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The role of immune system dysfunction in the appearance of autism spectrum disorder

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Abstract
Autism Spectrum Disorder is a childhood psychiatric disorder characterized by a variety of cognitive, language and social impairments that can adversely affect the family. ASD are typically characterized by a lack of communication and social skills, accompanied by repetitive patterns of behavior, interest, and activities, and limited communication skills. A new record prevalence rate for autism was released by the National Health Center for Health Statistics. This report said that 1-in-36 children have ASD. Boys' prevalence is four- to five-fold higher than girls. The exact cause of autism spectrum disorder is yet to be determined. Given the complexity of the disorder and the possibility of varying symptoms and severity, there could be many contributing factors. Genetics and environment may both play a role in the development of ASD. There are a variety of examination tools that pediatricians use in their practices, but the most commonly used one is the Modified Checklist for Autism in Children, Revised with Follow-up (M-CHAT-R/F). Immunological dysfunction has been linked to ASD in studies on peripheral immune components in people with the disorder. Study objectives were firstly to evaluate the immune system performance in children with ASD, and secondly to determine if some immunological parameters can be used to diagnose ASD. The results showed a significant increase in the level of IL-6 ($P<0.001$), IL-1β ($P<0.01$) in ASD children when comparing with the control group. The findings of the current study, suggest the presence of immune dysfunction associated with ASD.

Introduction

Autism spectrum disorder (ASD) is one the most common neurodevelopmental disorders, This condition is characterized by limitations on repetitive behavior, interests, or activities, in addition to ongoing difficulties in social connections and reciprocal communication[(1)].

ASD could be present in as many as 1 in 36 children, according to the National Center for Health Statistics' most recent prevalence figure, which was issued in 2016 and revealed a new record high[(2)]. Boys seem to have a four- to five-fold higher frequency of ASD than girls. Although autism has been identified as a separate disorder it begins to appear early in life since the 1960s, its exact cause is still unknown but what is currently known beyond a reasonable doubt is that it is caused by the interaction between nature and nurture. Genetic susceptibility in the form of multiple genes leads to many biological disorders (nature) in favor of the negative neurological influence of many environmental hazards[(3)]. The etiology of autism spectrum disorders is yet unknown. Given the complexity of the disorder and the possibility of varying symptoms and severity, there could be many contributing factors. Genetics and environment may both play a role in the development of autism spectrum disorder[(3)]. Pregnant women may be unaware of the many factors that can increase the risk of autism in their unborn child. According to studies, getting the flu, getting a fever, or gaining too much weight while pregnant can all increase their chances of giving birth to a child with autism. Pregnant women who take valproate, an epilepsy drug, are up to seven times more likely to have children with autism than women who don't[(4)]. In spite of the fact that ASD is one of the most common neurodevelopmental disorders, it is also one of the most heterogeneous, which complicates the identification and diagnosis of this condition. In addition to the variability of symptoms and developmental changes that occur over the lifespan, the diagnostic evaluation can be quite complex. A stable and consistent diagnosis is essential, since it allows us to understand the individual and develop an individualized treatment plan based on that understanding. In the case of autism spectrum disorders, early diagnosis is crucial to tailoring early intervention strategies, which

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have proven to produce better long-term outcomes for the majority of children. Furthermore, diagnosis often determines access to service programs. Consequently, an age-appropriate and comprehensive diagnosis is the first step in understanding and treating ASD.

