Immune Dysregulation and Cytokine Imbalance in Children with Autism Spectrum Disorder: A Case-Control Study

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Abstract
Background: Autism spectrum disorder (ASD) is a complex neurodevelopmental disorder characterized by deficits in social interaction, communication difficulties, and repetitive behaviors. The etiology of ASD is multifactorial and involves genetic, environmental, immunological, and neurological factors. Increasing evidence suggests that immune dysregulation plays an important role in the development and progression of ASD.
Aim of the Study: This study was conducted to evaluate the serum levels of pro-inflammatory (IL-21 and IL-22) and anti-inflammatory (IL-27 and IL-33) cytokines in children with ASD compared with healthy controls and to investigate their potential role in the pathogenesis of ASD.
Materials and Methods: This case-control study included 300 children, comprising 200 patients diagnosed with ASD and 100 healthy controls. Venous blood samples were collected from all participants at the Professor Dr. Arafat Al-Djalili Center in Al-Najaf, Iraq. Serum was separated from 5 mL of venous blood using gel tubes and stored at −20°C until analysis. Serum levels of the pro-inflammatory cytokines IL-21 and IL-22 and the anti-inflammatory cytokines IL-27 and IL-33 were measured using enzyme-linked immunosorbent assay (ELISA). The study protocol was approved by the Medical Ethics Committee of the Ministry of Health, Iraq.
Results: Children with ASD exhibited significant immune dysregulation characterized by markedly elevated serum levels of the pro-inflammatory cytokines IL-21 and IL-22 and significantly reduced serum levels of the anti-inflammatory cytokines IL-27 and IL-33 compared with healthy controls ($P < 0.0001$ for all comparisons). These findings indicate a pronounced imbalance between pro-inflammatory and anti-inflammatory immune responses in ASD.
Conclusion: The findings of this study demonstrate a significant imbalance in cytokine profiles among children with ASD, characterized by increased levels of IL-21 and IL-22 and decreased levels of IL-27 and IL-33. These alterations support the hypothesis that immune dysregulation contributes to the pathogenesis of ASD. Furthermore, these cytokines may serve as promising biomarkers for ASD, although additional large-scale longitudinal studies are required to confirm their clinical utility.

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Keywords: Autism spectrum disorder, ASD, Cytokines, Immune dysregulation, IL-21, IL-22, IL-27, IL-33

1. Introduction

Autism spectrum disorder (ASD) is a complex neurodevelopmental disorder characterized by persistent deficits in social communication and interaction, together with restricted and repetitive patterns of behavior, interests, or activities. Although genetic susceptibility is considered a major contributor to ASD, increasing evidence indicates that environmental,

immunological, and neurological factors also play important roles in its pathogenesis, Environmental and psychosocial factors may also influence endocrine and metabolic homeostasis through stress-related biological pathways, highlighting the potential importance of interactions between environmental exposures, psychological stress, and metabolic regulation in complex disorders, Advances in early diagnosis, behavioral interventions, and supportive therapies have substantially improved the long-term outcomes of affected individuals, enabling many children with ASD to achieve better educational attainment, social integration, and quality of life than was previously possible [1,2].

The prevalence of ASD has increased markedly over recent decades, rising from approximately 0.04% in the 1970s to nearly 2.8% in recent years. In developed countries, the average age at diagnosis ranges from 38 to 120 months. Current evidence highlights the multifactorial nature of ASD, with genetic, congenital, immunological, neuroanatomical, biochemical, and environmental factors all contributing to disease development. Furthermore, the identification of early clinical manifestations and multiple risk factors has emphasized the importance of early diagnosis and timely intervention. The global prevalence of ASD also varies considerably among countries because of differences in diagnostic practices, public awareness, healthcare accessibility, and study methodologies. Current estimates indicate that approximately one in every 100 children worldwide is affected by ASD [2,3].

