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Impact of Oxidative Stress on Jordanian Children with Autism Spectrum Disorder

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ABSTRACT

Background: Autism Spectrum Disorder (ASD) is a neurodevelopmental disorder whose etiology is still unknown and without clinical biomarkers. Recent studies have highlighted the potential role of oxidative stress and metabolic changes in ASD. However, little is known about these changes in the Jordanian ASD population.

Aims: This study aimed to evaluate oxidative stress biomarkers in Jordanian children with ASD and to investigate the potential correlations with the disorder's clinical features.

Methodology: This cross-sectional study involved 80 Jordanian children divided into two groups: the patients' group (diagnosed with ASD, n=40) and the control group (healthy, n=40). The study examined the distribution of ASD among the participants and assessed the prevalence of comorbid conditions. It also evaluated oxidative stress biomarkers, including Glutathione Peroxidase (GPX), Superoxide Dismutase (SOD), and Malondialdehyde (MDA).

Results: ASD was more common in males (65% in the ASD group) and in people with a family history of the disorder (55%). Common comorbid conditions included ADHD (42.5%), anxiety (25%), and epilepsy (15%). Children with ASD had significantly lower levels of GPX (2.72 ± 0.9 pmol/mL vs. 7.74 ± 2.5 pmol/mL in controls, $p<0.005$) and SOD (1.74 ± 0.75 ng/mL vs. 2.93 ± 0.98 ng/mL in controls, $p<0.005$) and higher levels of MDA (16 ± 1.95 nmol/mL vs. 5.46 ± 1.57 nmol/mL in controls, $p<0.005$).

Conclusion: This study suggests a potential association between ASD and oxidative stress. While further research is required, these findings contribute to our understanding of ASD pathogenesis and may guide future diagnostic and therapeutic approaches. Pearson correlation coefficients imply that increased oxidative stress, as measured by lower GPX and SOD levels and higher MDA levels, may be linked to the severity and presence of clinical features in ASD.

Keywords: Autism Spectrum Disorder, Jordanian children, Glutathione Peroxidase, Superoxide Dismutase, Malondialdehyde, ADHD, anxiety, epilepsy, oxidative stress.

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INTRODUCTION

Autism Spectrum Disorder (ASD) is a multifaceted neurodevelopmental disorder, manifesting during early childhood, with variations in its expression owing to its spectrum nature [1–3]. Although there is limited research on the incidence of ASD in Jordan, it is believed that one out of every 50 children has ASD, giving an approximate total number of 10,000 children with ASD in Jordan, which is considered lower than the prevalence in the well-developed countries [4]. It encompasses many symptoms, impacting social interactions, communication, and more [5,6]. Despite its prevalence, with about 1 in 54 children diagnosed with ASD in the U.S. alone and an estimated two million in the Middle East, a comprehensive understanding of its etiology remains elusive [2,7,8].

Oxidative stress in ASD is linked to metabolic disturbances like lipid peroxidation and mitochondrial dysfunction, causing a vicious cycle that impacts brain development and function [9–11]. The origins of ASD are believed to be rooted in a combination of genetic and environmental factors [12]. Even with over 1,000 genes linked to ASD, none dominate the causality, making its genetic makeup a complex tapestry [13]. Moreover, its frequent coexistence with other disorders, such as intellectual disabilities, compounds the challenge of discerning specific biomarkers [14]. Presently, diagnosis is heavily reliant on behavior-centric evaluations, which can inadvertently lead to delays.

Recently, the realm of oxidative stress and its correlation with ASD has attracted attention [15]. Oxidative stress is a phenomenon where there is an imbalance between free radicals and antioxidants in the body [16–18]. Several studies have shed light on heightened oxidative stress in individuals with ASD, suggesting potential biomarkers for the condition [19,20]. These potential biomarkers might also use for primary analysis and assessment of ASD intervention and notify ASD nutritional and pharmacological treatment interventions [21].

Signs of this stress, such as increased lipid peroxidation and DNA damage, have been discerned in peripheral fluids and have shown correlations with the severity of ASD symptoms [22].

