Overview in the etiopathogenesis of different phenotypes of autism

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The purpose of this study was to determine the evolution of autism by characterization of the validated biomarkers implicated in the pathophysiology of this disease to help in the diagnosis and treatments of ASD children. This study was conducted on 90 autistic children aged 2-7 years old. These children were divided into two main groups; the atypical autism group of 30 children and the childhood autism group of 60 children. The childhood autism group then divided into two groups of 30 children according to the severity of the disorder to mild-moderate autism group and severe autism group. The study also included 30 healthy children served as a control group. All participants were subjected to full psychiatric examination and psychological investigations. Biochemical measurements (TNF-α, GABA, GSH in plasma, glutamate in serum, and hair mercury) were done. The autistic groups showed a highly significant difference in both CARS and Vineland scores, a highly significant increase in hair mercury and in plasma TNF-α, GABA and glutamate in serum versus control. Plasma GSH level and glutamate/GABA ratio were significantly decreased in childhood autism group. The level of hair mercury, plasma TNF-α, serum glutamate and Glutamate/GABA ratio were significantly decreased with the increased disease severity. While plasma GABA level was significantly increased with the increased disease severity. Conclusively, excessive burden on the developing brain with mercury plays the critical role in the etiology of ASD. Neuroinflammations have a protective role during acute inflammation and a destructive role in chronic inflammation. These key elements would affect the brain homeostasis which could most probably lead to the pathogenesis of autism.

Keywords: Childhood autism, Atypical autism, Neuroinflammations, Excitatory/inhibitory balance, Brain homeostasis.

INTRODUCTION

Autism spectrum disorder (ASD) is a neurological disorder with a spectrum of qualitative aberrations in social interaction, qualitative impairments in communication, and restricted stereotyped patterns of behavior, activities and interests, with an onset acknowledged prior to 3 years of age. ASD includes not only classical autism (autistic disorder) according to American Psychiatric Association (DSM IV) or childhood autism according to the World Health Organization (ICD 10) but also Asperger’s disorder and pervasive developmental disorder not otherwise specified (PDD-NOS) or atypical autism (Cacola et al., 2017).

Pervasive developmental disorder not otherwise specified (PDD-NOS) according to (DSM IV) or atypical autism according (ICD 10) is often thought to convey the less severe end of ASD severity and is occasionally loosely interchanged with the label of high functioning autism to signify a milder version of autism. A diagnosis of atypical autism is regularly used as a
diagnosis of exclusion and is frequently detected as a “catch all” diagnostic category used when criteria for other PDD diagnoses are not met (Tidmarsh and Volkmar 2003).

Autism has become one of the most widespread childhood epidemics in recorded history. Apart from some infectious diseases epidemics of the past, no other critical condition has ever altered so many of our children (Sears, 2010). The incidence of autism has elevated rapidly since 1970s, when prevalence evaluation suggested that 1 in 2000 children were affected. Autism rates had increased from 1 in 200 by the late 1990 to 1 in 86 by 2007 and recently to 1 in 50, as published by the Centers for Disease Control and Prevention for 2011-2012 (CDC, 2013). Autism is 4 times more prevalent in males than in females, and the females have a higher ratio to the milder forms of the disorder (Landa, 2008). Atypical autism (PDD-NOS) emerges to be at least as common as childhood autism (Volkmar et al., 2007).

The ASD represents a highly heterogeneous group of disorders with various phenotypes and subgroups that receive behavioural commonalities. This inherent complication has made interpreting the etiology of the broad spectrum of ASD becomes greatly complex (Ashwood et al., 2006). Perinatal and obstetric conditions have been connected with many neurological and psychiatric disorders, involving Down syndrome, dyslexia, mental retardation and schizophrenia, as well as developmental disorders as ASD. Despite the studying of the potential part of pregnancy and birth difficulties in the origin of autism, the causal nature of these associations is still disputed (Kolevzon et al., 2007). A growing body of evidence showed that a variety of factors may be included in the etiopathogenesis of autism, as oxidative stress, antioxidant defense system, brain mitochondrial dysfunction, impaired immune reactions, environmental toxins, and genetic dysfunctions. However, the etiology of this ailment is still inadequately understood (Napoli et al., 2013).

Mercury in both its inorganic and organic chemical forms is a neurotoxic candidate able of inducing equally permanent and temporary destruction to the central nervous system of young and adult subjects (Dorea et al., 2011). In the CNS, this metal is able to influence vital developmental processes, involving cell proliferation, migration, differentiation, synaptogenesis, myelination and apoptosis (Grandjean and Landrigan, 2006). Joined with this, the sensitivity of the developing brain makes young children extremely susceptible to mercury insult, because both methyl mercury and ethyl mercury are able of disturbing the brain at small doses (Dorea, 2007). A study investigated mitochondrial respiration in lymphoblastoid cell lines (LCLs) extracted from children who have autism at baseline and following contact to the environmental toxins like ethylmercury showed significant impairment in mitochondrial respiration at baseline with this abnormality aggravated subsequent to contact to ethyl mercury (Rose et al., 2014).

Glutathione (GSH) is a tripeptide consisted of cysteine, glycine and glutamate that is produced de novo in all cells of the body and acts as the major intracellular redox buffer. The sulfhydryl group of the cysteine moiety supplies the reducing equivalents of the GSH molecule. The reducing environment is preserved within the cell by the high ratio of reduced GSH to the GSH oxidized disulfide (GSSG) (Schafer and Buettner, 2001). GSH is crucial for the removal of xenobiotics and heavy metal ions from the body (Herbert, 2010). Decreased GSH concentration and GSH/GSSG ratio, and elevated GSSG concentration have been recorded in plasma, peripheral blood mononuclear cells, lymphoblastoid cell lines, brain tissue and mitochondria in patients who have an ASD (Rose et al., 2012).

TNF-α is a cytokine implicated in systemic inflammation and it is a part of a group of cytokines that arouse the acute phase response. TNF-α is formed mostly through activated macrophages, even though it can be formed by other cell types, such as CD4+ lymphocytes and natural killer cells (NK) cells. TNF-α triggers the accumulations of neutrophil and monocytes to the location of inflammation and enhances their phagocytes function to remove pathogens and tissue remains; this effect cannot just be neuroprotective but as well be neurotoxic (Jyonouchi, 2010). TNF is able to bind two receptors; TNF receptor type-1 (TNF-R1) and TNF receptor type-2 (TNF-R2). So by binding with its receptors, TNF-α can activate the apoptosis signaling pathway (Xu et al., 2015).

Glutamate is the major excitatory neurotransmitter in the CNS. Discharging of glutamate from neuronal synaptic vesicles within the synapse motivates post-synaptic receptor reaction inducing fast depolarization, calcium uptake, and signal transduction. The glutamate ought to be quickly clear from the synapse to decrease background noise, which would damage
transmission efficiency (Daikhin and Yudkoff, 1998). It has been suggested that an important element of the neuronal deficits in ASD includes a dysregulation of glutamatergic neurotransmission in the brain (Blaylock and Strunecka, 2009). It is thought to be induced by antibodies against NMDA receptor proteins, which produce an inhibition of NMDA reaction (Creten et al., 2011).

In the adult CNS, GABA is the main inhibitory neurotransmitter, and it modulates glutamatergic activity. Modern researches have displayed that GABA acts as an excitatory transmitter in the immature CNS, and functions as a trophic factor for brain growth. Moreover, normal development of GABAergic synapses is vital for the expression of high brain functions as memory, learning and motor coordination. A variety of psychiatric disorders such as anxiety disorders, epilepsy, schizophrenia and autism are partially caused by the impairment of GABA in the developing and mature brain (van Kooten et al., 2005).

