Archives of Psychiatry and Psychotherapy, 2022; 3: 20–24

10.12740/APP/144882

# Comparison of serum IGF1, IGF2 and IGFBP1-6 concentration in the children with different stages of autism spectrum disorders

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## Abstract

**Aim of the study:** Autism spectrum disorder (ASD) is a neurodevelopmental condition considered by early-onset difficulties in social communication. Insulin-like growth factors (IGFs) play crucial roles in synapse formation. Most of circulating IGFs are bound to IGF-binding proteins (IGFBPs) that modify IGF action. IGFBPs prolong the plasma half-life of IGFs. In this project we studied the association of IGF1/2 and IGFBP1-6 serum concentration with the severity of ASD (Levels 1-3; Mild, Moderate and severe, respectively).

**Materials and Methods:** A hundred and eighty patients with ASD (Mild; n=69, Moderate; n=58 and Severe; n=53) and 118 controls age matched were used in this project and IGF1/2 and IGFBP1-6 serum concentration were measured by ELISA.

**Results:** The results demonstrated that IGF1, IGF2 and IGFBP1-6 were present in all serum samples. The results showed that the concentration of IGF1 and IGF2 was significantly higher in ASD patients when compared to controls, starting from stages I to III ASD, a significant increase of IGF1 and IGF2 serum concentration was observed. Results obtained also showed that IGFBP1-6 concentration in the ASD group were lower when compared to controls and low serum IGFBP1-6 concentration is associated with advanced stages of ASD.

**Discussions:** IGFs plays important role in synapse formation and changes in IGFs expression may be important in the pathogenesis of ASD.

**Conclusion:** It is suggested that IGFs and IGFBPs may be involved in the pathogenesis of ASD. Therefore, the detection of serum soluble IGFs and IGFBPs may be useful in classifying ASD.

**serum; IGF1/2; IGFBP1-6; concentration; autism spectrum disorders**

## INTRODUCTION

Autism spectrum disorders (ASD) is defined as a neurodevelopmental condition, a term that indicates impairment in the normal development and/or growth of the brain [1]. The incidence of ASD has been increased in the past decades and the average spread of 0.62% has been reported around the world [2].

Autism is a heterogeneous and multifactorial disease that results from the interaction between genetic susceptibility and environmental factors. It was documented that almost 10 percent of infants exposed prenatally to the medication valproic acid develop autism [3]. Nutrition including folate and vitamin D deficiency were shown

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to be involved in the pathogenesis of ASD [4]. Many genes that play role in autism are component of signaling networks that regulate growth and synaptic plasticity, play a central role in the etiology of autism. A lot of data suggests that the causes of ASD are multifactorial and differentiated in given persons [5].

ASD is supposed to stem from defects in the formation and maintenance of functional neuronal networks due to synaptic dysfunction. Growth factors including IGFs have been shown to play important role in the synapse formation [6]. The IGFs, IGF receptors and IGF-binding proteins (IGFBPs) have been demonstrated to be implicated in the regulation of somatic growth and cellular proliferation. There are six IGFBPs (1-6) that bind to IGFs with high affinity and specificity. The main functions of IGFBPs include: 1. prolongation of IGFs half-life in the circulation, 2. regulation of the passage of IGFs from the intravascular to the extravascular space, 3. control of the bioavailability of free IGFs to interact with the IGF receptors, 4. enhancement of IGFs actions by the formation of a pool of slow release IGFs [7]. About 99% of circulating IGFs are bound to IGFBPs that modify IGF action. IGFBP-3 is the quantitatively predominant IGFBP in circulation. This large complex is essentially limited to the intravascular space and prolongs the plasma half-life of IGFs from a few minutes to several hours [8].

IGF-I plays key role in neurogenesis and synaptic plasticity, facilitates oligodendrocyte development, promotes neuron and oligodendrocyte survival, and stimulates myelination [9]. The important role of IGFs on synaptic function, maintenance, and plasticity make it a potential target for treating ASD. In several mice model of ASD, IGF-II has been suggested as a potential novel therapeutic target for ASD [10]. IGFs have been shown to play important role in the survival of neurons [11]. Many studies in both animal and human ASD models have proven IGF1 as one of the most promising ASD therapeutic interventions to date. It has been recently shown that IGF1 can be capable of the treatment of numerous neurodevelopmental disorders including ASD [12]. IGF1 has been shown to recover symptoms of autism through its positive effect on synaptic function, maintenance, and plasticity. Furthermore, administration of IGF1 to children at early stages of ASD has been shown to decrease neuronal excitability. IGF1 may also increase oligodendrocytes function which targets myelination defects and plays key role in in synapse formation in the CNS [13]. Due to potential central roles of IGFs signaling in combination with IGFBPs in normal development of the central nervous system including synaptic plasticity and spatial learning function of the brain, this project aimed to study the serum levels of IGF1, IGF2 and IGFBP1-6 in different stages of ASD in a population-based case-control study.

