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## Enzymatic Studies in Autism Spectrum Disorder from a Psychiatric Research Unit in Mosul, Iraq

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دراسات إنزيمية لاضطراب طيف التوحد لوحدة البحوث النفسية في الموصل - العراق

صفاء عبدالعزيز الأمين، إلهام خطاب الجماس، فدوى خالد توفيق، طارق يونس أحمد

### Abstract

**B** **Background:** Autism spectrum disorder (ASD) is 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 ASD remains poorly understood. Oxidative stress and environmental toxicants exposure might contribute in ASD pathophysiology. **Objectives:** (1) Studying the oxidative stress effect of ASD patients; (2) assessment of the dipeptidyl peptidase-4 (DPP-4) activity of ASD patients and its critical role in gastrointestinal disorder and inflammation; and, (3) studying the relationship between DPP-4 activity and related oxidative stress enzymes. **Method:** Thirty seven children diagnosed with ASD (31 boys and 6 girls; age range 2-12 years) were selected randomly from the Psychiatric Research Unit-Mosul University. Diagnosis by specialist psychiatrists followed the DSM-IV criteria with the group classified into mild, moderate and severe categories of symptom severity. A control group consisted of 30 healthy children (20 boys and 10 girls). Glutathione-S-Transferase(GST), Acetyl cholinesterase(AchE), Myeloperoxidase (MPO), Xanthine Oxidase (XO) and aryl esterase activities were assayed. Statistical tests were used to calculate the differences in enzymatic activities; to study the DPP-4 effect on various inflammations; and, to clarify the correlation between DPP-4 with the studied enzymes. **Results:** There was a significant ( $p \leq 0.05$ ) decrease in DPP-4 activity of mild, moderate and severe ASD group (-15.2%, -37.2%, -48%) respectively compared to the control group. Activities of MPO, Ach E, Aryl esterase and GST were significantly ( $p \leq 0.05$ ) decreased in severe ASD group. XO activity was significantly ( $p > 0.05$ ) increased in severe ASD group. The present study indicated a significant ( $p > 0.05$ ) relationship between DPP-4 activity and gastrointestinal disorder. The incidence of GI disorder was (69.7%). A significant ( $p > 0.05$ ) relationship between DPP-4 and inflammation with Incidence (87.9%) was observed. There was a positive significant correlation ( $p > 0.05$ ) between DPP-4 activity and activities of MPO, Ach E, Aryl esterase and GST while a negative significant correlation with XO activity was shown. **Conclusion:** Oxidative stress is a potential risk factor in ASD with effects on several enzymatic activities. DPP-4 might be a good marker in some individuals with ASD especially in those having gastrointestinal disorder and various inflammations. The correlation results suggest that the relationships between DPP-4 activity and studied related enzymes.

**Keyword:** Autism spectrum disorder, Dipeptidyl peptidase-4, Gastrointestinal disorder, Inflammation, Enzymes.

**Declaration of interest:** None

### Introduction

Autism spectrum disorder (ASD) is characterized by impairments in social communications and repetitive or restricted patterns of interests or behaviors appearing during the first three years of life.<sup>1</sup> Despite extensive research, the etiology and natural history of ASD remains poorly understood.<sup>2</sup> Recent research indicates that several prenatal and perinatal factors are associated with ASD<sup>3</sup>. Other studies suggest that ASD might result from interaction between genetic, environmental and immunological factors with oxidative stress as a mechanism linking these risk factors.<sup>4</sup> Growing evidence suggests that redox imbalance and oxidative stress might contribute to ASD pathophysiology.<sup>5</sup> Physiological