Accordingly, the main target of this study was to gain better understanding of the etiopathogenesis of autism by means of targeted parameters which play a relevant role in the development of this disease. Tracking of the pathophysiological axis of this disease may help in diagnosis of ASD children and may be one of the acceptable strategies to treat this disease.

**MATERIALS AND METHODS**

The present study was conducted on 90 autistic children [66 males (73.3%) and 24 females (26.7%)] aged 2-7 years old. All the diagnosed children were regularly attending the outpatient clinic of Center for Care of Children with Special Needs; Institute of Postgraduate Childhood Studies, Ain Shams University, Egypt, during the period from January 2014 - January 2015. Autistic children were assigned into two main groups; the atypical autism group consisted of 30 children diagnosed with atypical autism and the childhood autism group consisted of 60 children diagnosed with childhood autism. The childhood autism group was further classified into two groups according to the severity of the disorder measured by CARS (mild-moderate autism group) and (severe autism group); each group consisted of 30 children. The study also included 30 healthy children (15 males and 15 females) as a control group, they were age and sex matched and they were relatives of children going to Pediatric Surgery Clinics, Ain Sham University Hospitals, Cairo, Egypt.

All patients were medication free for at least one month with the exclusion of cases diagnosed with any syndromes associated with autistic features as Down syndrome and Fragile X syndrome, cases diagnosed with other pervasive developmental disorders as Rett’s disorder, Asperger disorder and Childhood Disintegrative disorder, cases with Cerebral Palsy, CNS diseases and sensory impairments, cases with childhood autism or atypical autism that have epilepsy, cases with childhood autism or atypical autism associated with autoimmune diseases or any inflammatory conditions. This study was approved by the “Ethical Committee” of National Research Centre, Giza, Egypt and the written informed consent was obtained from the parents of the studied patients after explanation of the aim of the study and its possible benefits on their children and other children who have the same conditions (Ethical approval number 11023).

**II-Methods**

All the children included in the study (patients and controls) were subjected to the following: Full Psychiatric History and Complete Psychiatric Examination; each child from the study groups received a confirmed diagnosis according to World Health ICD-10 criteria. Each child was submitted to Full Medical History and Clinical Examination with particular emphasis on complete neurological examination, and any immune activation such as elevated temperature, infectious or inflammatory diseases. Also, EEG was done to exclude the presence of epilepsy in the subjects included in this study. Psychological assessment was performed to confirm the diagnoses of both childhood autism and atypical autism and to detect the severity of the disease by using Childhood Autism Rating Scale Second Edition (CARS-2). It is subjectively rate 15 items; relationship to people, imitation, emotional response, body use, object use, adaptation to change, visual response, listening response, taste-smell-touch response and use, fear and nervousness, verbal communication, nonverbal communication, activity level, level of consistency of intellectual response and general impressions. The Second Edition of CARS expands the test’s clinical value, making it more responsive to individuals on the “high functioning” end of autism spectrum disorders. The clinician rated the individual on each item, using a 4-point rating scale. Ratings were based on frequency of the behavior in question, its intensity, peculiarity, and duration (Schopler et al., 2010). The scores of our patients and control groups were estimated and
then categorized in severity according to CARS scores into: non autistic with scores less than 25, autistic features with scores (25-29), mild-moderate autism with scores (30-36) and severe autism with scores (37-60). Vineland Adaptive Behaviour Scale (VABS) Second Edition was applied to measure the personal and social skills since birth, diagnosing and classifying mental retardation and autistic disorder. It assesses adaptive behavior in four domains: communication, daily living skills, socialization, and motor skills. It also provides a composite score that summarizes the individual's performance across all four domains (Sparrow and Crchetti, 1984). The scores of our patients and control children were estimated and their Social Intelligent Quotient (IQ) was detected according to the Vineland IQ scores which are divided into: borderline IQ with scores (80-71), mild deficient IQ with scores (70-51), moderate deficient IQ with scores (50-35), severe deficient IQ with scores (35-20) and profound deficient IQ with score less than 20. The low average IQ was estimated with scores (80-90), the average IQ with scores (90-100), the above average IQ with scores (100-110) and the superior IQ with scores above 110. The Socioeconomic Status was assessed by using El Shakhs Socioeconomic Status Scale in which five primary categories were estimated and scored; education and employment of both parents as well as the family income. Then, the socioeconomic status were divided into 7 levels according to their scores; very low socioeconomic status with scores (10-19), low socioeconomic status with scores (20-29), low middle socioeconomic status with scores (30-39), middle socioeconomic status with scores (40-48), high middle socioeconomic status with scores (49-58), high socioeconomic status with scores (59-68) and very high socioeconomic status with scores (69-77). The primary assumption of the scale is that the higher levels of education and employment indicate higher levels of socioeconomic wellbeing, and the higher levels of poverty with low income, low education and employment indicate lower levels of socioeconomic wellbeing (Al Shakhs, 2006).

Biochemical determinations

Determination of mercury in hair:

Hair samples were collected from the autistic groups and the control group. The hair samples were cut close to the scalp from the occipital area for testing. All the samples were transferred to the National Institute for Standards and the analysis of mercury was performed following the standard analytical procedure. Before testing, the hair samples were cut into pieces (about 1 cm), then they were washed with nonionic detergent. They were soaked in deionized water for 10 minutes followed by soaking in acetone for other 10 minutes, then washed by deionized water to remove any contaminations. Finally, they were dried at 70 ºC over night and prepared using microwave digestion system; 100 mg of each sample was weighed and put into the digestion vessel, then 6 ml of nitric acid and 3 ml of hydrogen peroxide were added. The mixture was stirred carefully and left for 10 minutes before closing the vessel. The microwave digestion instrument (TOP wave Analytik Jena, Germany) was heated in steps to 190 ºC under pressure before inserting the digestion vessels. After the digestion protocol was finished (30 minutes), the vessels were left to cool at room temperature to avoid foaming and splashing. After cooling, the digested samples were diluted to a specified volume with deionized water (20 ml). Ultrapure water was used for final sample dilution and the analysis was performed via Atomic Absorption Spectrometer (ZEEmit 700, Analytik Jena, Germany) equipped with a hollow cathode of the element operated at a current recommended by the lamp and instrument manufacturer. An automatic deuterium background-correction was used for hydride system measurements. Parallel with the sample preparation, the same procedure was used to prepare a blank sample using the same quantities of all reagents. Also, a spike sample was used to check the recovery of the measurements. Test values were reported in µg/kg (mcg/g).

Determination of circulating markers:

From all the autistic groups and the control group, 5 ml of blood samples were withdrawn by venous arm puncture and partitioned into two tubes; a plain tube left to clot at room temperature for separation of serum and a heparinized tube for separation of plasma. Both the serum and the plasma were separated by centrifugation at 1800xg under cooling (4 ºC) for 15 minutes.

Tumour necrosis factor-α (TNF-α), Gamma-Aminobutyric Acid (GABA) plasma levels and Glutamate (Glu) serum level were measured using an enzyme-linked immunosorbent assay (ELISA) kit (Glory Science Co., Ltd, USA) according to the manufactured procedure.

Reduced glutathione (GSH) plasma level was determined by colorimetric method using...
glutathione reduced assay kit (TBAR) purchased from Biodiagnostic Co. Cairo, Egypt according to the manufactured instructions.

