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Serum homocysteine, vitamin B12 and zinc level in autism spectrum disorder children in Dhaka City

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Abstract

Background: Autism spectrum disorder (ASD) is a complex neurodevelopmental disorder with genetic and enviromental etiology. Homocysteine (Hcy) is a non-protein á-amino acid, its overproduction could add to the pathophysiology associated with autism. Serum homocysteine metabolism is closely related with vitamin B12 and zinc level for proper enzymatic function. **Objectives:** To evaluate the serum homocysteine, vitamin B12 and zinc level in children with autism spectrum disorder. **Methods:** This cross-sectional observational study was conducted on 35 diagnosed ASD children enrolled from parent's forum of autistic children, Mohakhali, Dhaka.Age BMI mached apparently healthy children were control. Serum homocysteine, vitamin B12 and zinc level were estimated by automated analyzer. For statistical analysis students unpaired "t" test was done. **Results:** The mean value of serum homocysteine was significantly higher whereas the Vitamin B12 and Zinc level were lower in ASD than the control group. **Conclusion:** ASD children are prone to have higher homocysteine with lowered vitamin B12 and zinc level.

Key words: ASD, Homocysteine, Vitamin B12, Zinc

Introduction

Autism spectrum disorder (ASD) is a complex neurodevelopmental disorder, characterized by symptoms of inattention, impulsivity and hyperactivity.[1] Autism is a pervasive disorder during infancy, occurs mostly during the first three years of life.[2-3] Because of debilitating and lifelong nature of autism spectrum disorder, these children are burden for both family and society.[4]The global burden of autism is 7.6 per 1000 population or 1 in every 132 persons.[4] In Bangladesh, the prevalence of ASD is 0.15 to 0.8%.[5-6]In 2013, survey of autism and neuro developmental disorder in Bangladesh was conducted by Non-Communicable Disease Control Program (NCDC) DGHS, were found that the prevalence of any neurodevelopmental disorder is 71/1000 in Bangladesh and prevalence of autism spectrum disorder in Dhaka city is 3% and rural population is 0.07%.[6]

The etiology of ASDs is complex and multifactorial. Neurochemical, neuroanatomic, genetic and environmental factors are thought to play a role in the etiology. Abnormalities involving vitamin B complex depended homocysteine methylation reactions, oxidative stress and genetic predisposition have been implicated as potential causes. Some previous studies reported a relationship between zinc, vitamin B12 and homocysteine and various psychiatric diseases including ASD.[7-11]

Homocysteine is an essential amino acid, metabolized by the remethylation pathways which requires zinc and Vitamin B12 together as a cofactor. Homocysteine is also a powerful excitotoxin and its metabolic products may cause neuronal damage and disrupt the synthesis of proteins and neurotransmitters which are required for the structural integrity of the brain. Normal levels of vitamins inside the body helps in the body's maintenance process and better performance.[11]Vitamin B12is necessary for protein synthesis and formation of the myelin sheath throughout the central nervous system.[12] A study on Egyptian children with ASD found significantly lower serum zinc and vitamin B12.[14] Low serum vitamin B12 levels in ASD patients suggested increased level of oxidative stress and impaired DNA methylation which can be a leading factor in the pathophysiology of ASD.[15-16]In a study investigating the role of the vitamin B12-homocysteine metabolic pathway in the etiology of attention deficit hyperactivity disorder (ADHD), it was shown that vitamin B12, homocysteine pathway gene variants could affect the etiology of ADHD through mild hyperhomocysteinemia and vitamin B12 deficiency.[18] Little is known about this relationship in ASD. This study was designed to explore this relationship in ASD children.

Methods

Design and setting

This cross-sectional observational study was carried out in the Department of Physiology, Bangabandhu Sheikh Mujib Medical University (BSMMU), Dhaka from March 2023 to February 2024.

Study participants

In this study 35 ASD boy aged 3-8 years were recruited from parents' forum of children with ASD in Dhaka city as study group. The control groupincluded 35 age and BMI matched apparently healthy children.

Exclusion criteria

All participants were free from acute illness, liver diseases, anticonvulsants and hepatotoxic drug.

Sampling

Purposive sampling was adopted to select the participants.

Data collection

After selection of the subjects,thorough information was given to their parents about the objectives and study procedure. An informed written consent was obtained from parents. Detail personal, medical, dietary, familial and socioeconomic history of the subjects from their

parents were documented. After selection, the parents were requested to report at the Department of Physiology, BSMMU on examination day at 8 am, with their child in fasting condition. Under aseptic precautions, 4ml venous blood from anti-cubital vein of all children was collected and was immediately sent to the laboratory of the Department of Biochemistry and Molecular Biology, BSMMU for estimation of serum homocysteine, vitamin B12 and Zinc by an autoanalyzer.

