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RESEARCH PAPER

Study of Homocysteine, Vitamin B12 and Folate in Children with Autism Spectrum Disorder

Ayatun Nesa, Gazi Sharmin Sultana

Laboratory Medicine, BIRDEM General Hospital, 122, Kazi Nazrul Islam Avenue, Shahbag, Dhaka.

Abstract

Background: Autism spectrum disorder (ASD) is a complex neurodevelopmental disorder that usually begins at or before the age of 3 years. The etiology of these disorders, includes environmental factors such as vitamins which have important role in central nervous system functioning. Vitamin B12 and folate are involved in the methionine-homocysteine pathway and some studies have shown a relationship between folate, vitamin B12 and homocysteine and various psychiatric diseases including ASDs.

Objective: To evaluate the homocysteine, vitamin B12 and folate status in children with ASDs in Bangladesh.

Methodology: This case control study included 50 ASD cases and 50 age matched healthy controls, according to inclusion criteria. After taking informed written consent from guardian, a structured questionnaire was filled up for each subject including socio-demographic history, family history, birth history and milestone of development. 5cc venous bloods were collected with all aseptic precautions in clot activator tube and serum homocysteine, vitamin B12 and folate levels were estimated. Collected data was checked, edited and processed with the help of SPSS (23).

Results: The mean age of autism children was 5.71±2.06 years and in control group, it was 6.00±1.99 years. Mean S.Vit B12 (pg/ml) and S.Folic acid (ng/mL) were found significantly lower in autism children compared to control children (241.46±60.51 Vs 302.58±76.66 and 7.12±2.16 Vs 9.72±2.96). On the other hand, the mean S. Homocystiene (µmol/L) was found significantly higher in cases than controls (8.08±2.11 Vs 6.12±1.71). Conclusion: This study revealed that serum vitamin B12 and folate levels were significantly decreased, and serum homocysteine were significantly increased in children with autism spectrum disorders (ASDs) compared to normal healthy children. So, screening of serum homocysteine, vitamin B12 and folate in children with autism spectrum disorders is recommended for early management of complications related to the disease process.

Key word: Autism spectrum disorders, Homocysteine, Vitamin B12 , Folate.

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]The global burden of autism is 7.6 per 1000 population or 1 in every 132 persons.[4]According to WHO fact sheet January 2016, it was estimated that worldwide 1 in 160 children has an autism spectrum disorder. 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 Programme (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 folate depended homocysteine methylation reactions, oxidative stress and genetic predisposition have been implicated as potential causes. Some studies have shown a relationship between folate, vitamin B12 and homocysteine and various psychiatric diseases including ASDs.[7-11]

Homocysteine is an essential amino acid,metabolized by the remethylation pathways which requires folate and Vitamin B12 together as a cofactor. Homocysteine is also known to be a powerful excitotoxin, and its metabolic products may cause neuronal damage and disrupt the synthesis of proteins and neurotransmitters which are important for the structural integrity of the brain. Recent clinical studies reported higher serum and urine homocysteine levels in children with ASDs when compared to healthy controls.[12-13]Some

***Correspondence:** Dr. Ayatun Nesa, Laboratory Medicine, BIRDEM General Hospital, 122, Kazi Nazrul Islam Avenue, Shahbag, Dhaka.*

Email: dipa2801@yahoo.com

ORCID ID: 0000-0002-2825-0314

researchers assessed dietary adequacy of Egyptian children with ASD and found that serum folate and vitamin B12 were significantly lower.[14] Low serum vitamin B12 levels in ASD patients were interpreted to reflect increased levels of oxidative stress and impaired DNA methylation which can be an important factor in the pathophysiology of ASDs.[15-16] Developmental delays, cognitive impairment and reduced memory function have been shown to be related to cerebral folate deficiency of abnormal folate transfer to the fetal CNS.[17] In a study investigating the role of the folate homocysteine metabolic pathway in the etiology of ADHD (attention deficit hyperactivity disorder), it was shown that folate, homocysteine pathway gene variants could affect the etiology of ADHD through mild hyperhomocysteinemia and vitamin B12 deficiency.[18]

Because of debilitating and lifelong nature of autism spectrum disorder these children as burden for individual, family and society.[19] Previous studies reported that, oppositionality and hyperactivity/ impulsivity symptoms in ASD and ADHD group were related to vitamin B12, folate and homocysteine levels.[20-21] Some preliminary studies suggest that vitamin and micronutrient supplements may reduce emotional liability, aggression and oppositional behaviors in children with ADHD and ASDs.[22-23] So this study had been planned to evaluate the level of homocysteine, vitamin B12 and folate in children with ASDs in Bangladesh. Outcome of this study may help for better understanding, improvement and management of children with ASDs.