oxidative stress is caused by an imbalance between the production of reactive oxygen species (ROS) and the ability to detoxify the reactive intermediates or repair the resulting damage.<sup>6</sup> This might suggest that Oxidative stress can increase via environmental toxicants exposure. Toxicants such as heavy metals pesticides and chemicals can damage cells and impair cellular signaling.<sup>7</sup> A prominent feature of children with autism is gastrointestinal (GI) disorders, which seems to occur in approximately one third of patients. GI symptoms include chronic constipation, diarrhea and abdominal pain.<sup>8</sup> Several studies indicate that enzymatic activities of some enzymes might change in ASD patients.<sup>9,10</sup> One of these enzymes is Dipeptidyl peptidase-IV (DPP-4),

which is a protein with multiple functions. It is known under different names depending on where it is found. DPP-4 is easily deactivated by small amounts of toxins, including mercury and organophosphates. Its function is needed to digest some peptides from gluten, casein and other substances that can have an opioid-like effect.<sup>11</sup> Insufficient production of the DPP-4 enzyme on the inner surface of the small intestine results in opioid effects of casomorphin and gluteomorphin structures, especially in people with autism.<sup>12</sup> Glutathione-S-transferase (GST), an enzyme which conjugates glutathione (GSH) to toxic electrophile, is reduced or absent in individuals with autism.<sup>9,13</sup> Aryl esterase activity detoxifies organophosphate pesticides and its activity is reduced in children with ASD when compared with healthy controls<sup>14</sup> although some studies have identified similar activity in both groups.<sup>15</sup> Organophosphates also function to irreversibly inhibit the activity of acetyl cholinesterase (AChE).<sup>16</sup> AChE, a marker of the central cholinergic system, is responsible for hydrolysis of acetylcholine.<sup>17</sup> Acetylcholine regulates aspects of nerve excitation. It plays a critical role in regulating muscle contraction, learning, attention, cognition and memory throughout adulthood.<sup>16</sup> Since children with ASD frequently have accompanying gastrointestinal symptoms,<sup>18</sup> including inflammation of the GI tract and fungal infection, myeloperoxidase (MPO) was assessed. MPO belongs to oxidoreductase enzymes family<sup>19</sup> and is secreted from active neutrophils.<sup>20</sup> Studies have shown that children with autism who have severe GI also have low serum levels of MPO.<sup>21</sup> MPO might serve as a biomarker for oxidative stress and MPO deficiency might also be associated with an increase incidence of inflammation.<sup>22</sup> Finally, xanthine oxidase (XO), is an endogenous pro-oxidant that produces superoxide radicals during conversion of xanthine to uric acid. Increased XO activity has been reported in the erythrocytes of patients with autism.<sup>23</sup> The present study assessed the activity of some enzymes which are related to oxidative stress in patients with ASD and used a control group for comparison in order to find a relationship between DPP-4 and other enzymes related to oxidative stress.

## **Materials and Methods**

### **Subjects**

Thirty seven children with ASD (31 boys and 6 girls; ranging in ages 2-12 years) were randomly selected from the psychiatric research unit in the College of Medicine, University of Mosul. By way of comparison, 30 healthy

children (20 boys and 10 girls) in the same age range were randomly selected from the same catchment area via nursery and primary schools. The diagnosis of ASD was applied by specialist psychiatrists following the criteria for ASD in the Diagnostic and Statistical Manual of Mental Disorder, Fourth Edition (DSM-IV). Patients with ASD were classified into three subgroups: mild, moderate and severe according to symptoms severity grade and PDD scale. Scores > 50 were considered normal; scores from 50-100 were mild; scores from 100-150 were moderate, and scores < 150 considered severe.<sup>24</sup>

### **Ethical issues**

Prior to commencement, the present study was reviewed and agreed by the research scientific committee in the College of Science, Department of Chemistry. Details of the study and related testing were shared with all families whose children were involved and their oral ascent was obtained.