Growing evidence suggests that immune dysregulation is closely associated with the development of ASD, growing evidence suggests that immune dysregulation is closely associated with the development of ASD. Cytokines and other immune mediators act through interconnected signaling pathways to regulate immune activation, inflammatory responses, and tissue homeostasis. Dysregulation of these pathways may therefore contribute to persistent inflammatory signaling and altered neuroimmune interactions in susceptible individuals [4]. Large population-based epidemiological studies indicate that maternal immune activation during pregnancy may increase the risk of ASD in offspring, while the increased prevalence of autoimmune diseases among family members of affected individuals further supports the autoimmune hypothesis. Cytokines, including interleukins, chemokines, interferons, tumor necrosis factors (TNFs), and growth factors, are low-molecular-weight signaling proteins that regulate neuronal development, immune function, and inflammatory responses. These molecules play essential roles in coordinating host defense against infection, tissue injury, and immunopathological processes, making them potential mediators of neuroimmune interactions in ASD [5,6].

Accumulating evidence from both human and animal studies has demonstrated that abnormalities in immune function contribute to the pathophysiology of ASD. Previous investigations have identified brain-specific autoantibodies, alterations in lymphocyte populations, imbalances in T-helper cell subsets, and disruption of blood-brain barrier integrity in a subset of children with ASD. In addition, elevated circulating levels of pro-inflammatory cytokines, anti-neuronal antibodies, and immune-related disorders including allergies, recurrent infections, and autoimmune diseases—have been consistently reported in affected individuals. Collectively, these findings support the hypothesis that dysregulated immune responses play an important role in the initiation and progression of ASD and provide a strong rationale for investigating cytokine profiles as potential biomarkers of disease pathogenesis [7,8].

2. Materials and Methods

2.1. Subjects and Study Design

This case-control study was conducted between February 2023 and October 2025 and included 300 children. The study population comprised 200 children diagnosed with autism spectrum disorder (ASD) and 100 age- and sex-matched healthy controls. Participants were recruited from the Professor Dr. Arafat Al-Djalili Center, Al-Najaf, Iraq. Venous blood (5 mL) was collected from each participant using sterile gel tubes. Following centrifugation, serum samples were separated and stored at −20°C until analysis. Serum concentrations of the pro-inflammatory cytokines IL-21 and IL-22 and the anti-inflammatory cytokines IL-27 and IL-33 were quantified using enzyme-linked immunosorbent assay (ELISA) according to the manufacturer's instructions.

2.2. Inclusion Criteria

Children with a confirmed diagnosis of autism spectrum disorder (ASD) established by a qualified pediatric neurologist or psychiatrist according to the diagnosed by specialist physicians' criteria were eligible for inclusion in the study.

2.3. Exclusion Criteria

Children with other neurological, psychiatric, genetic, autoimmune, or chronic inflammatory disorders, including attention-deficit/hyperactivity disorder (ADHD), bipolar disorder, major depressive disorder, Down syndrome, Fragile X syndrome, and eating disorders, were excluded. Children with acute infections or receiving immunomodulatory therapy at the time of blood collection were also excluded.

2.4. Ethical Considerations

The study protocol was reviewed and approved by the Ethics Committee of the College of Science, University of Kufa, and the Ministry of Health, Iraq. Written informed consent was obtained from the parents or legal guardians of all participants before enrollment. The study was conducted in accordance with the ethical principles of the Declaration of Helsinki.

2.5. Statistical Analysis

Statistical analyses were performed using IBM SPSS Statistics version 26. Data distribution was assessed using the Shapiro-Wilk normality test. Since cytokine concentrations were not normally distributed, continuous variables were expressed as the median and interquartile range (IQR). Comparisons between the ASD and control groups were performed using the Mann-Whitney U test. Categorical variables were compared using the Chi-square test or Fisher's exact test, as appropriate. A two-tailed P-value < 0.05 was considered statistically significant [9].

3. Results

3.1. Demographic Characteristics

Table 1: Demographic Characteristics of Children with Autism Spectrum Disorder (ASD) and Healthy Controls

Variable	ASD (n=200)	Control (n=100)	*P* value
Age (years), Mean ± SD	6.8 ± 2.1	6.5 ± 2.3	0.421
Male, n (%)	158 (79.0)	77 (77.0)	0.712
Female, n (%)	42 (21.0)	23 (23.0)	0.712
BMI (kg/m²)	17.4 ± 2.5	17.1 ± 2.2	0.389
Family history of ASD, n (%)	38 (19.0)	3 (3.0)	<0.001
Duration since diagnosis (years)	2.8 ± 1.5	—	—

Note: The ASD and control groups were comparable with respect to age and sex, with no statistically significant differences between the two groups (P > 0.05).