Methodology

This case control study was conducted with 50 diagnosed cases of ASDs and 50 age matched healthy children as controls. According to inclusion criteria, cases were collected from the Child Development Centre of BIRDEM -2 Hospital and controls were collected from outpatient department of Pediatrics, who came for routine checkup. A structured questionnaire was filled up for each study subject after taking informed written consent from their guardian. With all aseptic precaution 5 ml venous blood sample was collected in plain test tube from each study subject. Collected bloods were sent immediately to laboratory department and serums were separated after centrifugation at 3000 rpm. Serums were stored in refrigerator at -80^{0}c until biochemical analysis. Serum homocysteine was measured using an automated immunoanalyzer (ARCHITECT i2000SR immunoassay analyzer) and, serum vitamin B12 & folate were measured by a quantitative sandwich enzyme immunoassay technique (ELISA) using commercial kits from CALBIOTECH Inc.,El Cajon, CA 92020, USA (Catalog no: VB369B and FA370A). Statistical analyses were performed using the statistical package, SPSS version 23.0. Descriptive statistics were presented as Mean + SD. Differences in baseline variables between patients and control subjects were tested using Student's *t* test. $p<0.05$ was considered statistically significant. Prior to the commencement of the study, the research protocol was approved by the Ethical Institutional Review Committee of BMRC, Dhaka. The aims and objectives of the study, along with its procedure, risks, and benefits of this study, were explained to the parents of study subjects in easily understandable local language and then informed written consent was taken from each of them. Strict confidentiality was maintained regarding the information of the patients during and after the study.

Results

In this study, a total 100 study subjects were taken including, 50 Autism Spectrum Disorders (ASD) children as cases and 50 age matched healthy children as controls. Table I shows the baseline characteristics of the study subjects.

Table I: Baseline Characteristics of study subjects

Variables	Cases(n=50)	Controls(n=50)	P-Value
Age(year) †	5.71±2.06	6.00±1.99	0.485
BMI(kg/m²)†	15.23±1.59	15.42±1.18	0.502
Gender, n (%)			
Male	33 (66)	31 (62)	0.835
Female	17 (34)	19 (38)	
Maternal age†	26.90±3.71	23.58±3.21	0.000*
Paternal age†	34.14±4.68	30.48±2.99	0.000*
H/O Consanguinity, n (%)YesNo	10(20)40(80)	4(8)46(92)	0.148
H/O Maternal diabetes, n (%)YesNo	17 (34)33 (66)	07 (14)43 (86)	0.034*

Results are expressed as number and percentage (%). (†): values are expressed in Mean±SD, (*): p<0.01, p<0.05 was taken as level of significance.

This study found that Mean±SD of age of autism children was 5.71±2.06 years and in control group, it was 6.00±1.99 years. The majority of the study subjects were male (66% cases and 62% control). Mean±SD of BMI of autism children and controls were 15.23±1.59 and 15.42±1.18 respectively. No significant differences were observed for the mean age, gender and BMI, in between the study groups. Family history of autism revealed 22% of cases had family history of autism, whereas none of the control had family history autism, which was statistically significant (p<0.001). In this study, maternal and paternal age (Mean±SD) of autism children and healthy children were 26.90±3.71 & 23.58±3.21 and 34.14±4.68 & 30.48±2.98 respectively. Parents mean age of autism children was found significantly higher than that of control group (p<0.001). This study also found that H/O consanguineal marriage was comparatively higher in case group (20%) than control group (8%) but which was not statistically significant.

Natal and postnatal history of the study subjects has shown in the Table II. Mean±SD of Gestational age and Birth weight of ASD children were 37.22±1.80 & 2.93 ± 0.41 and in healthy children were 38.08±1.87 & 2.90± 0.34. Gestational age was found significantly lower in cases than controls. It was found that frequency of low birth weight was slightly higher in case group than controls but it was not statistically significant. This table also shows the mode of delivery and H/O prolonged labor of study subjects. 66% cases and 46 % healthy children were delivered by LUCS and H/O prolonged labor was also found slightly higher in cases than controls, but none of the findings were statistically significant. Frequency of neonatal jaundice was significantly higher in autism children than normal healthy children (54% Vs 16%, p=0.023). 24% cases & 8% control had H/O neonatal convulsion and 36% cases & 18% control had H/O delay in cry after birth, but none of them showed statistically significant differences.

Table III shows the Biochemical findings of serum Homocysteine, Viamin B12 and Folic acid levels in the study subjects. We observed that Mean S. Vit B12 (pg/ml) and S. Folic acid (ng/mL) were found significantly lower in autism children compared to control children (241.46±60.51 Vs 302.58± 76.66 and 7.12± 2.16 Vs 9.72 ± 2.96). On the other hand, the mean S. Homocystiene (µmol/L) was found significantly higher in cases than controls (8.08±2.11 Vs 6.12±1.71).

