ORIGINAL ARTICLE

Is Serum TGF-β1 and TGF-β2 levels Correlated to Children with Autism Intensity?

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Abstract

Objective

Transforming growth factor-beta (TGF-β), a group of multifunctional growth factors, plays an important role in the neuron survival and neurodevelopmental functions. Some studies have evaluated the correlation between TGF-β1 and TGF-β2 abnormalities and autism spectrum disorders. In this study, we compared the TGF-β1 and TGF-β2 levels between autistic and intellectually normal individuals.

Materials & Methods

The study population consisted of 39 autistic and 30 age-matched intellectually normal individuals (control group). Blood samples were taken from all individuals, and all patients were divided into 2 groups (mild-to-moderate and severe) according to the childhood autism rating scale. The cytokines levels were measured by Enzyme Linked Immunosorbent Assay (ELISA).

Results

The mean concentration of TGF-β1 was significantly lower ($P < 0.0001$) in children with autism compared to the control group (25.3 ± 6.5 versus 35.1 ± 9.4 ng/mL, respectively). Also, the mean concentration of TGF-β2 in children with autism (32.35± 7.75 ng/mL) was higher compared to those in the control group (30.47± 4.36 ng/mL); however, this difference did not reach statistical significance ($P = 0.21$). A positive correlation was observed between TGF-β1 concentration and autism severity ($r = 0.41$; $P = 0.02$), whereas a negative correlation was found between TGF-β2 concentration and autism severity ($r = -0.41$; $P = 0.02$).

Conclusion

The results of the present investigation suggest that there is a decrease in the levels of TGF-β1 in the serum of patients with autism and this cytokine may be effective in the treatment of the pathophysiological aspects of autism.

Keywords: Autism spectrum disorder; Neurodevelopmental factors; Transforming growth factor-Beta; Autism intensity.

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Introduction

Childhood autism, characterized by stereotypical behaviors, and social and communication impairments, is a condition with neurodevelopmental etiology that occurs under 3 years of age (1, 2). The prevalence of this disorder has increased considerably over the past 3 decades. Many of the recent epidemiological reviews estimate a prevalence of 1 per 88 children in the United States (3). Although the exact nature of autism is still not fully understood, genetic abnormalities, perinatal events (e.g. vaginal bleeding after the first trimester, presence of meconium in amniotic fluid, respiratory distress syndrome, and anemia in neonatal period), neuroanatomical abnormalities (e.g. enlargement of occipital, parietal, and temporal lobes), and biochemical factors (e.g. hyperserotonemia in the presence of mental retardation) are proposed to play a role in the pathophysiology of this condition (4).

It has been hypothesized that some mechanisms of autism act through inflammatory, oxidative, and autoimmune processes (5, 6). In addition, it has been proposed that immunological factors have a key role in the pathophysiology of this condition (7). Several previous studies have illustrated a correlation between abnormalities of the immune system and autism spectrum disorders (ASDs). These immune system abnormalities may include abnormalities in the functional immune cell subsets and autoimmunity caused by inappropriate immune regulation such as autoantibodies generated against the central nervous system (CNS) (8, 9). It has been assumed that the development of neurological disorders may be caused by the abnormal immune responses during the critical neurodevelopmental period (10). The validity of the hypothesis of the immune system abnormalities in autistic patients has been derived from the critical role of the immune system in neurodevelopment and the ability of these changes to influence the CNS (10, 11). Both the immune and nervous systems are evolved systems that cross-talk via cytokines and neuromediators such as neuropeptides (12). Changes in the ratios of excitatory to inhibitory signaling in the brain may also contribute to ASD (13).