A twins study that was published in the 1970s was the one that initially suggested that the genetic etiology of ASD. Comparing the phenotypic concordance of dizygotic twins with around 50% genetic similarity to that of monozygotic twins with 100% genetic similarity is one way to assess the trait. In general, It was found that in monozygotic twins (60-90%) autism was more prevalent than in dizygotic twins (5- 40%) due to a greater genetic effect[5]. Known as a complex and genetically heterogeneous disease, autism spectrum disorder is characterized by multiple inheritance patterns and genetic variants. While environmental factors, such as infections and the use of certain drugs during pregnancy, are believed to contribute to its pathogenesis, its heritability has been estimated to be 50 to 90 percent, suggesting genetic factors contribute to its pathogenesis[6]. In 1976, Stubbs suggested that the immunity could play a role in autism spectrum disorders after he observed undetectable rubella antibody titers in autistic children who had been challenged with the rubella vaccine. Immunological challenges during pregnancy result in behavioral abnormalities, according to several studies in animal models[6]. It is well acknowledged that the causes of ASD are multifaceted. Immune-inflammatory processes occur throughout pregnancy and after birth in many classical neurological diseases, including schizophrenia, bipolar disorder, depression, post-traumatic stress disorder, and Alzheimer's disease. The immune system controls inflammation through a number of signaling pathways involving pro- and anti-inflammatory mediators, as well as long-term molecular/cellular effects. This may ultimately contribute to the behavioral and cognitive symptoms of ASD. A number of exogenous and endogenous stressors cause inflammation. Brain tissue post-mortems, cerebrospinal fluid (CSF) samples, and blood samples all show that immune system disorders are linked to ASD[7]. Immunological dysfunction has been linked to ASD in studies on peripheral immune components in people with the disorder. Children with ASD have been found to have elevated levels of monocytes, myeloid dendritic cells, natural killer (NK) cells, and abnormalities in NK cell cytotoxicity. Variation in frequencies and response to in vitro stimulation among T cells, frequencies of both mature and activated B cells have also been reported. Higher levels of pro-inflammatory cytokines including interleukin-1 beta (IL1- β), IL-6, IL-8, IL-17 and interferon-γ (IFN-γ) are reported in subjects with ASD. Cytokine mediators of Th17 cells have been suggested to have a role in ASD. For example, elevated levels of IL-17A, the predominant Th17 cytokine, has been detected in the serum of autistic children. It is hypothesized that changes in the fetal immunological environment increase the likelihood of developing ASD. The levels of inflammatory cytokines in the prenatal and neonatal brains are found to change in mice models of ASD due to maternal immune activation (MIA) brought on by inflammatory stimuli and microbial pathogens. These immunological changes cause aberrant CNS development in the offspring, which results in behavior similar to ASD[8]. Several complex illnesses, including ASD, are linked to abnormal quantities of these chemicals. In general, elevated levels of IL-5, IL-8, IL-13, IL-17, IL-12, IL-21, IL-22,IFNγ , TNF-α, TNF-receptor II17,IL-1β, and IL-6 have been seen. Memory and learning problems are linked to IL-1β, while stereotyped behavior and synapse development are linked to IL-6. These cytokines can alter how the brain processes cognitive and emotional information, as well as mood and sleep issues in ASD. TNF-α-levels and the severity of ASD symptoms are strongly correlated. It has been discovered that IL-1β increases the production of IL-17, an IL-8 mediator. As well, higher levels of IL-8 have been linked to a range of deviant behaviors in patients with autism spectrum disorders, including hyperactivity, low language skills, and cognitive impairment[7].

Materials and methods

This observational case control study was conducted From September 2021 to April 2022 and included the collection of serum samples for twenty autistic children and 20 healthy children ranging in age from three to ten years. Samples were collected from the Autistic Children's Care Center in Najaf and from Al-Hali laboratories to conduct tests for children with autism in Baghdad. The process of collecting samples from children with autism was based on some approved diagnostic criteria (autism spectrum examination questionnaire). After centrifugation, serum samples were saved at A (-20) °c in the blood bank at Al-Manathera General Hospital. All children participating in this study were Iraqi children, regardless of geographical location, and written consents were taken from their parents for the purpose of collecting blood samples.

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Inclusion criteria

This study included children with autism spectrum disorder ranging in age from three to ten years

Samples Collection

A sample of 3 ml of blood was collected from each patient and placed in a special test tube. Serum was separated and kept at -20°C for further analysis. The collection of blood samples was conducted according to medically recommended methods.

Methods

The micro ELISA plate provided in the kit has been pre coated with an antibody specific to Human(IL6, IL-1β).Samples (or Standards) are added to the micro ELISA plate wells and combined with the specific antibody. Then a biotinylated detection antibody specific for Human (IL6, IL-1β) were added, then Avidin-horseradish peroxidase (HRP) conjugate are added successively to each micro plate well and incubated. Free components are washed away. The substrate solution is added to each well. Only those wells that contain Human (IL6, IL-1β), biotinylated detection antibody and Avidin-HRP conjugate will appear blue in color. By adding stop solution, the enzyme substrate reaction is stopped, and the color changes to yellow. At a wavelength of 450 nm + 2 nm, the optical density (OD) is determined spectrophotometrically. The OD value is proportional to the concentration of Human (IL6, IL1Beta)(9).

