Profile of Some Cytokines in Sera of Children with Autism Syndrome

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Abstract

Autism spectrum disorder (ASD) is a spectrum of behavioral anomalies characterized by impairment in social interactions and communication deficits. A potential role for immune dysfunction has been suggested in ASD. To test this hypothesis, certain cytokines: IL-2, IL-10, IL-12, IL-17A and IFN-γ were investigated in serum of all participants. The study includes: 39 child (male and female) aged < 5 to10 years with confirmed diagnosis of autism using standard assessment, age and gender matched 24 confirmed healthy children and 19 non autistic siblings used as controls. Serum was isolated and cytokines were detected using enzyme linked immunosorbent assay (ELISA). The observations indicate a significant increase ($P < 0.05$) in autistic patients serum levels of IL-10 compared with healthy control, but with lack of significant difference with their related non autistic siblings. Whereas detection of IL-12 and IFN-γ in the autistic patients serum showed significantly decrease level ($P < 0.05$) compared with healthy control, but with lack of significant difference with their related non autistic siblings. On the other hand, detection of IL-2 and IL-17A results showed no significant ($P > 0.05$) differences compared with healthy control and non autistic siblings.

Keywords: Autism spectrum disorder, cytokines, enzyme linked immunosorbent assay.

نسق بعض الحركيات الخلوية في مصول أطفال متلازمة التوحد

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الخلاصة:

أضطراب طيف التوحد (ASD) هو طيف من الشذوذ السلوكي الذي يتميز بضعف في التفاعل الاجتماعي واختلال التواصل. الدور المحتمل للخلل المناعي تم اقتراحه في اضطراب طيف التوحد. ولاختبار هذه الفرضية، أجري فحص مصول جميع المشاركين لتواجد الحركيات الخلوية IL-2, IL-10, IL-12, IL-17A, IFN-γ . شملت الدراسة: 39 طفل (ذكر وأنثى) تتراوح أعمارهم اقل من 5 إلى 10 سنوات بتشخيص مؤكد للأصابه بالتوحد وفق تقييم قياسي لمعهد الرحمن لرعاية التوحد، ومجاميع سيطرة بأجناس وفئات عمريه مماثله لإغراض المقارنة مكونه من (24 طفل سليم و 19 طفل من الأخوة غير المصابين بالتوحد). تم قياس تركيز الحركيات الخلوية IL-2, IL-10, IL-12, IL-17A,IFN-γ في مصول دم المشاركين باستعمال تقنية الأمتزاز المناعي المرتبط بالإنزيم (ELISA). أظهرت النتائج زيادة معنوية ($P < 0.05$) لمستوى IL-10 في مصول مرضى التوحد عند مقارنتهم مع الأطفال الأصحاء ، ولكن عدم وجود اختلاف معنوي مع أشقائهم غير المصابين بالتوحد. بينما الكشف عن مستويات IL-12 و IFN-γ في مصل أطفال

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التوحد أظهرت انخفاض معنوي ($P < 0.05$) عند مقارنتهم مع الأطفال الأصحاء، وعدم وجود اختلاف معنوي مع أشقائهم غير المصابين بالتوحد، بينما الكشف عن مستوى IL-2 و IL-17A أظهرت النتائج عدم وجود اختلاف معنوي عند المقارنة مع مجموعة السيطرة والأخوة غير المصابين.

Introduction

Autism spectrum disorders (ASDs), as termed in the International Classification of Diseases, 10th version [1], refer to a group of heterogeneous neurodevelopmental disorders characterized by qualitative impairments in social inter-action, communication and repetitive stereotypic behavior, with onset before 3 years of age[2,3]. Motor deficits, aggressive behavior, abnormal sleep patterns, gastrointestinal problems, epilepsy and intellectual disability are also observed [4, 5].