Immune mediators, known as cytokines, are the main facilitators of the cross-systemic communication. Cytokines, a broad category of small proteins, control the duration, nature, and intensity of an immune response. In addition, cytokines serve as a common language between the immune and the CNS (14). These mediators are essential in maintaining the normal aspects of neurodevelopment, such as progenitor cell

differentiation, cellular localization/migration within the CNS, and synaptic network formation, thus influencing both the developmental and functional aspects of the nervous system (15). Furthermore, neuroimmune interactions are highly diverse and vary between individuals (16). For example, the impact of the cytokines differ between the developing brain and the adult brain, and each require cytokines in different concentrations (15). Therefore, imbalanced cytokine production, signaling, and/or regulation may induce a wide range of neurological complications. Imbalances in the level of cytokine during development could affect neural activity and mediate behavioral disorders such as autism. In the following, we investigate the significance of several cytokines linked to ASD (14). Cytokine abnormalities in ASD are an important indication resulting from environmental and genetic factors, and may directly contribute to neurological dysfunctions (8, 14). However, the relationship between the neurological and immunological functions has not been highlighted enough (17).

TGF-β has a role to play in the immune system in addition to controlling proliferation, cellular differentiation, and other functions in the majority of cells (18). This cytokine exists in at least 3 isoforms: TGF-β1, TGF-β2, and TGF-β3 (19). Compared to other anti-inflammatory cytokines such as IL-10, higher expression of the genes encoding TGF-β exists in most tissues (20). TGF-β1 is an important immune regulator for immune homeostasis and orchestrates aspects of embryonic development (21). It has been suggested that TGF-β1 has an important regulatory role in CNS development and has potential implications for neurogenesis in a variety of TGF-β1-related CNS diseases (14, 15). Some investigators also found that TGF-β1 plays a crucial, suppressive role in early CNS development and has a major role in neuronal migration, survival, and synapse formation (22). Also, a TGF-β1 knockout mouse has been shown to have severe cortical developmental impairment with pervasive increased neuronal cell death and microgliosis complications; therefore, it is vital for proper neurodevelopment (23). However, the aforementioned investigations propose that immune system aberrations may cause abnormal immune responses, autoimmunity, or adverse neuroimmune interactions during brain development (22, 24). Several studies have demonstrated decreased TGF-β1 levels in the sera and cerebrospinal fluid (CSF) of autistic children (25, 26). Additionally, decreased levels of TGF-β1 in serum have been reported in autistic children (25). However, it remains unclear whether the serum levels of TGF-β1and TGF-β2 reflect their levels in the brain (26).

Many of the previous findings suggest that decreased levels of TGF-β1 may be implicated in the pathophysiology of autism (24, 25). There is little information about the role of TGF-β2 in autism. But the available data suggest that TGF-β2 has an anti-inflammatory effect on the inflammatory process. El-Ansary and Al-Ayadh (26) and Vargas et al (27) separately reported that the TGF-β2 level increases in autistic children. The purpose of the present study is to examine whether serum levels of TGF-β1 and TGF-β2 in autistic patients are altered as compared to age-matched normal controls. Furthermore, in humans, about 500 mL of CSF is absorbed into the blood daily, making blood a suitable source of neurodegenerative or neurodevelopmental disease biomarkers (28). Recently, researchers have shown interest in investigating the relationship between

serum or neurological biomarkers and autism intensity (29). For example, neuroinflammatory biomarkers such as heat shock protein-70, TGF-β, interferon-γ, and caspase7 have been shown to be correlated with autism intensity (26). However, it is unclear whether CNS immune activation contributes to the pathology of autism or whether it is an epiphenomenon (24). Furthermore, we have examined relationships between serum TGF-β1 and TGF-β2 levels and clinical degree in autistic patients.

Material & Methods

Study population and psychological measures

Thirty-nine autistic children between 7 and 13 years of age were included in this study population. Childhood Autism Rating Scale (CARS) has been performed to confirm the diagnosis of autism and to assess the severity (30). A 29.5 cut-off value was used to diagnose autism. Scores 30–36.5 and 37–60 indicated mild-to-moderate and severe autism, respectively. Moreover, Diagnostic Statistical and Manual of Mental Disorders 5th Edition (DSM-V) criteria were used to confirm autism and differentiate this condition from other developmental disorders such as Rett's disorder, Asperger's disorder, and childhood disintegrative disorder in those children with CARS > 30. All participants in both groups were Iranians. Children were excluded if they had any known chromosomal or neurological disorders. The control group included 30 randomly selected, age-matched, healthy children with intelligence quotient ranging from 90 to 110. All children in the control group had no neurological or psychological deficits. Informed consents were obtained from parents in both groups. Study design was in accordance with the tenets of the Helsinki Declaration, as revised in 2000, and was approved by the institutional review board and ethics committee of Azad University of Medical Sciences, Mashhad Branch. Questionnaires were used to collect information about demographics, past medical history, and clinical signs and symptoms of all participants.

