Correlation Between Hedgehog (Hh) Protein Family and Brain-Derived Neurotrophic Factor (BDNF) in Autism Spectrum Disorder (ASD)

Dost Muhammad Halepoto, Shahid Bashir, Rana Zeina and Laila Y. AL-Ayadhi

ABSTRACT

Objective: To determine the correlation of Sonic Hedgehog (SHH), Indian Hedgehog (IHH), and Brain-Derived Neurotrophic Factor (BDNF) in children with Autism Spectrum Disorder (ASD).

Study Design: An observational, comparative study.

Place and Duration of Study: Autism Research and Treatment Center, Al-Amodi Autism Research Chair, Department of Physiology, Faculty of Medicine, King Khalid University Hospital, King Saud University, Riyadh, Saudi Arabia, from October 2011 to May 2012.

Methodology: Serum levels of SHH, IHH and BDNF were determined in recently diagnosed autistic patients and age-matched healthy children (n=25), using the Enzyme-Linked Immunosorbent Assay (ELISA). Childhood Autism Rating Scale (CARS) was used for the assessment of autistic severity. Spearman correlation co-efficient ‘r’ was determined.

Results: The serum levels of IHH and SHH were significantly higher in autistic subjects than those of control subjects. There was significant correlation between age and IHH ($r = 0.176, p = 0.03$), BDNF and severe IHH ($r = 0.1763, p = 0.003$), and severe BDNF and severe SHH ($r = 0.143, p < 0.001$). However, there were no significant relationships among the serum levels of SHH, IHH and BDNF and the CARS score, age or gender.

Conclusion: The findings support a correlation between SHH, IHH and BDNF in autistic children, suggesting their pathological role in autism.


INTRODUCTION

Autism is a neurodevelopmental disorder of unknown origin defined by the presence of marked social deficits, specific language abnormalities, and stereotyped repetitive behaviors. There is growing evidence that autism may be influenced by genetic, neurological, environmental and immunological factors; however, its exact pathophysiology is unknown.

Hedgehog (Hh) proteins are involved in many essential developmental processes in vertebrates and invertebrates. Sonic Hedgehog (SHH) and Indian Hedgehog (IHH) belong to hedgehog family of proteins that play a critical role in the development of many organs, including the central nervous system. When SHH binds to its receptor Patched-1 (PTCH-1), it cannot interact with the transmembrane protein smoothened (SMO), resulting in activation of transcription factor GLI. The activated GLI regulates expressions of many target genes that control cell growth, survival, and differentiation in a wide variety of cells, including neurons. SHH signaling is activated in adult organism after injury and is involved in tissue repair mechanism. Indian Hedgehog (IHH) also plays very important role in vertebrate development including differentiation of the visceral endoderm, and to regulate the proliferation and differentiation of the gut epithelial stem cell. BDNF is a member of the neurotrophin family of growth factors, which are related to the canonical Nerve Growth Factor (NGF). Neurotrophic factors are found in the brain and the periphery. BDNF is candidate gene for modulating brain anatomy in ASD which plays a key role in neurogenesis, cortical lamination, synaptic plasticity, and neuron survival.

A correlation between SHH, Oxygen Free Radicals (OFR) and BDNF has already been reported in autistic children, suggesting a pathological role of oxidative stress and SHH in autism spectrum disorders. It was demonstrated that autistic children produced a significantly higher level of oxygen free radicals OFR and serum SHH protein in children with mild as well as severe form of autism. In addition, the serum level of BDNF was significantly reduced in autistic children with mild form of the disorder but not with severe form of the disorder. In another study, relationship between SHH,
BDNF and oxidative stress in autism spectrum disorders was also observed.\textsuperscript{10}

The excess of neurotrophins and neuropeptides in serum of newborn infants has predictive value in determining those children who would have disruption of intellectual and/or social development later. These facts have led to search for biological markers that may allow earlier detection of autism.

The rationale of the current study was to elaborate the authors’ previous work, related SHH, IHH and BDNF might be linked to ASD, by re-testing the subjects (patients and controls).

The aim of this study was to determine the correlation of Sonic Hedgehog (SHH), Indian Hedgehog (IHH), and Brain-Derived Neurotrophic Factor (BDNF) in children with Autism Spectrum Disorder (ASD).

**METHODOLOGY**

This study was conducted at the Autism Research and Treatment Center, Al-Amodi Autism Research Chair, Department of Physiology, Faculty of Medicine, King Khalid University Hospital, King Saud University, Riyadh, Saudi Arabia, from October 2011 to May 2012, on autistic children and their age and gender matched controls.