**Statistical analysis:**

Data obtained from this research are organized, tabulated and analyzed through IBM personal computer. Statistical analysis was performed using the SPSS statistical package software for Windows version 20 (SPSS Inc., Chicago, Illinois, USA). Parametric variables among the controls and the studied patient groups were analyzed using two tailed unpaired t-test. Qualitative variables were assessed by Chi-square test. A P value <0.05 was considered significant difference and P<0.005 was considered highly significant difference. Spearman correlation coefficient r measuring the strength and direction of a linear relationship between two variables on a scatter plot was done. The value of r is always between +1 and –1.

**RESULTS**

The data in Table (1) show that most of the autistic children in both the atypical autism group (70%) and the childhood autism group (75%) are males. The females are more likely to have atypical autism (30%) than childhood autism (25%). The old father and mother age 35+ risk factor is equally common in both the childhood and the atypical autism groups (20%) and (10%) respectively versus the control group (6.7%) and (3.3%). While + consanguinity is slightly common in the atypical autism group (50%) than the childhood autism group (48.3%) relative to the control group (16.7%). Regarding prenatal, perinatal complications and macrocephaly risk factors are more common in the childhood autism group (71.7%), (48.3%) and (11.7%) versus both the atypical autism (60%), (33.3%), (3.3%) and the control group (16.7%), (10%), (0%). Whereas, caesarean labour is more common in the atypical autism group (70%) than the childhood autism group (51.7%) versus the control group (33.3%). Whilst instrumental labour is only a risk factor in the childhood autism group (6.6%).

The results in Table (2) show a highly significant difference in respect to both CARS and Vineland scores between the autistic groups (atypical autism and childhood autism groups) as compared to the control group (P<0.0001) and also between the childhood autism group in comparison to the atypical autism group (P<0.0001). Regarding to the social status scores, the data also show a significant difference between atypical autism group and the control group (P=0.014) but no significant difference between childhood autism group compared to both atypical autism group and the control group.

The findings in Table (3) show a highly significant difference regarding to both CARS and Vineland scores between the mild-moderate autism group and the atypical autism group (P<0.0001). Also the tabulated results show a highly significant difference between the severe autism group and both the atypical autism and the mild-moderate autism groups (P<0.0001). However, there is no significant difference among all the autistic groups regarding to the social status scores.

The records in Table (4) show a highly significant elevation in both TNF-α plasma level and the level of Hg excreted in hair (P<0.0001) in comparing the atypical autism group to the control. Meanwhile, there is a highly significant reduction in both TNF-α plasma level and the level of Hg excreted in hair in comparing the childhood autism group to both the atypical autism group and the control group (P<0.0001). As regards GSH plasma level, there is insignificant difference between the atypical autism group and the control group (P=0.169). While there is a significant drop in GSH plasma level in comparing the childhood autism group to the control group (P=0.029) but a highly significant down regulation in GSH plasma level when comparing the childhood autism group to the atypical autism group (P<0.0001). As regard Glu serum level, there is a highly significant increase in comparing the atypical autism group to the control group (P<0.0001). There is only significant increase in Glu serum level in comparing the childhood autism group to the control group (P=0.01). However there is a highly significant decrease in Glu serum level in comparing the childhood autism group to the atypical autism group (P<0.0001). Concerning GABA plasma level, there is a highly significant amplification in comparing the atypical autism group to the control group (P=0.005) and also when comparing the childhood autism group to both the atypical autism group and the control group (P<0.0001). Glu/GABA ratio shows highly significant difference when comparing the childhood autism group and both the atypical autism group and the control group (P=0.719).
Table (1): Demographic data of the patients and the control groups

<table>
<thead>
<tr>
<th>Description</th>
<th>Control group No. 30</th>
<th>Atypical Autism group No. 30</th>
<th>Childhood Autism Group No. 60</th>
</tr>
</thead>
<tbody>
<tr>
<td>No.</td>
<td>%</td>
<td>No.</td>
<td>%</td>
</tr>
<tr>
<td>Male</td>
<td>15</td>
<td>50%</td>
<td>21</td>
</tr>
<tr>
<td>Female</td>
<td>15</td>
<td>50%</td>
<td>9</td>
</tr>
<tr>
<td>Children Age</td>
<td>6.6±0.7</td>
<td>5.11±1.5</td>
<td>6.1±2.1</td>
</tr>
<tr>
<td>Parental Age</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Old Father 35+</td>
<td>2</td>
<td>6.7%</td>
<td>6</td>
</tr>
<tr>
<td>Old Mother 35+</td>
<td>1</td>
<td>3.3%</td>
<td>3</td>
</tr>
<tr>
<td>+ Consanguinity</td>
<td>5</td>
<td>16.7%</td>
<td>15</td>
</tr>
<tr>
<td>Macrocephaly</td>
<td>-</td>
<td>-</td>
<td>1</td>
</tr>
<tr>
<td>Prenatal Problems</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Medical Problems</td>
<td>5</td>
<td>16.7%</td>
<td>18</td>
</tr>
<tr>
<td>Emotional Problems</td>
<td>3</td>
<td>10%</td>
<td>9</td>
</tr>
<tr>
<td>Both</td>
<td>1</td>
<td>3.3%</td>
<td>5</td>
</tr>
<tr>
<td>Normal Labour</td>
<td>20</td>
<td>66.7%</td>
<td>9</td>
</tr>
<tr>
<td>Caesarean Labour</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Instrumental Labour</td>
<td>10</td>
<td>33.3%</td>
<td>21</td>
</tr>
<tr>
<td>Perinatal Complications.</td>
<td>3</td>
<td>10%</td>
<td>10</td>
</tr>
</tbody>
</table>

Table (2): Comparison between autism groups (atypical autism and childhood autism) and the control group according to their scores of psychological tests.

<table>
<thead>
<tr>
<th>Groups Psychological tests</th>
<th>Control group</th>
<th>Atypical Autism group</th>
<th>Childhood Autism group</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>P-value</td>
<td>P-value</td>
<td></td>
</tr>
<tr>
<td>CARS</td>
<td>20.1±1.15</td>
<td>29.9±2.12&lt;sup&gt;a&lt;/sup&gt;</td>
<td>36.9±3.6&lt;sup&gt;ab&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.00</td>
<td>a&amp;b: 0.00</td>
</tr>
<tr>
<td>Vineland</td>
<td>87.0±5.36</td>
<td>63.2±14.26&lt;sup&gt;a&lt;/sup&gt;</td>
<td>43.4±15.7&lt;sup&gt;ab&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.00</td>
<td>a&amp;b: 0.00</td>
</tr>
<tr>
<td>Social Status</td>
<td>39.1±6.1</td>
<td>45.3±11.6&lt;sup&gt;a&lt;/sup&gt;</td>
<td>42.8±10.0</td>
</tr>
<tr>
<td></td>
<td>0.014</td>
<td>0.086</td>
<td></td>
</tr>
</tbody>
</table>

Data were expressed as means ± standard deviation (SD)

<sup>a</sup>: significant difference vs the atypical autism group.

Table (3): Comparison between atypical autism group and the childhood autism groups (mild-moderate autism and severe autism) according to their scores of psychological tests

<table>
<thead>
<tr>
<th>Groups Psychological tests</th>
<th>Atypical Autism group</th>
<th>Childhood Autism group</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>P-value</td>
<td>P-value</td>
</tr>
<tr>
<td>CARS</td>
<td>29.9±2.12</td>
<td>33.83±1.51&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>0.00</td>
<td>a&amp;b: 0.00</td>
</tr>
<tr>
<td>Vineland</td>
<td>63.23±14.25</td>
<td>51.4±15.65&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>0.00</td>
<td>a&amp;b: 0.00</td>
</tr>
<tr>
<td>Social Status</td>
<td>45.33±11.65</td>
<td>44.3±10.74</td>
</tr>
<tr>
<td></td>
<td>0.679</td>
<td>0.117</td>
</tr>
</tbody>
</table>

Data were expressed as means ± standard deviation (SD).