Cytokine analysis

Blood samples of autistic and normal children were taken by venipuncture from cubital vein at 9 am, 11:00 a.m. and 12 noon (31). Afterward, cell-free serums were collected and stored at –70°C until the assay. The serum levels of TGF-β1 and TGF-β2 were measured using the TGF-β1 and TGF-β2 ELISA Kit separately (R&D Systems, Inc., Minneapolis, MN, USA), and the samples were activated before the assay. The mean detection limits were 4.60 pg/mL for TGF-β1 and <7.0 pg/mL for TGF-β2. All samples were measured in duplicate and the respective mean value was calculated.

Statistical analysis

Data (i.e. mean age, gender, family history, and clinical signs and symptoms of autistic children) were analyzed using the Statistical Package for Social Sciences (SPSS 18, IBM Corporation, New York, NY, USA). The Chi-square test and Student's t-test were performed to analyze qualitative and quantitative variables, respectively. For nonnormal distributions, the Mann–Whitney test was used. Correlation between serum TGF-β level and autism severity was determined using Pearson's rank correlation coefficient. A P-value < 0.05 was considered statistically significant.

Results

The demographic and clinical characteristics of both groups are summarized in Table 1. Mean body mass index (BMI) was 22.85 ± 2.19 kg/m^2 and 20.96 ± 3.25 kg/m^2 in autistic children and the control group, respectively. No statistically significant differences were noted in terms of BMI (P = 0.719) and mean age between the 2 groups (autism group: 8.54 ± 1.68 years and control group: 7.9 ±1.4 years, P = 0.330). There were 25 males (64.1%) and 14 females (35.9%) in the autism group. Clinical examination of autistic children revealed that communication problems (40%), poor concentration/attention deficit (40%), and hyperactivity (33%) were the most common signs of these children. Prematurity was noted in the past medical history of 5% of patients with autism. Based on CARS, 10 children (25.6%) had mild-to-moderate autism and 29 children (74.4%) were diagnosed with a severe form of this condition (Table 1).

As shown in Figure 1(a), the mean concentration of TGF-β1 in children with autism (25.30 ± 6.54p g/mL) was significantly (P < 0.0001) lower compared to those in the control group (35.09 ± 9.40 pg/mL). In addition, as shown in Figure 1(b), the mean concentration of TGF-β2 in children with autism (32.35± 7.75 pg/mL) was higher compared to those in the control group (30.47± 4.36 pg /mL); however, this difference did not reach statistical significance (P = 0.218). In mild-to-moderate and severe autisms, the mean concentration of TGF-β1 was 24.93 ± 6.90 pg/mL and 25.45 ± 6.50 pg/mL, respectively, and it did not show a significant difference between the 2 groups. Also, as shown in Figure 2(a), there is a positive correlation between TGF-β1 concentration and autism severity (r = 0.415; P = 0.022). Moreover, in mild-to-moderate and severe autisms, the mean concentration of TGF-β2 was 35.11 ± 9.52 pg/mL and 31.24 ± 6.83 pg/mL, respectively. TGF-β2 concentration was significantly (P = 0.008) higher in those with severe autism. Furthermore, as shown in Figure 2(b), there is a negative correlation between TGF-β2 concentration and autism severity (r =–0.412; P = 0.023).

