The Association of Stem Cell Factor and Soluble c-Kit (s-cKit) Receptor Serum Concentrations with the Severity and Risk Prediction of Autism Spectrum Disorders

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Abstract

Stem cell factor (SCF) and its receptor (c-kit) signaling play important role in normal brain physiology including neurogenesis, synapse formation and spatial learning function of the hippocampal region of the brain. Autism spectrum disorder (ASD) is believed to result from abnormal development of neuronal networks and synaptic function. The aim of this study was to evaluate the SCF and its soluble receptor (s-ckit) serum concentrations in ASD. We also studied the serum SCF and s-ckit concentration with the severity of ASD (Levels 1–3; Mild, Moderate and severe, respectively). Ninety five patients with ASD (Mild; n = 33, Moderate; n = 32 and severe; n = 30) and 82 normal controls age matched were included in this study. The serum concentration of SCF and s-ckit were measured by enzyme-linked immunosorbent assay (ELISA). The SCF serum concentration in control subjects was 3.45 ± 1.06 ng/ml and in ASD was 3.41 ± 0.92 ng/ml (P = 0.88). The serum levels of s-ckit in control and ASD groups were 56.82 ± 13.22 ng/ml and 67.11 ± 12.00, respectively (P = 001). We have also studied serum SCF and s-ckit concentrations with the severity of ASD. The serum concentration of SCF in mild, moderate and severe ASD groups was 3.45 ± 0.93, 3.4 ± 0.87 and 3.43 ± 0.98 ng/ml, respectively (P > 0.05) and for s-ckit was 48.77 ± 9.28, 61.66 ± 12.18 and 93.11 ± 14.81ng/ml, respectively (P < 0.05). The result of this study suggests that serum s-cKit concentrations may provide a reliable and practical indicator of ASD and positively correlated with disease severity. It is also concluded that s-cKit might be involved in the pathophysiology of ASD.

Introduction

Autism spectrum disorder (ASD) is believed to result from abnormal development of neuronal networks and synaptic function (Sacai et al., 2020). It is estimated that worldwide one in 160 children has an ASD (62/10000). Based on epidemiological studies conducted over the past 50 years, the prevalence of ASD appears to be increasing globally (Elsabbagh et al., 2012). Syndromic forms unlike idiopathic forms include only 10% of cases often associated with malformations and/or dysmorphic properties. Syndromic forms originate from well-known genetic or genomic disorders that include neurofibromatosis, fragile X, tuberous sclerosis, Angelman and Rett syndromes (Benvenuto et al., 2009).

Stem cell factor (SCF) also named kit ligand, steel factor (SLF), is a cytokine that binds to the c-kit receptor (CD117). Its receptor, encoded by the proto-oncogene, c-kit, is a member of the class III family of intrinsic tyrosine kinase growth factor receptor. Both SCF and c-kit mRNAs are expressed in cells of the nervous system during development and in adulthood (Kim et al., 2003). c-Kit is expressed in neural stem cells and in their differentiated progeny (Erlandsson et al., 2004). During embryonic development, SCF mRNA is detectable in neural tube as early as at mouse embryonic day 9.5 (Keshet et al., 1991). In the adult nervous system, high level of SCF transcripts was found in the thalamus, cerebral cortex and cerebellum (Zhang and Fedoroff, 1997). Furthermore, c-kit has been shown to be expressed in neuroproliferative zones in the adult brain and in neuronal cultures (Jin et al., 2002).