<sup>a</sup>: significant difference vs the atypical autism group.

<sup>b</sup>: significant difference vs the mild-moderate autism group.
Table (4): Comparison between the autism groups (atypical autism and childhood autism) and the control group regarding to the biochemical markers

<table>
<thead>
<tr>
<th>Groups</th>
<th>Control group</th>
<th>Atypical Autism group</th>
<th>Childhood Autism group</th>
</tr>
</thead>
<tbody>
<tr>
<td>Biochemical Markers</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hair Hg(µg/kg)</td>
<td>0.51±0.28</td>
<td>2.02±0.88&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.38±0.79&lt;sup&gt;ab&lt;/sup&gt;</td>
</tr>
<tr>
<td>GSH (mg/dl)</td>
<td>9.2±1.3</td>
<td>10.39±4.03</td>
<td>7.6±3.3&lt;sup&gt;ab&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TNF-α (ng/L)</td>
<td>420.0±125.7</td>
<td>6803.0±3434.1&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.00</td>
</tr>
<tr>
<td>Glu (µmol/L)</td>
<td>248.14±68.2</td>
<td>428.5±158.9&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.00</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>GABA (µmol/L)</td>
<td>0.38±0.09</td>
<td>0.56±0.24&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.005</td>
</tr>
<tr>
<td>Glu/GABA</td>
<td>726.7±413.5</td>
<td>757.7±375.7</td>
<td>0.719</td>
</tr>
</tbody>
</table>

Data were expressed as means ± standard deviation (SD)

<sup>a</sup>: significant difference vs the control group

<sup>b</sup>: significant difference vs the atypical autism group.

The data in Table (5) show a significant blunting in the level of Hg excreted in hair in comparing the mild-moderate autism group to the atypical autism group (P=0.02) and a highly significant blunting in Hg level excreted in hair in comparing the severe autism group to the atypical autism group (P<0.0001) but only significant blunting in comparing the severe autism group to the mild-moderate autism group (P=0.035).

Regarding GSH plasma level, the data show a significant reduction in plasma GSH level in comparing the mild-moderate autism group to the atypical autism group (P=0.024). However there is a highly significant reduction in plasma GSH level in comparing the severe autism group to the mild-moderate autism group (P<0.0001), while only significant reduction in plasma GSH level is recorded in comparing the severe autism group to the atypical autism group (P=0.03). Regarding TNF-α plasma level, there is a highly significant decrease in comparing the mild-moderate autism group to the atypical autism group (P<0.0001) and in comparing the severe autism group to both the atypical autism and the mild-moderate autism groups (P<0.0001). (P=0.005) respectively. Regarding Glu serum level and Glu/GABA ratio, a significant decrease is recorded in comparing the mild-moderate autism group to the atypical autism group (P=0.025) (P=0.024) respectively. However there is a highly significant decrease in Glu serum level and Glu/GABA ratio in comparing the severe autism group to the atypical autism group (P<0.0001). While only a significant decrease in Glu serum level and in Glu/GABA ratio is detected in comparing the severe autism group to the mild-moderate autism group (P=0.035) (P=0.002) respectively. Concerning GABA plasma level, there is a significant increase in the mild-moderate autism group relative to the atypical autism group (P=0.049). But a highly significant increase in GABA plasma level is recorded in comparing the severe autism group to both the mild-moderate and the atypical autism groups (P<0.0001).

The results in Table (6) represent the Spearman correlation between the psychological tests and the measured biochemical markers in a group of the atypical autism. The data show that there is a significant negative correlation between CARS and the social status scores (P=0.008).

The findings in Table (7) illustrate the Spearman correlation between the measured biochemical parameters with each other’s in the atypical autism group. The results show a highly significant negative correlation between GABA and Glu/GABA ratio (P<0.0001) and a highly positive correlation between Glu and both TNF-α (P<0.0001) and Glu/GABA ratio (P<0.0001). The data also show a significant positive correlation between Glu/GABA ratio and TNF-α (P=0.017).

The records in Table (8) constitute the Spearman correlation between the psychological tests and the measured biochemical parameters in a group of the childhood autism. The data show a highly significant negative correlation (P<0.0001) between CARS and Vineland scores and a highly significant positive correlation between CARS and GABA (P=0.002). The results also show a highly significant negative correlation between CARS scores and both Glu/GABA ratio (P<0.0001) and TNF-α (P<0.0001).
Table (5): Comparison between the atypical autism group and the childhood autism groups (mild-moderate autism and severe autism) regarding to the biochemical markers.

<table>
<thead>
<tr>
<th>Groups Biochemical Markers</th>
<th>Atypical Autism group</th>
<th>Autistic groups</th>
<th>Mild-Moderate group</th>
<th>P- value</th>
<th>Severe group</th>
<th>P- value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hair Hg (µg/kg)</td>
<td>2.02±0.88</td>
<td></td>
<td>1.58±1.01&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.020</td>
<td>1.18±0.4&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>0.00</td>
</tr>
<tr>
<td>GSH (mg/dl)</td>
<td>10.39±4.03</td>
<td></td>
<td>8.5±3.9&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.024</td>
<td>6.8±2.37&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>0.035</td>
</tr>
<tr>
<td>TNF-α (ng/L)</td>
<td>6803.03±3434.1</td>
<td></td>
<td>3252.58±1396.1&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.00</td>
<td>1854.09±635.01&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.00</td>
</tr>
<tr>
<td>Glu (µmol/L)</td>
<td>428.5±158.9</td>
<td></td>
<td>355.8±138.6&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.025</td>
<td>287.4±111.05&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>0.035</td>
</tr>
<tr>
<td>GABA(µmol/L)</td>
<td>0.56±0.24</td>
<td></td>
<td>0.67±0.044&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.049</td>
<td>1.04±0.33&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>0.00</td>
</tr>
<tr>
<td>Glu/GABA</td>
<td>757.7±375.7</td>
<td></td>
<td>579.7±180.9&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.024</td>
<td>334.4±137.4&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>0.00</td>
</tr>
</tbody>
</table>

Data were expressed as means ± standard deviation (SD). a: significant difference vs the atypical autism group. b: significant difference vs the mild-moderate autism group.

Table (6): Spearman correlation between the psychological tests and biochemical markers in the atypical autism group

<table>
<thead>
<tr>
<th>CARS</th>
<th>Vineland</th>
<th>Social Status</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>r</td>
<td>P-Value</td>
<td>r</td>
</tr>
<tr>
<td>------</td>
<td>----------</td>
<td>--------</td>
</tr>
<tr>
<td>CARS</td>
<td>1.0</td>
<td>-0.026</td>
</tr>
<tr>
<td>Vineland</td>
<td>-0.026</td>
<td>0.89</td>
</tr>
<tr>
<td>Social Status</td>
<td>-0.474</td>
<td>0.008</td>
</tr>
<tr>
<td>H Hg</td>
<td>0.354</td>
<td>0.055</td>
</tr>
<tr>
<td>GSH</td>
<td>-0.09</td>
<td>0.635</td>
</tr>
<tr>
<td>TNF-α</td>
<td>0.069</td>
<td>0.716</td>
</tr>
<tr>
<td>Glu</td>
<td>-0.089</td>
<td>0.639</td>
</tr>
<tr>
<td>GABA</td>
<td>-0.335</td>
<td>0.071</td>
</tr>
<tr>
<td>Glu/GABA</td>
<td>0.232</td>
<td>0.217</td>
</tr>
</tbody>
</table>

r: Correlation Coefficient
### Table (7): Spearman correlation between the measured biochemical markers with each other’s in the atypical autism group