#### **Inclusion criteria:**

1. ASD cases diagnosed using the DSM-IV criteria
2. Physically healthy
3. No history of brain injury

#### **Exclusion criteria:**

1. All cases with speech disorder other than PDD
2. History of intrauterine or postnatal insult
3. Physically disabled

### **Blood sample**

Venous blood samples were collected from all groups in sterile plain tubes. Serum was separated by centrifugation at 3000 rpm after blood coagulation for 15 min. Serum samples were divided into many aliquot tubes and stored at -20 °C for subsequent enzymatic activities.

### **Assay of enzymatic activities**

Dipeptidyl peptidase-IV (DPP-4) activity was estimated via fasting blood samples collected between 8:00 and 8:30 am. Serum was stored at -20°C until thawed to determine the enzymatic activity.

Serum DPP-4 activity was assessed at 405 nm according to the Kreisell method, which involved glycine-proline-p-nitroanilide hydrochloride as a chromogenic substrate from sigma–Aldrich Chemicals – Germany (G0513).<sup>25</sup> Glutathione-S-Transferase (GST) was assayed using conjugation reaction between GSH and 1-chloro-2, 4-dinitrobenzene (CDNB) at 340 nm.<sup>26</sup>

Aryl esterase activity was determined according to hydrolysis of phenyl acetate to form phenol and acetic acid at 270 nm<sup>27</sup>.

Acetyl cholinesterase (AChE): was estimated using Ellman reagent and iodide as a substrate.<sup>28</sup>

Myeloperoxidase (MPO): was measured by o-dianisidine as a substrate.<sup>29</sup>

Xanthine oxidase (XO) activity was determined according to oxidation of xanthine to uric acid.<sup>30</sup>

2. One way ANOVA (Duncan test) was used to compare between more than two parameters.
3. Pearson correlation was performed to identify the relationship between different enzymatic activities.

Minitab program version 14 -proportional test- was used to:

- A. Evaluate the relationship between serum DPP-4 activity and incidence of various inflammation occurring in the ASD group.
- B. Evaluate the relationship between serum DPP-4 activity and incidence of gastrointestinal disorder occurring in the ASD group.
- C. Find the percentage of various inflammation and gastrointestinal disorders which occurred among those in the ASD group.

### Statistical analysis

Data obtained in the current study were analyzed using Statistical Package for Social Sciences (SPSS) version 11.5.

1. Standard statistical methods were used to determine the mean and standard error.

**Table 1. Enzymatic activities as mean ± S.E and change percentage for mild, moderate and severe autism spectrum disorder patients compared to control group**

Enzymes	Control mean ±S.E	Autism spectrum disorder patients					
		Mild mean ±S.E	% change	Moderate mean ±S.E	% change	severe mean ±S.E	% change
Dipeptidyl peptidase-4 µmol/L	C 68.23±3.6	b 57.85±2.35	-15.2	A 42.86±0.7	-37.2	a 35.47±0.94	-48.0
Myeloperoxidase U/L	B 58.90±4.71	b 54.31±2.68	-7.8	A 28.21±0.97	-52.1	a 17.04±0.39	-71.1
Acetylcholine esterase U/L	B 8.13±0.39	b 8.00±0.38	-1.6	A 5.54±0.06	-31.9	a 4.16±0.21	-48.8
Aryl esterase U/L	C 84.10±2.09	c 84.4±1.31	+0.3	B 78.05±0.5	-7.2	a 66.13±2.35	-21.4
Glutathione-S-Transferase U/L	C 148.60±5.71	c 147.79±3.92	-0.5	B 110.0±1.76	-25.9	a 83.67±2.55	-43.7
Xanthine oxidase U/L	B 21.62±1.76	a 12.82±1.37	-40.7	C 28.20±0.97	+30.4	d 52.73±3.19	+143.9

S.E = Standard Error

Different letters horizontally a, b, c, d indicate that the mean is different significantly at p < 0.05 (in one way ANOVA- Duncan test).