The SCF or kit ligand is a cytokine mediating its biological effects by binding c-kit as its receptor. C-kit is a tyrosine 3 kinase receptor affecting downstream signaling pathways through its molecular functions, thereby inducing and enhancing the activity of these pathways (Lee et al. 2013). Moreover, activation of c-Kit signaling has been found to mediate cell survival, migration, and proliferation depending on the cell type. Signaling from c-kit is crucial for some aspects of the nervous system including neurogenesis and synapse formation (Lennartsson and Rönnstrand, 2012). Expression data also suggest that c-kit signaling may have important roles in the nervous system. Studies on mice carrying loss-of-function mutations in either c-kit or SCF have established a role for c-kit signaling in the spatial learning function of the hippocampal region of the brain (Katafuchi et al., 2000). Administration of SCF in animals leads to proliferation of primitive neurons. c-kit plays an important role in the migration of neuronal stem- and progenitor cells to sites of injury in the brain (Sun and Lee, 2004). It was shown that SCF induced functional improvement in chronic stroke is associated with a contribution to increasing neurogenesis through direct effects on stimulating neurons to form new neuronal networks. Systemic administration of SCF during the acute phase of experimental stroke reduces infarct size and ameliorates brain ischemia-induced neurological deficits (Zhao et al., 2007). Administration of anti-c-kit antibody into the cerebrospinal fluid leads to increased cell death in the developing cerebral cortex (Mashayekhi and Golizadeh, 2011). c-Kit knockout mice exhibit abnormalities in learning and memory (Katafuchi et al., 2000), suggesting potentially important roles for SCF/c-kit in normal brain physiology. In culture, recombinant SCF supports the survival of rat and chick neurons which express c-kit receptor (Hirata et al., 1993). Therefore, SCF/c-kit may act as a neurotrophic factor for c-kit expressing neurons.

A variety of integral membrane receptors, including c-Kit, can be released from the lipid bilayer by proteolysis to form soluble, truncated proteins. The soluble form of Kit (s-cKit) is produced by post-translational proteolytic cleavage of full length Kit (Kasamatsu et al., 2008). The proteases that generate soluble forms of membrane proteins are predominantly metalloproteinases or serine proteinases. The soluble receptors are smaller, consisting of the extracellular origin of the membrane-bound receptor and, in general, are able to bind to ligand with reduced affinity. The proteolytic cleavage of full-length Kit at the cell surface reduces the availability and density of full-length receptor and anchoring protein. s-Kit can bind both soluble and membrane-bound forms of the ligand (Merkwitz et al., 2011).

Mutations in genes involved in the regulation of the number, size, shape and strength of neuronal synapses and genes codifying for synaptic proteins were shown to be linked to ASD as well as to other neuropsychiatric and neurodevelopmental disorders (Sala et al., 2015; Verpelli and Sala, 2012). Due to potential important roles of SCF/c-Kit signaling in normal brain physiology including neurogenesis, synapse formation and spatial learning function of the hippocampal region of the brain, we aimed to study the serum concentrations of SCF and s-Kit in the risk prediction of ASD.

Patients And Methods

In this project, the peripheral blood was collected from children with ASD referred to pediatric neurology clinic, Mazandaran, Iran. The diagnosis was subsequently confirmed at the clinic according to the

parameters established in Diagnostic and Statistical Manual of Mental Disorders-DSM-5 (American Psychiatric Association, 2013). Key diagnostic features of children with ASD include deficits in social communication and restricted, repetitive patterns of behavior, interest, or activities. The diagnostic criteria exist on a continuum of severity and functional impairment. The severity of disease was divided to three levels: level 1 = Mild/ requiring support, level 2 = Moderate/ requiring substantial support and level 3 = Severe/ requiring very substantial support (American Psychiatric Association, 2013). Ninety five ASD patients (level 1; n = 33, level 2; n = 32 and level 3; n = 30) (7.4 ± 3.6 years) and 82 control subjects (6.9.±3.4 years) were enrolled in this study.

ASD patients on medication that could in any way interfere with the test result and children with atypical forms of ASD such; as Rett's syndrome, invasive developmental disorders without further specification, fragile X syndrome, Asperger's syndrome and Down's syndrome, use of any medication to address behavior/focus/attention during the previous six months, any food or drug allergy, use of any nutritional supplements during the previous six months were excluded from this project. The control group involved of children, with no clinical characteristics of ASD attended for routine blood tests (e.g., periodic control exams, acute infectious states, preoperative exams). All parents and guardians, regardless of child's age, signed the consent. The study was approved by the University of Guilan Ethics Committee (Code: 97-11065) and has been carried out in accordance with the Code of Ethics of the World Medical Association (Declaration of Helsinki).