<table>
<thead>
<tr>
<th>Markers</th>
<th>H Hg</th>
<th>GSH</th>
<th>TNF-α</th>
<th>GABA</th>
<th>Glu</th>
<th>Glu/GABA</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>r</td>
<td>P</td>
<td>r</td>
<td>p</td>
<td>r</td>
<td>P</td>
</tr>
<tr>
<td>GABA</td>
<td>-0.023</td>
<td>0.902</td>
<td>0.060</td>
<td>0.754</td>
<td>-0.171</td>
<td>0.366</td>
</tr>
<tr>
<td>Glu</td>
<td>0.196</td>
<td>0.298</td>
<td>-0.016</td>
<td>0.934</td>
<td>0.468&quot;</td>
<td>0.009</td>
</tr>
<tr>
<td>Glu/GABA</td>
<td>0.23</td>
<td>0.222</td>
<td>0.088</td>
<td>0.643</td>
<td>0.434&quot;</td>
<td>0.017</td>
</tr>
<tr>
<td>TNF-α</td>
<td>0.114</td>
<td>0.549</td>
<td>-0.151</td>
<td>0.425</td>
<td>-0.171</td>
<td>0.366</td>
</tr>
<tr>
<td>GSH</td>
<td>-0.15</td>
<td>0.43</td>
<td>-0.151</td>
<td>0.425</td>
<td>-0.060</td>
<td>0.754</td>
</tr>
<tr>
<td>H Hg</td>
<td>-0.15</td>
<td>0.43</td>
<td>0.114</td>
<td>0.549</td>
<td>-0.023</td>
<td>0.902</td>
</tr>
</tbody>
</table>

r: Correlation Coefficient
The results in Table (9) indicate the Spearman correlation between the measured biochemical parameters with each other in the childhood autism group. The findings show a highly significant negative correlation between GABA and Glu/GABA ratio (P<0.0001) and a significant negative correlation between GABA and TNF-α (P=0.047). However, a highly significant positive correlation between Glu and Glu/ GABA ratio (P<0.0001) is recorded. Meanwhile, a significant negative correlation between TNF-α and GABA (P=0.047) is detected. Additionally, There is a significant positive correlation between TNF-α and both GSH (P=0.013) and Hg excreted in hair (P<0.042).

**DISCUSSION**

In the current study, 33.3% of the autistic children were diagnosed as atypical autism and 66.7% were diagnosed with childhood autism. This comes in line with Hussein et al., (2011) study who reported that childhood autism was more common than the atypical autism in both Saudi and Egyptian groups. The preponderance of autistic patients in both atypical autism and childhood autism groups were boys (70%) and (75%) respectively. This is in alignment with Whiteley et al., (2010) who acclaimed a higher preponderance of males over females (approximately 4:1) in autistic children. Frequently in western researches, the number of the atypical autism is greatly more than that of autistic disorders than autism. Cross
cultural causes might perform a part in this finding. Further causes can be the altered sampling methods used in various researches (Fombonne, 2003). The present results also pointed to increase the hazard of autism in both atypical and childhood autism groups with high paternal age at birth (father ≥ 35 years) (20%) in both groups versus (6.7%) in the control group and the maternal age at birth (mother ≥ 35 years) (10%) in both groups versus (3.3%) in the control group. This finding is congruous with that presented by El-Baz et al., (2011) who stated that 23% of autistic children have advanced maternal age at birth. The study demonstrated that parents of both atypical autism group (50%) and childhood autism group (48.3%) have positive consanguinity versus the control group (16.7%). This is consistent with Mamidula et al., (2015) who did their study on a large population in India and concluded that consanguinity elevates the hazard for ASD. In the present work, (60%) of atypical autism and (71.7%) of childhood autism had prenatal problems compared with (16.7%) in the control group. This finding is in keeping with Gardener et al., (2009) who examined more than 50 prenatal causes for ASD. The most causes correlated with autism risk in the meta-analysis were prenatal medication use, bleeding and gestational diabetes. There was also a high incidence of caesarean labour (70%) in atypical autism group and (51.7%) in childhood autism group versus (33.3%) in the control group. This coincides with Matthiesen et al. (2001) who detected that cesarean section may be accompanied with anomalies in oxytocin secretion due to delivery under local anesthesia or general anesthesia. This modern hospital obstetric procedure interferes with infant suckling responses and maternal milk let down, both of which are related to oxytocin release. In the current study, (33.3%) of atypical autism and (48.3%) of the childhood autism versus the control group (10%) had perinatal complications. These results are in concordance with Burd et al., (1999) who mentioned an association between autism and perinatal risk factors as breech presentation, low Apgar score (<7) at 5 min, low birth weight (<2500 g), gestational age at birth of less than 35 weeks. It has been found that small for gestational age was congruent with a statistically significant enlarged hazard of ASD. In the present study, (3.3%) of atypical autism and (11.7%) of childhood autism versus 0% of the control group had macrocephaly at birth. This fits with the study done by Lainhart et al., (1997) which recorded that macrocephaly is common in autism and 14% of the autistic individuals have macrocephaly.

Results of the present study come in line with the previous findings indicating that the CARS total score changes significantly by diagnostic group, with children diagnosed as childhood autism having significantly higher (P<0.0001) total CARS scores than children diagnosed with atypical autism, and both of them had significantly higher (P<0.0001) scores than those in healthy controls. Also, significantly higher (P<0.0001) total CARS scores have been found in severe autism group versus mild-moderate autism and also when compared both with atypical autism group. The clinical significant differences recorded in CARS total scores among the diagnostic groups are in keeping with the previous data reported by Chlebowski et al., (2010) who supported the utilization of CARS as a stable measure of autism severity.

The current results also showed that there are significantly lower scores in Vineland Adaptive Behaviour Scale with the enhancement of the disease severity. Greatly significant (P<0.0001) Vineland Adaptive Behavioural Scale low scores have been found in comparing the childhood autism group with the atypical autism group and in comparing both with the control group. As well a highly significant (P<0.0001) low scores have been detected in comparing severe autism with mild-moderate autism and in comparing both to the atypical autism group. These results are in conformity with those of Paul et al., (2004) who stated that individuals with atypical autism scored higher than those with autism in Vineland Adaptive Behaviour Scale. This is in agreement with the results in the current study, as it has been found a significantly (P=0.02) negative correlation between CARS and Vineland IQ in childhood autism group. Also, the study of Perry et al., (2005) recorded a moderate negative correlation of CARS scores and the developmental levels (both cognitive and adaptive).

