Journal of Pioneering Medical Sciences
Received: October 22, 2025 | Accepted: March 15, 2026 | Published: April 05, 2026
Volume 15, Issue 03, Pages 157-161

DOI https://doi.org/10.47310/jpms2026150324

Growth Hormone, Cytokine and Vitamin D3 Profiles in Children with Autism Spectrum Disorder: Evidence of Immune-Endocrine Dysregulation

Shilan Anwar Mawlood[1*] and Hiwa Ramadhan Fatah[2]
[1,2]Department of Biology, Faculty of Science and Health, Koya University, Koya, KOY45, Iraq

Author Designation: [1]PhD Student, [2]Assistant Professor

*Corresponding author: Shilan Anwar Mawlood (e-mail: shilananwarmawlood23@gmail.com).

©2026 the Author(s). This is an open access article distributed under the terms of the Creative Commons Attribution License (http://creativecommons.org/licenses/by/4.0

Abstract **Background:** Autism Spectrum Disorder (ASD) is a neurodevelopmental disorder with growing evidence implicating immune, endocrine and metabolic dysregulation in its pathogenesis. Despite advances in genetic research, the roles of inflammatory cytokines, Growth Hormone (GH) and vitamin D3 (Vit D3) in ASD to elucidate the pathophysiology of this disease have not studied properly. **Objective:** To investigate the serum inflammatory biomarkers (IL-6, IL-10, IL-17 and CRP), endocrine regulators (GH) and Vit D3 in children with ASD. **Methods:** A total of 100 children aged 6-12 years were enrolled, including 70 with ASD and 30 neurotypical controls. ASD diagnosis was confirmed by a Pediatric neurospecialist using the Diagnostic and Statistical Manual of Mental Disorders, Fifth Edition (DSM-5) criteria. Then, venous blood samples were collected and analyzed using enzyme-linked immunosorbent assay and automated immunoassay analysers to determine the level of serum biomarkers. **Results:** Children with ASD exhibited no significant changes in serum levels of inflammatory/regulatory cytokines (IL-6, IL-10 and IL-17), while CRP levels were increased compared to controls ($p>0.05$). GH levels and Vit D3 concentrations were significantly lower in the ASD group than in the control ($p<0.0001$). **Conclusion:** The immunological involvement, endocrine imbalance and Vit D3 deficiency might be related to the development of the ASD in children.

Key Words Autism Spectrum Disorder, Cytokine Profile, Growth Hormone, Vitamin D3, Immune Dysregulation

INTRODUCTION

Autism Spectrum Disorder (ASD) is a multifaceted neurodevelopmental condition marked by challenges in social interaction, communication difficulties and restricted or repetitive behaviours [1]. While substantial advances have been made in identifying the genetic and behavioural underpinnings of ASD, emerging evidence highlights the significant contribution of immune and endocrine dysregulation to its pathophysiology [2]. These biological disturbances appear to be especially influential during critical periods of neurodevelopment and may contribute to the wide variability observed in ASD phenotypes [3].

Among the endocrine abnormalities implicated in ASD, dysregulation of the Growth Hormone (GH) axis has garnered attention, as it plays a fundamental role in brain maturation, synaptic development and cognitive functioning [4]. Alterations in GH activity, ranging from hypersecretion to deficiencies in its downstream effector, insulin-like growth factor 1, have been reported in individuals with ASD [5]. Numerous studies have documented elevated levels of proinflammatory biomarkers, including interleukin-6 (IL-6) and IL-17, which suggest chronic, low-grade inflammation and ongoing neuroimmune activation [6]. These cytokines interfere with neural connectivity, synaptic homeostasis and neurotransmission central to ASD neurobiology. Furthermore, decreased levels of regulatory cytokines such as IL-10 may indicate an impaired anti-inflammatory response, thereby supporting the notion of persistent neuroinflammatory damage [7].

Vitamin D3 (Vit D3) has emerged as a crucial modulator of neuroimmune interactions, particularly in the context of ASD. It is synthesised through ultraviolet B radiation and activated via hepatic and renal pathways. Vit D3 is involved in calcium homeostasis, gene expression, immune regulation and brain development [8]. Hypo-vitaminosis D is frequently reported in children with ASD and may result from limited sun exposure, restrictive diets, gastrointestinal comorbidities and impaired metabolic conversion [9]. Also, genetic variants in

the Vitamin D receptor gene have been associated with ASD, suggesting a heritable component that may influence both Vitamin D metabolism and immune function [10].