Blood samples, from both ASD and controls, were collected in the morning, between 8:00 and 11:00 AM, centrifuged within the first 30 min and the resulting sera were stored at -80°C to avoid possible changes in s-Kit and SCF concentrations. All samples were evaluated in duplicate using a commercially available ELISA kits (Human SCF ELISA Kit) (ab176109) for SCF and Human c-Kit ELISA Kit (CD117) (ab45924) for soluble c-kit, according to the manufacturer's instructions.

Statistical analysis

For statistical analysis the values were calculated by one-Way ANOVA and were presented as mean ± standard deviation (SD). $P < 0.05$ was regarded as statistically significant. Graphs were made using Excel.

Results

Serum SCF and s-ckit concentration in ASD and controls

The serum concentration of SCF and s-ckit were measured by ELISA. The mean±SEM for SCF serum concentration in control subjects was 3.45±1.06 ng/ml and in ASD was 3.41±0.92 ng/ml (Figure 1). No significant change in SCF serum concentration was seen between ASD and control groups (P=0.88).

The mean±SEM serum levels of s-ckit in control and ASD groups were 56.82±13.22 ng/ml and 67.11±12.00, respectively (Figure 2). Significant increase in s-ckit serum concentration was seen between ASD and controls (P=001).

Serum SCF and s-ckit concentration in different ASD groups

We have also studied the SCF and s-ckit serum concentrations with the severity of ASD. The mean±SEM serum concentration of SCF in levels 1 to 3 ASD groups was 3.45±0.93, 3.4±0.87 and 3.43±0.98 ng/ml, respectively (Figure 3). No significant changes in serum SCF concentration was seen between three groups (P>0.05).

The serum concentration of s-ckit in levels 1 to 3 ASD groups was 48.77±9.28, 61.66±12.18 and 93.11±14.81 ng/ml, respectively (Figure 4). Significant changes in serum SCF concentration was seen between three groups (level 1 v level 2: p=0.02, level 1 v level 3: P<0.0001 and level 2 v level 3: P=0.0001). Thus, s-ckit was increased in patients with ASD and positively correlated with disease severity.

Discussion

Growth factors (GFs) are cytokines that regulate the neural development. Recent evidence indicates that alterations in the expression level of cytokines including stem cell factor during embryogenesis are linked to the pathophysiology and clinical manifestations of ASD. Synaptic defects have been shown to be implicated in ASD. Mutations in a large number of genes codifying for synaptic proteins have been identified in patients with ASD (Verpelli and Sala 2012).

It has been shown that c-Kit receptor tyrosine kinase and its ligand SCF are present on the pre- and postsynaptic sides of inhibitory synapses on Purkinje cells (Kim et al., 2003). It was reported that levels of numerous cytokines and growth factors are altered in the serum of ASD patients, implying that these factors may play a role in the development and progression of this disease (Russo et al., 2009). It was also suggested that SCF/c-Kit signaling plays role in synapse formation (Kim et al., 2003). Transgenic mice with a deletion of the c-kit gene display marked defects in learning and memory, suggesting an important function in normal brain physiology (Katafuchi et al. 2000). SCF and c-kit may be involved in the response to CNS damage, as both are up-regulated following brain injury and are correlated with neurogenesis (Jin et al., 2002), a mechanism for brain repair. Furthermore, overexpression of c-kit in a number of malignancies, including neuronal tumors, correlates with cellular survival (Vitali et al., 2003), further indicating SCF/c-kit may enhance cytoprotection in a variety of cell types, including neurons. SCF is a nerve regeneration factor, stimulating nerve regeneration *in vivo* and *in vitro* (Jin et al., 2002). The SCF/c-kit pathway is also involved in the migration of neural stem/progenitor cell to sites of brain injury. The SCF/c-kit pathway induces progenitor cell recruitment to ischaemic areas of the brain (Sun et al., 2004). Changes in the PGF2α, inflammatory-related neuropeptides and adiponectin serum concentrations have been observed in ASD patients (Pop et al., 2017; Mostafa et al., 2016; Quan et al., 2021).

