

STUDY ON SERUM MAGNESIUM, CALCIUM AND IRON IN AUTISM SPECTRUM DISORDER (ASD) CHILDREN

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Abstract

Background: Autism is a complex neurodevelopmental disorder and one of the major cause of lifelong disability. Magnesium, calcium and iron are important minerals of biological system. Hypomagnesaemia, hypocalcaemia and iron deficiency has been found to be associated with abnormal metabolic functions resulting in autistic spectrum disorder.

Hypothesis: Serum magnesium, calcium and iron levels are lower in ASD children than healthy control.

Objective: To measure serum magnesium, calcium and iron in ASD children and to compare them with healthy control.

Method: This observational type of analytical study with case-control design was conducted in the Department of Physiology, Bangabandhu Sheikh Mujib Medical University, Shahbag, Dhaka from March 2014–January 2016. For this study, 80 children aged 3-10 years were randomly selected, among which 40 were apparently healthy and 40 were diagnosed as ASD. The study group was selected from the Parents' Forum (Mohakhali DOHS, Dhaka) for ASD children and the control group was selected from some regular schools. 5ml venous blood was collected from both groups for analysis of fasting serum magnesium, calcium and iron. Fasting magnesium, calcium and iron were estimated in all children by standard laboratory method. Independent sample 't' test and proportion (Z) test were used for statistical analysis. P value ≤ 0.05 was accepted as significant.

Result: The mean serum magnesium, calcium and iron were significantly lower ($p \leq 0.001$) in cases as compared to controls. The frequency of hypomagnesaemia, hypocalcaemia and iron deficiency were significantly higher in early diagnosed ASD children, which was 7 (17.5%), 22 (55%) and 5 (12.5%) out of 40 ASD children respectively. The mean values of all the biochemical variables in normal children were within normal ranges.

Conclusion: The results indicate that hypomagnesaemia, hypocalcaemia and iron deficiency are common in ASD children.

Keywords: Autistic spectrum disorder, magnesium, calcium and iron deficiency.

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Introduction:

Autism spectrum disorder (ASD) is behaviorally defined by an extensive deficits in social interaction, impairment in verbal and nonverbal communication, and stereotyped patterns of interests and activities¹. According to the American Psychiatric Association, ASD is associated with behavioral, developmental, neuropathological and sensory abnormalities. The word spectrum refers to a wide range of disorders having different domains according to the severity of features. Autism is the most familiar diagnosis among the Autism Spectrum Disorders, which also includes pervasive developmental disorder not otherwise specified and Asperger's disorder^{2, 3}. ASD is usually diagnosed between the ages of 2 to 10 years of age, with peak prevalence between 5 to 8 years of age⁴.

During the first description by Kanner⁵, ASD was a rare disorder but in last three decades, its prevalence increased several folds. Autism may occur in any race, class and religion in all countries of the world. Now global burden of autism is 7.6 per 1000 population or one in every 132 persons⁶. In Bangladesh the prevalence of ASD is 0.15 to 0.8%. In rural area it is 0.075% but in Dhaka city it is alarmingly high 3%^{7, 8}.

ASD is a complicated condition in which along with genetic factor, nutritional and environmental factors play major roles⁹. The core feature of autism might play a role in dietary selectivity that result in less consumption of fruits, dairy products, vegetables, proteins and starches by the autistic group. Several studies informed that there were significantly lower levels of various nutrients in the blood of autistic children such as calcium, magnesium, and iron^{9, 10, 11}.

Magnesium (Mg), calcium and iron are the most important parts of biological system. Magnesium plays important roles in more than 300 enzyme systems significantly involved in energy production, synthesis of essential molecules and regulation of ion transport across the cell membranes. Bradstreet et al. (2010) reported that about 95% of autistic children had magnesium deficiency¹². In addition, Priya and

Geetha observed that serum magnesium significantly lower in autistic children compared to age and sex matched controls, which may lead to anxiety, depression, agitation, irritability, nervousness, and learning disability, impairment of social interaction and memory impairment¹¹. Peck and Meltzer reported that magnesium ions exert a depressant effect on neuromuscular transmission of central nervous system¹³. On the other hand, Al Farsi et al. informed that serum magnesium was higher in autistic children¹⁴.