Statistical analysis

All data were expressed as mean with standard deviation (mean±SD), range and percentage. For statistical analysis,Shapiro Wilk test and independent sample 't' test were done, as applicable, by using SPSS (Version 27) for Windows. In the interpretation of results, p value ≤0.05 was accepted as significant.

Result

All the subjects of the study were matched for age and BMI (Table -I). Here, the mean serum homocysteine was significantly higher and the mean serum vitamin B12 and zinc level was significantly lower in ASD children compared to controls ($p<0.001$) (Figure 1).

Table I: General characteristics of ASD Children and healthy controls (N=70)

Characteristics	ASD (n=35)	Controls (n=35)	p value
Age (years)	5.38± 1.45 (3-8)	5.38 ± 1.68 (3-8)	0.950
BMI (kg/m^2)	15.25 ± 1.22 (11.35-22.22)	(13.66-20.17) (13.66-20.17)	0.101

Data were expressed as mean± SD (range). Statistical analysis was done by independent sample t-test. N= total number of children; n= number of children in each group; ASD=Autism Spectrum Disorder; BMI=Body Mass Index (kg/m^2)

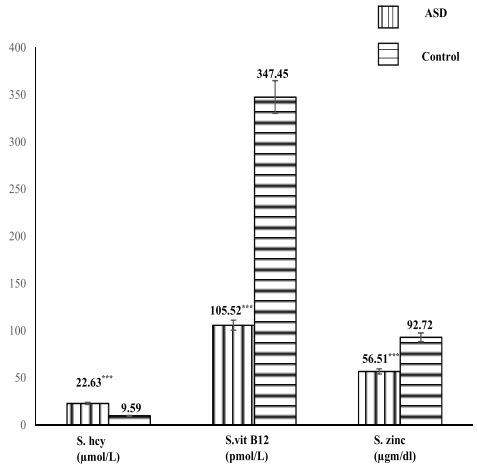

Figure 1: Each bar symbolizes mean± SD for 35 subjects. ASD: children with autism spectrum disorder (study group); Control: apparently healthy individual (control group).n=Number of subjects. ***p<0.001.

Discussion

In the present study, significantly higher serum homocysteine level in children with ASD was in agreement with other investigators.[14-16]

Again, the results of significantly lower serum vitamin B12and zinc (Zn) in these ASD children compared to healthy control also agree to other investigators.[13]On the contrary, one group of researchers found no significant difference of serum vitamin B12 level in ASD children compared to healthy control.[16] In the present study this observation further confirm to the association of low vitamin B12 and zinc to the current series of autistic children.

Dietary history of our ASD children reveals that 48% of them (data not shown) avoid all kinds of dairy foods and eggs which are the rich sources of vitamin B12 and zinc[19]. Moreover, they also have restrictive type of food habit and did not take enough vegetables. Therefore, their regular diet may fail to meet the demand of vitamin B12 and zinc in developing age. In agreement, Herdon et al. reported that ASD children have dietary selectivity which results in less consumption of vitamin B12 and other minerals.[21]

Several authors have suggested that high homocysteinemia and lowered Vitamin B12 and zinc during early brain development could be a risk factor for the altered neurobehavioral outcome.[22] Moreover, low plasma zinc may increase the possibility of blood lead and arsenic accumulation and toxicity, which were associated with pathophysiology of ASD.[23, 24]

Excess homocysteine causes direct damage to lipid, protein, mitochondria and DNA of neurons by unbalancing the antioxidant system.[16]An aggravation of homocysteine also causes excess glutamate which causes decreases the glutathione level specially to hippocampus; thus increases oxidative damage.It can be suggested that highhomocysteinemia can be a risk factor for altered neurobehavioral outcome.[17]

Again, Vitamin B12 is necessary for protein synthesis and formation of myelin sheath throughout the central nervous system. Moreover, vitamin B12 deficiency may cause of mitochondrial dysfunction and development of the sign symptoms of ASD[21-22]. The present result supports the role of vitamin B12 for the poor neural development in ASD children. In this particular investigation, the mean plasma zinc level of the study group was significantly lower than that of the control group in children who were between the ages of 3 and 8 years old. Decreased zinc causes copper level to increase that directly damages neurons by unbalancing the antioxidant system[28]. It causes Zn deficiency, dysregulates dopamine, serotonin and epinephrine levels, interferes with adrenal hormone production, decreases zinc absorption in pre and postsynaptic neurons, and decreases the conversion of glutamate to gamma amino butyric acid (GABA)[29]. Therefore, in this present study, a lower Zn and vitamin B12 in ASD children with raised homocysteine level may reflect decreased efficiency of the antioxidant system in autism spectrum disorder.

Conclusion

From the result of this cross sectional type study it may be concluded that higher serum homocysteine level and lower vitamin B12 and zinc level may be a risk factor for autism spectrum disorder children.

Conflict of interest

Authors did not have any conflict of interest

Ethical aspects

Ethical aspects of this study involving minor age group was approved by the Institutional Review Board of BSMMU.

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