Changes in the SCF and c-kit expression have been seen in many diseases. It has been shown that serum levels of SCF and s-ckit increased in pre-hypertension and are especially increased in hypertensive patients. SCF/c-kit levels are also positively correlated with serum levels of TNF-α (Zhong et al., 2009). It was shown that SCF mRNA expression is higher in asthmatic patients compared to controls (Tayel et al., 2017). It was suggested that serum SCF may be regarded as a predictor of *in vitro* fertilization and

embryo transfer (IVF-ET) outcome (Salmassi et al., 2011). Kalmarzi and colleagues suggested that serum concentration of SCF and s-ckit receptors have a direct effect on the severity of aspirin-induced asthma (Kalmarzi et al., 2019). Increased serum SCF levels was seen esophageal cancer patients and suggested that the potential role of serum SCF in the diagnosis of esophageal cancer patients, especially in combination with the classical tumor marker (Łukaszewicz-Zając et al., 2017). Bowen et al have shown that SCF serum concentration reduced in some patients with myelodysplasia (Bowen et al., 1993). Changes in serum concentrations of SCF and its soluble receptor c-kit seem to be reflecting asthma severity suggesting its role in asthmatic inflammation (Makowska et al., 2009). Moreover, changes in hepatocyte growth factor (HGF) serum concentration has been reported in ASD patients (Khalili et al., 2020).

It has been shown that soluble Kit binds SCF with high affinity, and can specifically block SCF, suggesting that one function of soluble Kit may be to modulate SCF bioactivity (Dahlen et al., 2001). Soluble Kit is generated by proteolytic cleavage of cell surface Kit. It has been suggested that shedding of Kit may modulate SCF bioactivity in two ways: by diminishing the density of Kit displayed on the cell surface, and by converting cell surface Kit to soluble Kit, which may serve as an antagonist of SCF bioactivity (Tajima et al., 2010).

The results of this study showed that serum s-kit concentration increased in ASD patients while there was no significant change in serum SCF levels in ASD patients as compared to control group. We have also shown that s-ckit concentration is higher in children with ASD compared to controls and associated with increased severity of ASD. As soluble Kit binds to SCF with high affinity which can specifically block SCF, suggesting that one function of soluble Kit may be to modulate SCF bioactivity (Dahlen et al., 2001). Increased s-cKit in the serum of patients with ASD in this study may bind to SCF and block SCF signaling.

It is concluded that s-cKit and SCF are a constant composition of human serum. The result of this study suggests that serum s-cKit concentrations may provide a reliable and practical indicator of ASD and positively correlated with disease severity. It is also concluded that s-cKit might be involved in the pathophysiology of ASD and the detection of serum s-ckit may be useful in classifying ASD.

Declarations

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Conflict of interest: The authors declare that they have no conflict of interest

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Author contributions

Conceptualization: [Farhad Mashayekhi], Methodology: [Somayeh Shabani and Soheila Talesh Sasani], Formal analysis and investigation: [Farhad Mashayekhi and Zivar Salehi], Writing - original draft

preparation: [Farhad Mashayekhi], Funding acquisition: [Farhad Mashayekhi], Resources: [Farhad Mashayekhi and Zivar Salehi], Supervision: [Farhad Mashayekhi].

Data Availability

A submission to the journal of Metabolic Brain Disease implies that materials described in the manuscript, including all relevant raw data, will be freely available to any researcher wishing to use them for non-commercial purposes, without breaching participant confidentiality.