Contacts to environmental toxicants such as mercury, lead, arsenic, polychlorinated biphenyls (PCBs) and toluene are identified to cause neurodevelopmental disorders (Rossignol et al., 2014). An epidemiological study published in 2006 announced 61% increases in Autism incidence rates per 1000 pounds of mercury discharged into the environment (Palmer et al., 2006). Many researchers have documented that several patients with autism assert polymorphism in genes implicated in the detoxification of the environmental pollutants. These genes have been
named ‘environmental reaction genes and more than one hundred of such genes may increase of the autism hazard (Faustman et al., 2000). In animal models and in human mercury poisonings, males were detected to be extensively more vulnerable to mercury toxicity (including neurotoxicity) than females. Furthermore, in a succession of tissue culture experiments with neurons, testosterone was capable to augment the neuronal toxicity of mercury, while estradiol drastically decreases the neuronal toxicity of mercury. Interestingly, it has been suggested that estradiol introduction probably implicates to maintaining normal neuronal cells and glutathione levels, therefore, helps to extensively decrease neuronal mercury toxicity (Olivieri et al., 2002). In the light of the current data, highly significant (P<0.0001) concentrations of mercury excreted in hair among both atypical autism group and childhood autism group compared with the healthy control group and compared with each other’s (P<0.0001). This agrees with Fido and AlSaad in Kuwait study which showed that the children with autism have significantly elevated levels of mercury concentration in hair (Fido and Al-Saad, 2005). Also the present data come in line with those of Al-Ayadhi (2005) who stated that hair samples from autistic patients living in Riyadh contain significantly higher concentration of the toxic heavy metals (mercury, lead and arsenic). Moreover El Sheshtawy et al. (2011) recorded highly significant changes in the level of mercury in hair between autism cases and controls in an Egyptian samples. The present findings showed also that the level of mercury excreted in hair is significantly decreased with increasing the severity of the disease as it is highly significantly decreased (P<0.0001) in childhood autism group in comparison with atypical autism group and it is also significantly dropped (P=0.02) in mild-moderate autism group as compared to atypical group. Moreover it is greatly significantly depleted (P <0.0001) in severe autism group in comparison to atypical autism group, but it is significantly diminished (P=0.03) in severe autism group versus the mild-moderate autism group. These results are greatly supported by the correlation results in the same study that there is a significant negative (P=0.01) correlation between CARS scores and level of mercury excreted in the hair and a highly significant (P<0.0001) positive correlation between Vinland Adaptive IQ scores and the level of mercury excreted in the hair (P=0.002) in the childhood autism group. These findings echo those of the previous study of Holmes and her co-workers who reported that autistic children have significantly lower levels of mercury in their hair comparative to non-autistic children, indicating high accumulation of mercury in the body due to decreased excretion facilities (Holmes et al., 2003). Nelson and Bauman (2003) also prescribed that autistic children preserve mercury in their body due to impairment in detoxification pathways. Another study on 78 children with autism and 31 control children recorded that, in comparison to the children who have greater levels of mercury in hair, children with lower levels of hair mercury are 2.5-fold more likely to have ASD (Adams et al., 2013). That study also documented that children diagnosed with autism have elevated average levels of several toxic metals, amongst which mercury is intensively connected with variations in the severity of the disorder. The current results are in compliance with those of the investigators who hypothesized that ASD is a mercurial syndrome and similarities are found between prenatal/infantile mercury exposure and delayed language, defective communication and repetitive behaviours (Muller, 2007). Contrary to the present results, El-baz et al., (2010) in their study which was conducted on Egyptian autistic children found that the level of mercury in hair is elevated in patients with the lowest mentality and also in patients with severe level of autism according to CARS. Meanwhile Majewska et al., (2010) stated that the impaired mercury excretion in young children diagnosed with ASD as less hair mercury (believed to be a marker of excretion) is correlated with a greater ASD severity or risk of ASD. Mercury can cause deficits in social interaction, communication difficulties, repetitive and stereotyped patterns of behaviour, which include the three DSM-IV autism diagnostic criteria. Additionally, mercury can produce characteristics noticeable in ASD, such as sensory abnormalities, emotional/psychological changes, movement disorder, impairments in abstract or complex thinking, severe sleep disorders, and self-injurious behaviour (Bernard et al., 2002). Mercury conjugates with thiol compounds such as glutathione and cysteine and induces depletion of glutathione, which is essential to mitigate oxidative damage (Nicole, 1998).

Glutathione is one of the most important candidates in the mechanisms of heavy metal detoxification and excretion in the body. Glutathione binds with these heavy metals resulting in water-soluble compounds which are more easily filtered out of the body (Stohs and
Bagchi, 1995). In the current study, there was insignificant (P=0.1) change in plasma levels of glutathione in the atypical autism group in comparison with the control group. While glutathione levels in childhood autism group showed a significantly (P=0.02) low levels versus the control group and highly significantly low levels (P<0.0001) compared with the atypical autism group. The proposed mechanism of the protective role of GSH in the atypical autism is that glutathione consists of glutamate, glycine, and cysteine (Ghanizadeh et al., 2012). Glutamate levels were remarkably high in the atypical autism group than the childhood autism group in the present study. Also, GSH acts as a carrier of mercury and has definite role in preserve the body from mercury toxicity. First, glutathione binds with methylmercury forms a complex that blocks mercury from binding to cellular proteins, producing damage to both enzymes and tissue and becoming intracellular toxins (Kromidas et al., 1990). Second, glutathione-mercury complex has been detected in the liver, kidney, and brain, and rise to be the primary form in which mercury is moved and eliminated from the body (Zalups, 2000). This is why the atypical autism group in the current study had the highest rate of mercury hair excretion. The present data revealed that plasma glutathione depletion increased with the increase in the severity of the disease, as glutathione plasma levels were significantly low (P=0.02) in mild-moderate autism group versus the atypical autism group. While it is highly significantly low (P<0.0001) in the severe autism group versus the atypical autism group and only significantly low (P=0.03) compared with the mild-moderate autism group. Also a significantly negative (P=0.01) correlation between GSH plasma levels and CARS scores in the childhood autism group has been observed. These outcomes coincide with Geier et al., (2009) and Alabdali et al., (2014) who also found a significant opposite relationship between blood GSH levels and autism severity measured with the CARS. The proposed mechanisms for the decreased glutathione plasma levels in severe autistic children are that the production of glutathione has been directly connected to the level of mercury excretion and cellular protection from mercury-induced damage. People with genetic lack in glutathione synthesis will be less capable to emit mercury and will be more vulnerable to its undesirable effects (Suh et al., 2008). The lowering levels of several metabolic precursors for glutathione synthesis have been detected in these individuals suggesting the insufficiency of glutathione synthesis (James et al., 2009). Inhibiting glutathione synthesis in the brain capillary endothelial cells (BBB cells) suppresses their capacity to release mercury as Kerper et al., (1996) has been found that these cells discharge mercury in a glutathione complex. Furthermore, Vargas et al. (2005) mentioned the existence of chronic inflammation in the autistic CNS that seems to be regulated by innate microglial activation and pro-inflammatory cytokines. The inflammatory reaction is enlarged when glutathione concentrations are low and chronic inflammation depletes more glutathione and stimulates a self-perpetuating cycle that might aggravate GI and CNS inflammation associated with ASD.

