Short Communication

Evaluation of Interleukin-35 and Serotonin Levels in Iraqi Children with Autism

Noor Tahreer Abd, Talib Abdullah Hussen
Department of Biology, College of Science for Women, Baghdad University, Baghdad, Iraq

Abstract

Background: Autism is a collection of conditions that cause a significant loss of ability to engage with, adhere to, or communicate with other kids or adults and maintain accurate records interaction with the outside world or society. A complex variety of neurological elements make up autism. These diseases are brought on by defects in a developing child's brain; these conditions typically endure a lifetime. Autism spectrum disorders are conditions that affect people of all ages. **Objectives:** The goal of this study is to compare the levels of the cytokine interleukin-35 (IL-35), cortisol, and serotonin in the serum of patients with autistic children to those of seemingly healthy controls due to their roles in the development of autism illnesses. **Materials and Methods:** The study included collecting blood samples from a group of autistic children that included 80 patients (60 men and 20women), and a healthy group that included forty individuals as a control group (20 men and 20 women). **Results:** The results showed that IL-35 had a low significant difference in autism patients compared to the control group by (126.50 ± 15.93, 484.00 ± 55.27), respectively, and some immune indicators that include serotonin. The results showed that there were low significant differences at the level of ($P \leq 0.05$) for patients. It reached (31.77 ± 6.55) compared to the control (138.21 ± 5.97). As for cortisol, it was highly significant and at a high level in autistic patients compared to the control by (21.70 ± 4.54) and (8.57 ± 0.74), respectively. **Conclusions:** Diminished serotonin, IL-35, and cortisol levels could potentially signify the involvement of these biomarkers in the development of autism, further endorsing the potential utility of serotonin, IL-35 and cortisol as diagnostic tools for assessing the severity of autism.

Keywords: Autism spectrum disorders, enzyme linked immunosorbent assay (ELISA) technique, interlukin-35

Introduction

The occurrence of autism spectrum disorders (ASD) has witnessed a substantial rise in the past 20 years.[1] This escalation is gauged using behavioral benchmarks that encompass impaired social interactions, communication challenges, and repetitive behaviors. Whereas the precise origins of ASD remain enigmatic, both genetic and environmental factors are deemed significant contributors.[2] Maternal immune activation has been linked to amplified behavioral deficits.[3] Additionally, ASD is correlated with an escalated prevalence of allergies, asthma, and autoimmunity, wherein pro-inflammatory elements such as cytokines, T cells, innate immune cells, and autoantibodies play a role.[4] Despite a wealth of evidence pointing toward various ASD triggers, a definite consensus regarding a precise immune response mechanism is yet to be achieved. Recent insights suggest that the absence of immune regulation might underpin certain facets. The functioning of the immune system relies on regulatory immune cells and the generation of immunosuppressive or anti-inflammatory cytokines.[5] Cytokines, which are small glycoprotein molecules produced by various cells in response to a range of immune triggers, play a vital role.[6] Research has revealed that there are diminished levels of regulatory cytokines like transforming growth factor β1 and interleukin (IL)-10 in ASD.[7]

A recent discovery, IL-35, a cytokine characterized by its immunosuppressive properties, has garnered attention.

Access this article online

Quick Response Code:

Website: https://journals.lww.com/mjby

DOI: 10.4103/MJBL.MJBL_1261_23

Address for correspondence: Noor Tahreer Abd, Department of Biology, College of Science for Women, Baghdad University, Baghdad, Iraq.
E-mail: noor.abd2102m@csw.uobaghdad.edu.iq

Submission: 23-Aug-2023 **Accepted:** 14-Sep-2023 **Published:** 23-Jan-2026

How to cite this article: Abd NT, Hussen TA. Evaluation of interleukin-35 and serotonin levels in Iraqi children with autism. Med J Babylon 2025;22:1574-8.