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Figures

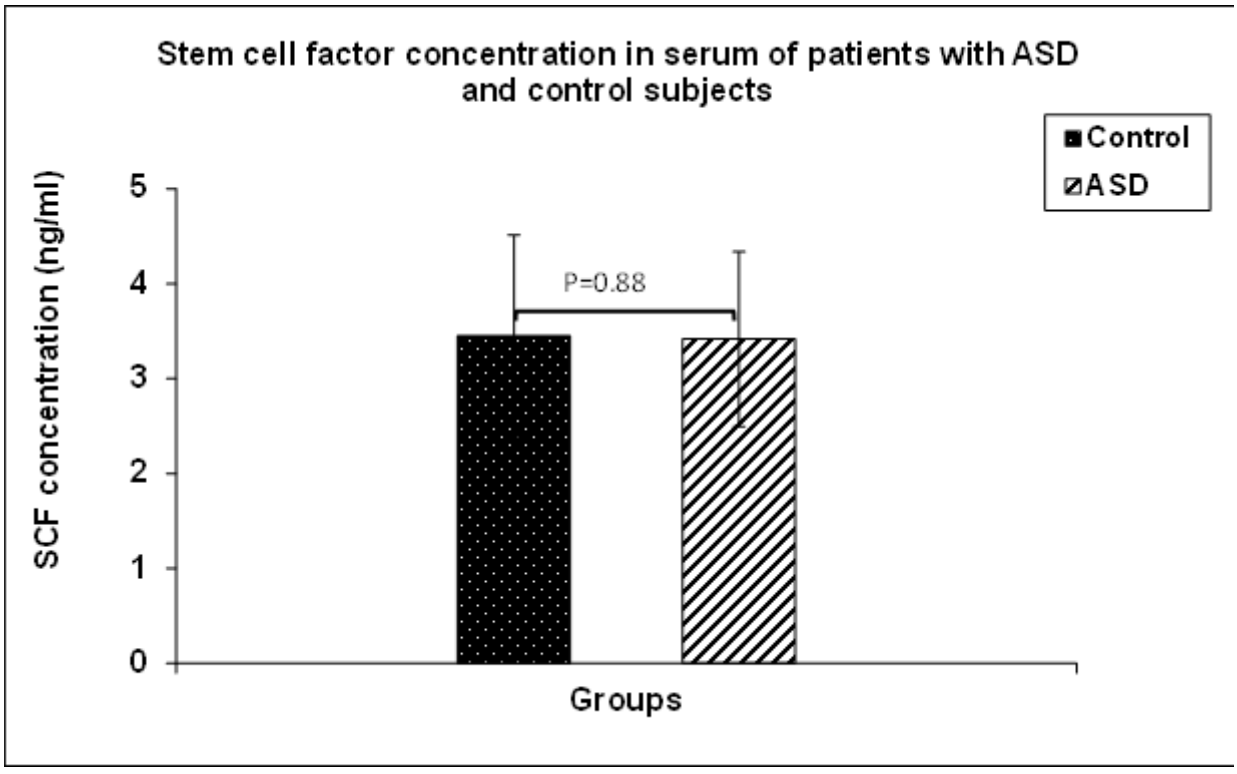

Figure 1

SCF serum level in the controls and patients with ASD (ng/ml). No significant change in serum SCF level has been seen in the patient's samples as compared with control group (P=0.88).