Statistical analysis

Graph pad prism 9.2.1 was used to analyze the data, and the results are presented as mean ± standard deviation. A T-test and correlation analysis were used to quantify the degree of significance, which is displayed through the estimation plot.

Result and Discussion

Estimation of serum interleukin-6 (IL-6)

According to the measurement of serum IL-6, the ASD group had a significant difference over the control group, It was found that serum IL-6 levels were significantly higher in the ASD group than in the control group ($P<0.001$) as shown in figure(1).

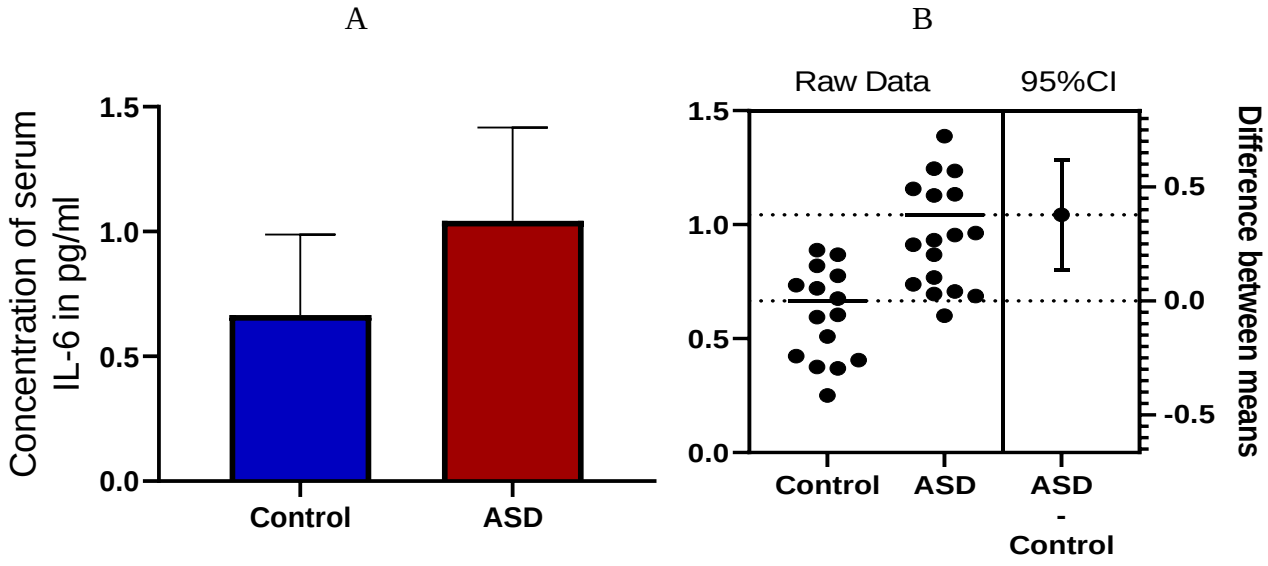

Figure (1): (A) comparing the levels of IL-6 in autistic children versus control children. (B) Estimation plot to illustrate the degree of significant

According to a comparison of IL6 level in the ASD group based on age, there is a significant difference between the males and females as shown in figure (2).

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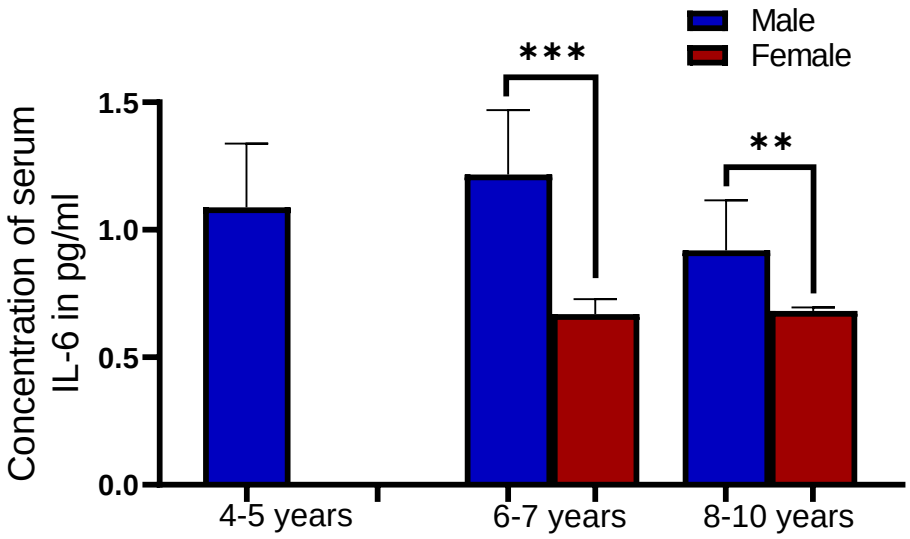

