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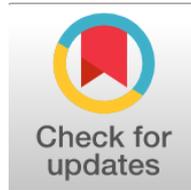How to submit to this journal (link)

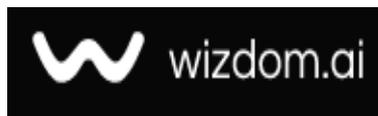# Article information

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Biological Pathways and Biomarker Patterns in Autism Spectrum Disorder: Jalur Biologis dan Pola Biomarker pada Gangguan Spektrum Autisme

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Abstract

General Background Autism spectrum disorder is a complex neurodevelopmental condition characterized by marked heterogeneity in clinical presentation and underlying biology. **Specific Background** Increasing attention has been directed toward biological mechanisms such as immune dysregulation, oxidative stress, mitochondrial dysfunction, and neuroendocrine rhythm disturbances. **Knowledge Gap** Despite extensive research, findings remain fragmented across biological systems, limiting integrative understanding. **Aims** This article aims to synthesize current evidence on key biological pathways and biomarkers associated with autism spectrum disorder. **Results** The review identifies consistent abnormalities in inflammatory markers, oxidative stress indicators, mitochondrial function, melatonin regulation, and serotonergic activity, suggesting interconnected physiological alterations. **Novelty** The study offers an integrative synthesis that brings together immune, metabolic, and neurobiological evidence within a single analytical framework. **Implications** These findings support the relevance of multi-system biological perspectives in autism research and provide a consolidated foundation for future investigations into diagnostic and therapeutic strategies.

Keywords: Autism Spectrum Disorder, Neuroinflammation, Oxidative Stress, Biomarkers, Biological Mechanisms

Key Findings Highlights:

Multiple physiological systems show recurrent abnormalities in autism spectrum disorder.

Immune and oxidative pathways appear closely interconnected across studies.

Neuroendocrine rhythm disturbances emerge as a consistent biological feature.

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Introduction

Autism spectrum disorder (ASD) was a complex neurodevelopmental disorder that was characterized by repetitive behavioral patterns, restricted interests, likewise persistent problems with social communication (1,2). Also , the prevalence of ASD has increased significantly in the past decade thanks to the increased diagnostic awareness and potential genetic and environmental factors (3,4). The increasing of research suggests that ASD was characterized by major physiological, metabolic, and immunological alterations that lead to it was diverse manifestation, in contrast the behavioral aspects of ASD were quite clear (5,6). Moreover , one of the disorders most frequently recorded in ASD was hormonal dysregulation (7). As an example, sleep disorders, impaired circadian rhythms modulation, also, elevated oxidative vulnerability were all associated with melatonin deficit in a number of children with autism, as demonstrated by various studies (8). Nevertheless , another initial biochemical predictor of ASD was high levels of serotonin or hyperserotonemia that influencing neurodevelopment, gastrointestinal, sensory and also, behavioral inflexibility (9,10). So, Dysregulation of the hypothalamic-pituitary-adrenal (HPA) axis has also been evidenced and increased cortisol response has been observed indicating a change in stress responses and constant physiological alertness (11,12). In addition, oxidative stress has been identified to have significant pathogenesis of ASD (13) . It has been discovered that autistic individuals show a great deal of elevated concentrations of total oxidant status (TOS) and lipid peroxidation product, malondialdehyde (MDA) which were indicative of increased production of reactive oxygen species (ROS) and also impaired redox balance in cells (14). Then ,these oxidative attacks were often accompanied by reductions in antioxidant defenses in the form of glutathione peroxidase (GPx) which was necessary to protect the brain tissue against oxidative damage(15,16). Furthermore , there has been a growing acceptance that dysregulation of the immune system in addition to hormonal and oxidative conditions was one of the core features of ASD (17). So, There was also an increase in pro-inflammatory cytokines such as interleukin-12 (IL-12), interleukin-8 (IL-8), and interleukin-1 β (IL-1 β) in serum, cerebral spinal fluid, and postmortem brain tissues of individuals with ASD (18). ASD neurobiology was believed to have a strong role played by the functions of these cytokines in promoting neuroinflammation, microglial activation, as well as abnormal neuronal connections (19). Thus , ASD pathogenesis was connected, immune activation in the chronic form can increase the severity of oxidative stress and disrupt hormonal mechanisms (20). Consequently , the entire available evidence indicates intricate relationships between oxidative imbalance, inflammatory cues, as well as hormonal disregulation as the source of ASD. So , the simultaneous study of these biomarkers may help to know better the causes of ASD and be more able to locate the objective diagnostic markers. Therefore to provide the light on the possible diagnostic and mechanistic implications of the hormonal, oxidative, and immunological indicators, the given study compares the autistic youngsters with the healthy ones.

Materials and Methods

Study Design and Population

A study was carried out between September 2024 up to June 2025 in a groups of privately and neurodevelopmental clinics in the Wasit Province , Iraq. In this study, fifty children with autism spectrum disorder (ASD) and fifty healthy controls were used, whereby they were matched in terms of age and sex. The conventional diagnostic criteria aided in checking all ASD diagnoses with the assistance of the pediatric and behavioral specialists. However , children that had any acute illness, autoimmune diseases, chronic inflammatory diseases as well as genetic syndromes and other neurological malformations were not allowed to participate.

Sample Collection and Analysis

In this study five milliliters of venous blood were collected on each of the children. After centrifugation, clotting, and isolation of serum samples was done; serum was stored at -20 C^0 pending analysis. Cortisol, serotonin and melatonin measured using ELISA kits (Elabscience, China). Also , the biomarkers of oxidative stress TOS were determined with ELISA kit (Elabscience, China; Rel Assay Diagnostics, Turkey) and MDA with a commercial kits MDA ELISA kit (Elabscience, China; MyBioSource, USA). The activity of glutathione peroxidase (GPx) was measured by an enzymatic kinetic kit (Randox, UK). And , immunological markers (IL-1β, IL-12 and IL-12) were determined using Sandwich ELISA kits (Elabscience, China). All the assays were performed according to the instructions of the manufacturer as well as analyzed twice to achieve precision**.**

Statistical Analysis

The data were analyzed on SPSS version 25. Categorical variables were presented in percentages and frequencies, but the quantitative values were presented in mean ± SD. Group differences between children with autism and controls were evaluated using the independent samples t-test . Also , to test the diagnostic performance of IL-1 β, IL-8 and IL-12 in terms of AUC, sensitivity, specificity and ideal cutoff values through ROC curve analysis. Pearson correlation was employed in order to determine correlations between cytokines and oscillators of oxidation/hormone. The significance was considered to be statistically significant with a p-value of less than 0.05.

Ethical approval

The ethical committee of the College of Science of the University of Wasit approved the protocol of the study (Approval No. BIO-2025-012).

Results

Population under examination characteristics

Table (1) shows the demographics of the patients, as well as the control persons. The age of the children with autism was 5.14±0.81 years, while the average age of the control was 4.64 ± 0.77 years. The two groups had no significant difference (P = 0.172). Also there was no significant difference in the frequency distribution of the patients and control subjects by age group. Nevertheless, the autistic children that took part in the current study were younger than or equivalent to the age of 5 (31(62.0%). Frequency distribution of patients and control subjects between male and female did not differ significantly (P = 0.410), 33 (66.0%) males and 17 (34.0%) females constituted the autistic children and 29 (58.0%) and 21 (42.0%) males and females in the control group, respectively.

Characteristic	Autistic children (n=50)	Healthy Control (n=50)	P
Age (years)	5.14 ± 0.81	4.64 ± 0.77	0.172
Gender			