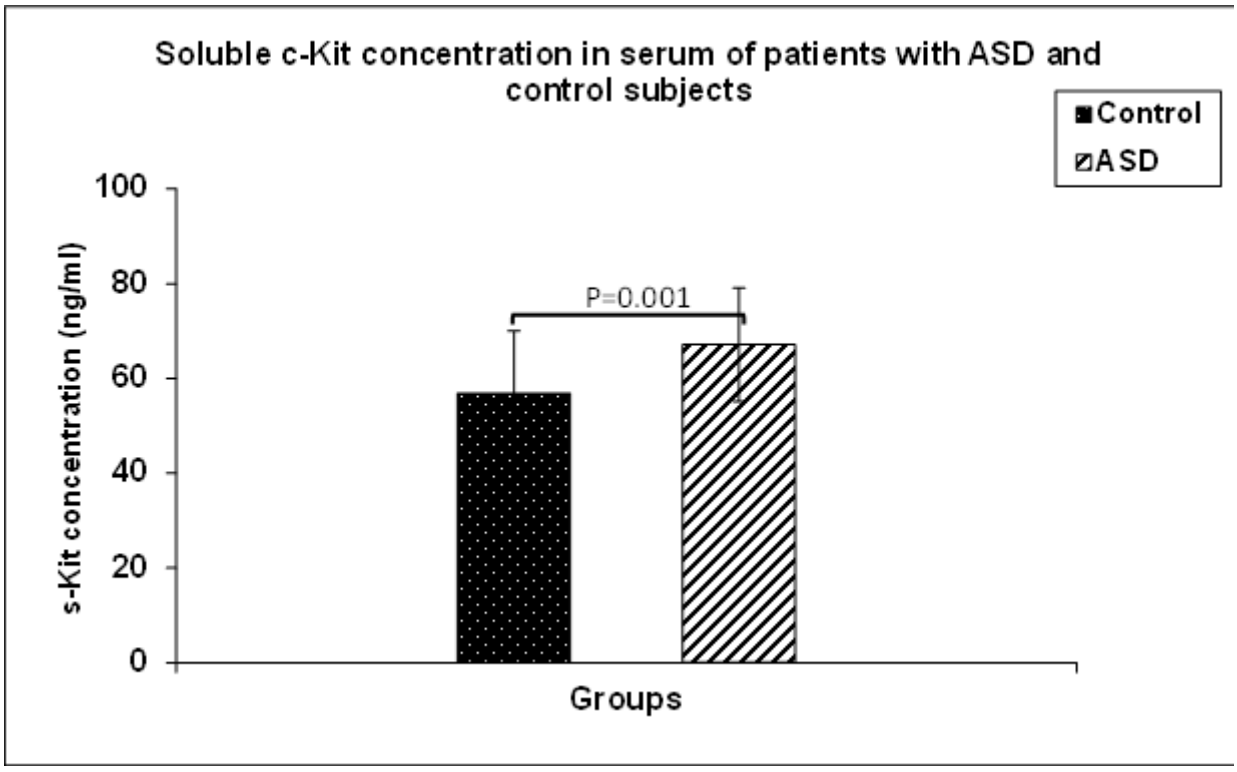

Figure 2

s-kit serum level in the control subjects and patients with ASD (ng/ml). Significant increase in serum SCF level has been seen in the ASD patients as compared with control group (P=0.001).