The diagnosis of ASD was made by child neuro-psychiatrist and pediatrician, based upon the criteria of ASD as defined in the Diagnostic and Statistical Manual of Mental Disorders (DSM-IV)\textsuperscript{1} on the day of screening test. The Childhood Autism Rating Scale (CARS) was completed as a further measurement of the severity of ASD\textsuperscript{11} which rates the child on a scale from one to four in each of fifteen areas (relating to people; emotional response; imitation; body use; object use; listening response; fear or nervousness; verbal communication; non-verbal communication; activity level; level and reliability of intellectual response; adaptation to change; visual response; taste, smell and touch response and general impressions). The scores on the CARS range from 15 to 60, whereas individuals with scores lower than 30 are categorized as non-autistic. A CARS score between 30 and 36.5 indicates mild to moderate autism and scores ranging from 37 to 60 indicate severe autism. Written consent was obtained from the parents of each subject, according to the guidelines of the Ethical Committee of King Khalid Hospital, King Saud University, Riyadh, Saudi Arabia. All procedures followed were in accordance with the Helsinki Declaration.

After an overnight fasting, blood samples (3 ml) were collected from subjects in both groups in plain test tubes. Blood samples were allowed to clot, and then centrifuged at 3,000 rpm to collect serum samples, which were stored in a freezer at -80°C until the time of analytical assays. All samples were assayed in duplicate and in a double-blind manner. The assay reproducibility generally ranged from 5 to 10% error.

Serum level of BDNF was measured using a commercially available sandwich enzyme immunoassay (ELISA) kit from Emax Immunoassay System (Promega Corp., Madison, Wisconsin). The detailed description of test is described in our previous study.\textsuperscript{9} Serum levels of SHH and IHH were measured using a commercially available sandwich enzyme immunoassay (ELISA) kit from Cusabio Biotech Co. Ltd (Wuhan, China).

All data are presented as mean ± standard deviation (SD). Statistical differences were ascertained by using the Student's t-test with significance set at a p-value of 0.05 or lower. Additionally, we computed the percent change (%) in serum level from mean data of patients and control subjects. Pearson's correlation coefficient “r” was used to determine the relationship between different variables.

**RESULTS**

Autistic children (n=60, mean age: 6 ±1.72 years; age range: 3 - 11 years) and age and gender-matched healthy controls (n=25, mean age: 7.04 ±1.74 years; age range 3 - 11 years) were recruited in the study.

There were no significant differences in the ages of autistic and control groups (p = 0.10). The resulting values of biomarkers SHH, IHH and BDNF from autistic and control subjects are shown in Table I.

**Table I:** Serum levels of brain-derived neurotrophic factor (BDNF), sonic hedgehog (SHH) and Indian hedgehog (IHH) in autistic and healthy children.

<table>
<thead>
<tr>
<th>Biomarkers</th>
<th>Healthy children (pg/ml)</th>
<th>Patients with autism (pg/ml)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean BDNF ± SD</td>
<td>290 ± 162*</td>
<td>392 ± 243*</td>
<td>0.474</td>
</tr>
<tr>
<td>Mean SHH ± SD</td>
<td>2.65 ± 2.72*</td>
<td>15.64 ± 10.53*</td>
<td>0.000</td>
</tr>
<tr>
<td>Mean IHH ± SD</td>
<td>0.79 ± 0.54*</td>
<td>1.63 ± 0.96*</td>
<td>0.002</td>
</tr>
</tbody>
</table>

* = Compare control subject with autistic children.

**Figure 1:** Serum level of Indian hedgehog (IHH) in severe autistic children (based on CARS score) were correlated with the brain-derived neurotrophic factor (BDNF) (r = 0.176, p=0.28).
The serum levels of BDNF 134% (the percent change (%) from mean data of patients and control subjects) (392 ±243 pg/ml, 290 ±162 pg/ml, p = 0.474), 590% (15.64 ±10.53 pg/ml, 2.65 ±2.72 pg/ml, p < 0.00) for SHH and 206% (1.63 ±0.96 pg/ml, 0.79 ±0.54 pg/ml, p < 0.002) for IHH of autistic, were significantly higher compared to control children. Figure 1 showed serum level of severe IHH was correlated with the BDNF in autism (r = 0.176). Relationship between IHH serum level and age was correlated in the autism (r = 0.176, Figure 2).