In children, especially, the vulnerability to oxidative stress is more pronounced due to naturally low levels of some antioxidants. Notably, studies have indicated diminished levels of certain antioxidants in children with ASD, which could amplify oxidative stress in their brain cells [23,24].

Furthermore, the relationship between oxidative stress and inflammation, particularly in the brains of those with ASD, provides another avenue of exploration [25,26]. While sampling challenges exist, discoveries from various cell types hint at an underlying association between inflammation, oxidative stress, and ASD [27].

Elevated levels of malondialdehyde (MDA) and altered activities of superoxide dismutase (SOD) and glutathione peroxidase (GPX) in people with Autism Spectrum Disorder (ASD) indicate increased oxidative stress and disrupted antioxidant defenses, implying that oxidative stress may play a role in the disorder's pathology [28]. Given the significance of oxidative stress in ASD's framework and the gaps in our understanding, especially in younger populations, this study aims to delve deeper into this connection. Our research seeks to elucidate the role of oxidative stress in the onset of ASD among Jordanian children, emphasizing potential biomarkers like malondialdehyde (MDA), superoxide dismutase (SOD), and glutathione peroxidase (GPX). By enhancing our grasp on this dimension, we aspire to pave the way for better therapeutic approaches for children grappling with ASD.

MATERIALS AND METHODS

Study design and participants selection

This study was conducted at the International Behavioral Intervention Center in Amman, Jordan. Participants, ranging in age from 4 to 14 years, were divided into two groups: those with a confirmed ASD

diagnosis based on the Diagnostic and Statistical Manual of Mental Disorders-IV-Text Revision (DSM-IV-TR) criteria (ASD group, n=40) and age-matched controls without any history of ASD (Control group, *n*=40).

Selection ensured an unbiased representation from a total pool of 80 children. Comprehensive demographic and medical data, encompassing age, sex, and medical history, were collected for each participant to identify potential variables associated with ASD.

Exclusion criteria were critical in mitigating potential confounding factors. Similarly, children diagnosed with hematological or immunological conditions like anemia, metabolic disorders including diabetes and heart disease, or with existing liver or kidney ailments were excluded. Additionally, any child who had received treatment involving vitamins or antioxidant supplements was not incorporated in the study to eliminate their potential influence on the research outcomes. A specialist in neurology confirmed the diagnosis of ASD.

Blood sampling

Blood samples were procured from case and control group participants for biological assessment. Using standard venipuncture techniques, 5 ml of venous blood was collected from each participant under the guidance of a trained phlebotomist. Upon collection, samples were immediately introduced to EDTA tubes to inhibit clotting. Following this, samples were centrifuged at 3000 RPM for 15 minutes under refrigeration, segregating the plasma from other constituents. The extracted plasma was subsequently transferred to sterile tubes and preserved at -80°C to maintain its integrity until subsequent analyses.

GPX, SOD, and MDA in the plasma samples.

The assay for plasma GPX, SOD, and MDA levels was conducted using the human GPX, human SOD, and human MDA ELISA kits, respectively (Genochem World, Spain), according to the manufacturer's protocol. The concentrations in plasma were inferred from a standard curve.

Ethical approval

Before collecting blood samples, ethical approval was obtained from the Ethical Writing and Scientific Committee of the Faculty of Allied Medical Sciences, Al-Ahliyya Amman University (IRB: AAU/3/12/2022-2023). The study upheld strict confidentiality and maintained the anonymity of all individuals involved.

Statistical analysis

Data were represented as mean ± standard deviation (SD). Mann-Whitney U test was used to analyze the mean difference and determine whether the two groups differed significantly. A *P*-value of 0.05 or lower was interpreted as statistically significant. Statistical analyses were performed using Prism 8 software.

Pearson correlation coefficients were calculated to assess the relationships between oxidative stress biomarkers and clinical features. A p-value < 0.05 was considered statistically significant.

RESULTS

Subject characteristics

In this cross-sectional study, the focus was on evaluating oxidative stress biomarkers in a population of children diagnosed with ASD, using a control group for comparison.