**Table 2. Relationship between DPP-4 activity and some pathological disorders which occurred in ASD patients and % of disorder incidence**

Relationship between DPP-4 with pathological disorders	P value	% incidence
DPP-4 & inflammation	0.0001*	87.9
DPP-4 & gastrointestinal disorders	0.035*	69.7

DPP-4, Dipeptidyl peptidase-4

\* Significant at (p ≤ 0.05).

**Table 3. Correlation between DPP-4 and the measured enzymes in ASD patients compared to control group**

r value	DPP-4	MPO	Ach E	Aryl esterase	GST	XO
<b>Patients group</b>	1	0.757**	0.760**	0.655**	0.742**	-0.713**
<b>control group</b>	1	0.131	-0.165	-0.017	-0.029	-0.055

\*\* Correlation is significant at the ( $p \leq 0.01$ )

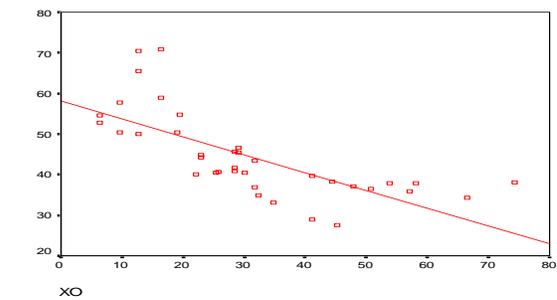
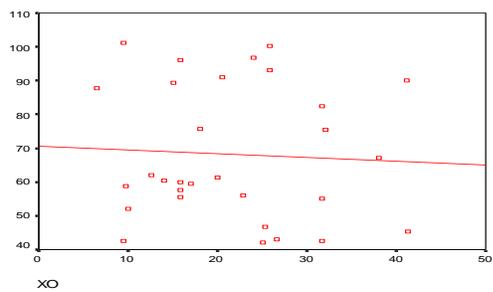
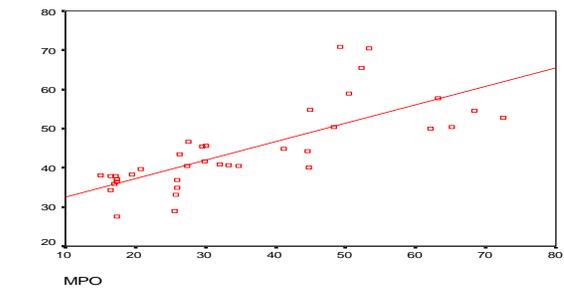
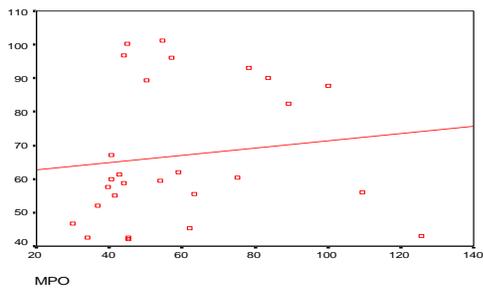
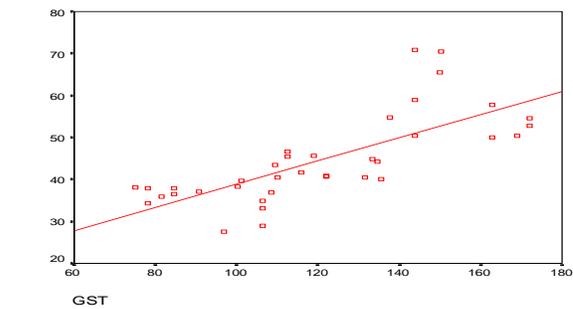
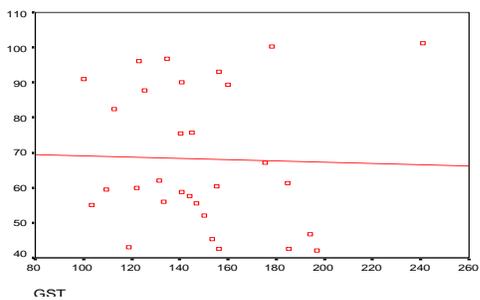
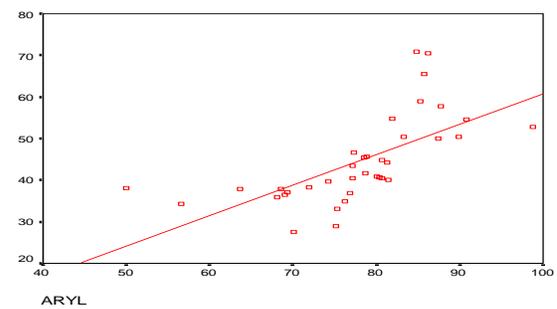
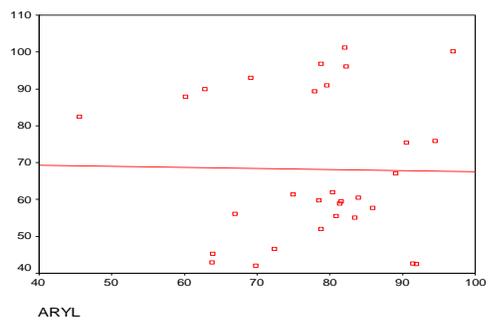
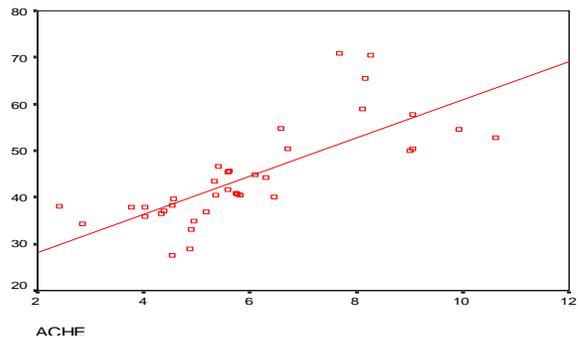
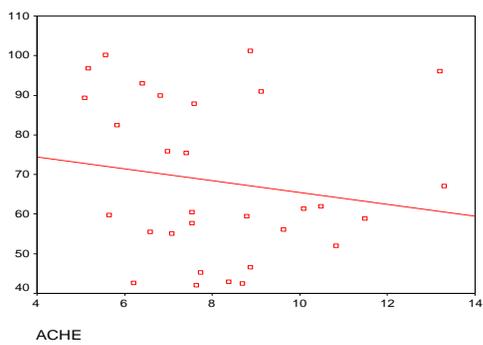
## Results