## MATERIAL AND METHODS

### Samples

A hundred and eighty patients with ASD (mild; n=69, moderate; n=58 and severe; n=53) ASD patients (8±3.8 years) and 118 control subjects (6.3.±3.7 years) were enrolled in this study. Controls were also evaluated to rule out neurological disorders (Table 1) The parents of both all subjects signed the informed consent form. The study was approved by the University Ethics Committee and has been carried out in accordance with the Code of Ethics of the World Medical Association (Declaration of Helsinki).

**Table 1.** Demographic characteristics of ASD patients and controls.

| Characteristics | Autism spectrum disorders (n=180) n (%) | Controls (n=118) n (%) |
|---|---|---|
| Gender<br>Male<br>Female | <br>146 (81.11)<br>34 (18.9) | <br>95 (80.50)<br>23 (19.50) |
| Age (years) | 8±3.8 | 7.3.±3.7 |
| Levels<br>1<br>2<br>3 | <br>69 (38.3)<br>58 (32.2)<br>53 (29.5) | <br>– |
| Intellectual disability<br>+<br>– | <br>18 (10)<br>162 (90) | <br>– |
| Sleep problems<br>+<br>– | <br>9 (5)<br>171 (95) | <br>– |

| | | |
|---|---|---|
| Multiple ASD in family<br>+<br>– | 36 (20)<br>144 (80) | – |
| Birth order<br>1st child<br>2nd child<br>3rd child<br>4th child | <br>118 (65.5)<br>46 (25.5)<br>12 (6.66)<br>4 (2.22) | – |
| Age of mother (years) | 20-34 (28.3±4.8) | 20-33 (27.8±5.1) |
| Race/Ethnicity | Iranian | Iranian |

The diagnosis of autism was made according to DSM-5 criteria for ASD patients [14]. Children visiting the Iran clinic, Iran, for a routine checkup and who were without a history or diagnosis of ASD were also asked from their parents as to whether their children could participate and donate blood for the study. These subjects were considered as control group. Controls were investigated to determine whether they or their first-degree relatives had psychiatric disturbances or previous psychiatric treatment through personal interviews. Only unaffected subjects with no psychiatric disorder or family history were regarded as controls. Inclusion criteria were meeting the DSM-IV diagnostic criteria for autism disorder and a score above cut off on each symptom domain of the Autism Diagnostic Interview Revised (ADI-R). Exclusion criteria were medical history or physical examination indicating physical or sensory impairment or significant medical problems.

## STATISTICAL ANALYSIS

All data presented are expressed as mean ± standard error of the mean (SEM). In all experiments, mean ± SEM was calculated. The one-way analysis of variance (ANOVA) was used to determine whether there are any statistically significant differences between the means of the groups and $P < 0.05$ was considered as significant.

### Analysis of IGF1, IGF2 and IGFBP1-6 serum concentration by ELISA:

IGF1 and IGF2 serum concentration was measured by Human IGF1 ELISA Kit (ab100545) and IGF2 ELISA Kit (LS-F11731) from LifeSpan BioSciences, respectively.

IGFBP1-6 concentration in serum was measured using the ELISA kits: Human IGFBP1 ELISA Kit (ab233618), Human IGFBP2 ELISA Kit (ab272207), Human IGFBP3 ELISA Kit (ab211652), Human IGFBP4 ELISA Kit (ab230936), IGFBP5 Human ELISA Kit, Invitrogen, and Human IGFBP6 ELISA Kit (ab240686) according their manufacturer instructions.

## RESULTS

In this study we measured serum concentration of IGF1, IGF2 and IGFBP1-6 in the children with different stages of ASD (Levels 1-3; Mild, Moderate and severe, respectively) using ELISA. We showed that IGF1, IGF2 and IGFBP1-6 were present in all serum samples of both ASD and control groups. The results showed that the serum concentration of IGF1 and IGF2 was significantly higher in ASD patients when compared to control group. We also showed that starting from stages I to III ASD, an increase of IGF1 and IGF2 serum concentration was observed (Table 2). Results obtained also shown that IGFBP1-6 concentration in the children with ASD were lower when compared to control group and low IGFBP1-6 serum concentration is associated with advanced stages of ASD (Table 2).