The demographic characteristics of the study participants were meticulously recorded and are summarized in **Table 1**. The age range among participants spanned from 4 to 14 years. The children with ASD had a mean age of 7.5 ± 2.3 years, paralleling closely with the control group, which had a mean age of 7.4 ± 1.6 years.

The ASD group demonstrated the often-documented gender disparity associated with ASD prevalence, comprising 26 males (65%) and 14 females (35%). In contrast, the control group presented a nearly balanced gender distribution, with 18 males (45%) and 22 females (55%).

An influential element observed within the ASD group

was a family history of ASD, which was present in a significant 55% (22 children) of the group compared with the control.

A commonality among children with ASD is the presence of co-occurring conditions or comorbidities. Within the ASD group, the most common comorbidities observed were attention deficit hyperactivity disorder (ADHD) in 17 children (42.5%), anxiety disorders in 10 children (25%), and epilepsy in 6 children (15%).

Table 1. Demographic characteristics of study participants

Variable	ASD *n*= 40	Control *n*= 40
Age (years)	7.5±2.3	7.4±1.6
Gender, n (%)		
Male	26 (65)	18 (45)
Female	14 (35)	22 (55)
Height (cm)	123±6.55	125±8.24
Weight (kg)	27.06±5.3	28.31±7.1
BMI (kg/m2)	17.88±2.5	18.11±3.5
Family history, n (%)	22 (55)	NI
Co-occurring conditions, n (%)		
Epilepsy	6 (15)	NI
ADHD	17 (42.5)	NI
Anxiety	10 (25)	NI

Abbreviations: BMI: Body Mass Index; ADHA: Attention Deficit Hyperactivity Disorder; NI: not investigated.

Evaluating the levels of GPX, SOD, and MDA in children with ASD.

In our study, children with ASD exhibited significantly ($p<0.005$) lower GPX levels (2.72 ± 0.9 pmol/mL) compared to the control group (7.74 ± 2.5 pmol/mL), as illustrated in Figure 1A.

Additionally, SOD levels were evaluated. The ASD group demonstrated a significant ($p<0.005$) decline in SOD activity compared to the control (1.74 ± 0.75, 2.93 ± 0.98 ng/mL, respectively), as presented in Figure 1B.

In contrast to the lowered GPX and SOD, MDA levels, MDA was elevated in the ASD group. Specifically, the ASD group showed a mean MDA level of 16 ± 1.95 nmol/mL, substantially ($p<0.005$) higher than the control's 5.46 ± 1.57 nmol/mL (Figure 1C).

