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Original Research Paper

Evaluation of serum level of IL-6 and TNF-α in children with autism

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Abstract: A wide range of diverse neuropsychiatric symptoms, such as difficulties with social communication, limited and specific interests, and repetitive behavior, are hallmarks of autism spectrum disorder (ASD), a collection of intricate multifactorial neurodevelopmental diseases. The immunological hypothesis is thought to have a significant role in the pathophysiology of autism and to explain the variations in clinical phenotypes and comorbidities that affect the severity and duration of the condition . In the current study, there were 90 Iraqi children (male and female), 60 children with ASD who visited Pediatric Teaching Hospital in Iraq (48 males and 12 females), and 30 children control (20 males and 10 females). They were between the ages of 1.5 and 12year. The children's peripheral venous blood was drawn in order to measure the concentrations of serum levels of IL-6, TNF-α and CBC. including total WBC (10^9/L) cells, differential WBC, platelet count, HGB, HCT levels. Cytokine levels were measured by enzyme-linked immunosorbent assay method. The study's findings revealed: 1) IL-6 levels were found to be significantly higher in children with autism than in control children. same that TNF-α levels were higher in children with autism than in control, however, with statistical significance ($P \leq 0.05$). But in CBC not statistical significance ($P > 0.05$). With regard to the influence of immunological factors on autism development, comorbidities affecting the course and severity of autism disease, and other aspects of particular significance, this review provides up-to-date information on immune dysfunction in ASD.

Keywords: Autism, interleukins -6, tumor necrosis factors (TNF-α)

1.Introduction

During the first three years of life, a complex group of multifactorial neurodevelopmental disorders known as autism spectrum disorder (ASD) can cause a wide range of neuropsychiatric symptoms, such as repetitive behavior, limited interests, and difficulties with social communication [1,2,3]. According to several early observations by writers in this field, the adaptive immune response is a crucial factor in the development of autism [4,5,6]. Cytokines are tiny proteins that are soluble and released [7], Its molecular weight is low [8]. Cytokines function as messengers of both innate and adaptive immunity in almost every facet of human immunology [9]. Inflammatory responses are significantly influenced by cytokines [10]; It controls the immunological response, facilitates cell-to-cell communication, and has a major impact on infection clearance and resistance to infectious diseases [11]. Mobile immune cells including B lymphocytes, T lymphocytes, mast cells, and macrophages are among the many immune cells that produce cytokines. These cells penetrate the blood-brain barrier and release cytokines based on the kind of target antigen that antigen-

presenting cells (APCs) recognize in the central nervous system (CNS)Variable cytokines secreted by T-cell subtypes counter-regulate one another [12], and an imbalance between pro- and anti-inflammatory pathways is observed, which is a significant factor in the pathophysiology of neuropsychiatric diseases like autism [13,14,15].Interleukins are a group of cytokines that discovered in 1980 [7]. Gp130 (CD130) mediates their signal by forming a complex with a distinct IL-6 alpha receptor (IL-6R / CD126). It regulates tissue homeostasis, hematopoiesis, immunity, cognitive function, and lipid metabolism [7]. IL-6 stimulates the growth of tumor-initiating cells and prevents apoptosis in intestinal epithelial cells, both normal and premalignant. It is also necessary for the macrophage-mediated healing of wounds [16]. TNF-α was initially identified in 1975 to encourage tumor hemorrhage, which aids in the classification of its subfamily [17]. TNF-α is a response molecular pattern associated with the pathogen that is produced by a variety of cell types, such as macrophages, natural killer cells (NK cells), monocytes, endothelial cells, white blood cells (neutrophils), smooth muscle cells, activated lymphocytes, astrocytes, and adipocytes [18]. The inflammatory cytokines TNF, IL-1, and IL-6 can change neurodevelopment and have a direct impact on the brain, which can influence behavior [19].