Statistical analysis of all studied enzymatic activities on mild, moderate and severe autism spectrum disorder according to AL-Hayaly<sup>24</sup> compared to control group were listed in Table 1 as mean  $\pm$  S.E for each enzyme. The results indicated a significant ( $p > 0.05$ ) decrease in DPP-4 activity in mild, moderate and severe compared to control group. Also, there was a significant ( $p > 0.05$ ) decrease in Ach E, MPO, GST and aryl esterase activities in patients with ASD especially within the severe group while a significant ( $p > 0.05$ ) increase of XO activity in the ASD severe group was also observed. Table 2 revealed a statistically significant ( $p > 0.05$ ) relationship between DPP-4 activity and the incidence of various inflammation such as urinary tract infection, nasosinusitis, amygdalitis, bronchitis and between DPP-

4 activities with the incidence of gastrointestinal disorders which occurred in patients with ASD. Furthermore, the results showed the percentage of inflammation (87.9%) and the gastrointestinal disorders (69.7%) which occurred in patients with autism.

Pearson correlation between DPP-4 and other enzymes was achieved indicating r values (Table 3) and explained a significant ( $p \leq 0.01$ ) relationship between DPP-4 activity and Ach E, MPO, GST, Aryl esterase and xanthine oxidase activities in the ASD group compared to the control group.