**Table 2.** IGF1, IGF2 and IGFBP1-6 serum concentration in different stages of Autism spectrum disorders and healthy control groups.

| Serum concentration (Mean±SD) ng/ml | ASD Mild | ASD Moderate | ASD Severe | Controls |
|---|---|---|---|---|
| IGF1 | 39.06±14.76 | 35.46±14.92 | 26.46±13.77 | 45.2±17.69 |
| IGF2 | 1490.6±159.90 | 1455.93±142.56 | 1380.133±178.55 | 1546.07±166.88 |

| | | | | |
|---|---|---|---|---|
| IGFBP-1 | 9.8±4.2 | 9.4±6.5 | 9.1±3.4 | 11.8±4.6 |
| IGFBP-2 | 175.13±47.40 | 167.53±51.27 | 150.8±40.28 | 178.6±60.43 |
| IGFBP-3 | 2867.33±496.49 | 2883.46±472.37 | 2214.86±543.20 | 3185.73±559.67 |
| IGFBP-4 | 397.86±91.642 | 387.8±77.96 | 357.13±75.05 | 409.6±102.06 |
| IGFBP-5 | 216.8±61.36 | 182.2±45.18 | 161.26±45.60 | 224.86±70.99 |
| IGFBP-6 | 189±60.009 | 176.93±50.15 | 165.06±47.742 | 200.53±64.72 |

## DISCUSSION

Autism spectrum disorder (ASD) is a neurodevelopmental condition characterized by defects in the establishment and maintenance of functional neuronal networks due to synaptic dysfunction [15]. Many genes and environmental factors have been suggested to play role in the pathogenesis of ASD. Growth factors including IGFs were shown to be associated with brain growth in children suffering from ASD [16].

Disturbances in neuronal development and synaptic plasticity have been documented in ASD patients. The physiological development, regulation and survival of specific neuronal populations shaping neuronal plasticity involve the so-called neurotrophic factor (NTFs) [17]. These regulate cellular proliferation, migration, differentiation and integrity, which are also affected in ASD [18]. Therefore, NTFs have gained increasing attention in ASD research.

The IGF pathway has been shown to play key role in a molecular mechanisms possibly leading to ASDs [19]. Steinmetz and colleagues showed that IGF2 signaling via its receptors target both basal and learning-dependent molecular abnormalities found in numerous ASD mice models, including those of identified genetic mutations and suggested that IGF2 represents a possible novel therapeutic target for ASD [10]. Provenzano and colleagues in 2014, using a mouse model of ASD, they suggested that altered GH/IGF levels in the hippocampus may play key role in learning disabilities associated to ASD [20]. It was suggested that increased IGF1 concentration in the CSF may have a neuroprotective effect against dopamine-induced neuro-toxicity in autistic children [21]. The deletion of IGFBP3 results in behavioral impairments that is associated with abnormal synaptic function associated with the pathogenesis of ASD and shows the critical role of IGFBP3 in the brain. IGF1 directly affects the rate at which oligodendrocytes promote myelination in the CNS, especially in the brain. Factors which reduce the production or availability of IGF could retard normal nerve programming in the fetus or neonate. Thus, it would be desirable to arrest the pathologic processes of autism in the early neonatal stage before irreversible nerve damage occurs [17].

It has been reported that the concentration of IGF1 in cerebrospinal fluid (CSF) is significantly decreased in ASD children [22]. Kimoto and colleagues showed that that lower serum IGF1 was related to cognitive impairment, suggest that metabolism of IGF1 may be involved in the pathogenesis of cognitive deficits in Alzheimer's disease [23]. It has been reported that the level of CSF and serum IGF1 and IGFBPs in the patients with Alzheimer's disease (AD) and Parkinson's disease (PD) is higher than in normal control. They suggested that IGF1 is a constant component of human serum and CSF and high levels of CSF IGF1 may be partly related to AD and PD pathophysiology [24, 25]. In this study for the first time we report that the serum concentration of IGF1, IGF2 and IGFBP1-6 has significantly decreased in ASD patients as compared to control group.

It is concluded that a low expression of IGFs and IGFBPs is correlated with advanced stages of ASD. It is also suggested that IGFs and IGFBPs system may be involved in the pathogenesis of ASD. Therefore, the detection of serum soluble IGFs and IGFBPs may be useful in classifying ASD.

**Acknowledgements:** This study was supported by the University of Guilan, Rasht, Iran.

**Conflict of interest:** None declared.

**Funding:** This research did not receive any specific grant from any funding agency in the public, commercial or not-for-profit sector.

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