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Serum Levels of S100b, Interleukin-6 and Anti-Transglutaminase Ii IgA as Immune Markers in a Sample of Egyptian Children with Autistic Spectrum Disorders

Shaker NM[1], Taha GRA[1]*, Kholeif H[2], Sayed NM[1], El-Sheikh MM[1] and Abulmagd ML[3]

[1]*Ain Shams University, Cairo, Egypt*
[2]*Department of Medicine, Al Azhar University, Cairo, Egypt*
[3]*Institute of Childhood Studies, Cairo, Egypt*

Abstract

Background: Autism spectrum disorder (ASD) is a severe neuro-developmental disorder. Various immune components and mediators have been investigated in ASD with controversial results. The purpose of this study was to: 1) investigate the levels of S100B protein (as a marker of neuronal damage), IgA autoantibodies to transglutaminase II (TG2) (as an indicator for presence of autoimmunity) and interleukin 6 (IL-6) (a pro-inflammatory cytokine), in sera of a group of autistic children, 2) explore the relation between serum levels of these parameters and severity of autism, 3) find out if there is any association between serum levels of S100B protein, IL-6 and TG2 IgA which might give clue to their pathogenic role in ASD.

Methods: The levels of S100B protein, IL-6 and TG2 IgA were measured in the sera of 30 autistic children aged from 3 to 14 years. These levels were compared to those of 22 matched healthy children aged from 3 to 13 years. Assessment of clinical parameters and severity of autism was done using Gilliam Autism Rating Scale.

Results: Autistic children showed higher significant serum S100B protein and IL-6 levels compared to healthy controls (P=0.003 and 0.002 respectively). No significant correlations were found between serum levels of S100B, IL-6, TG2 IgA and clinical parameters/severity of autism. Serum levels of S100B had significant negative correlation with TG2 IgA levels (P=0.037) and marginally significant positive correlation with IL-6 levels (P=0.05).

Conclusion: The significant elevations of S100B and IL-6 levels in sera of autistic children possibly imply an underlying neuropathological condition in autistic patients. Anti-TG2 antibodies may not have a possible contributing role in some ASD children. More research is needed to investigate any possible link between serum S100B protein, IL-6 levels and other brain autoantibodies as potential indicators of brain autoimmunity in ASD patients.

Keywords: ASD; Autoimmunity; S100B protein; Interleukin-6; Anti-transglutaminase antibody

Introduction

Autism spectrum disorder (ASD) is a severe neuro-developmental disorder that is increasing dramatically. It is defined by deficits in social development and communication and repetitive and stereotypic behaviours. It usually starts in early childhood and it is a lifelong condition for most [1].

Despite the unknown aetiology of the disorder, studies have suggested that the susceptibility to autism is attributable to genetic factors [2,3]. In addition, emerging evidence for the involvement of altered immune response in the pathogenesis of ASD is now gaining strength [4] and could be appropriate potential targets for pharmacological intervention [5]. The immune system plays an important role in neurodevelopment, regulating neuronal proliferation, synapse formation and plasticity, as well as removing apoptotic neurons [6]. Various components and mediators of the immune system including immunoglobulin levels, cytokines, cellular numbers and responsiveness, monocytes/macrophages and natural killer cells were repeatedly investigated in ASD [7-11]. Despite the controversial results, there is a wide agreement that a subgroup of autistic patients demonstrate abnormal or dysregulated immunity [12].

One of the frequently described immune abnormalities in patients diagnosed with ASD is altered cytokine profile [13,14]. Numerous studies showed that different cytokines were increased in the blood, brain, and cerebrospinal fluid of autistic patients [9,14-23]. Recent evidence emphasizes a crucial role for Interleukin 6 (IL-6) in the central nervous system (CNS). IL-6 is expressed normally at relatively low levels and increases under pathological condition [24]. Findings from post-mortem and animal studies point to the possibility that brain IL-6 may be involved in the mediation of autism-like behaviours via impairing neuronal plasticity and neuroanatomical structures [5].

Another component recently highlighted in autistic patients is S100B proteins, which are low molecular weight, calcium-binding proteins that interact with other proteins to modulate biological processes [25]. The clinical significance of S100B protein stems from the fact that it is primarily produced by brain astrocytes. In addition, it is an established biomarker of altered permeability of the blood brain barrier (BBB) associated with various CNS diseases [26,27]. Different studies reported that elevated S100B protein levels indicate neuronal damage [28-30]. Therefore, increased levels of S100B protein in ASD

***Corresponding author:** Taha GRA, Ain Shams University, Cairo, Egypt, Tel: 0020 1115681197, Fax: 00202 26824738, E-mail: ghadarefaat@gmail.com

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patients may indicate the presence of an underlying neuropathological condition [31].

On the other hand, a possible role of autoimmune abnormalities was frequently speculated in the pathogenesis of autism [32-35]. Several brain autoantibodies were reported with high levels in some autistic children [31,33,36]. Till now, the reasons underlying the formation of these autoantibodies in ASD subjects are not fully understood. It is postulated that an immune reaction might be trigged off by a cross reaction with antigens in the environment causing the release of some self-antigens, which in turn may result in induction of autoimmune reactions through activation of inflammatory cells in genetically susceptible subjects [32,33]. The core concept to establish an immunopathogenic role of the brain auto-antibodies is to prove their effects on specific brain functions [37].