Table II: Natal and postnatal history of study subjects

Variables	Cases (n=50)	Control (n=50)	p-value
Gestational age†	37.22±1.80	38.08±1.87	0.021*
Birth weight†	2.93 ± 0.41	2.90± 0.34	0.736
LBW, n (%)Yes No	16 (32)34 (68)	04 (08)46 (92)	0.003*
Mode of delivery, n (%)LUCSNVD	33 (66)17 (34)	23 (46)27 (54)	0.440
Prolonged labor, n (%)YesNo	12 (24)38 (76)	5(10)45 (90)	0.060
Neonatal jaundice, n (%)YesNo	19 (54)31 (46)	08 (16)42 (84)	0.023*
Neonatal convulsion, n (%)Yes No	12 (24)38 (76)	04 (08)46 (92)	0.054
Cry after birth, n (%)Immediate Delay	32 (64)18 (36)	41(82) 09 (18)	0.070

Results are expressed as number and percentage (%). (†): values are expressed in Mean±SD,(*): p<0.05. p<0.05 was taken as level of significance.

Table III: Comparison of Biochemical parameters of study subjects

Variables	Case(Mean±SD)	Control(Mean±SD)	p-value
S. Homocysteine (µmol/L)	8.08±2.11	6.12±1.71	<0.001*
S. Vit B12 (pg/ml)	241.46± 60.51	302.58± 76.66	<0.001*
S. Folic acid (ng/ml)	7.12± 2.16	9.72 ± 2.96	< 0.001*

Values are presented as Mean ±SD, (*): p<0.05, p<0.05 was taken as level of significance.

Discussion

This case-control study was undertaken to evaluate the homocysteine, vitamin B12 and folate status in children with ASDs in Bangladesh. The results of basic demographic data showed no significant differences in age, gender, height, weight and BMI in between the cases and control except the family history of autism, parent's age and H/O maternal diabetes.In this study, the mean age of autism children was 5.71 ± 2.06 years and in control group, it was 6.00 ±1.99 years, which is similar to that of the age group reported by Al-Farsi et al.,2012 and Omotosho et al.,2018.[24-25.] Similar to the study in Egyptby El.Baz et al.,2018, this study also found that family history of autism is strongly associated with autism.[26] The mean age of parents of autism children was found significantly higher than that of control group. Similar findings was also reported by Bhuiyan et al.,2017.[27]In accordance to Xiang et al.,2015, this study also found that ASD cases had significantly higher history of maternal diabetes than healthy children.[28]

Gestational age was found significantly lower and frequency of low birth weight was found significantly higher in case group than the controls, in this study.Whereas, H/O prolonged labor was found slightly higher in case group than control, but it was not statistically significant. Ferdousy et al.,2019 also did not found any significant association of prolonged labor with autism.[29]The present study found a significant link between autism and neonatal jaundice. Similarly, Lozada et al.,2015, in their study found that children who develop ASD are more likely to have admission with diagnosis of jaundice in their neonatal period, which may be due to bilirubin is a neurotoxin.[30]

The Biochemical findings of serum Homocysteine, Vitamin B12 abd Folic acid in the study subjects showed that, mean S.Vit B12 (pg/ml) and S.Folic acid (ng/mL) were significantly lower in autism children compared to control children. These finding were consistent with the report done by Ali et al.,2011, Altun et al.,2018.[12,31] In a study in Bangladesh by Mahruba et al.,2019 also reported that, serum Vit B12 and S. Folic acid were significantly reduced in ASD children compared to control group.[32] Folate, vitamins B6 and B12 play an important role in the metabolism of homocysteine and any defect in homocysteine turnover due to either dietary deficiencies or mal-absorption or inappropriate metabolic utilization of these nutrients can lead to accumulation of homocysteine in the body.[33-34] In this study, the mean S. Homocystiene (µmol/L) was found significantly higher in cases than control. These finding were consistent with the report done by Ali et al., 2011, Altun et al., 2018.[12, 31]

There are several limitations to this study. Our study had a relatively small sample size and consisted of only the Bangladeshi children. Our data did not reflect the dietary habits and lifestyles of the subjects, which may affect the symptoms of ASDs. Further prospective studies on a large scale with more rigorous study designmay eventually lead to a better comparative understanding of the possible role of homocysteine, Vit B12 and folic acid in ASDs.

Conclusions

This study revealed that serum vitamin B12 and folate levels were significantly decreased, and serum homocysteine levels were significantly increased in children with autism spectrum disorders (ASDs) compared to normal healthy children.

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