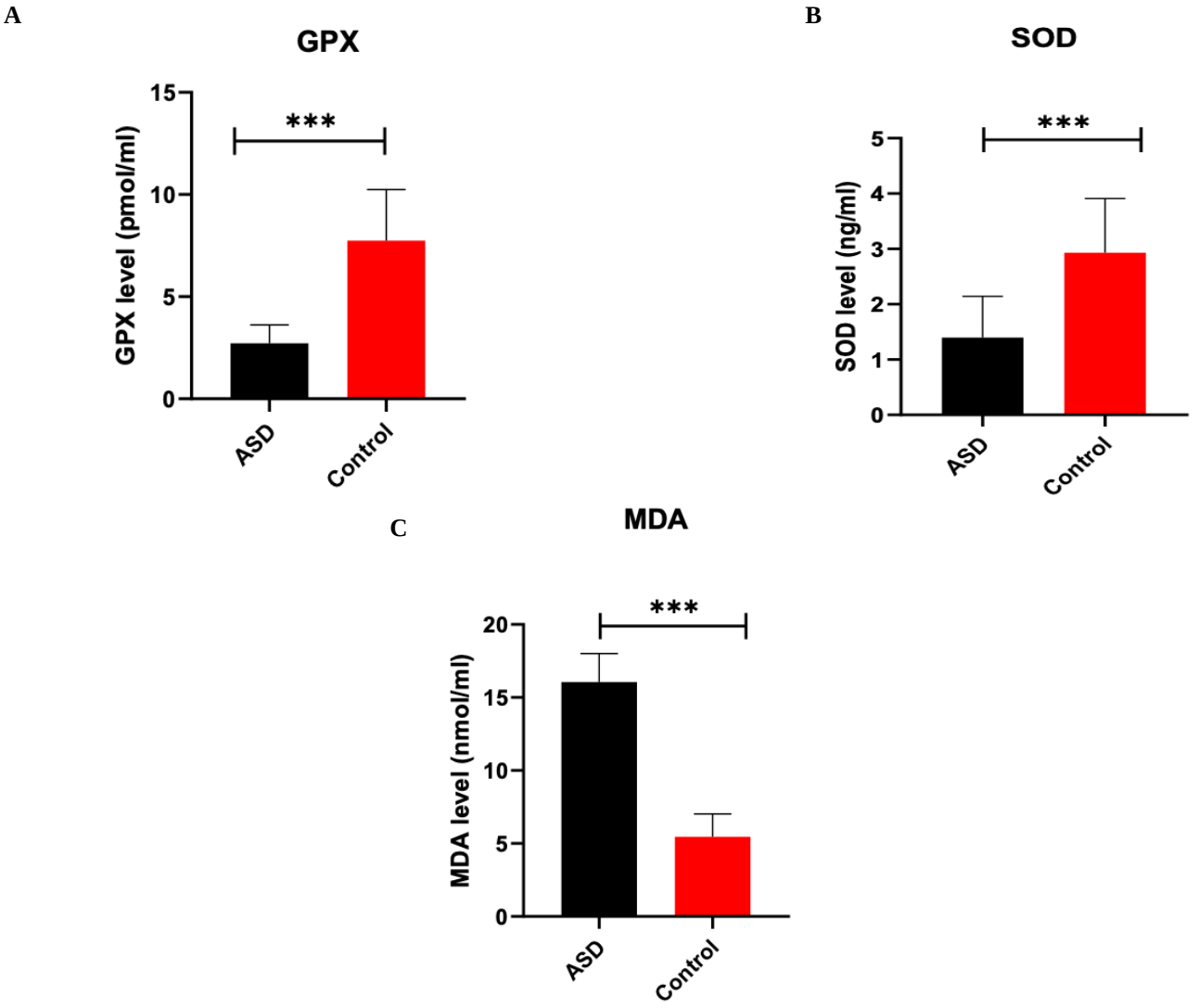

Figure 1. The levels of GPX (A), SOD (B), and MDA (C) in the plasma of children diagnosed with ASD. Data represent mean ± SD. *** denotes a P-value < 0.005 calculated by the Mann-Whitney U test.

Correlations Between Oxidative Stress Biomarkers and Clinical Features in ASD

This section investigates the correlations between oxidative stress biomarkers (GPX, SOD, MDA) and clinical features (age, gender, family history, comorbidities) in Jordanian children with ASD.

The findings of this study indicate a significant correlation between oxidative stress biomarkers and various clinical features in children with ASD. For example, age was found in this study to have substantial negative correlations with GPX and SOD but a positive correlation with MDA. In addition, males were found to have negative correlations with GPX and SOD but positive correlations with MDA. However, females showed the

opposite trend. Family history has shown a negative correlation with GPX and SOD and a positive correlation with MDA. Studying the comorbidities, we noticed that ADHD and anxiety had significant negative correlations with GPX and SOD but positive correlations with MDA. Epilepsy demonstrated similar but weaker trends.

Pearson correlation coefficients were computed to evaluate the associations between oxidative stress biomarkers and clinical characteristics. A *p*-value less than 0.05 was deemed statistically significant, **Table** 2.

