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## Article

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## Serum vitamin B<sub>12</sub> and folic acid status in Autism spectrum disorder children

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### Abstract

**Background:** Autism spectrum disorder (ASD) is a complex neurodevelopmental disorder. The etiology of ASD involves gene-environmental interaction. Vitamin B<sub>12</sub> and folic acid have important roles as methyl donor in many biosynthetic pathways, protein synthesis and formation of myelin sheath throughout the central nervous system. Therefore, deficiency of vitamin B<sub>12</sub> and folic acid may act as environmental risk factor for ASD.

**Objective:** To evaluate serum vitamin B<sub>12</sub> and folic acid levels in ASD children. **Methods:** This cross-sectional study was conducted in the Department of Physiology, Bangabandhu Sheikh Mujib Medical University, Shahbag, Dhaka, from 2018 to 2019. Total 100 children 3-10 years of age were enrolled for this study. Among them fifty (50) diagnosed children with ASD were included in the study group. Fifty (50) healthy children constituted the control group. ASD children were selected from the Parent's Forum for autistic children. No children were included receiving any vitamin supplementation or had acute illness. For this study, serum level of vitamin B<sub>12</sub> and folic acid were measured by automated analyzer. For statistical analysis unpaired "t" test and chi square test were done. **Result:** The mean values of vitamin B<sub>12</sub> and folic acid were significantly lower in ASD children than those of control group (p value <0.05). In addition 4% ASD children had vitamin B<sub>12</sub> deficiency. **Conclusion:** Low serum vitamin B<sub>12</sub> and folic acid was associated with ASD.

**Key words:** ASD, Vitamin B<sub>12</sub>, Folic acid.

## Introduction

**A**utism spectrum disorder (ASD) is a neurodevelopmental disorder characterized by impairment in reciprocal social communication and a tendency to engage in repetitive stereotyped patterns of behaviors, interests and activities<sup>1</sup>. By the age of three, all typical symptoms such as impaired social reciprocity, poor communication skills and restricted or repetitive behavior, affecting three major domains are observed<sup>2</sup>. Along with the comorbidity such as epilepsy, bowel disorder, intellectual disability and type -I diabetes, the social and economic impact of autism is devastating<sup>3</sup>.

The global burden of autism is 7.6 per 1000 population or 1 in every 132 persons<sup>4</sup>. In Bangladesh, the prevalence of ASD is 0.15 to 0.8%<sup>5,6</sup>. Scientists have found an interaction between genetic and environmental factors in ASD<sup>7,8</sup>. Prenatal environmental risk factors such as advance paternal age, being male, obstetric complications, maternal infections, stress during pregnancy and post-natal risk factors such as nutritional deficiency, metabolic imbalance may be associated with ASD<sup>9,10</sup>.

Normal levels of vitamins inside the body helps in the body's maintenance process and better performance<sup>11</sup>. Plants or animals cannot synthesize vitamin B<sub>12</sub>. It is naturally found in foods including meat, eggs and milk products. Vitamin B<sub>12</sub> is necessary for protein synthesis and formation of the myelin sheath throughout the central nervous system<sup>12</sup>. Some researchers assessed dietary adequacy of Egyptian children with ASD and found that serum folate and vitamin B<sub>12</sub> were significantly lower<sup>13</sup>. Folic acid is crucial for proper brain functioning and plays an important role in mental and emotional health. Folic acid has been linked to nucleic acid synthesis and to methylation of monoamines in many biosynthetic pathways<sup>14</sup>. If the body becomes deficient in folic acid, all cycles will become ineffective and lead to many problems such as megaloblastic anemia, cancer and neural tube defects<sup>15</sup>.

There is less published data available regarding serum vitamin B<sub>12</sub> and folic acid levels in ASD children of Bangladesh. Therefore, this study has been designed to explore serum vitamin B<sub>12</sub> and folic acid status in children with ASD. It is expected that the result of this study may aware the clinicians about the importance of assessment of serum vitamin B<sub>12</sub> and folic acid status in ASD children and thus, early detection of any deficiency may ensure better management of this group of children.

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## Methods

This cross sectional study was done in the Department of Physiology, BSMMU, Dhaka from September 2018 to August 2019. The research work was carried out after obtaining ethical clearance from Institutional Review Board of BSMMU. For this study 50 diagnosed ASD children 3-10 years of age of both boys and girls were randomly selected on the basis of exclusion and inclusion criteria. The study group was selected from Parent's forum for autistic children, Mohakhali Directorate of Health Service, Dhaka. Fifty (50) healthy children with similar age and sex were selected from personal contact and hospital staff's children for control.

All children were free from any acute illness and vitamin supplementation. After selection, the nature, purpose and benefits of the study were explained to the gurdians and informed written consent was taken from them. Before taking blood, detailed family and medical history were taken. Anthropometric measurement of the subjects was recorded and blood pressure was measured. All the information was recorded in a data sheet. With aseptic precaution, 5 ml of venous blood was collected from ante-cubital vein by a disposable plastic syringe from each subject after overnight fasting for biochemical tests. Serum vitamin B<sub>12</sub> and folic acid were measured by automated analyzer in the Department of Biochemistry and Molecular Biology, BSMMU. For statistical analysis,

unpaired student's 't' test and chi square test were performed as applicable using SPSS for windows version 16.0. All data were expressed as mean  $\pm$  SD&%. The p value of  $\leq 0.05$  was accepted as level of significance.