The demographic characteristics of the study participants are summarized in Table 1. No statistically significant differences were observed between the ASD and control groups regarding age or sex distribution (Table 1).

3.2. Serum Levels of Pro-inflammatory Cytokines

Table 2: Comparison of Serum Cytokine Levels Between Children with Autism Spectrum Disorder and Healthy Controls

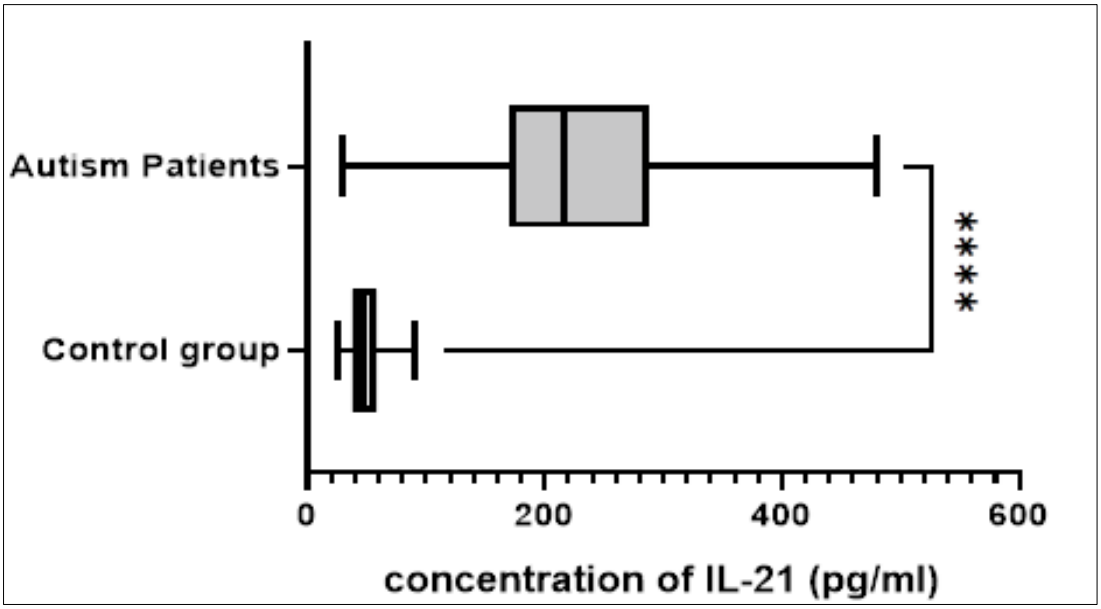
Biomarker	Control Group Median (IQR), pg/mL	ASD Group Median (IQR), pg/mL	P-value
IL-21	47.05 (37.45–58.61)	216.6 (170.2–288.3)	<0.0001
IL-22	57.1 (38.58–79.5)	882.3 (784.4–971.4)	<0.0001
IL-27	282.1 (242.6–344.4)	166.1 (121.9–198.5)	<0.0001
IL-33	532.6 (400.0–788.2)	268.4 (189.6–376.7)	<0.0001

Values are presented as median (IQR). Comparisons between groups were performed using the Mann–Whitney U test. Statistical significance was defined as $P < 0.05$.

As presented in Table 2, children with ASD exhibited significantly higher serum levels of the pro-inflammatory cytokines IL-21 and IL-22 than healthy controls ($P < 0.0001$ for both comparisons). In contrast, serum levels of the anti-inflammatory cytokines IL-27 and IL-33 were significantly lower in the ASD group than in the control group ($P < 0.0001$). These findings indicate a marked imbalance between pro-inflammatory and anti-inflammatory cytokine responses in children with ASD.