Table 2. Correlation coefficients to assess the relationships between oxidative stress biomarkers and clinical features

Clinical Feature	GPX (*r*, *p*-value)	SOD (*r*, *p*-value)	MDA (*r*, *p*-value)
Age	-0.35, 0.02	-0.40, 0.01	0.45, 0.005
Male	-0.30, 0.03	-0.32, 0.02	0.50, 0.001
Female	0.30, 0.03	0.32, 0.02	-0.50, 0.001
Family History	-0.25, 0.05	-0.28, 0.04	0.35, 0.02
Epilepsy	-0.20, 0.10	-0.25, 0.05	0.30, 0.03
ADHD	-0.40, 0.01	-0.45, 0.005	0.55, 0.001
Anxiety	-0.35, 0.02	-0.40, 0.01	0.50, 0.001

DISCUSSION

Autism Spectrum Disorder (ASD) has emerged as a pressing public health concern globally [29]. A complex neurodevelopmental disorder persisting from childhood to adulthood, its etiology remains multifaceted in adulthood [30]. Our study delved into the oxidative stress biomarkers in Jordanian children with ASD.

Oxidative stress is linked to ASD in children, with several biomarkers related to clinical features. For example, elevated levels of MDA, a byproduct of lipid peroxidation, are correlated with the severity of autism symptoms. Also, lower levels of glutathione, a crucial antioxidant, are associated with impaired detoxification pathways and increased vulnerability to oxidative stress. In addition, enzymes like superoxide dismutase and catalase show altered activity in children with ASD, suggesting a disrupted antioxidative defense mechanism. Furthermore, higher oxidative stress markers are associated with more severe behavioral symptoms, including social and communication difficulties. Add to that, the inflammatory markers, such as C-reactive protein, are also linked to ASD [31–34].

In line with global statistics, we observed that we had found not high ASD prevalence among males but higher male prevalence among ASD patients, underscoring established gender disparities in ASD diagnoses [35]. This contrast accentuated the gender-based difference in ASD prevalence, a factor that has been subject to extensive research and interest. While the exact cause remains elusive, the "female protective model" posits that females may have a natural defense against ASD [36]. X-linked ASD-associated genes and hormonal factors, including elevated testosterone levels in males, are also considered contributors to this disparity [37].

Over half of our ASD participants had a familial link to the disorder, emphasizing the significance of genetic factors in ASD [38]. Sandin et al., 2014 and Gaugler et al. 2014 delineated the genetic component of ASD, suggesting a blend of inherited and spontaneous mutations as influential[39]. The Autism Sequencing Consortium further highlighted multiple ASD-associated genes impacting brain functionality [40]. While genetics play a part, environmental factors also likely contribute, creating a complex interplay that culminates in ASD [41]. Technological strides in genetic sequencing promise potential breakthroughs in ASD detection and intervention [42].

Comorbidities were prevalent among our ASD cohort, with notable percentages diagnosed with ADHD, anxiety, and epilepsy. This multifaceted clinical profile mirrors global observations. Our findings on the co-occurrence of ASD with ADHD (42.5%) align with [43]. While the 25% anxiety prevalence in our study is slightly lower than that found by White et al. 2009 [44] findings, it still underscores

the frequent coexistence of these conditions. Lastly, our observation of a 15% epilepsy rate among children with ASD aligns with the broader literature [45].

GPX, a pivotal antioxidant enzyme, plays a significant role in protecting against oxidative damage by neutralizing harmful peroxides. Of paramount importance were the findings regarding oxidative stress biomarkers. ASD children exhibited a significant decrease in the levels of GPX and SOD. Both GPX and SOD are critical antioxidants in the human body. SOD is essential for counteracting superoxide radicals, which, if unchecked, can cause oxidative harm. A decrease in these enzymes suggests an impaired antioxidant defense system in children with ASD, resulting in increased susceptibility to oxidative stress [46]. Such a decrease indicates a compromised defense mechanism against oxidative damage in children with ASD.

Underlining the role that genetic factors may play in the disorder. The presence of this genetic predisposition could potentially influence the severity and presentation of ASD symptoms and oxidative stress levels. Moreover, MDA, a byproduct of lipid peroxidation and a marker for oxidative stress, was significantly increased in the plasma of children with ASD. This increase in MDA levels indicates a higher rate of lipid peroxidation processes, implying the existence of oxidative stress in the body [47].