Different brain auto-antibodies were investigated in autistic patients with controversial results. High serum levels of IgA autoantibodies directed against tissue transglutaminase II (TG2), has been reported in some autoimmune disorders as celiac disease [38,39], several neurodegenerative diseases [40] and recently in ASD [41]. TG2 is a widely distributed multifunctional enzyme expressed throughout the body, including brain and small intestine. The TG2 expressed in the brain has been shown to be important in cell adhesion and synaptic stabilization [41]. Previously, TG2 was known to be important for normal neural development as some studies showed elevated transglutaminase activity during early development, especially in the cerebellar cortex [42]. During development, TG activity mediates formation of protease resistance cross-links between synaptic proteins that enhance neurite outgrowth [43,44] and causes stabilization of neuronal synapses [45]. Boscolo et al. [46] have further shown that anti-TG2 antibodies alone are sufficient to induce ataxia in mice, supporting the notion that an autoimmune response targeting TG2 can have neurological consequences.

Based on the growing evidence suggesting that researchers should be looking into issue of autoimmunity in ASD with much greater assiduity and whether markers of autoimmunity might be related to presented symptoms, the authors propose that a subset of autistic children may show altered immunological function and response of the neuronal tissues. These responses may be correlated with features and severity of autism. Consequently, they can be used as biomarkers for this subset and can be also a target for new interventions. Therefore, the aims of the current work were: (1) investigate various immune markers as serum levels of S100B protein (marker of neuronal damage), TG2 IgA (indicating the presence of autoimmunity) and IL-6 (one of the pro-inflammatory cytokines), in a group of children with autism spectrum disorders. (2) Explore the relationship between levels of S100B protein, IL-6 and TG2 IgA in serum and clinical features/ severity of autism. (3) Find out if there is any association between levels of S100B protein, IL-6 and TG2 IgA in serum that could give a clue to their pathogenic role in ASD patients.

Materials and Methods

Participants

This cross-sectional case- control study included 52 children; 30 autistic patients and 22 healthy matched free of any medical or psychiatric illness. The autistic children were diagnosed according to ICD-10 criteria and selected from the child psychiatry outpatient clinics of the Institute of Psychiatry and the Institute of childhood studies, Ain Shams University, Cairo, Egypt. They were comprised of 27 (90%) males and 3 (10%) females. Their age ranged from 3 to 14 years [median (IQR)=4 (2)]: 83% of the patients (n=25) aged from 3-5 years, 6% (n=2) aged from 6-7 years, 6% (n=2) aged 9-10 years and only one patient (3.3%) aged 14 years (Figure 1). Patients under medication, those with neurological diseases or metabolic disorders were not included in the study. Patients having past history or family history of autoimmune disorders, allergic disorders or inflammatory disorders were also excluded (Figure 1).

The healthy matched control group was comprised of 20 (90.9%) males and 2 (9.1%) females. Their age ranged from 3 to 13 [median (IQR)=4 (3)]: 45.5% (n=10) of the controls were of 3 years old, 45.5% (n=10) aged from 4 to 6 years, only one patient (4.5%) aged 10 years and another patient aged 13 years (4.5%) (Figure 2). Matched controls were randomly selected from relatives of children attending the pediatric out-patient clinics of Ain Shams University hospitals, Cairo, Egypt. Full clinical and psychiatric history was taken from caregivers of controls using the child psychiatry and pediatric clinical sheets of Ain Shams University hospitals to exclude any medical or psychiatric illness (Figure 2).

Subjects participated in the study after obtaining an informed consent from their parents. The research was approved by "ethics of academic research committee" at Ain-Shams University.

Clinical assessment of autistic patients

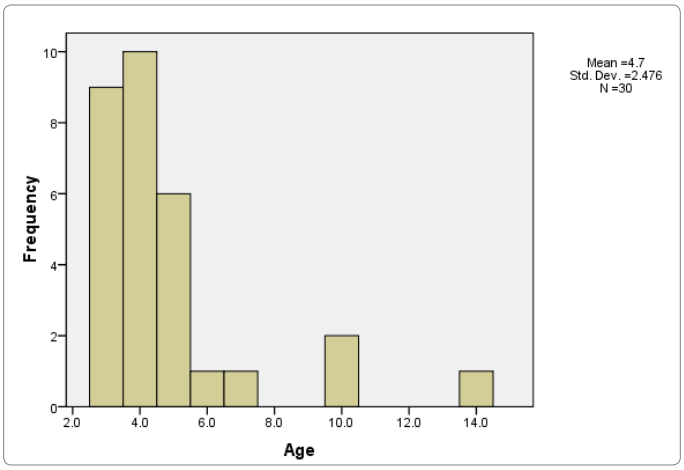

Figure 1: Histogram showing age distribution of cases.

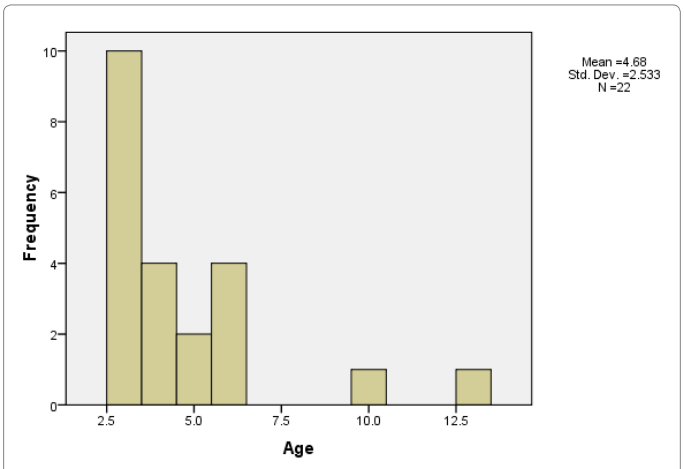

Figure 2: Histogram showing age distributions of controls.

Patients were diagnosed according to ICD-10 criteria for autism. Full clinical and psychiatric history was taken from caregivers using the child psychiatry and pediatric clinical sheets of Ain Shams University hospitals including: age, developmental history; educational level; socio-economic status; history of allergy; autoimmune disease or neurological disorder. Gilliam autism rating scale (GARS) was used to assess the severity of autistic symptoms [47], Arabic version [48]. This test is used for diagnosis and assessment of the severity of autistic features for ages 3-22 years with good reliability and validity. It consists of 56 items, subdivided into 4 subscales: communication, social interaction, stereotyped behaviors, development and total score. The lower the score, the worse the condition.