Figure 1 (below) demonstrated a positive significant ( $P \leq 0.01$ ) correlation between DPP-4 and Ach E, MPO, GST and aryl esterase activities. It showed a negative significant ( $p \leq 0.01$ ) correlation with xanthine oxidase activity in patients to the (right) compared to control group to the (left).



## Discussion

The reduction percentage of DPP-4 activity level was 48% in the severe ASD group compared to control group as Table 1 demonstrates. The possible causes for the activity reduction are inconclusive. There are at least four different factors which could be proposed to reduce serum DPP-4 activity in patients with ASD, such as genetic; mercury and organophosphates exposure, gut flora and dysbiosis were associated in patients with ASD. Also it was reported that drugs which are used in type-2 diabetes mellitus treatment might act as DPP-4 inhibitors<sup>31</sup>.

Many people with autism suffer from digestive complaints which might make them susceptible to casomorphin, partially digested from casein, absorption reaching the brain by crossing the blood brain barrier and leading to autistic type behavior.<sup>32</sup> There is an evidence of increasing intestinal permeability in people with ASD.<sup>33</sup> Gastrointestinal permeability allows larger molecules that would normally stay in the gut to cross into the blood stream.<sup>34</sup>

The present study revealed a significant ( $p \leq 0.05$ ) relationship between DPP-4 activity and gastrointestinal disorders which occurred in ASD groups. Data revealed the GI disorders incidence among the ASD groups was 69.7% as indicated in Table 2. It appears that DPP-4 activity was affected by metallothionine levels.<sup>35</sup> In persons with normal functioning, metallothionine donates zinc, which activates DPP-4. However, in people with autism, morphine like peptide produced from partially digested gluten and casein could be reacted as ligand for opioid receptor in different areas of the brain such as areas in the temporal lobes involving in speech and auditory integration.<sup>36</sup>

Organophosphates and mercury were shown to inhibit metallothionine, which in turn inhibits DPP-4 activity. Children with autism show a high prevalence of DPP-4 enzyme damage<sup>9</sup> and these findings are in agreement with our present results. Another significant ( $p \geq 0.05$ ) relationship between DPP-4 activity and various inflammatory conditions which occurred in patients with autism was found in the present study as illustrated in Table 2. The data in the Table revealed the incidence of inflammation between patients as 87.9%. These results confirmed that patients with autism suffered from immunological disorder or immune dysfunction. The role and origin of soluble DPP-4 is not completely understood, but it is clear that the enzymatic activity or at least the catalytic domain of DPP-4 was involved in immune regulation by cleaving cytokines and influencing T-cell activation.<sup>37</sup> Previous studies

suggested that serum DPP-4 activity could be an additional marker that confirms the inflammatory processes, especially in the bowel,<sup>38</sup> in those with autism. Other studies suggest that the persisting immune dysbalance has significant impact on the pathogenesis of ASD.<sup>34</sup> It was concluded that serum DPP-4 activity in ASD patients correlated inversely with ASD severity grade. Also previous researcher indicated a significant correlation between ASD symptoms and impaired ability to adequately digest peptides and protein from wheat and dairy sources.<sup>39</sup> Reduced levels of DPP-4 could manifest as autistic symptoms. Indeed DPP-4 is thought to be down regulated in children with autism and is currently being used as a diagnostic marker for ASD.<sup>40</sup> Researchers believe that mutated gene is responsible for DPP-4 expression which was down regulated or silenced. For this reason, the addition of galactose appears to be able to increase normal DPP-4 gene expression, and the enzymatic activity becomes more than present.<sup>40</sup>

Significant ( $p > 0.05$ ) reduction in myeloperoxidase (MPO) activity in moderate and severe ASD groups compared to control and mild ASD group was shown in Table 1 as 52.1% and 71.1%, respectively. These results are in agreement with previous studies which showed a significant MPO activity reduction in children with autism who had GI disorders.<sup>18</sup>