Krey and Dolmetsch¹⁵ reported that calcium is an important mineral which plays an important role in the regulation of synaptic transmission and also in the regulation of neuronal excitability. Magnesium deficiency may lead to increased intracellular calcium. Laumonnier et al. suggested that increased intracellular calcium in ASD children compared to control was due to inactivation of ionized Ca^{2+} -activated K^+ channel (BK_{Ca}) which lead to neuronal hyperexcitability¹⁶.

In autism, low plasma Ca^{2+} and high brain Ca^{2+} readily leads to oxidative stress as mitochondrial oxygen radical is stimulated by increased intracellular Ca^{2+} ^{17, 18}. Calcium plays an important role in the activation of different essential enzymes in energy producing pathways, such as α -ketoglutarate dehydrogenase, isocitrate dehydrogenase and pyruvate dehydrogenase^{19, 20}.

In a recent study, it was reported that about 33.33% of ASD children had decreased serum calcium compared to matched controls¹⁷. Another study reported that calcium was significantly lower in autistic children than in the control group, probably due to nutritional deficiency and exposure to environmental toxins, especially during early development¹⁴. Another study reported that children with ASD had significantly lower levels of serum calcium²¹. On the other hand, another study reported no significant differences in serum calcium levels in ASD and control group²².

Iron plays an important role in the development and functioning of brain, especially during the early stages of its development²³. According to Lozoff et al. iron deficiency early in life may

cause permanent damage to nervous system development and function, resulting in impaired cognition, diminished learning capacity, attention deficit, and neuromotor dysfunction²⁴. Iron deficiency affects enzymatic activity, oxygen transport and its cellular utilization, energy utilization, hormonal regulation, muscle function, defense mechanisms, intestinal function and absorption, growth and development. According to Horowitz and Greenamyre numerous proteins in almost all compartments of the human cell – mitochondria, cytosol, nucleus, endoplasmic reticula – require iron-sulfur complex and/or heme prosthetic groups to support enzymatic and structural functions such as oxidative phosphorylation (complex I-III), TCA cycle (aconitase), DNA repair and heme synthesis²⁵.

Iron deficiency is the most common deficiency disease worldwide. In a study Latif, Heinz and Cook suggested that about 52% of autistic children were iron deficient, which might cause learning and behavioral impairment²⁶. According to Cornish about 29% of autistic children aged 3.5-9.5 year showed low dietary intake of iron²⁷.

In this study, we estimated the serum magnesium, calcium and iron levels of ASD children and healthy control and compared the status of these minerals. Our aim is to create consciousness among parents about the detrimental effects of selectivity in feeding and its resultant mineral deficiencies in ASD children.

Methods:

40 diagnosed children of autism spectrum disorder with age range 3-10 years were taken for this analytical type of case-control study. 40 age and BMI matched apparently healthy children were taken as control. All subjects were excluded for conditions like, epilepsy, Turner syndrome, Down syndrome and children who take any medication by history taking. This study was conducted in Department of Physiology of Bangabandhu Sheikh Mujib Medical University (BSMMU), Shahbag, Dhaka. The study group was selected from Parents Forum (DOHS, Mohakhali) for Autism spectrum

disorder children and control group was collected from some normal school. After selection of the subjects, thorough information was given to their parents about the objectives and study procedure. Their parents were encouraged for voluntary participation of their children. Their parents were also allowed freedom to withdraw their children from the study even after participation whenever they feel like. When their parents agreed for participation, an informed written consent was taken from them. The parents of subject were requested to attend the Department of physiology of BSMMU, Dhaka for examination of their children. Detail personal, medical, family, socioeconomic, occupational and dietary history of the children were recorded in a data schedule from their parents. Thorough physical examinations of the subjects were done. Then 5 ml of venous blood was collected from ante-cubital vein of each subject of both groups for estimation of biochemical variables. After collection of blood samples, serum calcium, magnesium and iron were measured in the laboratory of Biochemistry department, BSMMU. Data were expressed in mean \pm SE and also in percentage. Statistical analysis was done by SPSS for Windows version 16. Independent samples 't' test were used as the tests of significance. P value < 0.05 was accepted as significant.