Sample collection and processing

Three mL of venous blood samples were collected aseptically in plain sterile vacutainer tubes from each subject in this study. Coagulated blood was then centrifuged and sera were separated and stored at -20°C until use. Each sample was subjected to following measurements:

Human tissue transglutaminase II immunoglobulin A (TG2 IgA): INOVA Quanta lite ™ h-TG2 IgA ELISA test was used. It is a semi-quantitative detection of IgA antibodies to tissue transglutaminase in human serum.

S100B concentration in human serum, DRG, Inc. USA ELISA kit was used Avi Bion Orgenium laboratories. This kit allows the determination of S100B from 10 to 5000 pg/ml.

Human IL-6 by Avi Bion Human IL-6 ELISA kit, Vantaa Finland: Standard curve was used to determine the amount of IL-6 pg/ml in each sample.

In all ELISA tests, samples were run in duplicate as recommended in the user manual of each kit to establish the accuracy of every assay.

Statistical Analysis

The computerized version of the Statistical Package for Social Sciences, 17th version (SPSS 17) was for statistical analysis of the data. Using the One-Sample Kolmogorov-Smirnov normality test, data of age, S100B protein and IL-6 were non-parametric (skewed); other data including serum TG2 IgA were normally distributed. Quantitative data were presented as means ± SD for parametric data; medians, range and interquartile range (IQRs) for nonparametric data. Pearson Chi Square Test (X^2) and Fisher exact test were used to detect presence or absence of significant association between two categorical variables. Student's t-test and Mann- Whitney U test were used to compare the mean scores of 2 independent groups (2-tailed). Correlations (2-tailed) were calculated using Pearson coefficient and Spearman's rho correlation coefficient. The level of statistical significance was $P<0.05$ for all tests. Serum levels of S100B protein or TG2 IgA or human IL-6 were considered elevated if their levels were above the following cutoff values (123.4 pg/ml, 4.4 U/ml, 7.8 pg/ml, respectively). The cutoff value of serum S100B protein was defined by the 95th percentiles of healthy controls; IL6 and serum TG2 IgA cut-off scores were calculated according to the manufacturer.

Results

Description of the sample

Autistic children (n=30) and controls (n=22) were matched regarding their age, gender and socioeconomic status (Table 1). Most autistic children were typical autism according to ICD-10 criteria. The mean score of autistic symptom severity measured by GARS was 96.8 ± 9.1 (average severity). The majority of the sample (90%, 27/30) had average to below average severity of autism.

S100B protein, TG2 IgA and IL-6 serum levels in patients with autism compared to healthy controls

Patients with autism showed significantly higher S100B protein serum levels [median (IQR)=123.8 (48.8)] than healthy controls [median (IQR)=112.8 (26)] (P=0.003) (Table 2). According to the previously determined cutoff value for positivity (95th percentile of the controls), increased serum S100B protein levels were found in 50% of autistic patients (15/30).

Autistic children also showed significantly higher IL-6 serum levels [median (IQR)=7.59 (24.4)] than healthy controls [median (IQR)=4.61 (3)] (P=0.002) (Table 2). Increased serum IL-6 levels were found in 43% (13/30) of autistic patients compared to 18.2% of controls (4/22) (P=0.05).

Although 50% of autistic patients (15/30) had increased serum TG2 IgA, however, they did not show significant difference compared to controls neither in frequency (36.4%, 8/22) ($x^{2=}$0.96, P=0.33) nor in the mean serum levels (4.87 ± 2.1, 4.31 ± 0.85 respectively, P=0.19) (Table 2).

Relationship between serum levels of S100B protein, IL-6 and TG2 IgA and clinical severity and parameters of autism

Patients with atypical autism recorded higher serum levels of S100B protein and serum IL-6 than those with typical autism, however without statistical significance (P=0.5, 0.33, respectively). On the

	Cases (n=30)	Controls (n=22)	Test	P-value
Age [median (IQR)]	4 (2)	4 (3)	Z=-0.14 #	0.89
Gender				
Male	27 (90%)	20 (90.9)	X2=0.01≠	2.6
Female	3 (10%)	2 (9.1)		
Socioeconomic status				
High	3 (10%)	2 (9.1%)	X2=0.26≠	0.9
Middle	17 (56.7%)	14 (63.6%)		
Low	10 (33.3%)	6 (27.3%)		
Type of autism				
Typical	24 (80%)			
Atypical	6 (20%)			
Severity of autism				
Average	21 (70%)			
Below average	6 (20%)			
Above average	2 (6.6%)			

IQR: Interquartile Range, ≠ Pearson Chi Square Test (X2), # Mann- Whitney U test

Table 1: Sample characteristics.

	S100B	TG2 IgA	IL-6
	Median (Range, IQR)	Mean (SD)	Median (Range, IQR)
Cases (n=30)	123.8 (493.6, 48.8)	4.87 (2.1)	7.59 (995.4, 24.4)
Controls (n=22)	112.8 (37.1, 26)	4.31 (0.85)	4.61 (53.1,3)
Test	Z=-2.96 #	*t*=1.32 ≠	Z=-3.09 #
P-value	0.003*	0.19	0.002*

TG2 IgA: Tissue Transglutaminase Immunoglobulin A; IQR: Interquartile Range; SD: Standard Deviation, ≠ Student's t-test, # Mann-Whitney U test, * P<0.05

Table 2: S100B protein, TG2 IgA and IL-6 serum levels in patients with autism compared to healthy controls.

contrary, serum TG2 IgA level was higher in typical autistic patients, also without statistical significance (P=0.18) (Table 3).