The exact aetiology of ASDs is unknown; likely it results from a complex combination of genetic, environment, and immunological factors [6,7]. Correlations between pro-inflammatory cytokine levels and autistic symptoms have been reported [8]. An early inflammatory process has been proposed as the potential etiology of ASDs [9]. The immune system is one such network that has been shown to be very important during neurodevelopment as well as in adult brain homeostasis [10], and perturbations of which have been linked to autism.

While the etiology and pathogenesis of autism are poorly understood, there is evidence that immune system abnormalities are associated with symptoms in a substantial number of affected individuals [11]. Immune dysfunction plays a major role in the pathophysiology of ASD [12]. Inflammatory changes in the central nervous system (CNS) [13], and the peripheral immune system [14], have been repeatedly reported in different biologic samples of individuals with ASD. Interestingly, such dysfunctional immune profiles have been reported during pregnancy, after birth and post mortem which may indicate an ongoing immune dysfunctional profile in individuals with ASD [15]. The presented introductory theme promoted the present study to be carried out with the aims to evaluate some cytokines that may have an impact on the aetiopathogenesis of ASD in a sample of Iraqi patients.

Materials and methods

The study was carried out on 39 Iraqi children (male and female) aged > 5 - 10 with autistic disorder who were registered in AL-Rahman Specialist Institute of Autism Care/ Baghdad. The diagnosis was made by the medical staff responsible at the Institute on the basis of international criteria. All patients were not under drugs and they did not suffer from any other disease. For the purpose of comparison, 43 age and gender matched children were enrolled as a control including: 19 non autistic siblings and 24 healthy individuals. From each participating subject, 3-5 ml of blood was obtained by venipuncture. The collected blood was transferred to a plain tube and left to clot at room temperature (20-25°C) for 15 minutes. The clotted blood was centrifuged at 2000 rpm for 15 minutes; and by then, serum was collected and distributed into aliquots of (200 µl) in Eppendorf tubes, which were frozen at -20°C until laboratory assessments. Serum samples were collected from all study individuals to determine the seropositivity levels of certain cytokines: IL-2, IFN-γ, IL-12, IL-10 and IL-17A using enzyme linked immunosorbent assay (ELISA) and laboratory kits used by PeproTech-USA Company, during the period from January to March 2013. Furthermore, an informed written consent of participation in the study was signed by the parents or the legal guardians of the all studied subjects.

Statistical analysis

Data were entered and analyzed by using SPSS (Statistical Package for Social Sciences) version 21.0 for Windows. Descriptive statistics (frequencies, percentages, tables, graphs) and inferential statistical were used. Independent T-test used to compare mean between cases and controls. P-value < 0.05 was considered Statistically Significant [16].

Results and Discussion

Interleukin-2

Data observed no significant difference (P>0.05) between the mean serum level of IL-2 in the autistic children compared with the non-autistic siblings and the healthy control children table 1.

Interleukin-10

The study observations indicate significant increase in autistic children serum level of IL-10 compared with the healthy control ($p < 0.05$), but with lack of significant difference with their related non-autistic siblings table 2.

Table 1-Mean serum levels (pg/ml) of IL-2 in study samples.

Parameter	Case-control	N	Mean ± SE*	95% Confidence Interval for Mean	
				Lower Bound	Upper Bound
IL-2	Autism	39	14.47 ± 1.23 [a]	11.97	16.97
	non autistic(siblings)	19	14.45 ± 1.51 [a]	11.26	17.64
	Healthy control(non relatives)	24	16.52 ± 1.61 [a]	13.18	19.87

* Similar letters = No significant difference ($P > 0.05$) between means.
LSD value = 2.398
$\alpha = 0.05$

Table 2-Mean serum levels (pg/ml) of IL-10 in study samples.