In our locality, the ASD is growing dramatically with little research on its cause, mechanism, pathway, genetic relationships and influencing factors [11]. Thus, this study aimed to examine the complex interplay between GH signaling, immune dysregulation, cytokine imbalance and Vit D3 metabolism in the pathogenesis of ASD. Consequently, by integrating perspectives from endocrinology, immunology and neurodevelopment, this study seeks to identify potential biomarkers and therapeutic targets that could improve diagnostic precision and clinical management in individuals with ASD [12].

METHODS

Study Design and Setting

This comparative analytical study enrolled participants from five autism centres in Sulaimaniyah, Iraq, including Redox Centre, Raparin Centre, Lutfy Mala Galb Centre, Kareza Wshk Centre and Amin Centre, from October to December 2024. Participants were 70 children diagnosed with ASD and 30 neurotypical children (healthy controls). ASD diagnosis was confirmed by a Pediatric neurospecialist using the Diagnostic and Statistical Manual of Mental Disorders, Fifth Edition (DSM-5) criteria. Additionally, this study adheres to the Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) checklist guidelines for the transparent and standardized reporting of observational research.

Inclusion Criteria

Children aged 6-12 years with confirmed ASD regardless of gender, nationality or ethnicity.

Exclusion Criteria

Children diagnosed with other neuro disorders rather than ASD, those on chemotherapy or chronic medications for different purposes and individuals who had undergone a major surgery.

Specimen Collection

Approximately 4.0 mL of venous blood was drawn aseptically from the antecubital vein of each participant, collected into clot activator gel tubes and allowed to clot at room temperature. The samples were then centrifuged at 3500 rpm for 10 minutes. The serum was separated and stored in plain tubes at -70°C until further immunological and serological analyses were performed. Laboratory analyses were performed at Mercy Private Health Laboratory, Sulaimaniyah, Iraq.

Immunological Analysis

Serum cytokine levels were quantified using the Enzyme-Linked Immunosorbent Assay (ELISA), following the manufacturer's protocol. The tests were done using the Chromate ELISA machine (Model 4300, USA) and the kits were purchased from Elabscience, China. The process was based on the double-sandwich technique with a sensitivity of <9.375 pg/mL.

Serological Analysis

GH and Vit D3 levels were measured using the Cobas e411 analyser (Roche Diagnostics, Germany), employing enzyme immunoassay techniques. Calibration utilised two calibrators (Calibrator 1 and 2) and two control sera (Universal Control 1 and 2). C-Reactive Protein (CRP) levels were determined using the Cobas c311 analyser (Roche/Hitachi 902). The test was calibrated with one standard and monitored using C5 protein control. Both procedures adhered strictly to the manufacturer's protocols.

Statistical Analysis

Data were entered and analysed using GraphPad Prism software (version 8.0.2, 263). Descriptive statistics were used to summarise categorical variables (frequencies and percentages) and continuous variables (mean±standard deviation). Group differences were assessed using the independent samples t-test and a $p<0.05$ was considered statistically significant.

RESULTS

A total of 70 children with ASD and 30 age-matched healthy controls were enrolled. Serum levels of inflammatory and endocrine markers were analysed and compared between groups. CRP levels were higher in the ASD group (3.059±5.459 mg/dL) than the control group (1.321±1.311 mg/dL) without a significant difference ($p = 0.088$). Vitamin D3 levels were significantly lower in the ASD group (16.57±5.606 ng/mL) than in the control group (33.36±4.335 ng/mL), with a highly significant difference ($p<0.0001$). Similarly, GH levels were markedly reduced in children with ASD (0.676±0.944 ng/mL) compared to controls (2.873±2.735 ng/mL), with a very highly significant difference ($p<0.0001$). In contrast, no significant differences were observed in the levels of IL-6, IL-10, or IL-17 between the ASD and control groups. The mean IL-6 level in the control group was 0.540±0.492 pg/mL, whereas in the ASD group, it was 0.475±1.558 pg/mL ($p = 0.825$). IL-10 levels were 0.5157±1.056 pg/mL in the ASD group and 0.643±1.265 pg/mL in the control ($p = 0.603$). IL-17 levels in the ASD group were 7.274±4.697 pg/mL and in controls, they were 6.979±4.88 pg/mL ($p = 0.780$) (Table 1 and Figure 1).