Belonging to the IL-12 cytokine family, it consists of an alpha chain (p35) and a beta chain encoded by the Epstein-Barr virus-induced gene 3 (EBI3) subunit IL-27. Regulatory T cells (Treg), encompassing CD4+ and CD8+ Tregs, dendritic cells, and regulatory B cells, produce IL-35. The primary role of IL-35 lies in suppressing T helper type 17 (TH17) cells and promoting Treg proliferation. Conditions marked by auto-inflammatory responses, such as multiple sclerosis, inflammatory bowel disease, systemic lupus erythematosus, rheumatoid arthritis, and psoriasis, exhibit diminished levels of IL-35.[8]

Furthermore, this study delves into the role of neurotransmitters, specifically serotonin. Serotonin, a pivotal neurotransmitter and intrinsic active agent, exerts a pivotal influence on brain growth and development, profoundly affecting the central nervous system.[9] Its sphere of influence extends to various physiological functions, encompassing sleep-wake cycles, memory, behavior, and even aggression, altered serotonin systems have been associated with atypical brain development. In ASD patients, irregularities in serotonin transporters, fluctuating serotonin levels, decreased serotonin synthesis, and modified serotonin receptor responses have been detected.[10] In addition, a heightened or attenuated hormone of stress (cortisol) response to exposure of stress has been observed in individuals with ASD in childhood stage, which may have contributed to their irritability and anxiety. As it has been demonstrated that individuals with ASD have higher anxiety levels than normal subjects, higher trait anxiety levels are associated with increased stress hormones.[11]

Materials and Methods

The current study included 120, in whom 80 patients (20 girls and 60 boys) with autistic children, aged between (5 and 15 years), during the period from March to June, 2023, from Al-Safaa Center for Autism, Baghdad. All samples were taken with the patients' consent before inclusion in the study. Forty samples from healthy subjects for comparison (3 mL) were collected and placed in a gel tube for 30 min for clot formation at room temperature. To collect serum, the tubes were centrifuged at 3000 rpm for 10 min to separate the serum from other blood components, using micropipettes, and then the serum was transferred into Eppendorf test tubes and kept in deep freeze at −20°C until use, for the purpose of immunological and serological tests (serotonin, IL-35, cortisol). When quantitating cytokines using the sandwich enzyme-linked immunosorbent assay (ELISA) technique, the resulting serum was chilled by freezing at (-20°C), and the optical density used was 450 nm.

Ethical approval

The study was conducted in accordance with the ethical principles that have their origin in the Declaration of Helsinki. It was carried out with patients verbal and analytical approval before sample was taken. The study protocol and the subject information and consent form were reviewed and approved by a local ethics committee according to the document number (22/2078 in April 9, 2023) to get this approval.

Statistical analysis

Patient and control group differences in the study's variables were identified using the Statistical analysis was performed by using statistical package of social science (SPSS) version 26. The data were expressed as (mean ± SD). Statistical comparison between groups were made using student's Chi-square test and a P value of ≤0.05 was considered significant; while the differences between more than two groups were analyzed using an analysis of variance (one – way ANOVA). Also, Pearson correlation coefficients were calculated to check the relationship between the studied parameters.[12]

Results

The comparison of the mean ± standard error (mean ± SE) of the IL-35 levels in the serum of children with ASD and control groups was shown in Table 1. The results in this study elucidated the highly significant ($P < 0.05$) reduction in the means of IL-35 levels in patients compared to control. Table 2 shows that the age group did not affect the level of IL-35 concentration as the differences were not significant.

The comparison of the mean ± standard error (mean ± SE) of the serotonin levels in the serum of children with ASD and control groups was shown in Table 3. The results in this study elucidated the highly significant ($P < 0.05$) reduction in the means of serotonin levels in patients compared to control. Table 4 shows that the age group did not affect the level of serotonin concentration as the differences were not significant.

The comparison of the mean ± standard error (mean ± SE) of the cortisol levels in the serum of children with ASD and control groups was shown in Tables 5 and 6.