### Results

Both groups were similar in respect of age, BMI & gender (table I and table II). Vitamin B<sub>12</sub> and Folic acids were found significantly ( $p < 0.05$ ) lower in ASD children (Table III). Moreover, vitamin B<sub>12</sub> deficiency was found in 4% ASD children (Figure 1).<sup>16</sup> No control children had any deficiency of vitamin B<sub>12</sub>.

**Table I:** Mean Age and BMI in both groups (N=100)

	Control (n = 50)	ASD (n = 50)
Age (years)	6.11 $\pm$ 1.92 (3-10)	6.16 $\pm$ 1.99 (3-10)
BMI (kg/m <sup>2</sup> )	16.50 $\pm$ 1.58 (13.66-20.17)	16.02 $\pm$ 2.75 (11.35 – 25.45)

Data were expressed as mean  $\pm$  SD. Values in parentheses indicate ranges; statistical analysis were done by independent sample t-test. BMI-Body Mass Index ( Kg/m<sup>2</sup>). N = Number of subjects.

**Table II:** Distribution of subjects according to sex (N=100)

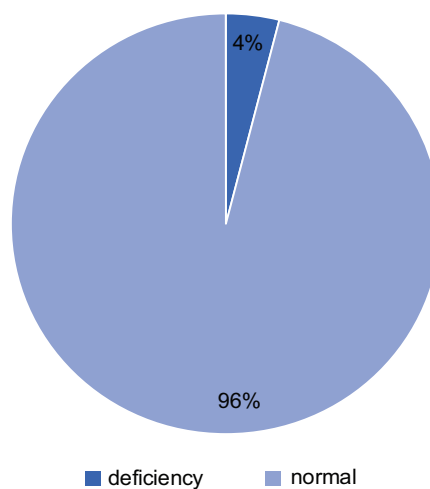
Sex	Control	ASD
	No. (%)	No. (%)
Male (n=73)	34 (68%)	39(78%)
Female(n=27)	16(32%)	11(22%)

Data were expressed as number. Values in parentheses indicate percentage. Statistical analysis was done by Chi-Square test. N = Total number of subjects.

**Table III:** Mean serum vitamin B<sub>12</sub> and folic acid in different groups (N=100)

Parameters	Control (n= 50)	ASD (n= 50)
Vitamin B <sub>12</sub> (pg/ml)	690.54 $\pm$ 237.06 (386-1280)	552.30 $\pm$ 246.56** (154-1086)
Folic Acid (ng/ml)	12.63 $\pm$ 4.46 (5.7-20)	9.19 $\pm$ 3.13** (3.2-17.5)

Data were expressed as mean  $\pm$  SD. Values in parentheses indicate ranges; Statistical analysis was done by Independent sample t-test; N= Number of subjects\*\*- $p < 0.01$



**Figure 1:** Frequency distribution of serum vitamin B<sub>12</sub> deficiency in ASD children (n=50)

### Discussion

The present study was undertaken to observe vitamin B<sub>12</sub> and folic acid status of ASD children. The mean serum vitamin B<sub>12</sub> and folic acid were significantly lower in ASD children compared to healthy control. Similar findings were reported by other investigators<sup>13</sup>. A cross sectional study on Egyptian children found significantly positive correlation between serum B<sub>12</sub> and folic acid with their food content. They suggested nutritional deficiency was associated with autistic disorder in Egyptian children<sup>13</sup>. In

this study, in addition to lower vitamin B<sub>12</sub> and folic acid level, 4% of ASD children were affected with vitamin B<sub>12</sub> deficiency whereas no control children had any deficiency of vitamin B<sub>12</sub> and folic acid. In the present study this observation further confirm to the association of low vitamin B<sub>12</sub> and folic acid to the current series of autistic children.

Literature review suggested that, the etiology of ASD involves gene environment interaction. Therefore, when genetically susceptible children are exposed to the nutrient deficiencies in early age of life, it may act as an environmental trigger for ASD<sup>27</sup>. Therefore deficiency of the nutrients have been implicated as a risk factor for autism. Several authors have highlighted the role of vitamin B<sub>12</sub> and folic acid deficiency in ASD<sup>17,18</sup>. Vitamin B<sub>12</sub> is necessary for protein synthesis and formation of myelin sheath throughout the central nervous system. Folic acid has role as a methyl donor in many biosynthetic pathways<sup>23,24</sup>. Moreover vitamin B<sub>12</sub> and cerebral folate deficiency may cause of mitochondrial dysfunction and development of the sign symptoms of ASD<sup>21-22</sup>. The present result supports the role of vitamin B<sub>12</sub> and Folic acid for the inadequate/poor neural development in ASD children.

### Conclusion

After analyzing the results of the study, it is concluded that vitamin B<sub>12</sub> and folic acid was lower in ASD children. Therefore, early diagnosis of the deficiency may be helpful for the clinicians and parents to maintain proper diet of this group of children.

**Conflict of interest:** None

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