Table 1: Comparison of different studied biomarkers between the control and ASD groups

Variable	Control (n = 30) Mean±SD	ASD (n = 70)	p-value
CRP (mg/dL)	1.321±1.311	3.059±5.45	0.0889
Vitamin D3 (ng/mL)	33.36±4.335	16.57±5.60	<0.0001****
GH (ng/mL)	2.87±2.735	0.67±0.944	<0.0001****
IL-6 (pg/mL)	0.540±0.492	0.475±1.558	0.8258
IL-10 (pg/mL)	0.643±1.265	0.515±1.056	0.6034
IL-17 (pg/mL)	6.979±4.885	7.274±4.697	0.7800

****: Very highly significant using test, ASD: Autism Spectrum Disorder, CRP: C-Reactive Protein, GH: Growth Hormone, IL: Interleukin

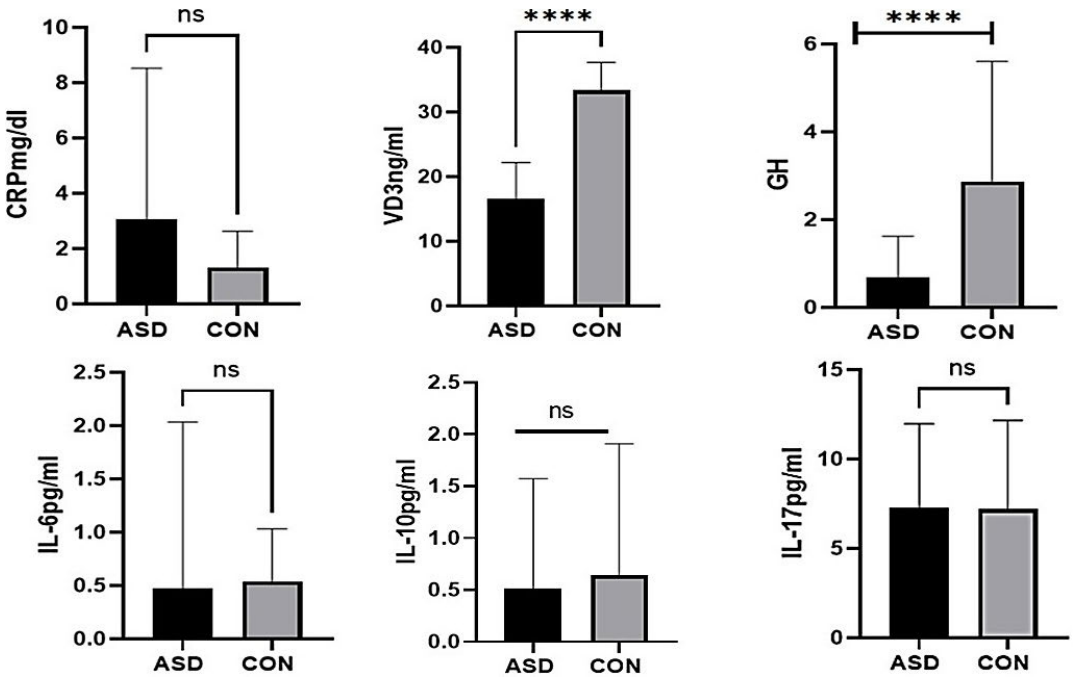

Figure 1: Comparison of Different Biomarkers between control and Autism Spectrum Disorder (ASD) groups. CON: Control Group, CRP: C-Reactive Protein, GH: Growth Hormone, IL: Interleukin, ns: Non- significant, VD3: Vitamin D3. ****: Very Highly Significant using Test

DISCUSSION

The present study extends the growing body of evidence implicating endocrine and immune dysregulation in the pathophysiology of ASD. These findings demonstrate significant alterations in serum concentrations of GH and vitamin D3 ($p \leq 0.05$), while the proinflammatory cytokines (CRP, IL-6 and IL-17) and the regulatory Cytokine (IL-10) did not exhibit significant changes ($p \geq 0.05$). The reduction in GH levels observed in children with ASD is consistent with previous investigations highlighting GH dysregulation in neurodevelopmental disorders [13-15]. GH is a critical regulator of neuronal development, synaptic plasticity and overall brain maturation, which are essential for expected cognitive and behavioural outcomes [4,16]. The observed reduction in GH activity may indicate a state of GH resistance, characterised by typical or elevated serum GH levels alongside diminished biological responsiveness, suggesting potential hormone insensitivity in this population. GH deficiency has been linked to impaired myelination, reduced neuronal survival and disruptions in prenatal and postnatal brain development, potentially contributing to the cognitive and motor delays frequently reported in ASD [17,18].