2.Methodology

In the cross-sectional and control-case study, 90 Iraqi youngsters participated; 60 of them had autism and visited the Iraqi Association for Psychotherapy (IAP), while the remaining 30 were control subjects of the same gender. The subjects were between 1.5 and 12 years old. The time frame for sample collection was April 2024–July 2024. According to the final diagnosis given by a specialized physician based on criteria regarding their clinical signs and symptoms, all children were diagnosed with autism at varying stages, ranging from moderate to severe. Every patient's medical history, including name, gender, age, height, weight, and disease stage, as well as any prior medical conditions, treatment type, and year of diagnosis, was extracted from their profiles. The control group's normal patients had no prior medical history of illnesses or other inflammatory conditions. After gaining the approval of their parents, blood was drawn from both patients and controls to assess a number of factors, including immunological. Four milliliters of peripheral venous blood were drawn with a sterile syringe. To calculate the complete blood count (CBC), One milliliter of blood was combined with ethylene diamine tetra acetic acid (EDTA) in an anticoagulated tube. Blood samples weighing four milliliters were placed in a gel activator tube and left at 40°C for fifteen minutes to coagulate. After that, the tubes were centrifuged for ten minutes at 3000 rpm in order to extract the serum. The serum was placed in sterile plain tubes and kept at -20 0C until it was time to measure IL-6 and TNF-α.

3.Results and discussion

Table 1 shows a significant increase ($p \leq 0.05$) in levels of IL-6 (pg/mL) in the serum of ASD children in female and male (27.48±3.827, 23.68±1.262), respectively as compared to children without ASD (8.548±0.458, 8.276±0.370), respectively in female and male.

Table 1: The levels of IL-6 in the serum of the ASD and control group depended on sex. Capital different letters: Significant difference ($P \leq 0.05$) between column. Small different letters: Significant difference ($P \leq 0.05$) between row.

Group / Sex	IL-6 pg/ml (mean± SE)			
	Autism	**Control**	**P-value**	**LSD**
Female	A, a 27.48±3.827	A, b 8.548±0.458	**0.0005**	**3.88**
Male	A, a 23.68±1.262	A, b 8.276±0.370	**0.00009**	**5.02**
P-Value	**0.234**	**0.663**		
LSD	**Non**	**Non**		

According to Table 2, there was a significant increase ($P \leq 0.05$) in IL-6 of the autism group (21.83±1.464, 24.54±1.800, 26.37±2.763), respectively as compared with the control group (7.999±0.277, 8.365±0.573, 9.168±0.770), respectively in (1.5-3.5,3.6-5.5,>5.5) years, but (1.5-3.5,3.6-5.5,>5.5) years children autism the result are close to each other not Significant difference ($P>0.05$) between them.

Table 2: The level of IL-6 in serum of the ASD group and control depended on age.
Capital different letters: Significant difference ($P \leq 0.05$) between column. Small different letters: Significant difference ($P \leq 0.05$) between rows.

Group / Age (year)	IL-6 Pg/ml (mean± SE)			
	Autism	**Control**	**P-value**	**LSD**
1.5-3.5	A, a 21.83±1.464	A, b 7.999±0.277	**0.00004**	**3.55**
3.6-5.5	A, a 24.54±1.800	A, b 8.365±0.573	**0.00057**	**4.12**
>5.5	A, a 26.37±2.763	A, b 9.168±0.770	**0.004**	**5.082**
P-Value	**0.363**	**0.332**		
LSD	**Non**	**Non**		

Table 3: The levels of TNF-α in the serum of the Autism and control group
Capital different letters: Significant difference (P≤0.05) between column. Small different letters: Significant difference (P≤0.05) between rows.

Table 3 shows levels of TNF-α (pg/mL) in the serum of ASD children high in female and male (259.4±37.729,264.1±14.058), respectively as compared to children without ASD (130.9±12.016,135±5.355), respectively in Female and male. Significant difference (P≤0.05).

Group / Sex	TNF pg/ml (mean± SE)			
	Autism	Control	P-value	LSD
Female	A, a 259.4±37.729	A, b 130.9±12.016	0.007	37.78
Male	A, a 264.1±14.058	A, b 135±5.355	0.00012	42.06
P-Value	0.889	0.720		
LSD	Non	Non		

The result show level TNF-α (pg/mL) there was a significant increase (P≤0.05) in the autism group (260.3±18.044,244.1±19.290,283.6±28.377),respectively compared with the control group (140.1±10.677, 123.05±4.549, 139.04±8.164) ,respectively in (1.5-3.5,3.6-5.5,>5.5) years, but in children autism the result are not significant difference (P>0.05) between the age (1.5-3.5,3.6-5.5,>5.5) same as in control children were shown in table 4.