1. Evaluation of Il-21 Concentration of Autism Patients Compared with The Healthy Group

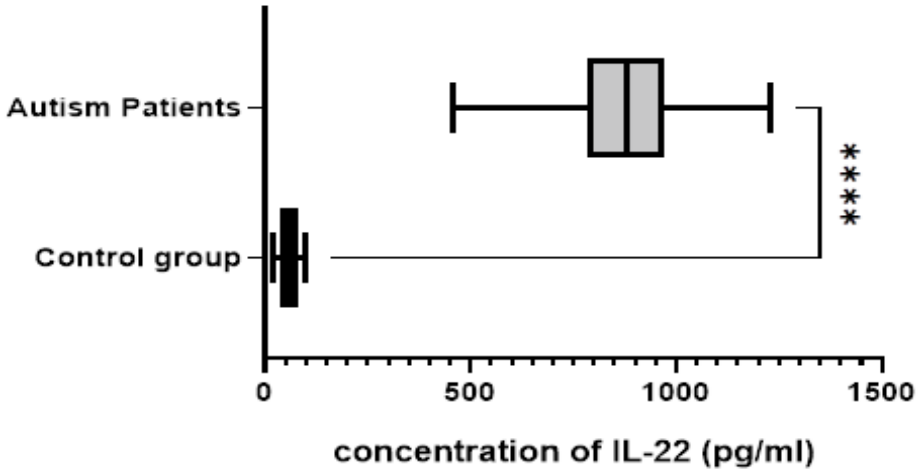
Figure 1 illustrates the significantly higher serum IL-21 concentration observed in children with ASD compared with healthy controls ($P < 0.0001$).



Note: Interquartile Range (25th–75th percentile)

Fig 1: Comparison of serum IL-21 concentrations between children with autism spectrum disorder (ASD) and healthy controls.

2. Evaluation of Il-22 Concentration of Autism Patients Compared with The Healthy Group
Serum IL-22 concentrations were significantly elevated in children with ASD compared with healthy controls (P < 0.0001).

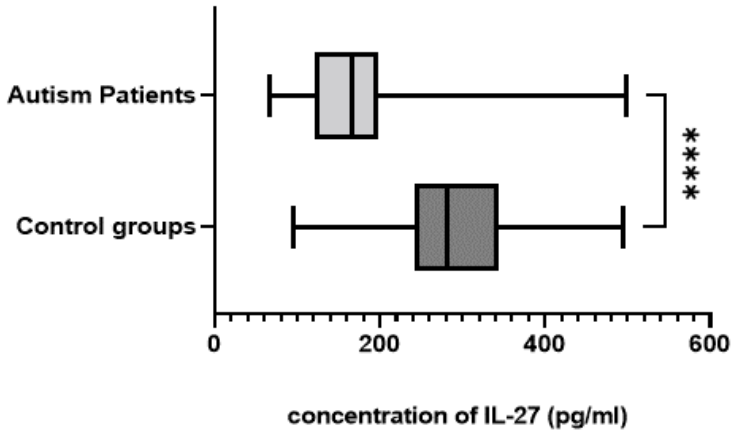


Note: interquartile range = percentile

Fig 2: Comparison of serum IL-22 concentrations between children with autism spectrum disorder (ASD) and healthy controls.

3.3. Serum Levels of Anti-inflammatory Cytokines

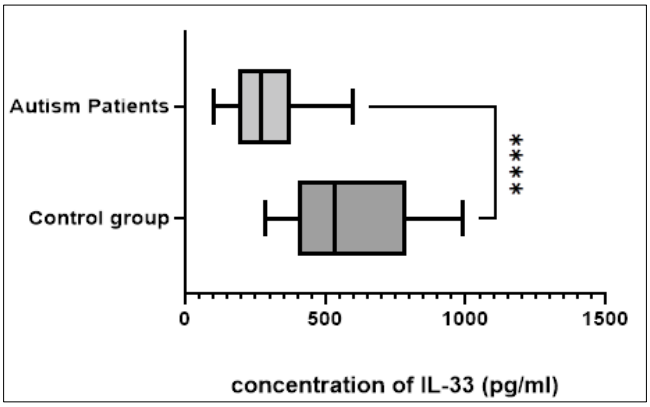
1. **Evaluation of Il-27 Concentration Of Autism Patients Compared With The Healthy Group**: Children with ASD exhibited significantly lower serum IL-27 concentrations than healthy controls (P < 0.0001).



Note: interquartile range = percentile

Fig 3: Comparison of serum IL-27 concentrations between children with autism spectrum disorder (ASD) and healthy controls.

