<td>0.357</td>
</tr>
<tr>
<td>ASD patients</td>
<td>P</td>
<td>Nil</td>
<td>0.098</td>
<td>0.710</td>
<td>0.977</td>
<td>0.013</td>
<td>0.053</td>
</tr>
<tr>
<td></td>
<td>R</td>
<td>1.0</td>
<td>0.823**</td>
<td>0.890**</td>
<td>0.705**</td>
<td>-0.791**</td>
<td>-0.626**</td>
</tr>
<tr>
<td></td>
<td>p</td>
<td>nil</td>
<td>0.001</td>
<td>0.001</td>
<td>0.001</td>
<td>0.001</td>
<td>0.001</td>
</tr>
</tbody>
</table>

*Correlation is significant at P ≤ 0.05, **Correlation is significant at p ≤ 0.01
Figure 1: Positive correlation between antioxidant activity and GSH, MEL, and NGB in ASD patients to the left compared to control group to the right.
Oxidative modification of proteins has been also implicated in ASD patients through advanced oxidation protein products determination. AOPP has been shown as an inflammatory marker in many diseases. However, there are no reports for AOPP roles in ASD. AOPP might contribute to disorder by activating monocytes and providing pro-inflammatory cytokine production. Acute inflammation that occurs during severe autism spectrum disorder might accelerate AOPP formation. In the present study, AOPP levels increased significantly (P≤0.05) in severe group (+120%) compared to mild, moderate and control group. AOPP high levels in those patients might suggest an active role of macrophage activation and inflammation in the oxidative stress through myeloperoxidase-derived hypochlorous acid (HOCl). It might cause tissue damage, lipid and protein oxidation. Oxidative stress occurrence is further documented by increase concentration of nitric oxide (NO) in ASD, measured as nitrite. Nitric oxide is a potent pleiotropic mediator of physiological processes such as smooth muscle relaxation, neuronal signaling, inhibition of platelet aggregation and regulation of cell mediated toxicity. NO has been recognized as biological neural messenger molecule, although it is known to affect development and function of the central nervous system. Furthermore, NO block energy production and was found to be increased in autism as compared to age and sex-matched controls. NO has been shown to damage the blood brain barrier. Excess NO in ASD is localized to specific organs or tissues. The brain and gut are plausible sources as both are often abnormal in ASD to gross, behavioral and gastrointestinal symptoms predominate. The current results indicated a significant (P≤0.05) increase level in severe group (+85.5%) which agreed with a previous study. This indicates a possible role of NO in the pathogenesis of autism. Too much NO deplete antioxidant defense, depressing levels of GSH, low GSH in turn increase NO. So, in the present study,

Figure-2
Negative correlation between antioxidant activity and AOPP, TBARS and NO in ASD patients to the left compared to control group to the right.
thiobarbituric acid reactive substances, advanced oxidative protein products and NO found to be higher in ASD patients and give negative significant correlation with antioxidant activity.

Some studies reported ASD children have a higher prevalence of sleep abnormalities than typically developing children with a percent ranging from 40% to 86%\textsuperscript{50}. Our results revealed that the mean melatonin levels among ASD groups were significantly (p≤0.05) decreased as compared to control group in early morning by (-25.5%, -57.5%, -81.8%) as indicated in Table 1. These data might be due to the melatonin production in ASD patients seems to be lower than in controls. These results concided with the previous studies which indicated a significant correlation between melatonin levels and ASD symptoms\textsuperscript{50}. So, causes might be attributed to abnormalities in ASMT gene that codes for acetyl serotonin methyl transferase, an enzyme involved in melatonin synthesis in some people with ASD\textsuperscript{50} or reduced levels of Tryptophan\textsuperscript{51}. Lower ASMT is associated with hyper activity, sleep disorder in ASD patients and ASD severity\textsuperscript{52}. These studies suggested that melatonin metabolism is directly or indirectly related to certain autistic behavior\textsuperscript{50}. A study indicated that neuroglobin, novel globin type, is an endogenous neuroprotection molecule against hypoxia (inadequate oxygen supply) and ischemic insult in the brain (inadequate blood supply)\textsuperscript{53}. It has been demonstrated that NGB could alleviate oxidative stress, eliminate reactive oxygen species\textsuperscript{54}, preserve mitochondrial function and resist apoptosis\textsuperscript{18}. Although, currently no literature showing a connection between ASD and NGB, the present study revealed a severity dependent significant (p≤0.05) in NGB levels in ASD patients (-42.9%,-75.3%,-84.9% ) in comparison with control group as indicated in Table 1. The reduction of NGB levels might cause increase oxidative stress and free radical production which accompanied with antioxidant depletion. These finding might affect brain tissue and stimulate lipid, protein oxidation besides neuroinflammation. In addition, the results might reveal the important role of NGB in purkinje cells survival and ASD pathophysiology. It was reported that, administration of antioxidant might protect purkinje cells against oxidative stress\textsuperscript{17}. Many studies suggest that NGB acts as NO scavenger because NGB can directly bind to NO with high intrinsic affinity and a low dissociation rate\textsuperscript{54}. Our results indicate low levels of NGB and high levels of NO in ASD patients in accord with the previous studies that explained the effect of oxidative stress on some biochemical parameters in autistic children.