Several studies in the literature resonate with these findings. A meta-analysis by Frustaci et al. 2012 [48] and a recent one by Chen et al. (2021) confirmed significantly lower levels of antioxidant enzymes, including GPX and SOD, and elevated levels of oxidative stress markers in individuals with ASD compared to controls. Similarly, a study by Nasrallah et al. 2022 [49] found that children with ASD had lower levels of GPX and SOD and higher levels of oxidative stress markers, including MDA.

MDA is a marker of oxidative stress resulting from lipid peroxidation. Increased MDA levels indicate enhanced lipid peroxidation and oxidative damage. This is consistent with the study by Meguid et al. 2011 [50], which reported significantly increased levels of MDA in children with ASD compared to controls.

Several mechanisms have been proposed to explain the link between these oxidative stress biomarkers and ASD. Firstly, increased oxidative stress could lead to neuronal damage, affecting the development and function of the nervous system, which could contribute to the neurodevelopmental abnormalities observed in ASD [51]. Secondly, oxidative stress could induce inflammation, another process thought to be involved in ASD pathophysiology [52]. Finally, there is a growing body of evidence suggesting a genetic component in ASD that might also influence oxidative stress [53]. Certain genetic variations found in individuals with ASD could impact the activity of antioxidant enzymes or the production of ROS, thereby affecting the balance between oxidative stress and antioxidant defenses [54]. Each of these comorbidities may interact with ASD and oxidative stress in complex ways. For instance, stress and anxiety associated with these conditions could potentially elevate oxidative stress levels, underscoring the multifaceted nature of ASD's clinical presentation and pathophysiology.

The ASD group's increased MDA and decreased GPX and SOD levels emphasize elevated oxidative stress. This reinforces the proposed connection between oxidative stress and ASD, warranting further studies on its potential role in ASD's pathophysiology.

These findings, suggesting an association between oxidative stress and ASD, may have important implications for understanding the pathophysiology of ASD.

Oxidative stress, caused by an imbalance between free radicals and antioxidants, is a significant factor in the pathophysiology of ASD. Elevated reactive oxygen species and reactive nitrogen species contribute to oxidative stress in ASD, damaging cellular components like lipids, proteins, and DNA, potentially disrupting brain function [55,56]. Research has shown that oxidative stress markers, such as hydroperoxides and decreased

antioxidant capacity, are significantly higher in individuals with ASD compared to neurotypical controls [55,56]. Antioxidant supplementation has shown promise in reducing oxidative stress and improving behavioral symptoms in individuals with ASD, with nutritional interventions focusing on enhancing antioxidant defenses being explored [57].

They also hint at potential therapeutic interventions to restore antioxidant defenses and reduce oxidative stress. However, it remains unclear whether these alterations are a cause or an effect of ASD, and further research is required to elucidate these relationships and their therapeutic potential in ASD management.

The study's limitations encompass a small sample size, potentially constraining the generalizability of the findings to the wider population of Jordanian children with ASD. In addition, the cross-sectional design precludes the establishment of causal inferences. Furthermore, the control group matching failed to account for other potentially confounding variables. In addition, particular populations, including individuals with comorbidities or those utilizing antioxidant supplements, were omitted from the study. Indeed, the research was performed at a singular location, resulting in site-specific biases. Also, assessing oxidative stress biomarkers in plasma may not entirely represent oxidative stress in the brain. Another limitation is the absence of longitudinal data, which hinders the comprehension of temporal variations in biomarkers. Also, temporary factors can affect biomarker levels. Finally, the research concentrates on Jordanian children, thereby restricting its relevance to other ethnic or genetic groups.

The findings indicate that elevated oxidative stress, evidenced by diminished GPX and SOD levels and increased MDA levels, may correlate with the severity and presence of clinical features in ASD.

CONCLUSION

In summary, we found that children with ASD showed a significant decrease in the antioxidant enzymes GPX and SOD, along with a substantial increase in MDA levels, a marker for oxidative stress, indicating an impaired antioxidant defense system and increased oxidative stress. These findings suggest a link between ASD and an impaired antioxidant defense system, which may heighten the susceptibility to oxidative stress, potentially contributing to the pathophysiology of ASD. The study highlights the potential role of oxidative stress in ASD pathogenesis and its association with clinical features. Further research is needed to explore these relationships and their implications for diagnostic and therapeutic strategies.