Table 1. Comparison of demographic and clinical data as well as abnormalities in CT-scans between autism (n = 39) and control (n = 35) groups

Variables		Autism group	Control group	p-value
Mean age(Years)		8.54 ± 1.68	7.9 ±3.1	NSa
Gender (boys/girls)		25/14	20/15	NS
BMI (kg/m2)		22.85 ± 2.19	21.14 ± 2.45	NS
Age of diagnosis		2.8	–	–
Severity	Severe	29(74.4%)	–	–
	Mild/moderate	10(25.6%)	–	–
Obstetric complications		4(25%)	6(37.5%)	NS
Another disease		8(20.5%)	2(5.7%)	NS
Family history		8(20.5%)	0(0.0%)	0.008
Epilepsy		12(30.8%)	0(0.0%)	0.001
Hypoxia		3(7.7%)	0(0.0%)	NS
Abnormal CT-scan		1(2.6%)	0(0.0%)	NS

[a]Not signi

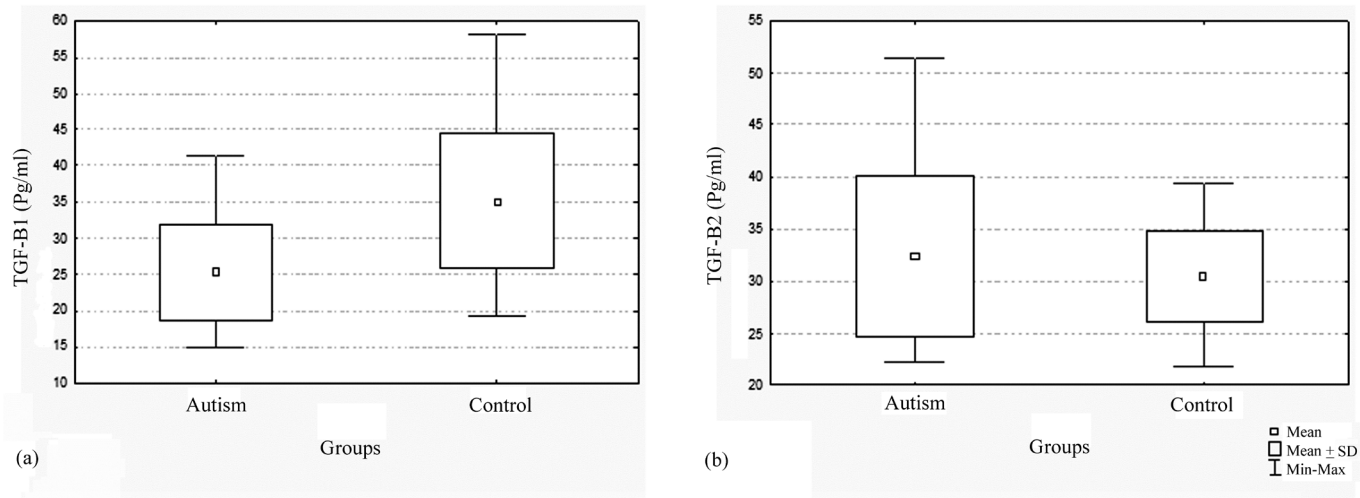

Fig. 1(a). Data are displays as mean ±SD. In autism group, TGF-β1 is significantly (P < 0.0001) lower compared to the control group (25.30± 6.54p g/ml vs. 35.09±9.40 pg/ml). (b). Data are displays as mean ±SD. In autism group, TGF-β2 is higher compared to the control group (32.35± 7.75 pg /ml vs. 30.47± 4.36 pg /ml). However, did not show significant difference between two groups (P-Value=0.218)