Table 4: The level of TNF-α in serum of the Autism and control children according in years.
Capital different letters: Significant difference (P≤0.05) between column. Small different letters: Significant difference (P≤0.05) between rows.

Table 5: Comparison between Autism children and control groups in WBC and its differentia. Capital different letters: Significant difference (P≤0.05) between column.

Results of Hematology (Complete Blood Count CBC).
According to Table 5, there was a significant decrease (p≤ 0.05) in the monocytes counts (x10^9 /L) in the serum of ASD children when compared to the control group (7.182±2.704 and 10.163±3.355x10^9/L), respectively. while there were no significant (P>0.05) differences in the levels of total WBC, lymphocyte and neutrophils (x10^9/l) between the ASD group (8.942±3.9, 47.85±12.65 and 44.96±12.96x10^9/L, respectively) compared to control group (8.083±1.279 ,42.49±10.81 and 42.49±10.81x 10^9 /L, respectively).

Group / Age (year)	TNF Pg/ml (mean± SE)			
	Autism	Control	P-value	LSD
1.5-3.5	A, a 260.3±18.044	A, b 140.1±10.677	0.00021	42.31
3.6-5.5	A, a 244.1±19.290	A, b 123.05±4.549	0.0019	37.66
>5.5	A, a 283.6±28.377	A, b 139.04±8.164	0.015	38.09
P-Value	0.462	0.319		
LSD	Non	Non		

The Red Blood Cell Indices

	WBC 10^9/l	**lymphocyte %**	**monocytes%**	**Neutrophilia %**
Autism	A 8.942±3.9	A 47.85±12.65	B 7.182±2.704	A 44.96±12.96
Healthy	A 8.083±1.279	A 42.49±10.81	A 10.163±3.355	A 47.24±10.63
P-Value	0.245	0.551	0.0026	0.407
LSD	**Non**	**Non**	**1.144**	**Non**

The comparison of the red blood cell indices in the in ASD group compared with the control group, including the hemoglobin (HGB) level, packed cell volume (HCT%), PLT x10^9/l were shown in table 6 ; that were

no significant (P>0.05) differences in the number of (HGB g/dl, HCT and PLTx10^9/l)in the blood of ASD children (11.793±1.14, 34.7±3.037and312.5±91.7, respectively) compared to the control group (11.713±0.831, 35.827±2.311 and 326.5±69.4), respectively.

Table 6: Comparison between ASD and control groups in The HGB, HCT, PLT.

No significantly different between ASD children and control groups**.**

	HGB g/dl	HCT %	PLT 10^9/l
Autism	A 11.793±1.14	A 34.7±3.037	A 312.5± 91.7
Healthy	A 11.713±0.831	A 35.827±2.311	A 326.5± 69.4
P-Value	0.734	0.077	0.464
LSD	Non	Non	Non