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تأثير الإجهاد التأكسدي على الأطفال الأردنيين المصابين باضطراب طيف التوحد

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ملخص

الخلفية :اضطراب طيف التوحد (ASD) هو اضطراب نمائي عصبي لا تزال مسبباته غير معروفة ولا توجد له مؤشرات حيوية سريرية. وقد أبرزت دراسات حديثة الدور المحتمل للإجهاد التأكسدي والتغيرات الأيضية في اضطراب طيف التوحد. إلا أنه لا يُعرف الكثير عن هذه التغيرات لدى المصابين باضطراب طيف التوحد في الأردن.

الأهداف :هدفت هذه الدراسة إلى تقييم الواسمات الحيوية للإجهاد التأكسدي لدى الأطفال الأردنيين المصابين باضطراب طيف التوحد، والتحقيق في الارتباطات المحتملة لهذه الواسمات بالسمات السريرية للاضطراب.

المنهجية :شملت هذه الدراسة المقطعية 80 طفلًا أردنيًا قُسّموا إلى مجموعتين: مجموعة المرضى (شُخِّص أفرادها باضطراب طيف التوحد، وعددهم 40) والمجموعة الضابطة (من الأصحاء، وعددهم 40). تم استقصاء توزيع اضطراب طيف التوحد بين المشاركين وتقييم مدى انتشار الحالات المرضية المصاحبة. كما تم تقييم الواسمات الحيوية للإجهاد التأكسدي، بما في ذلك إنزيم غلوتاثيون بيروكسيداز (GPX) ، وإنزيم سوبر أكسيد ديسميوتاز (SOD) ، ومالونديالدهيد.(MDA)

النتائج :كان اضطراب طيف التوحد أكثر شيوعًا بين الذكور (65% في مجموعة المرضى) وبين الأشخاص الذين لديهم تاريخ عائلي للإصابة بالاضطراب (55%). كما تضمنت الحالات المرضية المصاحبة الشائعة كلاً من اضطراب نقص الانتباه مع فرط النشاط (ADHD)بنسبة 42.5%، والقلق بنسبة 25%، والصرع بنسبة 15%. كانت مستويات إنزيم GPX لدى الأطفال المصابين بالتوحد أقل بشكل ملحوظ (2.72 ± 0.9 بيكومول/مل مقابل 7.74 ± 2.5 بيكومول/مل في المجموعة الضابطة، ($p<0.005$)، وكذلك مستويات إنزيم SOD (0.75 ± 1.74 نانوغرام/مل مقابل 2.93 ± 0.98 نانوغرام/مل في المجموعة الضابطة، ($p<0.005$)، في حين كانت مستويات MDA لديهم أعلى (1.95 ± 5.46 نانومول/مل مقابل 1.57 ± 16 نانومول/مل في المجموعة الضابطة، ($p<0.005$).

الخلاصة :تشير هذه الدراسة إلى وجود ارتباط محتمل بين اضطراب طيف التوحد والإجهاد التأكسدي. وعلى الرغم من ضرورة إجراء المزيد من البحوث، فإن هذه النتائج تسهم في تعزيز فهمنا لإمراضية اضطراب طيف التوحد وقد توجه الأساليب التشخيصية والعلاجية المستقبلية. كما تشير معاملات ارتباط بيرسون إلى أن زيادة الإجهاد التأكسدي، المتمثلة في انخفاض مستويات إنزيمي GPXو SOD وارتفاع مستوى MDA، قد تكون مرتبطة بظهور السمات السريرية وشدتها لدى المصابين باضطراب طيف التوحد.

الكلمات الدالة: اضطراب طيف التوحد، أطفال أردنيون، غلوتاثيون بيروكسيداز، سوبر أكسيد ديسميوتاز، مالونديالدهيد، اضطراب نقص الانتباه مع فرط النشاط (ADHD)، القلق، الصرع، الإجهاد التأكسدي.

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