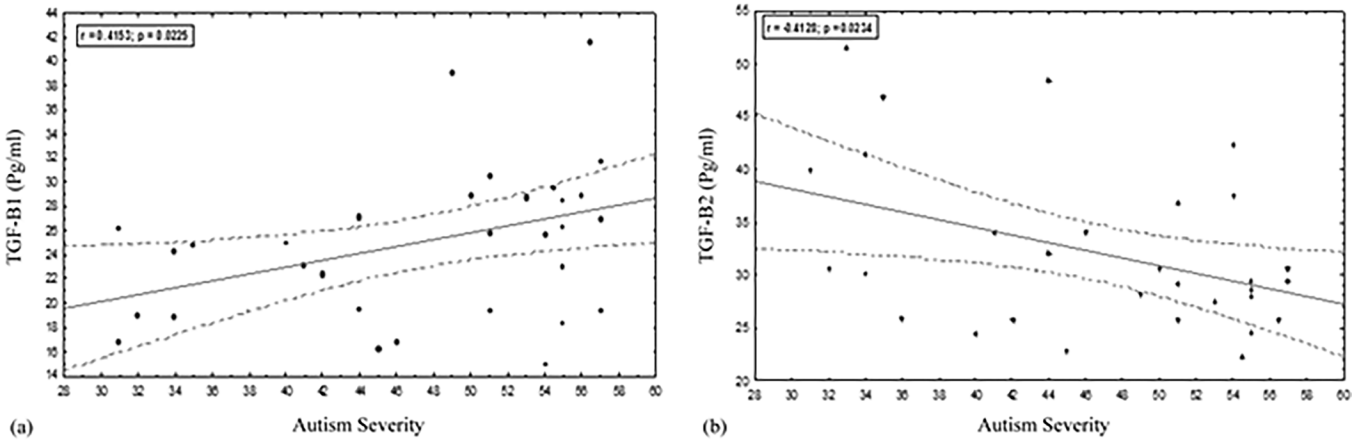

Fig 2. (a). A positive correlation(r = 0.415; P = 0.022) was found between mean mild-to-moderate and severe autistic children. (b). A negative correlation(r =−0.412; P = 0.023) was found between mean mild-to-moderate and severe autistic children. CARS: childhood autism rating scale

Discussion

In this study, TGF-β1 level was significantly lower in autistic children compared to the control group. Also, our results show that the mean concentration of TGF-β2 in children with autism was higher compared to that in the control group. However, it did not show any significant difference with the control group. A few previous studies have evaluated the correlation between TGF-β1 and TGF-β2 levels and autism severity (8, 25, 26). Similar to previous findings, results of the present study show decreased levels of TGF-β1 in blood samples from individuals with ASDs. However, Vargas et al (27) and El-Ansary and Al-Ayadh (26) have, respectively, reported an increased level of TGF-β1 and TGF-β2 in the brain of autistic patients compared to normal individuals. The nervous and immune systems communicate with each other through multiple neuroanatomical and secretory routes, in addition to the molecular mechanisms (15, 24). Pathophysiological changes may occur due to the imbalance of the neuroimmune axis associated with neurodevelopmental diseases such as autism (27). TGF-β1 has been shown to play a key role in early CNS development and during brain development, which is essential in the regulation of early CNS development such as astrocyte differentiation, synaptogenesis, neuronal migration in the cerebral cortex, neuronal survival, neuronal death, microgliosis control, wound healing, and immunosuppression (27). These findings are in agreement with the hypothesis that reduced levels of this cytokine may lead to an inappropriate regulation of immune responses, and hence the development of neuroinflammation disorders such as ASDs. However, it has not been shown that whether reduction of TGF-β1 is a primary cause of autism or simply a secondary reflection of this condition.

Our results demonstrate a mild increase in mean concentration of TGF-β2; however, this increase did not reach statistical significance. It should be noted that, although TGF-β2 may have a role to play in brain injury and some neuroinflammations, it has been illustrated that TGF-β2 is often considered as an anti-inflammatory and wound-healing molecule. It has been proposed that enhanced TGF-β2 expression may play a role in promoting

inflammation in brain injury associated with autism (32). Therefore, TGF-β2 is an immunomodulatory cytokine that has different functions based on its microenvironment.(33). Generally, TGF-β plays a dual role in the inflammation phase by exerting proinflammatory effects during the early stages and contributing to the resolution of inflammation and anti-inflammatory effects in later stages (32). We could not confirm the correlation between increase in the serum level of TGF-β2 and neuroinflammations in autistic children. However, controversies remain about the role of TGF-β2 and autism.