Result:

This study included 40 ASD children as study group and 40 age and BMI matched healthy children as control group. The characteristics of study group and control group are shown in Table 1. The mean serum magnesium levels were significantly lower in children with ASD compared with the normal cases [2.03 (SE =0.03)mg/dl and 2.16 (SE =0.02) mg/dl, respectively; P< 0.01]. The mean serum calcium levels were significantly lower in children with ASD compared with the normal cases [8.90 (SE =0.06) mg/dl and 9.32 (SE =0.06) mg/dl, respectively; P< 0.001]; Accordingly, the mean serum iron levels were also significantly (P <0.001) lower in children with ASD than the normal cases [71.45 (SE = 4.42) μ g/dl and 90.10 (SE =2.67) μ g/dl, respectively; P <0.001]. Out of 40 early diagnosed ASD children,

hypomagnesaemia was found in 7 (17.5%) children, hypocalcaemia in 22 (55%) children and iron deficiency in 5 (12.5%) children respectively which was significantly higher than the healthy control children. Among the healthy control children, only one (2.5%) boy had hypocalcemia (Table 2).

Table-I
Characteristics of the study subjects

Parameters	Study group n = 40	Control group n = 40	P value
Serum Magnesium (mg/dl)	2.03±0.02 (1.7-2.5)	2.14±0.02 (1.9-2.6)	<0.01
Serum Calcium (mg/dl)	8.90±0.06 (7.2-9.6)	9.40±0.07 (8.2-10.4)	<0.001
Serum Iron (µg/dl)	71.45±4.42 (19-124)	90.97±3.01 (58-138)	<0.001

Data are expressed as mean ± SE. For statistical analysis, independent student 't' test was performed for comparison between study (autism spectrum disorder children) and control (healthy children) group. Figures in parentheses indicate ranges. P < 0.05 considered as significant, n = number of subject in each group.

Table-II
Distribution of hypomagnesaemia, hypocalcaemia and iron deficiency in ASD children of 3-10 years.

Characteristics	Study group n = 40	Control group n = 40
Hypomagnesaemia	7 (17.5%)	0 (0%)
Hypocalcaemia	22 (55%)	1 (2.5%)
Iron deficiency	5 (12.5%)	0 (0%)

Data are expressed in number (percentage)

The lower limit of normal level for serum magnesium was 1.9 mg/dl, for serum calcium was 9 mg/dl and for serum iron was 30 µg/dl.

Discussion:

The present study was undertaken to observe some biochemical variables in children with

autistic spectrum disorder in order to evaluate the alterations in nutritional deficiency. Serum nutritional deficiency were assessed by estimating serum magnesium, calcium and iron levels. All these variables were also studied in apparently healthy age, height, weight and BMI matched children for comparison. In this study, our data indicated a significant decrement in the serum levels of all the variables in ASD children. Whereas mean values of all the biochemical variables of normal children were within physiological limits and were almost similar to those reported by different investigators^{28, 29, 30, 31, 14, 32, 18, 21, 33, 12, 34}. Both the groups were comparable, as there were no significant differences in the confounding variables such as age, height, weight and BMI between the two groups. The mean values of serum magnesium and calcium were below the lower limit of normal range, whereas those of total iron were within the normal range.

In our present study, a significantly lower serum magnesium level was found in the study group than those of control group. The observed decrease in serum magnesium is in agreement with many previous findings by^{34, 12, 22}. In addition, serum magnesium level was found abnormally low in 17.5% of children of study group whereas none of them in control group had abnormally low serum magnesium levels. Similarly, Kozielc and Hermelin observed that 33.6% of autistic children had magnesium deficiency³⁵.

This study also showed that serum calcium levels were significantly lower in the study group than in the control group. These findings are consistent with the findings that were observed by El-Ansary et al., Al-Farsi et al., Yasuda et al., Meguid et al. and Sun et al.^{18, 14, 36, 21, 37}. Surprisingly, 55% of children of the study group were found abnormally low serum calcium levels. Whereas only 2.5% of children of the control group had serum calcium levels below the lower limit of normal which may be due to nutritional deficiency and or vitamin D deficiency due to inadequate exposure to sun light. In contrary to this finding, Yasuda et al. observed that, only 5.8% of autistic children had calcium deficiency³⁶.

Decreased levels of serum iron was also observed in this study. Serum iron was significantly lower in the study group than those of control group. Almost similar findings were observed by Cornish, Latif, Heinz and Cook, Bilgic et al. and Konofal et al.^{27, 26, 33}. In addition, serum iron level was found abnormally low in 12.5% of children of study group. But none of them in control group had abnormally low serum magnesium levels. Similar findings were observed in 84% of ASD children by Konofal et al. in 29% by Cornish); in 52% by Latif, Heinz and Cook and in 32.3% by Bilgic et al.^{27, 26, 33}.

Conclusion:

The results indicate that hypomagnesaemia, hypocalcaemia and iron deficiency are common in ASD children.

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