Parameter	Case-control	N	Mean ± SE*	95% Confidence Interval for Mean	
				Lower Bound	Upper Bound
IL-10	Autism	39	13.35 ± 1.00 a	11.32	15.37
	non autistic(siblings)	19	10.61 ± 1.25 ab	7.97	13.25
	Healthy control(non relatives)	24	8.82 ± 1.25 b	6.22	11.41

* Different letters = Significant difference ($P \leq 0.05$) between means.
LSD value = 1.927
$\alpha = 0.05$

Interleukin-12

The serum level of IL-12 was significantly decreased ($P \leq 0.05$) in autistic children group (38.67 ± 4.82 pg/ml) as compared with the healthy control group (59.53 ± 8.82 pg/ml), while no significant difference ($P>0.05$) was seen between the autistic children and the non autistic siblings, and that of the non autistic siblings and the healthy control children table 3.

Interleukin-17A

The detected results indicate that the children with autism and their non autistic siblings had no significant difference ($P>0.05$) in serum IL-17A level compared with the healthy control children table 4.

IFN- γ

It was noticed that serum level of IFN- γ was significantly decreased ($p < 0.05$) in autistic children (18.77 ± 1.19 pg/ml) as compared with the healthy control (24.77 ± 1.80 pg/ml), whereas no significant difference ($P>0.05$) was observed between the autistic children and their non autistic siblings table 5.

Despite the large number of data reported on autism til lately [3], and the possible involvement of ASD with immune dysfunction [17], the results are still inconclusive. This is probably attributable to the large phenotypic and genetic heterogeneity of ASD [18].

In the present study as mentioned earlier serum levels of IL-2 and IL-17A in the subjects with autism revealed no statistical difference than those of control subjects (healthy siblings and healthy individuals). While IL- 12 and IFN- γ showed a significant decrease ($P \leq 0.05$) than those of healthy control individuals. Whereas IL-10 was the only detected cytokine that showed significantly higher mean concentration value than those of healthy control individuals.

Earlier studies have reported altered results in cytokine patterns in the sera or plasma of patients with ASD [19-25]. Some of these studies have demonstrated increased levels of inflammation inducing cytokines such as IFN- γ or IL-12[24]. Others with increased levels of IL-12, IL-17A and IFN- γ [25].

Later, AL-Ayadi and Mostafa[26], confirms the presence of high levels of the pro-inflammatory cytokine IL-17A in the serum of autistic patients. Whereas others indicate significantly lower ($p < 0.05$)values of cytokines (IL-2, IL-10, IL-12, IL-17A and IFN- γ) in autistic children [27], While earlier Enstrom *et al*. [23], reported no difference in the level of IL-17A. This difference in the results was attributed by some to the age of the subjects (2-5 years) involved which they may be too young to reliably detect elevated levels of IL-17A [23].This explanation does not fit with the results obtained in this study, since the subjects enrolled were at age of > 5 to 10 years, but it may be due to the status of the patients, since the elevated level of IL-17A was correlated significantly with the severity of autism [26], whereas the subjects in this study were not identified to mild, moderate, and severe autistic patients. Collectively, the shift in cytokine balance in ASD is linked with severe impairments in key autism behavioral domains, including social interaction and communication, as well as associated features such as aberrant behaviors [28, 24].

Table 3-Mean serum levels (pg/ml) of IL-12 in study samples.

parameter	Case-control	N	Mean ± SE*	95% Confidence Interval for Mean	
				Lower Bound	Upper Bound
IL-12	Autism	39	38.67 ± 4.82 a	28.89	48.45
	non autistic(siblings)	19	50.81 ± 8.65 ab	32.64	68.98
	Healthy control(non relatives)	24	59.53 ± 8.82 b	41.28	77.78

* Different letters = Significant difference ($P \leq 0.05$) between means.
LSD value = 2.564
$\alpha = 0.05$

Table 4-Mean serum levels (pg/ml) of IL-17A in study samples.