A connection between immune system malfunction and ASD was proposed for the first time in over 40 years. Numerous studies have proposed a likely connection between the autistic phenotype and different aspects of compromised immunity, including cytokine levels. Patients with ASD have been found to have immune system problems [20]. Research in years has demonstrated that ASD is linked to a highly inflammatory condition, which is often linked to immune system dysfunction [21]. According to experimental research, cytokines like TNF-α are essential for the development of ASD and are connected to its detection [22,23]. In this study, we found that children with ASD had significantly higher serum levels of TNF-α than controls. This result supports other studies that discovered elevated serum levels of certain cytokines, such as TNF-α [23,24], plasma [25]. However, another study found no difference in the TNF-α levels between the control group and autistic patients [26], supporting that heightened innate immune responses, particularly TNF-α production, may contribute to the pathophysiology of ASD. However, the majority of autistic children in the study exhibited overactive or poorly controlled innate immune responses, especially those with increased TNF-α, the researchers discovered [22], Moreover, it has been shown that excessive TNF-α contributes to autistic characteristics such repetitive behaviors and a reduction in social engagement [27]. Furthermore, we discovered that children with ASD had significantly different serum levels of IL-6 than controls. Interleukin-6 levels in ASD participants were found to be rising in certain studies [21,27] which agreed with our results. Our findings were consistent with Ricci et al. (2013), who found no relationship between the cytokine level and autism severity, as the levels of both cytokines did not vary according to the severity of autism. However, our findings indicate that there is no discernible difference in the levels of the pro-inflammatory cytokines TNF-α and IL-6 between male and female autistic children. This is consistent with other research on autistic children that found no relationship between the cytokine levels and gender [28]. while contradicting research by Suzuki et al. (2011) and Singh (1996), which revealed no discernible variations in serum IL-6 levels between children with ASD and the control group [26,23], Although the reason for this difference in cytokine levels among studies is likely related to a number of limitations, such as sample quality, changes in the cytokine profile with time, statistical modifications [21] and inflammatory response [27]. It is thought that a disruption of the blood-brain barrier and an increase in neuro-inflammatory processes are linked to ASD. Because of the barrier membrane's enhanced permeability, proinflammatory signaling molecules produced by circulating monocytes can freely enter and change how neurons operate. Numerous brain and immune cell types are involved; mast cells are triggered to release TNF-α and IL-6, which causes microglia and localized brain inflammation to become activated and multiply. This disrupts neural plasticity and results in deficiencies in behavior, communication, and social interaction [21] Accordingly, peripheral cytokines can communicate with the brain and traverse the blood-brain barrier, and they may be essential for brain development [27]. According to D'Mello and Swain (2009), peripheral cytokines and the signals they mediate enter the brain through the following biological mechanism: It's possible that TNF-α and other cytokines encourage microglia to draw monocytes into the brain by boosting the production of monocyte chemoattractant protein [29]. The proinflammatory cytokine TNF-α, on the other hand, influences the pathophysiology and homeostasis of the central nervous system. Microglia produce high amounts of TNF-α in pathological situations; this de novo production of TNF-α is a crucial component of the so-called neuroinflammatory response linked to a number of neurological disorders [30]. Although many research have found that children with ASD have higher levels of inflammatory cytokines, few have isolated and stimulated circulating monocytes to find out how these cells respond dynamically. The results of these investigations showed that children with ASD have higher than normal levels of monocytes. [31,32] and neutrophils [33,34], contradicting the findings of the current study and Ellul et al., 2022, which showed no differences between the neutrophils from ASD and controls. However, our results demonstrated that there were no differences in total WBCs and

lymphocytes between the ASD group and the control group [35]. refuting the conclusions of Ashwood et al., 2011, and Harutyunyan et al., 2021, who discovered that ASD patients had greater median lymphocyte and total WBC counts than control individuals [36,37]. In addition, Robinson Agramonte et al. (2022) demonstrated that lymphocyte subsets were implicated in the pathophysiology of ASD and identified a decreased lymphocyte response to mitogens in children with ASD [38]. Ellul et al. (2022) recommended considering the discrepancy in findings among studies that were connected to various sources of heterogeneity. First, the heterogeneity of results may be influenced by differences in staining methods. Second, more homogeneous subgroups need to be researched because the ASD community is inherently heterogeneous [35]. This suggests that the humoral immune response of individuals with ASD is more impacted than their innate immune response. Therefore, the calculation of the platelets, lymphocyte ratio is important to prove this fact and as expected, Because platelets play a part in the immune response, our data showed that the ASD subject's ratio was similar to the control's. There is only one study that we are aware of that examined PLRs in ASD and found that they were comparable to those in healthy controls [36]. This study agreed with ours, but they did not explain their results, so this result needs more research to prove it in addition to the outcomes in the table (6) is non-significant (P>0.01) between Autism children and control, found in the current study disagreed with (Bijl et al.,2015), who also discovered that, in comparison to controls, ASD patients had higher average platelet counts [39].

Conclusion

Raise the levels of IL-6 and TNF-α in children with autism; this rise in TNF-α is a crucial part of the so-called neuroinflammatory response linked to a number of neurological conditions. Consequently, a comprehensive assessment of peripheral cytokine levels might provide a fresh approach to improving the prognosis of autistic people.

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Author's Contributions

Samar Abdul Raheem AL-Gharrawi, luma Qasim Ali suggested the research idea, performed the practical section, and Doaa Abd Al-hassan Abboud organized all the results, data, wrote and revised the manuscript. All authors agreed to the final version of this manuscript.

Ethics

The study was approved by ethics committee of the department of biology, college of science, mustansiriyah university , baghdad , iraq, as did the iraqi ministry of health and environment . committee number dated BCSMU/4024/0054Z written informed consent was obtained from all patients .

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