Table 1 : The concentration of IL-35 with respect to age groups

Study population			IL-35 concentration (pg/mL)		*P* value ($P \leq 0.05$)
Age group	Type	No.	Mean	Std. deviation	
4-6	Patient	19	126.50	15.93	0.000*
	Control	7	480.00	56.02	
7-9	Patient	34	128.76	14.57	0.000*
	Control	11	477.11	38.43	
10-12	Patient	16	126.13	19.80	0.000*
	Control	13	475.29	36.07	
13-15	Patient	11	130.81	16.48	0.000*
	Control	9	484.88	55.27	

*Highly significant difference under $P \leq 0.05$ by one way ANOVA

Table 2 : Intensity of IL-35 in patients

IL-35 intensity	Age group/year (%)				Total	*P* value ($P \leq 0.05$)
	4-6	7-9	10-12	13-15		
Sever	10 (30.3)	13 (39.4)	7 (21.2)	3 (9.1)	33 (41.2)	0.719 NS
Mild	4 (23.5)	6 (35.3)	3 (17.6)	4 (23.5)	17 (21.2)	0.734 NS
Moderate	5 (16.7)	15 (50)	6 (20)	4 (13.3)	30 (37.5)	0.383 NS
Total	19	34	16	11	80 (100)	

NS: non-significant difference at the 0.05 level by chi-square test

Table 3: Evaluation of serotonin with respect to age groups

Study population			Serotonin (nmol/L)		*P* value ($P \leq 0.05$)
Age group	Type	No.	Mean	Std. deviation	
4-6	Patient	19	31.77	6.55	0.084 NS
	Control	7	137.64	5.29	
7-9	Patient	34	34.57	6.95	0.058*
	Control	11	134.94	4.16	
10-12	Patient	16	34.00	7.24	0.000**
	Control	12	138.21	5.97	
13-15	Patient	11	35.72	5.98	0.000**
	Control	9	136.95	10.33	

* Significant difference under $P \leq 0.05$ by one way ANOVA

** HS: highly significant, NS: non-significant difference

Table 4: Intensity of serotonin in patients

Serotonin intensity	Age group/year (%)				Total	*P* value ($P \leq 0.05$)
	4-6	7-9	10-12	13-15		
Sever	8 (32)	9 (36)	6 (24)	2 (8)	25 (31.2)	0.790 NS
Mild	3 (15)	10 (50)	4 (20)	3 (15)	20 (25)	0.776 NS
Moderate	8 (22.9)	15 (42.9)	6 (17.1)	6 (17.1)	35 (43.7)	0.450 NS
Total	19	34	16	11	80 (100	

NS: non-significant difference at the 0.05 level by chi-square test

Table 5: The concentration of cortisol with respect to age groups

Study population			Cortisol concentration (μg/dL)		*P* value ($P \leq 0.05$)
Age group	Type	No.	Mean	Std. deviation	
4-6	Patient	19	21.32	3.24	0.000*
	Control	7	8.10	1.79	
7-9	Patient	34	20.75	4.19	0.000*
	Control	11	9.20	1.43	
10-12	Patient	16	21.70	4.54	0.000*
	Control	13	8.76	1.37	
13-15	Patient	11	19.38	4.07	0.000*
	Control	9	8.57	0.74	

* Highly significant difference under $P \leq 0.05$ by one way ANOVA

The results in this study elucidated the highly significant ($P < 0.05$) increase in the means of cortisol levels in patients compared to control. Table 4 shows that the age group did not affect the level of serotonin concentration as the differences were not significant.

Correlation between IL-35, serotonin and cortisol

In our investigation, the link between parameters was found to be non-significant for cortisol and serotonin, but significant for IL-35. All parameters in control are non-significant Tables 7.