The results demonstrated near to significant correlation between severity of autism and serum levels of IHH, SHH and BDNF in autistic subjects. Furthermore, serum levels SHH and BDNF of autistic subject had no significant correlations with the age of autistic subjects (p = 0.51). 

DISCUSSION

In the current study, it was speculated an important link among SHH, IHH and BDNF interactions and how this link could help us understand the ASD. The authors demonstrated higher serum level of SHH, which was positively correlated with the degree of autism. Furthermore, there was a statistically significant correlation of BDNF with severe SHH and IHH in ASD patients. The etiology of ASD is not well understood, though it likely involves genetic, immunologic, and environmental factors. The dramatic increase in reported prevalence has encouraged an intense effort to identify early biological markers. Such markers could allow earlier identification and therapeutic intervention, contributing to improved prognosis. 

In addition, BDNF participates in neurotransmitter release. BDNF is involved in the survival and differentiation of dopaminergic neurons in the developing brain and plays an important role in the formation and plasticity of synaptic connections. Within the nervous system, SHH protein is associated with development and patterning of the CNS. Latest reports have signified a critical role played by SHH pathway in many neurological diseases. However, its exact role and the underlying mechanisms are still unclear. It has been reported that SHH expression is up-regulated prior to the induction of BDNF mRNA, and blocking SHH signal suppresses BDNF expression. Considering the protective role of BDNF against oxidative stress, activation of the SHH pathway induces the increase of BDNF and results in neuroprotective to oxidative stress.

BDNF belongs to the neurotrophin family that may affect neuronal survival and differentiation. SHH is a morphogen important for the embryonic development. Possible correlation between BDNF and SHH is less studied. Hashimoto and colleagues demonstrated up-regulation of SHH expression, prior to the induction of BDNF mRNA in Schwann cells, adjacent to the injured site in an animal model of sciatic nerve injury. The same research group demonstrated causative relationship between the induction of SHH and BDNF, continuous administration of hedgehog inhibitor CPM to the injured site suppressed the increase of BDNF expression and, notably, deteriorated the survival of motor neurons in lumbar spinal cord. Wu et al. demonstrated BDNF-induced up-regulation of SHH at both mRNA and protein levels, suggestive of involvement of a transcriptional mechanism.

In addition, pretreatment of SHH has been shown to protect cardiomyocytes against hydrogen peroxide-induced cytotoxicity in vitro. As a result, SHH may offer both anti-oxidative and anti-apoptotic actions under appropriate circumstances. Metabolic stress induced by compromised mitochondria has been implicated in both acute and chronic neurodegenerative disorders such as ischemic stroke, Alzheimer's Disease (AD), Parkinson's Disease (PD), and Huntington's Disease (HD).

An increase in SHH protein was initially reported in the gray matter from multiple sclerosis brain lesions or in animal models of this pathology including experimental autoimmune encephalomyelitis and cuprizone-induced demyelination. It points out towards a possible protective effect exerted by SHH and it is with agreement to our findings in autism. The most likely explanation for higher level of SHH in autistic, examined in this study, is due to increased oxygen free radicals production as a protective mechanism secondary to increase oxidative stress inside the autistic. In severe ASD, further increase in IHH and SHH produced a negative feedback response on the production of BDNF, as demonstrated by lower level of BDNF in severe but not in mild ASD. Existing data provide support for considering SHH signaling as an important mechanism in tissue-repair process in brain diseases, and as a target for novel therapeutic approaches for the treatment of brain disorders and particularly in ASD.
Overall, converging evidence from several domains of autism research, points toward the involvement of widespread abnormalities in brain structures and functions in autism. As autism is such a heterogeneous disorder, searching for the underlying causes is like looking for a lost needle in a haystack. One of the most important things to do in future research might thus be decreasing sample heterogeneity, which will increase the power to detect brain abnormalities, specific to autism. Defining the underlying brain abnormalities and its causes, it will certainly help in developing better, may be even curative (pharmacological) treatment of autism.

**CONCLUSION**

The reported findings provide a preliminary as well as direct evidence of altered IHH, SHH and BDNF proteins in subject with ASD, which may contribute to the early pathogenesis of ASD, offer valuable biomarkers, and point to novel therapeutic interventions. However, these data should be treated with caution until further investigations are performed, with a larger subject population, to determine whether the decrease of serum SHH, IHH and BDNF levels is a mere consequence of autism or has a pathogenic role in the disease.

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**REFERENCES**