2. **Evaluation of IL-33 concentration of Autism Patients compared with the healthy group:** Serum IL-33 concentrations were significantly reduced in children with ASD compared with healthy controls (P < 0.0001).



Note: interquartile range = percentile

Fig 4: Comparison of serum IL-33 concentrations between children with autism spectrum disorder (ASD) and healthy controls.

Overall, children with ASD demonstrated significantly elevated serum levels of the pro-inflammatory cytokines IL-21 and IL-22 together with significantly reduced levels of the anti-inflammatory cytokines IL-27 and IL-33 compared with healthy controls. These statistically significant differences highlight distinct alterations in cytokine profiles between the two study groups.

4. Discussion

The present study demonstrated a significant imbalance in serum cytokine profiles among children with autism spectrum disorder (ASD), characterized by markedly increased levels of the pro-inflammatory cytokines IL-21 and IL-22 and significantly decreased levels of the anti-inflammatory cytokines IL-27 and IL-33 compared with healthy controls. These findings support the growing body of evidence indicating that immune dysregulation plays a central role in the pathophysiology of ASD. The observed alterations in cytokine expression suggest a shift toward a pro-inflammatory immune response, which may contribute to the persistent neuroinflammatory processes implicated in ASD development [10].

In the present study, serum IL-21 levels were significantly higher in children with ASD than in healthy controls. This finding is consistent with previous studies that reported elevated IL-21 concentrations in individuals with ASD, supporting the hypothesis that immune activation contributes to disease pathogenesis [11,12]. IL-21 is a pleiotropic cytokine produced primarily by activated $CD4^+$ T cells and plays a critical role in regulating both innate and adaptive immune responses. Increased IL-21 production may promote persistent inflammatory activity by enhancing T-cell activation, B-cell differentiation, and cytokine production, thereby contributing to the chronic neuroinflammatory environment associated with ASD [13].

The present study demonstrated significantly elevated serum IL-22 levels in children with ASD compared with healthy controls. This observation is consistent with previous reports that identified increased IL-22 concentrations in individuals with ASD, further supporting the presence of an activated inflammatory response in affected children [14,15]. IL-22 is a cytokine mainly produced by T helper 17 (Th17) cells and innate lymphoid cells, where it contributes to tissue inflammation, epithelial barrier function, and immune regulation. Persistent elevation of IL-22 may reflect continuous immune activation and inflammatory signaling, which have been implicated in the neuroimmune abnormalities associated with ASD [16].

In the present study, serum IL-27 levels were significantly lower in children with ASD than in healthy controls. This finding is consistent with previous studies reporting reduced IL-27 concentrations in individuals with ASD, suggesting impairment of anti-inflammatory immune regulation [17,18]. IL-27 is an important immunoregulatory cytokine that suppresses excessive inflammatory responses and modulates T-cell differentiation. Therefore, reduced IL-27 levels may weaken anti-inflammatory mechanisms, allowing persistent activation of pro-inflammatory pathways and contributing to the chronic neuroinflammatory state associated with ASD [19].

The present study also demonstrated significantly lower serum IL-33 levels in children with ASD than in healthy controls. This observation is in agreement with previous reports describing reduced IL-33 concentrations in individuals with ASD, supporting the hypothesis of impaired anti-inflammatory immune responses [20,21]. IL-33 functions as an important immune regulatory cytokine involved in maintaining tissue homeostasis and limiting excessive inflammatory responses. Therefore, reduced IL-33 expression may contribute to inadequate control of inflammation and further exacerbate neuroimmune dysfunction in ASD [22].

Collectively, the simultaneous elevation of the pro-inflammatory cytokines IL-21 and IL-22 together with the reduction of the anti-inflammatory cytokines IL-27 and IL-33 demonstrates a clear imbalance in immune regulation among children with ASD. This cytokine profile suggests a shift toward a sustained pro-inflammatory immune environment, which may contribute to neuroinflammation, altered neuronal signaling, and disease progression. These findings support the growing evidence that immune dysregulation is closely involved in the pathophysiology of ASD and highlight the potential value of cytokine profiling as a complementary biomarker for disease assessment [23,24].

Conflict of Interest: None.
Funding: None.

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