Serum levels of S100B protein, IL-6 and TG2 IgA were not correlated with the severity of autistic symptoms measured by GARS (r=-0.19, P=0.3; r=0.18, P=0.4; r=-0.13, P=0.5, respectively). Also, they did not show significant correlations with any of the clinical domains of GARS (Table 4). However, patients who showed high S100B protein levels had worse development [median (IQR)=9 (2)] than those with serum S100B protein levels below cut-off for positivity [median (IQR)=10 (1)] with marginal significance (Z=-1.92, P=0.05) (Table 4).

Relationship between elevated S100B protein, TG2 IgA and IL-6 serum levels in autistic group

Using Spearman's rho correlation test, serum levels of S100B protein were negatively correlated with TG2 IgA levels (r=-0.29, P=0.037) (Figure 3) and had marginally significant positive correlation with IL-6 levels (r=0.27, P=0.05). Moreover, the TG2 IgA mean scores of patients showing high S100B protein serum levels were lower than those with serum S100B protein levels below cutoff for positivity (4.08 ± 1.5, 5.72 ± 2.2, t=2.3, P=0.03). Patients with high IL-6 levels recorded higher rates of elevated S100B protein serum levels (61.5%, 8/13) than patients with normal serum IL-6 levels (38.5%, 5/13), however without statistical significance (P=0.46). TG2 IgA serum levels were not correlated with IL-6 levels (r=0.12, P=0.42) (Figure 3).

Discussion

The pathogenic role of immune system abnormalities in some psychiatric disorders, including ASD, is a new idea with growing interest among neuroscientists [49]. It may represent new horizon in the treatment of autism [50]. In this context, various immune hypotheses have been attempted, such as maternal autoantibodies, childhood vaccinations, and increased incidence of autoimmunity and increased expression of inflammatory cytokines [24].

	S100B	TG2 IgA	IL-6
	Median (Range, IQR)	Mean (SD)	Median (Range, IQR)
Typical autism (n=24)	121.5 (165.7, 50.9)	5.14 (2.2)	7.4 (995.4, 24.7)
Atypical autism (n=6)	126.45 (448.8, 145.2)	3.84 (0.96)	23.2 (339.9, 102.6)
Test	Z=-0.67 #	*t*=1.38 ≠	Z=-0.97 #
P-value	0.5	0.18	0.33

TG2 IgA: Tissue Transglutaminase Immunoglobulin A; IQR: Interquartile Range; SD: Standard Deviation, ≠ Student's t-test, # Mann-Whitney U test

Table 3: S100B protein, TG2 IgA and IL-6 serum levels in typical and atypical autistic patients.

		Stereotyped behaviors	Communication	Social interaction	Development
S100B#	r	-0.05	0.14	0.18	-0.17
	P	0.76	0.47	0.36	0.38
TG2 IgA ≠	r	-0.01	0.22	0.15	0.12
	P	0.95	0.26	0.45	0.55
IL-6	r	-0.1	-0.19	-0.07	-0.15
#	P	0.6	0.3	0.71	0.43

GARS: Gilliam Autism Rating Scale, TG2 IgA: Tissue Transglutaminase Immunoglobulin A, ≠ Pearson coefficient and # Spearman's rho correlation coefficient

Table 4: Correlations between S100B protein, TG2 IgA and IL-6 serum levels and clinical domains of GARS.

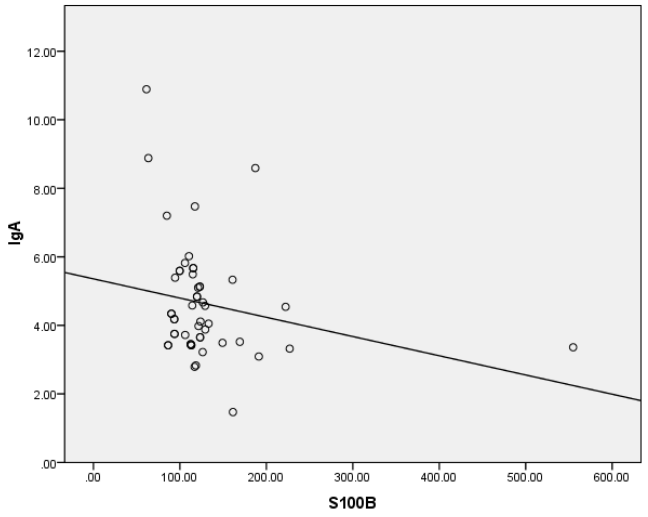

Figure 3: Negative correlation between S100B protein and TG2 IgA serum levels among autistic children using Spearman correlation coefficient (r=-0.29, P=0.037).

Consistent with the previous research, findings from the current study showed significant elevations of S100B protein and IL-6 serum levels in autistic subjects compared to healthy controls. Similarly, Al-Ayadhi and Mostafa [31] reported significant elevation of S100B in sera of 35.9% of their autistic sample versus 50% of the current autistic sample. S100B protein is chiefly found in glial and Schwann cells. It is also considered as a marker of neuronal damage and dysfunction [51]. Thus, increased serum levels of S100B protein can possibly indicate an underlying neuropathological condition in some autistic patients [31]. This may also contribute to the reporting of Quincozes-Santos et al. [26] that significant increment of 80% of the secreted S100B protein was induced by risperidone, a drug used to improve core features of autism, proposing that glial cells are targets of risperidone.