Figure (2): A t-test used to compare the ASD group between males and females revealed a significant difference by age

Estimation of serum interleukin-1 Beta (IL-1β)

In the ASD group, serum IL-1 beta levels were significantly differ when comparing with the control group, According to the results of serum IL-1beta measurements, there was a significant ($P<0.01$) increase in the ASD group as shown in figure(3).

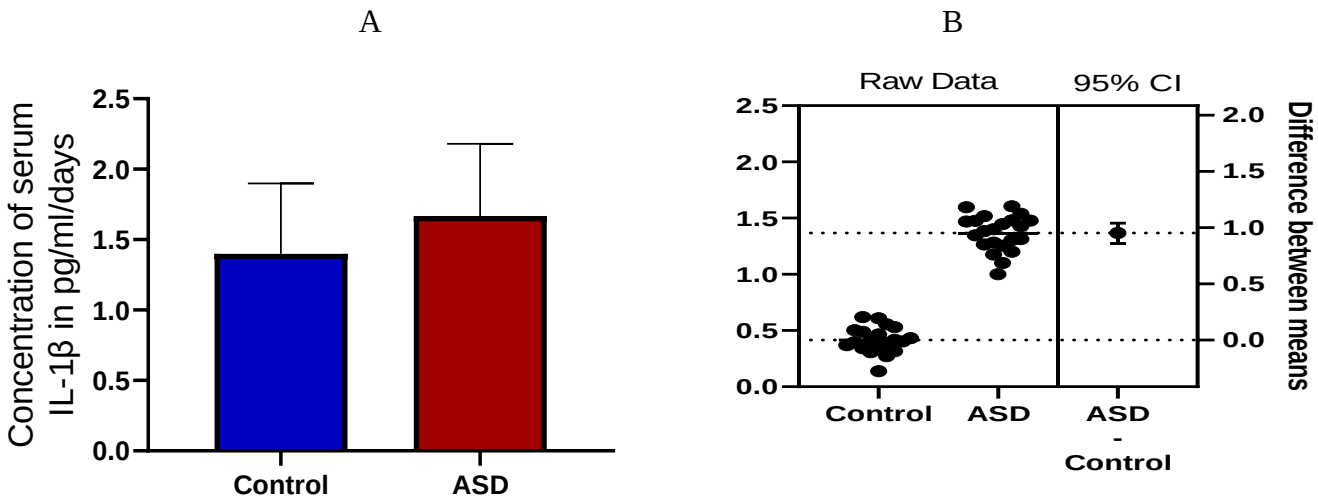

Figure (3): (A) Measurement of serum IL-1β showed a significant increase in ASD group more than control. (B) Estimation plot to illustrate the degree of significant

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A comparison of serum IL-1β level in the ASD group based on different age showed no significant difference between male and female as shown in figure (4).