A number of pre-clinical and clinical researches in different disease models and in chronic neurodegenerative disorders recommended that targeting TNF action in the brain may be an appealing disease-modifying process to slow progression or attenuate severity of the disease (Sharp et al., 1989). In light of the present data, a remarkably significant elevation (P<0.0001) of the plasma level of TNF-α in both atypical autism group and childhood autism group versus the control group has been recorded. Also, its level was significantly highly elevated (P<0.0001) in atypical autism group relative to the childhood autism group. These results fit with those of Croonenberghs et al., (2002) and El-Ansary et al., (2011) who observed significant increased production of TNF-α in the ASD group versus the control group and disagree with Jyonouchi et al., (2012) who found a decrease in the production of TNF-α in the autistic patients versus the control counterparts. Furthermore, our study showed that the remarkably elevated level of plasma TNF-α in both autistic groups decreases with the increase in the severity of the disease measured by CARS. As TNF-α plasma level showed a greatly significant (P<0.0001) negative correlation with CARS scores in the childhood autism group. The present findings also revealed a highly significant decrease (P<0.0001) in TNF-α plasma level in both mild-moderate and severe autistic groups in respect to the atypical autistic group. Additionally a significant decrease (P<0.005) in TNF-α plasma level has been observed in comparing severe autistic group with the mild-moderate autistic group. The explanation of the remarkably significant elevation of plasma TNF-α in all autistic groups in the present study.
may be due to high burden of the brain with high level of heavy metal (mercury) as shown in all the autistic groups in the current study and this suggests that the neuroinflammation is closely related to the pathophysiology of autism. This is because of TNF-α is one of the central regulators of tissue inflammation and has been implicated in the pathogenesis of several neurological disorders. In the CNS, TNF-α exerts both homeostatic and pathophysiological roles (Santello and Volterra, 2012). TNF-α has regulatory functions on vital physiological processes such as synaptic plasticity, learning and memory, sleep, food and water intake, and astrocyte-induced synaptic strengthening (Olmos and Lladó, 2014). In pathological conditions, astrocytes and mostly microglia discharge great amounts of TNF-α; this de novo synthesis of this cytokine is a critical constituent of the so-called neuro-inflammatory reaction that is connected with several neurological aberrations such as ASD (Montgomery and Bowers, 2012).

Several studies supported this explanation as Pickering et al., (2005) found an elevation of TNF-α in the brain of ASD patients suggesting that children diagnosed with ASD have a heightened immune response that may be connected with localized brain inflammation and tissue necrosis. Also Popa et al., (2007) mentioned that the increased concentration of TNF-α is found in acute and chronic inflammatory conditions. Despite all of these beneficial acute effects, the longer the persistence of inflammatory markers, such as TNF-α, the more will induce pathological changes (Popa et al., 2007). In view of the current data, the atypical autism group experienced the highest level of mercury excreted in their hair and the greater level of glutathione as well as the remarkably high level of TNF-α and they are the milder form of autism phenotype. This proved that the process of elimination of the heavy metals (mercury) from the brain (the highly complex and self-regulating system) stimulates the immune system trying to preserve body homeostasis by inducing a state of acute inflammation with the marked rise in the level of proinflammatory cytokine TNF-α which has a protective function in this stage. This explanation was proved by Feingold et al., (1998) who stated that during acute conditions, the organism reacts quickly through several mechanisms that are meant to set the different homeostatic systems. Also Nathan (2002) found that inflammation is a dynamic, highly-controlled process that is not inherently damaging, but rather essential for immune surveillance, optimal post-injury tissue repair, and regeneration, and is required for the elimination or lessening of challenges to the organism and successive restoration of homeostasis. Moreover, Luheshi (1998) found that TNF-α is closely related with elements of the acute phase immune reactions, including fever, and is responsible for lipopolysaccharides bacteria (LPS a potent bacteria-derived pyrogen-generated fever). This explains why the reported cases of autism improvement and cases of remission with fever (Herbert and Anderson, 2008). Herein the reason for the decreased plasma level of TNF-α in which its low level heightened the severity of the disease in both mild-moderate and severe autistic groups versus the atypical autistic group is most probably related to the higher concentration of the heavy metal (mercury) in their brain due to impairments of its detoxification mechanism. This is because of the low blood level of glutathione that causing chronic activation of the immune system producing a condition of chronic inflammation with a release of TNF-α in lower level than in acute inflammatory state which has a long term destructive effect on the brain tissues. The present results revealed a positive significant correlation (P=0.04) and (P=0.01) between plasma level of TNF-α with both the levels of mercury excreted in hair and glutathione plasma level. Also, there were also a highly significant (P=0.002) positive correlation between Vinland IQ scores and TNF-α plasma level in the childhood autism group. The present findings are greatly supported by several previous studies, as the elevated level of TNF-α in cerebrospinal fluid from some ASD patients with a history of regression supported the observation of the ASD brain as being in chronic, subclinical inflammatory condition (Chez et al., 2007). TNF signaling have been shown to have many pivotal roles inside the CNS including injury-mediated microglial and astrocyte stimulation, and arrangement of BBB permeability, febrile responses, glutamatergic transmission and synaptic plasticity and scaling (McCoy and Tansey, 2008). Herbet (2010) cited that chronic, constant change in neural function (cumulative toxic body burden and/or chronic neuroinflammation ) can in turn lead to alteration in brain tissues (e.g., mitochondrial damage that leads to cellular dysfunction and cell death), which subsequently may feedback to additional involve function. McGeer and McGeer (2002) stated that immune cytokines can function as neurotrophic substances, protecting and encouraging neurite.
growth. But when chronically stimulated, these cytokines can be very destructive. Also Kim et al. (2002) found that mercury can provoke TNF-α, deplete glutathione, and elevate glutamate, dopamine, and calcium-related toxicity, inducing inflammatory effects and cellular apoptosis in neuronal and immune cells.

Gamma-amino butyric acid (GABA) and glutamate are, respectively, the chief inhibitory and the major excitatory neurotransmitters in the mammalian CNS (Krnjevic, 2004). The brain, in general under the control of chemical neurotransmission, is mainly a glutamatergic excitatory system, regulated by a relatively smaller GABAergic inhibitory component and modulated by a much fewer number of neurons secreting a variety of other neurotransmitters (including monoamines) (Pralong et al., 2002). So they are thereby involved directly or indirectly in most aspects of normal brain function (Li et al., 2013).

The present work revealed a marked significant increase (P<0.0001) in the serum level of glutamate in atypical autism group versus the control group and a significant increase (P=0.01) in its serum level in the childhood autism group versus the control group. While, it recorded a highly significant decrease (P<0.0001) in comparing the childhood autism group with the atypical autism group. This finding is consistent with that of Aldred et al., (2003) who did their study on 23 of children with autism and 55 of their relatives, and found that all of them have a significantly higher plasma concentration of glutamate than age matched controls. As well Ghanizadeh (2013) reported that nearly, all the studies including his study recorded a higher level of glutamate in autism than those of the controls. The marked significant decrease in the serum level of glutamate with the increase in the severity of the disease has been recorded in this study, as its level was highly significantly low (P<0.0001) in both mild-moderate and severe autistic groups versus the atypical autistic group and significantly low (P=0.005) in comparing severe with the mild-moderate autistic group. This fits with the study done by Shinohe et al., (2006) who discovered that the elevated serum level of glutamate in autism is weakly associated with severity of autism. The explanation for the significant rise of serum level of glutamate in the autistic groups in the present work is positively associated to the same elevation in the level of TNF-α in these groups. Also, the current results showed a significantly positive (P=0.009) correlation between glutamate serum level and TNF-α plasma level in atypical autism group. Moreover the current results pointed to the more the decrease in the high level of TNF-α, the higher the severity of the disease. TNF-α can potentiate glutamate-mediated cytotoxicity using two approving mechanisms: indirectly, by inhibiting glutamate transport on astrocytes, and directly, by stimulating the localization of ionotropic glutamate receptors to synapses (Pickering et al., 2005). In addition, TNF-α-dependent increase in AMPA receptors at the cell surface and reduction in GABAα receptor cell surface expression indicates that TNF may control synaptic strength at excitatory synapses by increasing excitatory synaptic transmission and reducing inhibitory transmission. TNF was proved to heighten the mean frequency of miniature excitatory postsynaptic currents (mEPSCs). As TNF has been found to increase mEPSC amplitude and reduce mIPSC (miniature inhibitory postsynaptic currents) amplitude (McCoy and Tansey, 2008).