MPO-deficient neutrophils produce superoxide and  $H_2O_2$  but are unable to convert  $H_2O_2$  to the hypochlorous acid (HOCl). As a consequence, the ability of these cells to kill bacteria seems impaired and diminished early.<sup>41</sup> Their capacity to kill certain fungi seems completely absent in MPO-deficient neutrophils.<sup>18</sup> Thus, it could be concluded that children with autism who also have a GI disorder have MPO-deficiency. It is unclear whether this deficiency is acquired or inherited. Since MPO deficiency is associated with oxidative stress, increased inflammation and propensity for fungal infections; all of which are the mechanisms associated with ASD.

Pregnant women are exposed to organophosphates through a wide variety of sources. Environmental toxicants such as organophosphates insecticides were proposed as one causal factor for ASD.<sup>9</sup> Organophosphate will inhibit any enzyme with an active serine site including DPP-4.<sup>42</sup> Furthermore, products of organophosphates inhibit other enzymes such as acetyl cholinesterase, which breaks down the neurotransmitter acetylcholine. Organophosphates are exitotoxic to the central nervous system (CNS). They work primarily through phosphorylation of acetyl cholinesterase, which helps control impulse transmission in the CNS or at the synaptic junction.<sup>43</sup> Loss of enzyme function results in

accumulation of acetylcholine, which causes unregulated impulses; the major characteristic of neurotoxicity is over stimulation of the CNS.<sup>44</sup> In the present study, significant reduction of acetylcholine esterase activity was shown among the ASD group when compared with the control. In addition, a positive significant ( $p \leq 0.01$ ) correlation between DPP-4 and AChE was shown. The causes might be due to opioids tending to inhibit neuronal transmission at both pre and post synaptic neurons.<sup>31</sup>

Organophosphates degraded by reacting with hydroxyl radical to form Oxon compounds.<sup>44</sup> The Oxon compounds are more reactive and stronger acetyl choline esterase inhibitors than their parent compounds. Organophosphates are generally highly lipid soluble and could be absorbed upon exposure by the skin, mucous membrane, gastrointestinal system and respiratory system.<sup>45</sup> Xenobiotic such as organophosphate also inhibit aryl esterase activity and glutathione-S-Transferase (GST), which plays an important role in detoxification system.<sup>46</sup> There is preliminary evidence that at least one of the genes controlling paroxonase1/aryl esterase activity production is aberrant in some subjects with ASD.<sup>47</sup> Our results indicated a significant ( $p > 0.05$ ) reduction in aryl esterase activity among the moderate and severe ASD groups by 7.2% and 21.4%, respectively compared to controls and those with mild ASD.

These findings are consistent with another study.<sup>14</sup> Aryl esterase activity is considered high density lipoprotein HDL-associated esterase and the key of organophosphorous compounds detoxification. Also, a significant reduction ( $p > 0.05$ ) in GST activity was observed among the moderate and severe ASD groups by 25.9%, 43.7%, respectively compared to controls. This could be attributed to lack of substrate availability in the participants with autism, i.e. reduced glutathione that was previously observed in the patients with autism.<sup>5,11</sup> The lowered activity of GST found in the present study was consistent with the previous studies by AL-Yafee et al. and Hermawati et al.<sup>11,13</sup> in which they demonstrated that GST activity was reduced in patients with autism when compared with controls.

The recorded reduction in the essential detoxifying enzymes could explain the observed poor detoxification ability in patients with autism.<sup>46</sup> Children can be more sensitive to environmental toxicants which induced free radical formation and oxidative stress.<sup>49</sup> Increased oxidative stress might lead to increase xanthine oxidase activity in ASD groups.<sup>48</sup> Our results were consistent with previous studies. Data in Table 1 indicated a

significant ( $p > 0.05$ ) increased XO activity level among ASD groups especially the moderate and severe ASD groups. These results might be attributed to superoxide anion production during conversion of xanthine to uric acid. This will induce oxidative stress and consequently inflammation, immune deregulation, membrane lipid abnormalities, mitochondrial dysfunction and be the predisposing factor that might occur in patients with autism. These abnormalities might contribute in behavior, sleep disorder and gastrointestinal disturbances in patient's group.<sup>7,49</sup>

