This journal is the official publication of Bangladesh Society of Physiologists (BSP)
Web URL: www.banglajol.info/index.php/JBSP

Abstracted /indexed in Index Copernicus, Director of Open Access Journal, HINARI Index Medicus for South East Asia Region, Google Scholar, 12OR, infobse index, Open J gate, Cite factor, Scientific indexing services

pISSN-1983-1213; e-ISSN-2219-7508

Article

Article information:
Received:10th August 2020
Accepted: 28th November /2020
DOI: https://doi.org/10.3329/jbsp.v15i2.50921

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Cite this article:
Afroz S, Siddiqi UR, Mahruba N, Shahjadi S, Begum S. Serum Calcium and Phosphate in Children with Autism Spectrum Disorder
J Bngladesh Soc Physiol 2020;15(2): 72-77

Serum Calcium and Phosphate in Children with Autism Spectrum Disorder

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Abstract

Background: Autism spectrum disorder (ASD) is a complex disorder of neuronal development which may cause lifelong disability. The etiology of ASD involves gene-environmental interaction. Calcium signal is crucial for neuronal communication and neuro-plasticity and phosphate is related to neural energy metabolism. Therefore, deficiency of these minerals may act as an environmental risk factor for the development of ASD. **Objective:** To assess serum calcium and phosphate in children with ASD. **Methods:** This cross-sectional comparative study was conducted in the Department of Physiology, Bangabandhu Sheikh Mujib Medical University(BSMMU) Dhaka from March 2018 to February 2019. For this, 50 ASD children of both sex (age 3 to10 years) diagnosed by pediatric neurologist were included as 'Study' participants through 'Parents Forum of differently abled children, Mohakhali, Dhaka' and for comparison, age, BMI and sex matched 50 apparently healthy children were enrolled, as 'Control'. Serum calcium and phosphate level of all children were estimated by colorimetric method. All data were expressed as mean±SD, range and percentage. For statistical analysis, Chi-Square test, Shapiro Wilk test and independent sample 't' test was done, as applicable. **Results:**The mean serum calcium was significantly ($p<0.01$) lower in ASD children compared to control, though the mean values were within normal reference range in both groups. However, hypophosphatemia was found

in 4% of ASD children. **Conclusion:**From this study, it may be concluded that ASD children was associated with lower serum calcium level and hypophosphatemia.Therefore, adequate dietary intake of calcium and phosphate is recommended for children with ASD.

Key words: ASD, Calcium, Phosphate

Introduction

Autism spectrum disorder (ASD) is a range of neurodevelopmental disorder of global concern. It is characterized by impaired reciprocal social communication and a tendency to engage in repetitive, stereotyped patterns of behaviors, interests and activities. ASD includes autism, pervasive developmental disorder not otherwise specified and Asperger's disorder.[1] The prevalence of autism has increased in last three decades. Now the global burden of autism is 7.6 per 1000 population or 1 in every 132 persons.[2] In Bangladesh, the overall prevalence of ASD is 0.15 to 0.8%[3]. Though in rural area, it is 0.075%, but it is alarmingly high (3%) in Dhaka city.[3-4]

In search of the etiology of autism, scientists found an interaction between genetic and environmental factors.[5,6] Evidences indicate that some of the prenatal environmental risk factors such as advanced paternal age, being male, obstetric complications, maternal infections, stress and several post-natal risk factors such as nutritional deficiency, metabolic imbalance may be associated with ASD.[7-8]

Calcium and phosphate ion are important parts of metabolism and biological signaling system. Of them, calcium is a key regulator of mitochondrial function. Calcium signals are required for cellular proliferation and differentiation during neurogenesis. During post-natal life, it is required for calcium-dependent gene expressions, neuronal communication, synaptic transmission and memory related synaptic plasticity.[9-12]

In addition, phosphate plays important role in several biological processes such as bone formation, cell signaling, energy metabolism, biochemical pathway and nucleic acid synthesis. In phosphate depletion metabolic derangement occurs which results in multiorgan dysfunction. In addition, low ATP content in cells impedes the function of the Ca^{2+} ATPase, which leads to elevated cytosolic calcium.[13]

To the best of our knowledge, a very few researchers reported lower calcium[14-16] as well as lower phosphate[16] in serum of the ASD children than their normal reference value. Whereas, two group of investigators reported no significant differences of these minerals in between ASD children and healthy control children.[8,17]

However, the volume of the information regarding serum calcium and phosphate in ASD children is not enough for any conclusive remark and there is a place of conceptual innovation. Therefore, this study aimed to assess serum calcium and phosphate in children with ASD.