Furthermore, this study identified a pronounced deficiency in Vitamin D3 among ASD patients, aligning with earlier studies that reported high rates of hypovitaminosis D within this demographic [19,20]. In addition to its well-established role in calcium homeostasis, Vit D3 is vital for neurodevelopment and modulation of immune mechanisms [21]. Factors such as limited exposure to sunlight, dietary restrictions and metabolic impairments may account for the observed deficiency in Vit D3 [22]. Previous studies have reported elevated levels of proinflammatory markers IL-6 and IL-17, indicative of chronic low-grade inflammation in ASD [23,24]. In contrast, the current study did not observe significant alterations in these cytokine levels. Notably, prior research has associated diminished IL-10, a key anti-inflammatory cytokine with impaired immunoregulation, which may exacerbate neuroinflammatory responses and contribute to increased neurotoxicity [25,26]. The limitations of this study include a small sample size, limited parameters, a limited timeframe and its cross-sectional design that prevents causal interpretation; however, it was a multicenter study that recruited patients from different hospitals. Additionally, its single-region recruitment limits generalizability and other limitations include lacking of ASD severity, serum biomarkers may not reflect CNS activity and exclusion of overlapping neurodevelopmental disorders limits scope.

CONCLUSION

These findings highlight the broad spectrum of biological and physiological disruptions associated with ASD, encompassing endocrine disturbances, immune dysregulation and Vit D3 insufficiency. Such abnormalities may have adverse effects on brain maturation and behavioural outcomes in affected individuals. Future investigations employing larger sample sizes and longitudinal designs are warranted to confirm these associations and explore the viability of these biomarkers as early diagnostic indicators or therapeutic targets. Interventions aimed at modulating immune responses, correcting hormonal

imbalances and replenishing Vitamin D3 levels hold potential for mitigating ASD severity and enhancing the quality of life for individuals with ASD. Future recommendations include larger, age- and sex-matched control cohorts and longitudinal studies assessing seasonal vitamin D variation. Also, integration of genetic and metabolic profiling is suggested, together with inclusion of gut inflammatory markers may clarify CRP findings. Whereas, interventional studies evaluating supplementation effects are required.

Acknowledgement
The authors express sincere gratitude to the staff of Mercy Private Laboratory and Autism Centers for their invaluable assistance in the execution of this study.

Patient Consent
Written informed consent was obtained from the participant's parent, guardian or relatives and they were informed about the nature of the study and that their data would be protected. The anonymity and confidentiality of the patients' data were kept throughout the study.

Funding
This project originated from a PhD thesis and was not funded by any organizer, university, or society.

Data Availability
The raw data of this study are available with the corresponding author and can be provided upon request.

Authors' Contributions
SAM: Data collection, data analysis, study registration, resources and manuscript drafting and writing. HRF: Conceptualization, supervision, methodology and manuscript edition and revision.

Conflicts of Interest
The authors declare no competing interests.

Ethical Statement
The study protocol was approved by the Scientific and Ethics Committees of the College of Science and Health, University of Koya, Koya Province, Sulaimaniyah, Iraq (No. 013Bio, on January 05, 2024). The parameters were conducted according to the Declarations of Helsinki, 2013.

REFERENCES
[1] Genovese, A. and M.G. Butler, The autism spectrum: Behavioral, psychiatric and genetic associations. *Genes*, vol. 14, no. 3, 2023, pp. 677.

[2] Uccella, S. *et al.* Inflammatory and infectious disorders in endocrine pathology. *Endocrine Pathology*, vol. 34, no. 4, 2023, pp. 406–436.

[3] Wang, L. *et al.* Autism spectrum disorder: neurodevelopmental risk factors, biological mechanism and precision therapy. International *Journal of Molecular Sciences*, vol. 24, no. 3, 2023, p. 1819.

[4] Bhalla, S. *et al.* Protective role of IGF-1 and GLP-1 signaling activation in neurological dysfunctions. *Neuroscience and Biobehavioral Reviews*, vol. 142, 2022, pp. 104896.

[5] van Nieuwpoort, I.C. Growth hormone: Beyond growth: The growth hormone/insulin-like growth factor-1 axis in healthy elderly, growth hormone deficient adults and adults with Prader-Willi Syndrome. Vrije University Amsterdam, 2024.

[6] Liakakis, G. *et al.* Exploratory analysis of cerebrospinal fluid IL-6 and IL-17A levels in subcortical small-vessel disease compared to Alzheimer's disease: A pilot study. *Diagnostics*, vol. 15, no. 6, 2025, pp. 669.