## Conclusion

Oxidative stress might be considered a risk factor in ASD and may impact upon several enzymatic activities. Attention has been focused on potential roles of DPP-4 activity. The correlation results suggest that there is a relationship between DPP-4 activity and studied enzymes. DPP-4 was considered to be a good marker and possesses a significant relationship with GI disorder incidence and inflammation in patients with ASD. Clearly, exposure to environmental toxicants might inhibit DPP-4 and associated enzymatic activities.

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## المخلص

الأوليات: يشخص اضطراب طيف التوحد على انه ضعف في التواصل الاجتماعي والقيام بأعمال روتينية او نمطية متكررة وتظهر هذه الحالات في السنوات الثلاثة الاولى من عمر الطفل. اكدت الدراسات ان الاضطراب في الذكور يكون اكثر شيوعا مما في الاناث بنسبة 4 الى 1 وبنسبة انتشار 10000/5 وبالرغم من الابحاث والدراسات الواسعة في هذا المجال فقد بقي اضطراب طيف التوحد على انه مرض غامض ومفهوم بشكل سيء. الهدف: 1- تقييم تأثير الاجهاد التأكسدي في مرضى اضطراب طيف التوحد 2- تقييم فعالية إنزيم داي بيتايديل بيتايديز 4 للأطفال المصابين باضطراب طيف التوحد ودوره في اضطرابات القناة الهضمية وتكرار مختلف الالتهابات 3- دراسة العلاقة بين الإنزيم ذاته والإنزيمات الأخرى التي تتأثر بالإجهاد التأكسدي. العينة وطرق البحث: أعتمد المعيار-(DSM IV) في تشخيص المرضى المصابين باضطراب طيف التوحد واختيرت عشوائيا عينات سيطرة اصحاء ومن ثم تم قياس فعالية انزيمات الداى بيتايديل بيتايديز-4 والكلوتاثاينون-S-ترانزفريز والاريل استريز والماليلوبروكسيديز فضلا عن الاستايل كولين استريز والزانتين اوكسيديز. استخدمت الطرق الاحصائية لبيان اختلاف فعالية كل انزيم بين المجاميع الاربعة وتأثير انزيم (DPP-4) في حدوث مختلف الالتهابات وكذلك تأثيره في اضطرابات القناة الهضمية. كذلك تم اختبار الارتباط ما بين الانزيم ذاته والانزيمات الأخرى قيد الدراسة. النتائج: أظهرت النتائج بوجود انخفاضاً معنوياً في فعالية إنزيم الداى بيتايديل بيتايديز-4 في المجاميع المصابة بالاضطراب للحالات الخفيفة والمتوسطة والشديدة عند المقارنة بالمجموعة الضابطة. كذلك أظهرت الدراسة بوجود انخفاضاً معنوياً في فعالية الانزيمات (MPO, Ach E, GST, Aryl esterase) خصوصا في المجموعة الثالثة من اضطراب طيف التوحد عند مقارنتها بالمجموعة الضابطة. كذلك لوحظ ارتفاعاً معنوياً في فعالية إنزيم الزانتين اوكسيديز للمجموعة الثالثة. أظهرت الدراسة أيضاً وجود علاقة ارتباط قوية بين فعالية إنزيم الداى بيتايديل بيتايديز-4 وفعالية الإنزيمات الأخرى قيد الدراسة. الاستنتاجات: يمكن اعتبار الإنزيم داي بيتايديل بيتايديز-4 مؤشر جيد للأطفال المصابين باضطراب طيف التوحد وخصوصاً الذين لديهم اضطرابات معوية و التهابات متكررة .

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