Finally, the term antioxidant denotes a compound which can delay or inhibit the oxidation of biomolecules by inhibiting the initiation or propagation of oxidative chain reactions and thus prevents damage done to the body’s cell by oxygen, i.e. reactive oxygen species (ROS)\textsuperscript{55}. Overproduction of such free radicals can cause oxidative damage to biomolecules, eventually leading to many chronic diseases\textsuperscript{56}. So, heavy metals are known to generate toxic ROS such as H$_2$O$_2$, O$_2$, OH,....etc. which degrade important cellular components by inducing oxidative stress\textsuperscript{57}. The current results demonstrated that antioxidant activity level is significantly (p≤0.05) decreased in ASD group especially moderate (-37.3%) and severe (-69.8%) compared to controls as illustrated in table 1. These findings suggested an increase oxidative stress occurrence and the oxidative compounds turns into toxic substances. When ROS effects cannot be prevented with endogenous and exogenous antioxidants, causing damage to the basic structural cellular elements including lipids, protein and nucleic acids, the process results in cell death via necrosis or apoptosis\textsuperscript{58}.

**Conclusion**

The results of the present study might conclude that oxidative stress with environmentaltoxicants exposure promotes autism spectrum disorder. Oxidative stress affects some biochemical parameters concentration and alterations might induce pathophysiology of ASD. Our team would like to advise the administration of specific antioxidants to prevent or to alleviate ROS effect. However, further in depth studies is required for the understanding ASD pathophysiology.

**References**


Autism spectrum disorder (ASD) was characterized by impairment in social communication and repetitive or restricted patterns of interest appearing during the first three years of life. It is four times more common in boys than girls with an overall incidence 5/10000. Despite extensive research, the etiology and natural history of autism spectrum disorder remains poorly understood. Oxidative stress and environmental toxicants exposure might contribute in ASD pathophysiology. Objective: 1- studying the oxidative stress effect of ASD patients. 2- Assessment dipeptidyl peptidase-4 (DPP- ASD) involves a complex interplay of both genetic and environmental risk factors, with immune alterations and synaptic connection deficiency in early life. In the past decade, studies of ASD have substantially increased, in both humans and animal models. In the present study we have carried out a clinical and descriptive study of autism spectrum disorder (ASD), and the involved risk factors and comorbidities in a developing country facing a severe and critical sociopolitical situation. Aggression is common in patients with autism spectrum disorders (ASD) along with the core symptoms of impairments in social communication and repetitive behavior. Risperidone, an atypical antipsychotic, is widely used to treat aggression in ASD [35]. Background Previous reports indicate an association between autism spectrum disorders (ASD) and disorders of mitochondrial oxidative phosphorylation. One study suggested that children with both diagnoses are clinically indistinguishable from children with idiopathic autism. There are, however, no detailed analyses of the clinical and laboratory findings in a large cohort of these children. Therefore, we undertook a comprehensive review of patients with ASD and a mitochondrial disorder. Methodology/Principal Findings We reviewed medical records of 25 patients with a primary diagnosis of ASD by