parameter	Case-control	N	Mean ± SE*	95% Confidence Interval for Mean	
				Lower Bound	Upper Bound
IL-17A	Autism	39	9.39 ± 1.33 a	6.68	12.10
	non autistic(siblings)	19	8.02 ± 1.27 a	5.35	10.69
	Healthy control(non relatives)	24	8.76 ± 1.43 a	5.79	11.74

* Similar letters = No significant difference ($P > 0.05$) between means.
LSD value = 2.361
$\alpha = 0.05$

Table 5-Mean serum levels (pg/ml) of IFN- γ in study samples.

parameter	Case-control	N	Mean ± SE*	95% Confidence Interval for Mean	
				Lower Bound	Upper Bound
IFN- γ	Autism	39	18.77 ± 1.19 a	16.35	21.18
	non autistic(siblings)	19	21.38 ± 1.90 ab	17.39	25.37
	Healthy control(non relatives)	24	24.77 ± 1.80 b	21.04	28.49

* Different letters = Significant difference ($P \leq 0.05$) between means.
LSD value = 2.564
$\alpha = 0.05$

The results observed suggest that IL-10 (anti-inflammatory and regulatory cytokine) may play a role in the pathogenesis of autism. According to the observations obtained no clear evidence was found for skewing toward either Th1 or Th2. Instead, our findings indicate statistically significant ($P \leq 0.05$) elevation of anti-inflammatory and regulatory cytokine IL-10 response.

Elevations of either Th1 or Th2 cytokines should produce a subsequent elevation of IL-10 to modulate the inflammatory response [29]. Whereas earlier Ashwood *et al.* [30], report an inverse relationships between raised pro-inflammatory and decreased regulatory $CD3^+$ $IL\text{-}10^-$ activities, which was consistent between sites and mucosal compartments for a children with autism and gastrointestinal symptoms. All these support the concept of an immune dysfunction in autistic children [31-32].

Increase in IL-10 cytokine has also been correlated in other neuronal loss or neurodegenerative disorder patients. Angelopoulos *et al.*[33] and Al-Ganzawi [34] reported elevated IL-10 in serum of their patients with dementia and Alzheimer by suppression of pro-inflammatory cytokines disease respectively. Hence, reducing inflammation by suppression of pro-inflammatory cytokines. Interlukin-10 is an inhibitor of activitated macrophages and dendritic cells and is thus involved in the control of innate immune reactions and cell-mediated immunity. Because of these actions IL-10 will inhibit the productions of IL-12 and this in turn will stop stimulating the secretion of IFN- γ [35].

Recently a study concerning the related behaviors on autism showed an association of IL-10 with lower levels of autism-related behavioral impairments. While increase in pro-inflammatory cytokines including IL-12p40, IL-6, IL-1β and IFN- γ levels were associated with more severe behavioral deficits in children with autism [36-37]. Moreover, Ashwood *et al.* [24] suggested that Th1 skewing may be associated with more impaired behaviors, whereas Th2 responses may be associated with improved developmental and adaptive function. These suggest that immune activation including activation of T- lymphocyte subsets may be important in modulating and potentially improving behaviors in some individual with autism.

Cytokines play an important role in the cross-talk between the immune and central nervous systems. Cytokines and products of the immune system have wide spread effects on neuronal pathways, and may potentially play a role in many common ASD features such as mood and sleep disturbances [30].

The study data did not reveale any significant difference in cytokine levels between children with autism and their non-autistic siblings. This result is in line with a previous studies [3, 38], which observed that the immune profile of children with autism did not differ from their typically developing siblings. Saresella *et al.* [38], indicate the presence of an" autism endophenotype" that expands immune dysfunction to family members who are seemingly not affected by the core symptoms of autism. Moreover, both children with autism and their unaffected siblings shared the presence of anti-brain antibodies [3, 39].

It is possible also that a common immunogenetic background shared by siblings might eventually lead to different clinical outcomes when an environmental stress (prenatal exposure to environmental toxins, viral and bacterial infection, parental microchimerism, etc.) occurs during the development [3].

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