Whereas significantly elevated levels of IL-6 (43% autistic versus 18.2% controls in the current sample) were similarly reported by several researchers [14,20-23,52-55]. The current result concurs with the report of Vargas et al. of increased IL-6 levels in the brain and CSF of individuals with ASD, characterized by chronic state of neuroinflammation [56]. In a series of studies investigating the role of IL-6 in autism, Wei et al. showed significantly elevated IL-6 in the fontal cortex and cerebellum of autistic subjects and that mice overexpressing IL-6 display changes in brain morphology similar to that found in autistic subjects indicating that elevated IL-6 in the brain act as a neuroimmune factor that could mediate neuroanatomical abnormalities [22]. They also proposed that IL-6 elevation in the autistic brain can alter neural cell migration and adhesion and can also cause an imbalance of inhibitory and excitatory circuits [57].

Interestingly, some studies have investigated the relation between S100B and IL-6 in the pathogenesis of different neurological and psychiatric disorders including autism [31,58-60]. S100B seems to be able to modulate cytokine secretion and may be also modulated by pro-inflammatory cytokines [61,62]. In vitro studies revealed that high levels of S100B protein can induce the neuronal expression and secretion of proinflammatory cytokine IL-6 [63,64]. These reporting may explain the marginally significant positive correlation between mean serum levels of S100B and IL-6 (p=0.05) in the current study. Moreover, de Souza et al. [63] demonstrated that S100B secretion, induced by IL-6, is prevented by haloperidol and risperidone and that

only risperidone was able to change basal S100B secretion, confirming the previous reporting of Quincozes-Santos et al. [26]. These reporting are also in favour of the speculation that the oxidative stress observed in some autistic patients [21] and other brain disorders could be induced by IL-6 as well as other cytokines and could consequently modulate S100B secretion [63].

Furthermore, substantial evidence has implicated a state of chronic neuroinflammation and immune dysregulation leading to upregulation of inflammatory cytokines in brain of autistic subjects, probably caused by alteration in BBB function [1]. This has been shown by prominent cytokine profile of ASD patients and microglia cell activation [9,15-17,19,56], together with elevated circulating brain autoantibodies [65]. S100B proteins are known to be key mediators in polymorphonuclear neutrophil migration involved in inflammation [66], thus S100B protein may probably act as a cytokine [67-69] and may be an important component in neuroinflammation [63]. Elevated S100B protein was also proposed as indicative of active cell injury [69]. Whereas several studies frequently reported increased IL-6 in the autistic brain together with other cytokines as TNF-α, IFN-γ, IL-1β and IL-12 [57] indicating an association with tissue inflammation and necrosis [70]. Taken together, the current reporting of significantly elevated S100B and IL-6 levels in the sera of some autistic patients and the potentially significant correlation between serum levels of S100B and IL-6 supports the implication of neuroinflammation in the development of autism and that elevation of S100B and proinflamatory cytokine IL-6 may contribute to the pathogenesis of autism. Consequently, measuring serum levels of both S100B protein and IL-6 can be useful in supporting clinical assessment of autistic patients and monitoring immunosuppressive therapy.

Nonetheless, one of the questionable findings in the current study is the failure to find any association between elevated serum S100B proteins, IL-6 levels and GARS assessment scores representing the phenomenology and severity of the current autistic subjects. Few studies addressed the relation between different immune parameters and severity of autism. Al-Ayadhi and Mostafa [31] reported that the extent of the elevation of serum S100B protein levels was associated with the degree of autism severity. Ashwood et al. [14] reported a borderline association between IL-6 and stereotype which could not be confirmed by the current findings. However, the current study reported a marginally significant relation between elevated serum S100B protein levels and worse development (P=0.05). Whereas, other studies reported significant positive correlation between elevated serum IL-6 and severity of autism [55]. In the current study, the relatively small number of autistic sample may explain the lack of association between the tested immune parameters and clinical features of autism especially that 90% of the current sample had average to below average severity of autism, which could not allow for finding significant correlations. This may raise a question; if the elevation of S100B protein and IL-6 serum levels is a mere consequence of autism or has a possible etiologic role in ASD. The answer to this question is not easy as several researches have implicated a pathogenic role for S100B protein and IL-6 in some autoimmune neuropsychiatric diseases [28-30]. However, another possibility that is dysfunction in common basic cellular processes, such as those involved in signaling, may be manifest as aberrations in both the immune and neuronal systems [14] can be considered. As stated above, the current findings are in favour of underlying neuroinflamation in a group of autistic patients. However, apart from animal studies, the relation between this proposed neuroinflammation and clinical features of autism is still lacking.

Another immune aspect that has been commonly proposed in a major subset of autism is autoimmunity to the CNS [71]. Different autoantibodies have been reported with high levels in the nervous system of children with ASD [72,73]. Unexpectedly, 50% of autistic patients had abnormal elevations in the serum level of TG2 IgA in comparison to 36.4% of controls, yet, without statistical significance. Moreover, the serum TG2 IgA levels in the autistic group did not show any association with autistic severity or with the clinical features of autism as indicated by GARS assessment scores. This may indicate that TG2 IgA may not have a possible contributing role to the pathogenesis of autism. This speculation can be further supported by the significant negative correlation between TG2 IgA and S100B levels (Figure 3). These results are unlike those reported by Rosenspire et al. [41] who found that children diagnosed with ASD are about 2.5 times more likely to express elevated serum levels of TG2 IgA, and that this elevated expression of anti-transglutaminase is linked to the (HLA)-DR3, DQ2 and DR7 haplotypes. They argued that the elevated levels of IgA autoantibodies recognizing transglutaminase are a phenotypic (as opposed to a purely genetic) risk factor for a subpopulation of autistic patients in whom immune system dysregulation may be integral to the etiology of their disease. However, another study by Lau et al. [74], investigating serum level of TG2 IgA in a subset of ASD patients with prominent gastrointestinal symptoms reported negative finding similar to the current results. These controversial results may be attributed to several factors such as using different experimental designs, ages of the probands and control (including the comparison of children with ASD with adult controls), different classification systems for diagnostic criteria as well as power of the statistical analysis, analytical techniques used and tissue/specimens types, all of which may have confounded the interpretation of results. Furthermore, the mere presence of autoantibodies is not abnormal as it can be found in normal, healthy controls like the current one. Also, it must be emphasized that these autoantibodies are not present in all subjects with ASD and that they are also found in other diseases than ASD. However, the elevated levels seen in some patients with ASD may possibly indicate an on-going immune activation [31] or may be an epiphenomenon, referring to an altered period in neurodevelopment [12]. This notion may also explain the current failure to find any association between TG2 IgA levels and severity of autism. Further research is needed to investigate if there is a possible link between serum S100B protein and/or IL-6 levels and other brain autoantibodies as possible indicators of autoimmunity to CNS, in autism.