Methods

This cross sectional comparative study was carried out in the Department of Physiology, Bangabandhu Sheikh Mujib Medical University (BSMMU), Dhaka from March 2018 to February 2019, with prior protocol approval from Institutional Review Board (IRB) of the University, following ethical rule of Helsinki.[18] For this purpose, 65 ASD children of both sex (3 to10 years), diagnosed by pediatric neurologist, were purposively enrolled, after getting informed

written consent from the parents of 'Parents Forum of differently abled children, Mohakhali, Dhaka'. Among them, ASD children with any acute illness, malabsorption, Down syndrome, cerebral palsy, epilepsy and renal insufficiency, were excluded. In addition, ASD children who were receiving multivitamins, calcium, vitamin D, Cyproterone acetate, Glucocorticoids, Bisphosphonate, were also excluded from the study and ultimately 50 ASD children were selected as study group. Furthermore, for comparison, age, BMI and sex matched 50 apparently healthy children were also enrolled as control. 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, 5ml venous blood of all children was collected and was immediately sent to the laboratory of the Department of Biochemistry and Molecular Biology, BSMMU. Then serum calcium, phosphate and albumin assay [by colorimetric methods using automated analyzer (Architect plus ci4100)] of all children were done. Then adjusted or corrected total calcium (mg/dL) was manually calculated as,= total calcium (mg/dL) + 0.8 x [4 – albumin (gm/dL)].[19]

All data were expressed as mean with standard deviation (mean±SD), range and percentage. For statistical analysis, Chi-Square test, Shapiro Wilk test and independent sample 't' test were done, as applicable, by using SPSS (Version 16) for Windows. In the interpretation of results, p value <0.05 was accepted as significant.

Results

General characteristics of the ASD children and control are shown in Table I. Here, the mean serum calcium was significantly ($p<0.01$) lower and the mean serum phosphate level was not significantly different in ASD children compared to controls (Table II). However, all the values of serum calcium were within normal reference range[20] in children of both groups. In addition, 4% of ASD children had hypophosphatemia[19] (Figure 1).

Table I: General characteristics of ASD Children and healthy controls (N=100)

Characteristics	ASD (n=50)	Controls (n=50)	p value
Age (year)[a]	6.16± 0.28 (3-10)	6.04 ± 0.27 (3-10)	0.77[α]
BMI (kg/m^2)[a]	15.96 ± 0.39 (11.35-22.22)	16.50 ± 0.22 (13.66-20.17)	0.284[α]
Male: Female[b]	3.5 : 1	2.12 : 1	0.260[β]

Data were expressed as mean±SD (range) (a), and ratio (b). Statistical analysis was done by independent sample t-test ([α]) and Chi square test ([β]). N= total number of children; n= number of children in each group; ASD=Autism Spectrum Disorder; BMI=Body Mass Index

Table II: Serum calcium and phosphate of ASD Children and healthy controls (N=100)

Serum variables (mg/dL)	Reference values (mg/dL)	ASD (n=50)	Controls (n=50)	P value
Calcium	8.5 – 10.5[20]	9.30 ± 0.04 (8.76-9.9)	9.48 ± 0.03 (8.84-10.06)	0.002**
Phosphate	4-7[19]	5.36 ± 0.08 (2.8-6.13)	5.42 ± 0.05 (4.60-6.10)	0.148

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;
** = statistically significant ($p<0.01$).

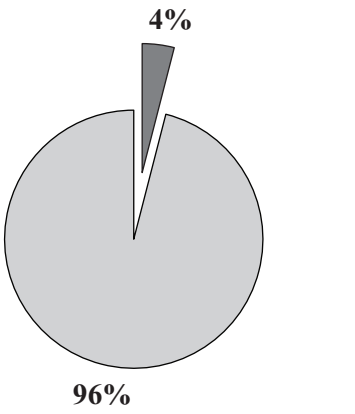

Figure 1: Frequency distribution of hypophosphatemia in ASD children (n = 50). ASD = autism spectrum disorder

Discussion

In the present study, significantly lower serum calcium level in children with ASD was in agreement with other investigators.[14-16] However, some researchers found no significant difference in serum calcium levels between these two groups.[8, 17]

Again, the results of serum phosphate in ASD were similar to other investigators.[8, 15,17] On the contrary, one group of researchers found significantly lower serum phosphate level in ASD children compared to healthy control.[16]

Dietary history of our ASD children reveals that 48% of them (data not shown) avoid all kinds of dairy foods (which are the rich sources of calcium and phosphate[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 calcium and phosphate in developing age. In agreement, Herdon et al. reported that ASD children have dietary selectivity which results in less consumption of calcium and other minerals.[21]

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

In addition, the lower serum calcium may be a consequence of dysregulated calcium homeostasis.[25] Mutations of several voltage-gated and ligand gated ion channels regulating calcium homeostasis found in ASD, which may result in decreased calcium concentration in ECF and increased cytosolic calcium concentration. Cytosolic calcium overload affects mitochondrial functions.[11, 26-27] Again, phosphate depletion may result in a reduction in ATP content of cells. Low ATP impedes the function of the Ca-ATPase, which leads to elevated cytosolic calcium and impairs mitochondrial function.[8] Therefore, apoptotic cellular death is accompanied by a burst of reactive oxygen species, collapse of the electrochemical proton gradient and bioenergetics catastrophe.[28] However, in our study, the lowered calcium and phosphate deficiency may be accountable to inadequate dietary intake of dairy foods, which is evident from their dietary history.

Conclusion

From this study, it may be concluded that, ASD was associated with lower calcium and phosphate deficiency.Therefore, we may recommend adequate dietary intake of calcium and phosphate rich foods for children with ASD.

Ethical Consideration: This study was approved by Institutional Review Board of BSMMU, Dhaka

Acknowledgement

This study was partly supported by Bangladesh Medical Research Council (BMRC), Dhaka.

Conflict of interest: Authors of this study have no conflict of interest.

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