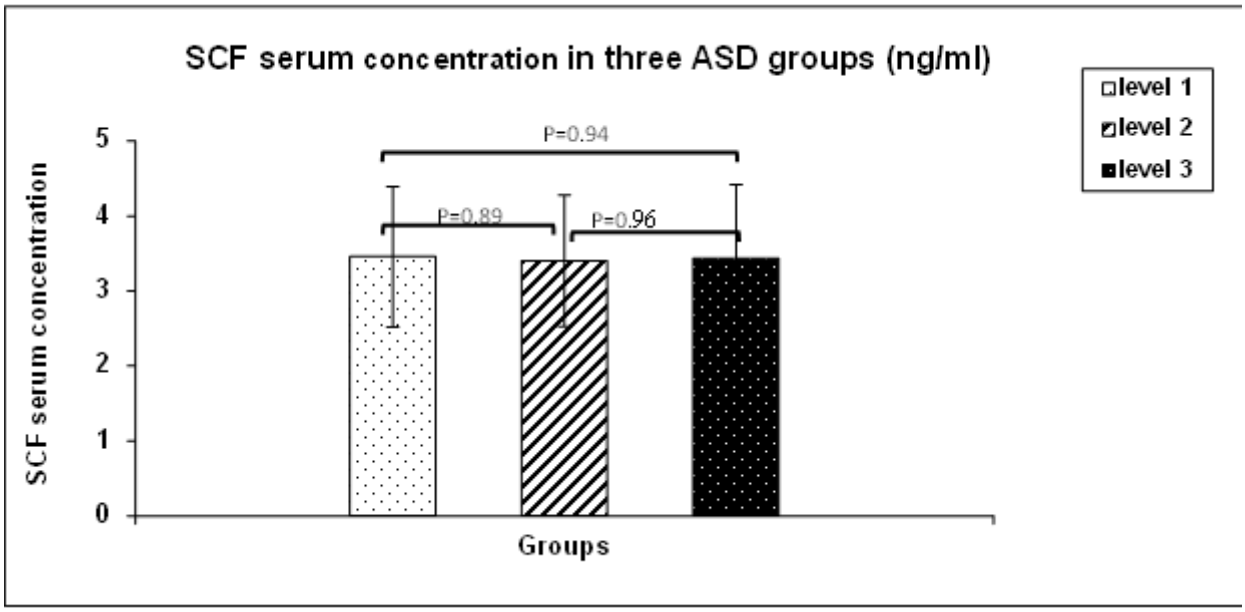

Figure 3

Association of SCF serum concentration with the severity of ASD. The serum concentration of SCF in level 1 (mild), level 2 (moderate) and level 3 (severe) ASD groups was 3.45±0.93, 3.4±0.87 and 3.43±0.98 ng/ml, respectively. No significant change in serum SCF concentration was seen between three groups (P>0.05).

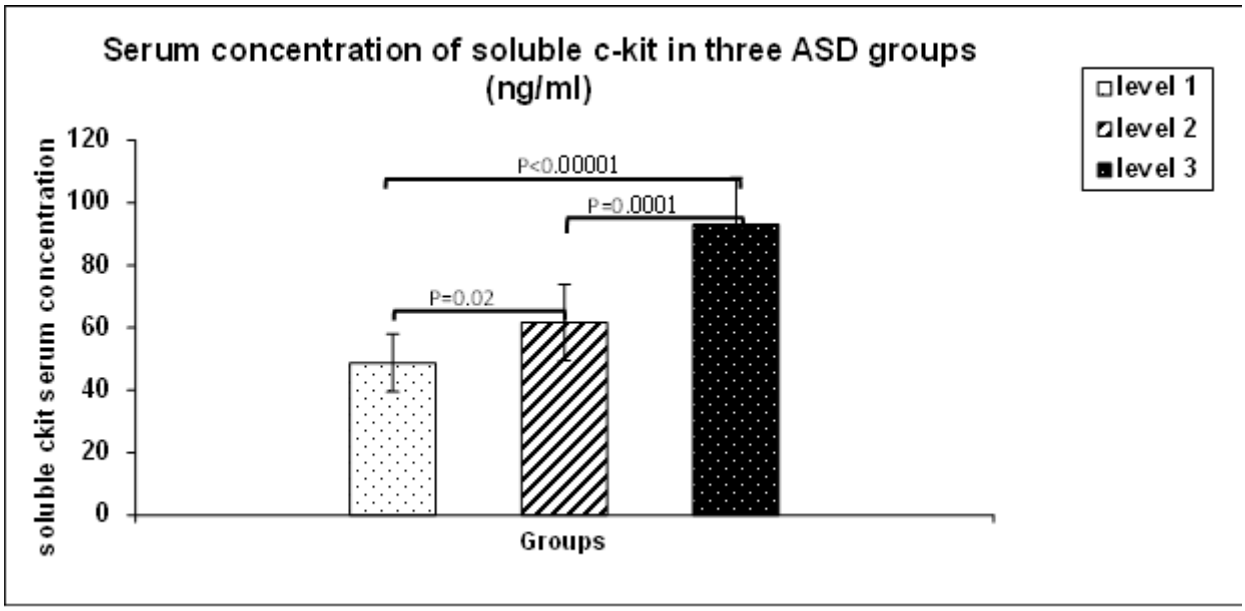

Figure 4

Association of serum s-kit concentration with the severity of ASD. The serum concentration of s-kit in level 1 (mild), level 2 (moderate) and level 3 (severe) ASD groups was 48.77±9.28, 61.66±12.18 and 93.11±14.81ng/ml, respectively (P<0.05). Significant change in serum s-kit concentration was seen between three groups (P<0.05).