In conclusion, the current data support the concept that alterations in the immune responses involving the brain are significant in a group of autistic children. Whether these abnormal responses clinically attribute directly to autism remains a controversial question. The pathological role of these abnormal responses in ASD need further studies involving larger number of patients and investigating other autoimmune antibodies with respect to clinical features of autism and functional neuroimaging in order to have more clarification in this area of research.

Strengths and Limitations

There are number of factors that may contribute to the results of the current research and should be mentioned. In order to lessen the bias in selection criteria, the research group used more or less homogeneous autistic group excluding other autistic diagnoses like Rett's syndrome or childhood disintegrative disorder which may have another underlying pathogenic mechanisms, thus contaminating the results. Also, autistic cases with history of allergy or autoimmune disorders

were excluded to avoid bias in results. In addition, the authors used GARS which is one of the strong sophisticated tools for assessment of clinical features and severity of autism. Again, to ensure increased accuracy and reproducibility of the observed results, all samples were analysed twice in two independent experiments. However, there were some limitations including small number of autistic sample which may attribute to non-significant elevation of TG2 IgA and negative correlations with clinical parameters and severity of autism. Also, it is uncertain whether biomarker profiles detected in ASD probands, compared to typically developing controls, are unique to ASD as they may be found in other neurodevelopmental disorders. Thus, using new methods to enhance biomarkers specificity in ASD, e.g. multiplex immunoassay will be more promising.

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Competing Interests

The authors declare that they have no conflicts of interest.