Table 6: Intensity of cortisol in patients

Cortisol intensity	Age group/year (%)				Total	*P* value ($P \leq 0.05$)
	4-6	7-9	10-12	13-15		
Sever	9 (24.3)	14 (37.8)	10 (27)	4 (10.8)	37 (46.2)	0.411 NS
Mild	3 (13.6)	10 (45.5)	5 (22.7)	4 (18.2)	22 (27.5)	0.304 NS
Moderate	7 (33.3)	10 (47.6)	1 (4.8)	3 (14.3)	21 (26.2)	0.469 NS
Total	19	34	16	11	80 (100)	

NS: non-significant difference at the 0.05 level by chi-square test

Table 7: Relationship between gender cortisol, IL-35, and serotonin in patients and control groups

Parameters	Patients			Control		
	Male (No = 60)	Female (No = 20)	*P*-value	Male (No = 20)	Female (No = 20)	*P*-value
Cortisol (µg/dL)	20.37 ± 0.60	21.41 ± 0.02	0.389 NS	8.53 ± 0.29	8.97 ± 0.34	0.595 NS
IL-35 (pg/mL)	124.13 ± 2.75	134.49 ± 3.92	0.050 *	484.25 ± 8.10	477.67 ± 11.95	0.757 NS
Serotonin (ng/mL)	35.54 ± 1.53	33.04 ± 1.61	0.379 NS	137.18 ± 1.66	136.76 ± 1.39	0.872 NS

* $P \leq 0.05$, NS: non-significant

Discussion

ASD results from a combination of abnormalities in the nervous system, environment, and genetics.[13] Despite nearly 50 years of pathogenesis research, the etiology of ASD remains unknown.[14] It is estimated that the disease's global common is about 1%, with a female-to-male 1:4 ratio.[15] This study agreed with[16] emerging research findings suggest a decline in plasma IL-35 levels within the realm of ASD-affected children, implying a plausible connection between IL-35 levels and neuronal functionality evident in the data presented in Table 1. This linkage appears to influence neurodevelopmental processes and the ensuing behaviors. These outcomes resonate with a study[17] evident in the data presented in Table 2. Regarding the severity gradations of ASD, we made subgroups for severity (mild, moderate, and severe) of disease according to their scores and to the results of their examination and relation to age, an analysis employing the chi-square test yielded a non-significant outcome ($P = 0.05$). This observation is documented in Tables 3 and 4. In contrast to the stance of study[18] where the influence of serotonin on various physiological functions such as mood, appetite, sleep, cognition, and anxiety is underscored, these multifaceted roles could possibly elucidate its association with characteristic autistic traits. Earlier investigations, exemplified by those of[19] contend that serotonergic systems might contribute to the predisposition towards autism. In Tables 5 and 6, our results cortisol agree with some studies which demonstrated a significantly higher cortisol level in the serum.[20] Another study using plasma cortisol collected in the morning hours found lower cortisol levels in children with ASD than controls in contrast to our findings. This pattern of hormonal fluctuation could point to autism-related abnormalities of the hypothalamic-pituitary-adrenal (HPA) axis. On the other hand, disruption of the HPA axis has been linked to both excessive and insufficient cortisol responses; it is commonly recognized that any kind of physical or mental stress can raise cortisol levels.[21]

Conclusion

The present study demonstrated that serotonin, IL-35 parameters showed reduced levels in autistic children and increase levels cortisol parameter in autistic children comparison to their healthy counterparts. This study did not conduct a statistical correlation existed between autism in children with autism. Conversely, substantial distinctions were identified between the groups regarding the distribution of age and gender.

Financial support and sponsorship

Nil.

Conflicts of interest

There are no conflicts of interest.

References

1. Baio J, Wiggins L, Christensen DL, Maenner MJ, Daniels J, Warren Z, *et al*. Prevalence of autism spectrum disorder among children aged 8 years—Autism and developmental disabilities monitoring network, 11 sites, United States, 2014. MMWR Surveill Summ 2018;67:1.
2. Shaker MA, Dawood AA. Emotions recognition in people with autism using facial expressions and machine learning techniques: Survey. JUBPAS 2023;31:128-36.
3. Reda NA. The impact of the educational program in the development of social and linguistic skills and cognitive sense in the sample of autistic children in the center of the province of Babylon. J Babylon Center Human Stud 2019;9:91-134.
4. Licata L, Briganti L, Peluso D, Perfetto L, Iannuccelli M, Galeota E, *et al*. MINT, the molecular interaction database: 2012 update. Nucleic Acids Res 2012;40:D857-61.
5. Omran RH, Ahmed ZA, Alrawi AA. Evaluation of some new cytokines in rheumatoid arthritis. J Fac Med Bagdad 2022;64: 159-62.