[7] Doganyigit, Z. *et al.* The role of neuroinflammatory mediators in the pathogenesis of traumatic brain injury: A narrative review. *ACS Chemical Neuroscience*, vol. 13, no. 13, 2022, pp. 1835–1848.

[8] Voiculescu, V.M. *et al.* Vitamin D: Beyond traditional roles-insights into its biochemical pathways and physiological impacts. Nutrients, vol. 17, no. 5, 2025, pp. 803.

[9] Jiang, Y. *et al.* Associations between vitamin D and core symptoms in ASD: An umbrella review. *Nutrition and Dietary Supplements*, 2024, pp. 59–91.

[10] Saechua, C. *et al.* Impact of gene polymorphisms involved in the vitamin D metabolic pathway on the susceptibility to and severity of autism spectrum disorder. *Scientific Reports*, vol. 14, no. 1, 2024, pp. 28333.

[11] Samadi, S.A. The Challenges of Establishing Healthcare Services in low-and Middle-Income countries: The case of Autism Spectrum Disorders (ASD) in the Kurdistan Region of Iraq-Report from the field. *Brain Sciences*, vol. 12, no. 11, 2022, p. 1433.

[12] Samadi, S.A. *et al.* Screening children for Autism Spectrum Disorders in low-and middle-income countries: Experiences from the Kurdistan region of Iraq. *International Journal of Environmental Research and Public Health*, vol. 19, no. 8, 2022, pp. 4581.

[13] Provenzano, G. *et al.* GH dysfunction in Engrailed-2 knockout mice, a model for autism spectrum disorders. *Frontiers in Pediatrics*, vol. 2, 2014, p. 92.

[14] Sethuram, S. *et al.* A proof-of-concept study of growth hormone in children with Phelan–McDermid syndrome. *Molecular Autism*, vol. 13, no. 1, 2022, p. 6.

[15] Abozaid, H. *et al.* Growth hormone and ghrelin status in autistic children. *QJM: An International Journal of Medicine*, vol. 113, Supplement 1, 2020, pp. hcaa063.

[16] Al-Samerria, S. and S. Radovick. Exploring the therapeutic potential of targeting GH and IGF-1 in the management of obesity: Insights from the interplay between these hormones and metabolism. *International Journal of Molecular Sciences*, vol. 24, no. 11, 2023, pp. 9556.

[17] Arjunan, A. *et al.* Identification of the molecular mechanism of Insulin-like Growth Factor-1 (IGF-1): A promising therapeutic target for neurodegenerative diseases associated with metabolic syndrome. *Cell and Bioscience*, vol. 13, no. 1, 2023, pp. 16.

[18] Dönmez, B. *et al.* The role of insulin-like growth factor on autism spectrum disorder. *Journal of Experimental and Basic Medical Sciences*, vol. 2, no. 3, 2021, pp. 430–435.

[19] Boyd, N.K. *et al.* Hypovitaminosis D in persons with Down syndrome and autism spectrum disorder. *Journal of Neurodevelopmental Disorders*, vol. 15, no. 1, 2023, pp. 35.

[20] Daniel, K.S. *et al.* The *Increasing Prevalence of Autism Spectrum Disorder in the US and Its Implications for Pediatric Micronutrient Status: A Narrative Review of Case Reports and Series*. Nutrients, vol. 17, no. 6, 2025, pp. 990.

[21] Eyles, D.W. Vitamin D: Brain and behavior. *Journal of Bone and Mineral Research Plus*, vol. 5, no. 1, 2021, pp. e10419.
[22] Dominguez, L.J. *et al.* Vitamin D sources, metabolism and deficiency: Available compounds and guidelines for its treatment. *Metabolites*, vol. 11, no. 4, 2021, pp. 255.
[23] Nadeem, A. *et al.* Imbalance in pro-inflammatory and anti-inflammatory cytokines milieu in B cells of children with autism. *Molecular Immunology*, vol. 141, 2022, pp. 297–304.
[24] Noori, A.S. *et al.* Correlation of biochemical markers and inflammatory cytokines in Autism Spectrum Disorder (ASD). BMC Pediatrics, vol. 24, no. 1, 2024, pp. 696.
[25] Zhao, H. *et al.* Association of peripheral blood levels of cytokines with autism spectrum disorder: A meta-analysis. *Frontiers in Psychiatry*, vol. 12, 2021, pp. 670200.
[26] Rahman, M.M. *et al.* Exploring the management approaches of cytokines including viral infection and neuroinflammation for neurological disorders. *Cytokine*, vol. 157, 2022, pp. 155962.