References

1. Noriega DB, Savelkoul HF (2014) Immune dysregulation in autism spectrum disorder. Eur J Pediatr 173: 33–43.
2. Folstein SE, Rosen-Sheidley B (2001) Genetics of autism: Complex aetiology for a heterogeneous disorder. Nat Rev Genet 2: 943-955.
3. Abrahams BS, Geschwind DH (2008) Advances in autism genetics: On the threshold of a new neurobiology. Nat Rev Genet 9: 341-355.
4. Onore C, Careaga M, Ashwood P (2012) The role of immune dysfunction in the pathophysiology of autism. Brain Behav Immun 26: 383–392.
5. Wei H, Alberts I, Li X (2013) Brain IL-6 and autism. Neuroscience 252: 320-325.
6. Mead J, Ashwood P (2015) Evidence supporting an altered immune response in ASD. Immunol Lett 163: 49-55.
7. Engstrom AH, Ohlson S, Stubbs EG, Maciulus A, Caldwell V, et al. (2003) Decreased expression of CD95 (FAS/APO-1) on CD4+ T-lymphocytes from participants with autism. Journal of Developmental and Physical Disabilities 15: 155–163.
8. Sweeten TL, Posey DJ, McDougle CJ (2003) High blood monocyte counts and neopterin levels in children with autistic disorder. Am J Psychiatry 160**:** 1691–1693.
9. Jyonouchi H, Sun S, Le H (2001) Pro-inflammatory and regulatory cytokine production associated with innate and adaptive immune responses in children with autism spectrum disorders and developmental regression. J Neuroimmunol 120: 170-179.
10. Warren RP, Yonk LJ, Burger RA, Cole P, Odell JD, et al. (1990) Deficiency of suppressor-inducer (CD4+CD45RA+) T cells in autism. Immunol Invest 19: 245-251.
11. Warren RP, Foster A, Margaretten NC (1987) Reduced natural killer cell activity in autism. J Am Acad Child Adolesc Psychiatry 26**:** 333–335.
12. Wills S, Cabanlit M, Bennett J, Ashwood P, Amaral D, et al. (2007) Autoantibodies in autism spectrum disorders (ASD). Ann NY Acad Sci 1107: 79–91.
13. Li X, Chauhan A, Sheikh AM, Patil S, Chauhan V, et al. (2009) Elevated immune response in the brain of autistic patients. J Neuroimmunol 207: 111–116.
14. Ashwood P, Krakowiak P, Hertz-Picciotto I, Hansen R, Pessah I, et al. (2011) Elevated plasma cytokines in autism spectrum disorders provide evidence of immune dysfunction and are associated with impaired behavioural outcome. Brain Behav Immun 25: 40–45.
15. Ashwood P, Wakefield AJ (2006) Immune activation of peripheral blood and mucosal CD3α lymphocyte cytokine profiles in children with autism and gastrointestinal symptoms. J Neuroimmunol 173: 126-134.
16. Croonenberghs J, Bosmans E, Deboutte D, Kenis G, Maes M (2002) Activation of the inflammatory response system in autism. Neuropsychobiology 45: 1-6.
17. Molloy CA, Morrow AL, Meinzen-Derr J, Schleifer K, Dienger K, et al. (2006) Elevated cytokine levels in children with autism spectrum disorder. Journal of Neuroimmunology 172: 198-205.
18. Chez MG, Dowling T, Patel PB, Khanna P, Kominsky M (2007) Elevation of tumor necrosis factor-alpha in cerebrospinal fluid of autistic children. Pediatr Neurol 36: 361–365.
19. Singh VK (1996) Plasma increases of interleukin-12 and interferon-gamma: Pathological significance in autism. Journal of Neuroimmunology 66: 143-145.
20. Goines PE, Ashwood P (2013) Cytokine dysregulation in autism spectrum disorders (ASD): Possible role of the environment. Neurotoxicol Teratol 36: 67-81.
21. Tostes MH, Teixeira HC, Gattaz WF, Brandão MA, Raposo NR (2012) Altered neurotrophin, neuropeptide, cytokines and nitric oxide levels in autism. Pharmacopsychiatry 45: 241-243.
22. Wei H, Mori S, Hua K, Li X (2012) Alteration of brain volume in IL-6 overexpressing mice related to autism. Int J Dev Neurosci 30: 554-9.
23. El-Ansary AK, Ben Bacha AG, Al-Ayadhi LY (2011) Proinflammatory and proapoptotic markers in relation to mono and di-cations in plasma of autistic patients from Saudi Arabia. J Neuroinflammation 8: 142.
24. Wei H, Chadman KK, McCloskey DP, Sheikh AM, Malik M, et al. (2012) Brain IL-6 elevation causes neuronal circuitry imbalances and mediates autism-like behaviors. Biochim Biophys Acta 1822: 831-42.
25. Tu CL, Chang W, Bikle DD (2001) The extracellular calcium-sensing receptor is required for calcium-induced differentiation in human keratinocytes. J Biol Chem 276: 41079-41085.
26. Quincozes-Santos A, Abib RT, Leite MC, Bobermin D, Bambini-Junior V, et al. (2008) Effect of the atypical neuroleptic risperidone on morphology and S100B secretion in C6 astroglial lineage cells. Mol Cell Biochem 314: 59-63.
27. Marchi N, Rasmussen P, Kapural M, Fazio V, Kight K, et al. (2003) Peripheral markers of brain damage and blood-brain barrier dysfunction. Restor Neurol Neurosci 21: 109-121.
28. Portela LV, Brenol JC, Walz R, Bianchin M, Tort AB, et al. (2002) Serum S100B levels in patients with lupus erythematosus: Preliminary observation. Clin Diagn Lab Immunol 9: 164-166.
29. Schenatto CB, Xavier RM, Bredemeier M, Portela LV, Tort AB, et al. (2006) Raised serum S100B protein levels in neuropsychiatric lupus. Ann Rheum Dis 65: 829-831.
30. Bartosik-Psujek H, Psujek M, Jaworski J, Stelmasiak Z (2011) Total tau and S100b proteins in different types of multiple sclerosis and during immunosuppressive treatment with mitoxantrone. Acta Neurol Scand 123: 252-256.
31. Al-Ayadhi LY, Mostafa GA (2012) A lack of association between elevated serum levels of S100B protein and autoimmunity in autistic children. J Neuroinflammation 9: 54.
32. Cohly HH, Panja A (2005) Immunological findings in autism. Int Rev Neurobiol 71: 317-341.
33. Vojdani A, Pangborn JB, Vojdani E, Cooper EL (2003) Infections, toxic chemicals and dietary peptides binding to lymphocyte receptors and tissue enzymes are major investigators of autoimmunity in autism. Int J Immunopathol Pharmacol 16: 189-199.
34. Al-Ayadhi LY, Mostafa GA (2011) Increased serum osteopontin levels in autistic children: Relation to the disease severity. Brain Behav Immun 25: 1393-1398.
35. Al-Ayadhi LY, Mostafa GA (2011) Low plasma progranulin levels in children with autism. J Neuroinflammation 8: 111.
36. Mostafa GA, El-Sayed ZA, Abd El Aziz MM, El-Sayed MF (2008) Serum anti-myelin associated glycoprotein antibodies in Egyptian autistic children. J Child Neurol 23: 1413-1418.
37. Greenwood DL, Gitlits VM, Alderuccio F, Sentry JW, Toh BH (2002) Autoantibodies in neuropsychiatric lupus. Autoimmunity 35: 79-86.
38. Alaedini A, Green PH (2005) Narrative review: Celiac disease: Understanding a complex autoimmune disorder. Annals of Internal Medicine 142: 289–298.

39. Sollid LM (2000) Molecular basis of celiac disease. Annual Review of Immunology 18: 53–81.

40. De Vivo G, Di Lorenzo R, Ricotta M, Gentile V (2009) Role of the transglutaminase enzymes in the nervous system and their possible involvement in neurodegenerative diseases. Current Medicinal Chemistry 16: 4767–4773.

41. Rosenspire A, Yoo W, Menard S, Torres AR (2011) Autism spectrum disorders are associated with an elevated autoantibody response to tissue transglutaminase-2. Autism Res 4: 242-249.

42. Hand D, Perry MJ, Haynes LW (1993) Cellular transglutaminases in neural development. International Journal of Developmental Neuroscience 11: 709–720.

43. Maccioni RB, Seeds NW (1986) Transglutaminase and neuronal differentiation. Molecular and Cellular Biochemistry 69: 161–168.

44. Mahoney SA, Perry M, Seddon A, Bohlen P, Haynes L (1996) Transglutaminase forms midkine homodimers in cerebellar neurons and modulates the neurite-outgrowth response. Biochemical and Biophysical Research Communications 224, 147–152.

45. Festoff BW, Suo Z, Citron BA (2001) Plasticity and stabilization of neuromuscular and CNS synapses: Interactions between thrombin protease signaling pathways and tissue transglutaminase. International Review of Cytology 211: 153–177.

46. Boscolo S, Lorenzon A, Sblattero D, Florian F, Stebel M, et al. (2010) Anti transglutaminase antibodies cause ataxia in mice. PLoS One 5: e9698.