The present study, showed a significantly elevated (P=0.005) plasma levels of GABA in comparing atypical autism group with the control group. Also a highly significant elevation (P<0.0001) in GABA levels has been observed in comparing childhood autism group with both the control and atypical autism groups. Additionally, it showed an increase of its level with the increase in the severity of the disease. As, it has been observed a marked significant increase (P<0.0001) in the plasma level of GABA in comparing both mild-moderate and severe autism groups with atypical autism group and in comparing the severe with mild-moderate autism groups. Also it has been found a marked significant (P=0.002) positive correlation between GABA plasma levels and CARS scores in the childhood autism group. These results echo the studies done by Dhossche et al., (2002) and Meguid el al. (2014) who recorded a significant increase in GABA plasma level in patients with autism. Also, Enticott et al., (2013) demonstrated the significantly elevated level of plasma GABA in patients with autism in comparison to the controls. These investigators stated that GABA, as the major inhibitory transmitter in the CNS, has been involved in the pathophysiology of ASDs. As well Mendez et al., (2013) reported the correlation between elevated plasma GABA and severity of autism by using CARS and the severe autistics show higher plasma GABA levels compared to mild-moderate patients. Furthermore, Russo (2013) found that autistic individuals have
significantly high plasma levels of GABA which correlates with increasing hyperactivity, impulsivity severity, tip toeing severity, light sensitivity and tactile sensitivity. Elevation of plasma GABA level in the autistic children in the current study can be easily correlated to the significant increase of glutamate (Han et al., 2014). This finding is supported by Guptill et al., (2007) who mentioned that the high plasma GABA levels are positively related to glutamate and glutamate/GABA levels which leading us to suggest that the unexpected increase in GABA concentration in autistic individuals is caused partly by glutamate bioavailability. Other mechanisms implicating the increased of GABA plasma level with the increase in the severity of the autism despite decrease of glutamate with the same severity may be linked to that the chronic contact to environmentally significant levels of mercury disturbs GABAergic signaling (Basu et al., 2010). This may be a defense mechanism of the brain to reduce the chronic burden of the destructive effect of the neuro inflammation in the CNS, as GABA can decrease inflammation, or, in contrary, lacking GABA function may correlate to uncontrolled inflammation. Such function could probably occur at a specific GABA receptors (GABAB) which are metabotropic and create extended inhibitory signals, which form a more probable candidate for impacting a chronic inflammatory reaction (Kelley et al. 2008). Also, it has been reported that GABA and GABAx receptor agonists decrease the cytotoxic immune reactions (Bhat et al., 2010), as GABA receptor transcripts are located in immune cells. Finally it has been found that the GABA treatment reduces inflammatory cytokine synthesis in peripheral macrophages (Reyes-Garcia et al., 2007).

In the brain, information processing depends on the integration of excitatory and inhibitory circuits which utilize glutamate and GABA as neurotransmitters, respectively. The so-called excitatory/inhibitory (E/I) balance expresses a vital state for the proper execution of neuronal networks and it is necessary for almost all brain functions, including expression of sensory information and cognitive processes. The E/I balance is preserved through extremely regulated homeostatic mechanisms (Turrigiano and Nelson, 2004). Stability between excitation / inhibition is critical for synaptogenesis and plasticity, specifically in the first 3 years of life (Oberman, 2012). A disturbance of the homeostatic control, due to the absence of compensatory changes, induces an imbalanced E/I ratio and the development of neuropsychiatric disorders including ASDs (Rubenstein and Merzenich, 2003). Even though greatly more work is required to recognize the cellular and molecular mechanisms modulating the E/I balance at synapses. It is apparent from the reviewed information that GABAergic signaling performs an important function in the formation of neuronal networks and any disturbances of GABAergic circuits can induce several neurodevelopmental disorders including mental retardation, epilepsy and ASDs (Pizzarelli and Cherubini, 2011). In the current study, the level of glutamate/GABA ratio showed insignificant change (P=0.7) in comparing atypical autism group to the control group, while the glutamate/GABA ratio revealed highly significant (P<0.0001) decrease in comparing childhood autism group with both the control and atypical autism groups. Furthermore, it has been observed that the reduction of the glutamate/GABA ratio is linked with the increase in the severity of the disease as a highly significant (P<0.0001) negative correlation between glutamate/GABA ratio and CARS scores in childhood autism group has been recorded. As well the present results showed that a highly significant decrease (P<0.0001) and (P=0.002) in glutamate/GABA ratio in comparing severe autism group with both atypical autism and mild-moderate autism groups, and only significant decrease (P=0.024) in comparing mild-moderate with atypical autism group. These results are in compliance with the two recent studies: El-Ansary and Al-Ayadhi (2014) who reported that the great decrease in the glutamate/GABA ratio in the autistic children compared to the healthy controls can be easily correlated to autistic characteristics. Also, Olmos and Lladó (2014) demonstrated that the increase of brain TNF-α is linked with the impaired glutamate/GABA ratio in ASD. These results are in agreement with the current results which found a highly positive correlation (P<0.0001) between glutamate and glutamate/GABA ratio and a highly negative correlation (P<0.0001) between GABA and glutamate/GABA ratio in both atypical and childhood autism groups. A disturbance of the GABAergic signaling early in development induces a severe E/I imbalance in neuronal circuits, a state that may be correlated to some of the behavioral deficits observed in ASD individuals (Pizzarelli and Cherubini, 2011). Such an imbalance would reduce the ratio signal to noise of the sensory and procedural information in mild cases (Casanova et al., 2006). The...
Excitatory/inhibitory balance is vital for the functioning of the synapse and multiple mechanisms may compromise this balance such as neuro inflammation which is highly contributed to the imbalance and consequently to the causes of autism (Ascoli et al., 2008). Alterations in the E/I balance could also occur when the development or maintenance of one class of synapses dominates over the others. The particular loss of excitatory or inhibitory synapses can occur during the initial period of synapse development and consolidation or late in development during activity-dependent refinement of neuronal circuits and may include mutations in genes encoding for ion channels or GABAA receptor subunits. These would lead to circuits with aberrant activity and vulnerable to seizures (Noebels, 2003).

CONCLUSION

In conclusion, the present study highlights that the excessive burden of the developing brain with heavy metals and or xenobiotics are most probably playing the critical roles in the etiology of ASD. The difference in the clinical pictures between the childhood autism as the severe form of autism and the atypical autism as the milder form of autism may be attributed to the ability of the developing brains to have a defensive mechanism in the form of antioxidants i.e. glutathione. These mechanisms will lead to neuro inflammation which shows a dual role in ASD; as a protective role during acute inflammation and sets an acute homeostatic state of the brain and might lead to the milder form of autism and as a destructive role in the form of chronic inflammation that causes more or less the severe form of autism. All of these processes would lead to changes in the most important two neurotransmitters, glutamate and GABA as well as glutamate/GABA ratio which are considered as the main homeostasis mechanisms of the brain. Thus, understanding the complex cross-talk between neuro inflammation and brain homeostasis would help in development of targeted therapy for autism with its different phenotypes.

CONFLICT OF INTEREST

The authors declared that present study was performed in absence of any conflict of interest.

AUTHOR CONTRIBUTIONS

Prof H.H.A participated actively in invention of the scientific idea, conducting all the biochemical analyses, revising the paper critically, editing and approving the final version and submission of the manuscript to the Journal. Dr. M.A.G participated actively in invention the scientific idea and in diagnosing the autism cases, conducting the psychological testing and the statistical analysis. Prof N.A.M, Prof O.M.A, Prof S.S.A and prof E.F.E, all of them contributed greatly in guidance, supervision and revising the paper and the results critically.

REFERENCES