Despite the large amount of research, it is still poorly understand why some of autistic children have a mild or severe score in behavioral findings. Also, we do not know what leads to certain changes to symptoms from those commonly known and the reason for the worsening of behavioral scores, while they have been calm throughout. Cytokine abnormality has been reported as a trigger of events leading to autism (14, 34). We found that reduced levels of TGF-β1 in autistic children are correlated to dysregulation of autism intensity. Our results have shown that there is a positive correlation between TGF-β1 concentration and autism severity. On the other hand, other factors such as defects in regulatory T-cell (T.reg) development may explain the decreased levels of TGF-β1 in the sera and the brain. T.regs are responsible for TGF-β1 production, self-tolerance, and immune homeostasis. TGF-β1 is required for the suppressive function of T.regs (35). The increased level of TGF-β in brain tissue may be an outcome of brain inflammation (20, 27). However, the relationship between CNS and peripheral TGF-β is unclear, and all these studies are in agreement that TGF-β dysregulation may have a role to play in ASDs (24). Many reports indicate that TGF-β1 plays a crucial role in the regulation of neurosurvival and neurodevelopmental function and limitiing the production of IL-2, IFN-ɣ, and TNF, all of which can act together to orchestrate the repair processes in CNS (1, 28). In fact, TGF-β1 plays a Janus-like dual role in the regulation of immune and inflammatory responses. Under normal conditions, TGF-β1 is secreted from microglia, to inhibit the neuroinflammation process in brain. But under inflammatory conditions, such as autism, TGF-β1, which is involved in inflammation, apoptosis, neurodegeneration, and other brain injuries, is secreted from different cell types (32). Thus, TGF-β1 is a multifunctional cytokine that depends on the location and surrounding milieu for its functions. In cases where a direct correlation between TGF-β1 and severity of autism is found, the TGF-β1 inflammatory pathway may be predominant in comparison to its suppressive role. Thus, there is a prevalence of a strong neuroinflammatory condition in children at a severe stage.

In conclusion

according to the results of this study, lower TGF-β1 was found to be correlated with more severe behavioral scores in ASD children. In addition, there was a positive correlation between TGF-β1 concentration and autism severity. An increased serum level of TGF-β2was also observed in ASD children compared to the control group, but the difference was not significant between the 2 groups. Furthermore, there was a negative correlation between TGF-β2 concentration and autism severity. Dysregulations of these cytokine levels were associated with severity level in our patients. The neurological consequences of cytokine imbalances

during early stages of brain development can lead to behavioral abnormalities in autistic children. Because autism is a multifactorial disorder, cytokine elevation cannot be considered as the only factor responsible for autism susceptibility. Considering the influence of serum levels of TGF-β1 and TGF-β2 on the intensity of autism, further studies are required to determine the effects of decreased levels of these cytokines. More importantly, due to the small sample size, we believe the findings of the present study should be confirmed through conducting studies based on a larger number of patients. Undoubtedly, more findings should be taken into account in the assessment of autism and in understanding the underlying clinical and molecular mechanisms of this disorder. The CARS criteria and other clinical findings related to cytokine variation in different groups must be evaluated separately. Also, postmortem and normal newborn infant's cytokine determinations/ assays may help enhance our understanding of the neuroscience behind autism.

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Author's Contribution

Jamshid yousefi and Mohammad Reza Khakzad carried out the hypothesis in this study.Seyed Ali Ebrahimi and Mohammad Reza Khakzad wrote the manuscript with support from Jamshid yousefi and Mohammad Reza Khakzad. Seyed Ali Ebrahimi and Maryam Hojati fabricated the sample. Mitra Hosseinpour and Mohammad Reza Khakzad designed and performed the experiments the project, derived the models and analyzed the data. Javad Akhondian supervised the project. Both Jamshid Yousef and Javad Akhondian contributed to the final version of the manuscript. All authors provided critical feedback and helped shape the research, analysis and manuscript.

Conflict of interest

The authors confirm there are no conflicts of interest.

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