47. Gilliam JE (1995) Gilliam autism rating scale. Austin.

48. Abdelrahman ME, Hasan MKA (2004) Gilliam autism rating scale arabic version. Cairo: Dar El-Sahab press.

49. Mostafa GA, AL-Ayadhi LY (2011) Increased serum levels of anti-ganglioside M1 auto-antibodies in autistic children: Relation to the disease severity. Journal of Neuroinflammation 8: 39.

50. Enstrom AM, Van de Water JA, Ashwood P (2009) Autoimmunity in autism. Curr Opin Investig Drugs 10: 463-473.

51. Jackel A, Deichmann M, Waldmann V, Bock M, Näher H (1999) S-100β protein in serum, a tumor marker in malignant melanoma–current state of knowledge and clinical experience. Hautarzt 50: 250-256.

52. Ross HE, Guo Y, Coleman K, Ousley O, Miller AH (2013) Association of IL-12p70 and IL-6:IL-10 ratio with autism-related behaviors in 22q11.2 deletion syndrome: A preliminary report. Brain Behav Immun 31: 76-81.

53. Ricci S, Businaro R, Ippoliti F, Lo Vasco VR, Massoni F, et al. (2013) Altered cytokine and BDNF levels in autism spectrum disorder. Neurotox Res 24: 491-501.

54. Zubarev OE, Klimenko VM (2011) Elevation of pro-inflammatory cytokines level at early age as the risk factor of neurological and mental pathology development. Ross Fiziol Zh Im I M Sechenova. 97: 1048-1059.

55. Yang CJ, Liu CL, Sang B, Zhu XM, Du YJ (2015) The combined role of serotonin and interleukin-6 as biomarker for autism. Neuroscience 284: 290-296.

56. Vargas DL, Nascimbene C, Krishnan C, Zimmerman AW, Pardo CA (2005) Neuroglial activation and neuroinflammation in the brain of patients with autism. Annals of Neurology 57: 67–81.

57. Wei H, Zou H, Sheikh AM, Malik M, Dobkin C, et al. (2011) IL-6 is increased in the cerebellum of autistic brain and alters neural cell adhesion, migration and synaptic formation. J Neuroinflammation 8: 52.

58. Ashraf S, Bhattacharya K, Tian Y, Watterson K (1999) Cytokine and S100B levels in paediatric patients undergoing corrective cardiac surgery with or without total circulatory arrest. Eur J Cardiothorac Surg 16: 32–37.

59. Steiner J, Walter M, Wunderlich MT, Bernstein HG, Panteli P, et al. (2009) A new pathophysiological aspect of S100B in schizophrenia: Potential regulation of S100B by its scavenger soluble RAGE. Biol Psychiatry 65: 1107–1110.

60. Potvin S, Stip E, Sepehry AA, Gendron A, Bah R, et al. (2008) Inflammatory cytokine alterations in schizophrenia: A systematic quantitative review. Biol Psychiatry 63: 801–808.

61. Edwards MM, Robinson SR (2006) TNF alpha affects the expression of GFAP and S100B: implications for Alzheimer's disease. J Neural Transm 113: 1709–1715.

62. Schmitt KR, Kern C, Lange PE, Berger F, Abdul-Khaliq H, et al. (2007) S100B modulates IL-6 release and cytotoxicity from hypothermic brain cells and inhibits hypothermia-induced axonal outgrowth. Neurosci Res 59: 68–73.

63. de Souza DF, Wartchow K, Hansen F, Lunardi P, Guerra MC, et al. (2013) Interleukin-6 induced S 100B secretion is inhibited by haloperidol and risperidone. Prog Neuropsychopharmacol Biol Psychiatry 43: 14-22.

64. Ponath G, Schettler C, Kaestner F, Voigt B, Wentker D, et al. (2007) Autocrine S100B effects on astrocytes are mediated via RAGE. J Neuroimmunol 184: 214–222.

65. Wills S, Cabanlit M, Bennett J, Ashwood P, Amaral DG, et al. (2009) Detection of autoantibodies to neural cells of the cerebellum in the plasma of subjects with autism spectrum disorders. Brain Behav Immun 23: 64–74.

66. Yano J, Lilly E, Barousse M, Fidel PL (2010) Epithelial cell-derived S100 calciumbinding proteins as key mediators in the hallmark acute neutrophil response during Candid vaginitis. Infect Immun 78: 5126-5137.

67. Donato R (2001) S100: A multigenic family of calcium-modulated proteins of the EF-hand type with intracellular and extracellular functional roles. Int J Biochem Cell Biol 33: 637-668.

68. Massaro AR, Michetti F, Laudisio A, Bergonzi P (1985) Myelin basic protein and S-100 antigen in cerebrospinal fluid of patients with multiple sclerosis in the acute phase. Ital J Neurol Sci 6: 53-56.

69. Michetti F, Massaro A, Russo G, Rigon G (1980) The S-100 antigen in cerebrospinal fluid as a possible index of cell injury in the nervous system. J Neurol Sci 44: 259-263.

70. Beutler B, Cerami A (1989) The biology of cachectin/TNFα a primary mediator of host response. Annu Rev Immunol 7: 625-655.

71. Singh VK (2009) Phenotypic expression of autoimmune autistic disorder (AAD): A major subset of autism. Ann Clin Psychiatry 21: 148–61.

72. Ashwood P,Van de Water J (2004) Is autism an autoimmune disease? Autoimmun Rev 3: 557–562.

73. Cabanlit M, Wills S, Goines P, Ashwood P, Van De Water J (2007) Brain-specific autoantibodies in the plasma of subjects with autistic spectrum disorder. Ann N Y Acad Sci 1107: 92-103.

74. Lau NM, Green PHR, Taylor AK, Hellberg D, Ajamian M, et al. (2013) Markers of celiac disease and gluten sensitivity in children with autism. PLoS ONE 8: e66155.