6. Ahmed MQ, Hussein TA. Evaluation of IL-4 and IL-35 levels in Iraqi fuel stations workers suffering from allergic rhinitis. AIP Conf Proc 2023;2820:050019.
7. Jyonouchi H, Geng L, Davidow AL. Cytokine profiles by peripheral blood monocytes are associated with changes in behavioral symptoms following immune insults in a subset of ASD subjects: an inflammatory subtype? J Neuroinflammation 2014;11:1-3.
8. Al-azzawi RH, Mohsen RT, Ad'hiah AH. Serum level of interleukin-35 in patients with chronic hepatitis B virus infection. Iraqi J Sci 2020;61:2860-5.
9. Teymouri M, Pirro M, Fallarino F, Gargaro M, Sahebkar A. IL-35, a hallmark of immune-regulation in cancer progression, chronic infections and inflammatory diseases. Int J Cancer 2018;143:2105-15.
10. Shanyoor SJ, Ali Naji ZM, Shanyoor GJ. Association of serum serotonin levels, and hyperglycemia with ageusia and anosmia in Iraqi COVID-19-infected patients. J Med Chem Sci 2022;6:1344-51.
11. Khudhiar NK, Saud AM. Genetic polymorphisms rs643627 in serotonin receptor gene (5-HTR2A) with schizophrenia. Iraqi J Sci 2019;60:2642-8.
12. Niazi AD, Isa SA. Gender differences in achievements among students of the Iraqi college of medicine. Baghdad Sci J 2004;1:300-5.
13. Sugaya N, Izawa S, Ogawa N, Shirotsuki K, Nomura S. Association between hair cortisol and diurnal basal cortisol levels: A 30-day validation study. Psychoneuroendocrinology 2020;116:104650.
14. Robinson-Agramonte MD, Noris García E, Fraga Guerra J, Vega Hurtado Y, Antonucci N, Semprún-Hernández N, *et al*. Immune dysregulation in autism spectrum disorder: What do we know about it? Int J Mol Sci 2022;23:3033.
15. Bozkurt H, Şimşek S, Şahin S. Elevated levels of cortisol, brain-derived neurotropic factor and tissue plasminogen activator in male children with autism spectrum disorder. Autism Res 2021;14: 2078-84.
16. Eissa N, Al-Houqani M, Sadeq A, Ojha SK, Sasse A, Sadek B. Current enlightenment about etiology and pharmacological treatment of autism spectrum disorder. Front Neurosci 2018; 12:304.
17. van't Hof M, Tisseur C, van Berckelear-Onnes I, van Nieuwenhuyzen A, Daniels AM, Deen M, *et al*. Age at autism spectrum disorder diagnosis: A systematic review and meta-analysis from 2012 to 2019. Autism 2021;25:862-73.
18. Sanctuary MR, Kain JN, Chen SY, Kalanetra K, Lemay DG, Rose DR, *et al*. Pilot study of probiotic/colostrum supplementation on gut function in children with autism and gastrointestinal symptoms. PLoS One 2019;14:e0210064.
19. Harutyunyan AA, Harutyunyan HA, Yenkoyan KB. Novel probable glance at inflammatory scenario development in autistic pathology. Front Psychiatry 2021;12:788779.
20. Oteify G, El-Ramly A, Samy A, Soliman E. Evaluation of oxytocin and serotonin levels in autism spectrum disorder. J Med Sci Res 2018;1:66.
21. Alshammary AA, Aljibori QN, Al-Jabory MA. Clinical and demographic characteristics of autistic children in Al-Hilla city. Med J Babylon 2024;12:195-9.