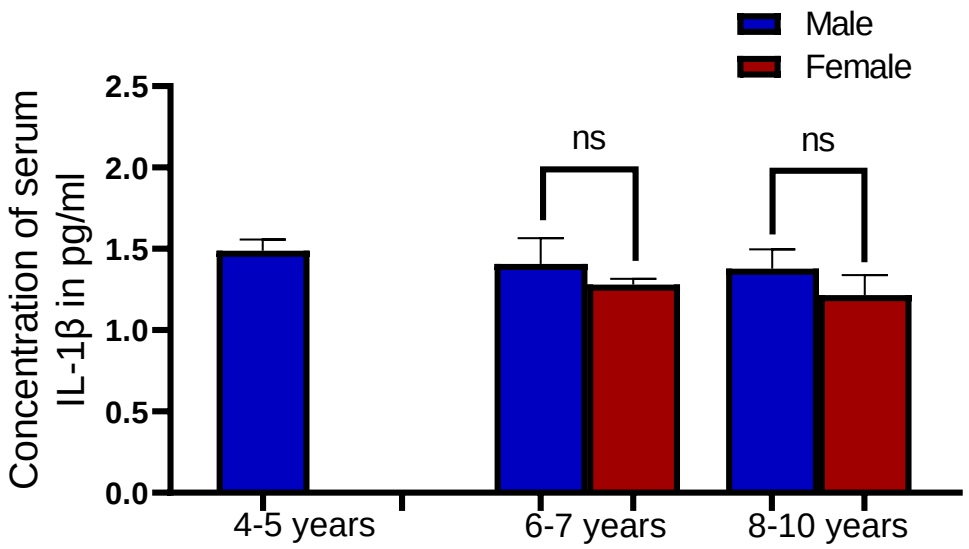

Figure (4): A comparison (IL-1β level) in the ASD group based on different age showed no significant difference

Discussion

ASD is a neurological condition caused by the degeneration of a complex neural system. As a result of the neural system's deformity, people with ASD have a variety of symptoms. It appears that at least some children with ASD suffer from immune system dysregulation. Defining the exact relationship between ASD symptoms and immune function is a challenge[10]. Researchers have shown alterations in the immune system in both animal models and humans as well as several studies have found a correlation between ASD symptoms and immunity[11],[12],[13]. These studies leave a lot of unanswered questions, including whether immune dysfunction is causal or rather a consequence of a larger problem. It is still unclear why individuals with ASD experience immune dysfunction and what role it plays in their aberrant behaviors[14]. Recently, there has been increased interest in immune system problems associated with autism spectrum disorders, including abnormalities in cytokine profiles and signaling. The goal is to determine whether cytokines can be used as biomarkers to determine subgroups of autism spectrum disorders, or as an objective measure of response to treatment[15].The high level of pro-inflammatory cytokines according to the data presented in this study is consistent with several studies that found a significant increase in the levels of pro-inflammatory cytokines[16]. In addition to being considered a pro-inflammatory cytokine, IL-6 has been identified as a molecular signal of sickness by the brain. It is also involved in metabolic and neural processes, as well as regenerative and anti-inflammatory activities. Stereotypical behavior has been associated with elevated levels of IL-1beta and IL-6. A pro-inflammatory cytokine called IL-1beta, expressed in the early stages of immune response, is implicated in learning and memory impairment. ASD can be associated with region-specific deviant brain growth due to IL-1beta ability to induce and inhibit neural progenitor cell proliferation in the CNS. Many studies reported an elevated level of IL-1β in subjects with ASD (children and adult) and claimed that the alteration is associated with behavioral severity. Many inflammatory states have been implicated in the release of IL-6, as previously highlighted[15],[17].

Conclusions

As a result, autism spectrum disorder can be caused by a variety of factors, including environmental, industrial, and agricultural pollutants, which disrupt the immune system. Thus, toxic metals increase autism symptoms. In spite of design issues or small sample sizes, extensive research indicates that a subgroup of

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individuals suffer from immune dysregulation. Alternatively, immune dysregulation may be comorbidly associated with autism spectrum disorders or impair neurodevelopmental processes directly, leading to ASD development. It is still not possible to cure ASD, so further research is needed to explore its etiology and the possibility of immune modulation. According to the findings of the current study, the identification of autistic individuals with immune dysfunction may help develop a biomarker to help develop diagnostic methods for this disorder and help develop accurate treatment approaches.

Recommendations

1. Conducting an extensive study that includes a large number of samples in order to obtain reliable information about children with autism, especially in Iraq.
2. The recommendation of the concerned authorities in this field on the work of confirmed and approved statistics showing the prevalence of autism spectrum disorder in Iraq because there are no approved statistics from the government or the Ministry of Health regarding the prevalence of autism spectrum disorder in Iraq.
3. Opening government specialized diagnostic and research centers in this field for understanding the mechanism of this disorder and for educating people to understand autism disorder.

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