

Study of Serum Calcium and Phosphorus Level in Autism Spectrum Disorder Patients and Its Correlation with Severity of Disease

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Abstract

Introduction: Autism spectrum disorders (ASD) a considerable health obstacle in kids characterized by compromised social collaboration, and stereotyped behavior. Autism is triggered by an interaction of environmental and genetic influences.

Aims: The Objective of this study is to evaluate calcium and phosphorus levels in patients with autism spectrum disorder and to assess their association with severity of the disease.

Materials and Methods: This case-control observational study includes 75 autism spectrum disorder patients of age Group 2-15 years as well as age, and gender, matched 25 healthy children as controls. The degree of ASD severity was diagnosed by clinical psychologist by using Indian scale for assessment of autism (ISAA Score). Blood samples were collected from all participants and analyzed for serum Calcium, Phosphorus. The Obtained Results were analyzed statistically to calculate significance of difference among them by calculating p-value. P-value less than 0.05 is considered significant.

Results: In this study macronutrient like serum calcium and phosphorus (P) levels were found significantly decreased in ASD patients as compared to controls. Further the levels of calcium and phosphorus decreased with increasing severity of ASD.

Conclusions: From this study we would like to conclude that Hypocalcemia and Hypophosphatemia are common in autistic children.

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Introduction

Autism is an extremely inconsistent neurodevelopmental disorder, which typically develops during infancy or early childhood, with a subsequently steady pattern of progression. There is generally no remission, and the presentation requires difficulties in social contact and communication with repetitive and

restricted patterns of behaviour. [1,2] Unusual eating habits a risk factor for nutrient deficiencies are common in ASD. Inadequate intakes of calcium, phosphorus and vitamins have been reported in children with autism [3] Atypical feeding behavior adoption of intentional diet restrictions and the peculiar lifestyle of

individuals with ASD (with not only different levels of physical activity, but also idiosyncratic Social Skill and poor social interaction) are factors that imply risk of both excessive and insufficient intakes [4]

The dietary intake of macronutrients and some micronutrients in children with Autism have been found to be lower & higher or similar compared to recommended amounts [5] Micronutrients and macronutrients are substances including vitamins and minerals that are required in small amount and are essential for proper growth and development. [6] They are found in foods and beverages. These intakes of food have been compared between children with ASD and their typically developing (TD) peers. Nearly three decades ago, a nutritional assessment of children with ASD found overall intake adequacy to be comparable to those of controls. [7]

Calcium and phosphate ions are important parts of metabolic and biological signaling system. Calcium is a key regulator of mitochondrial function. Calcium signals are required for cellular proliferation and differentiation during neurogenesis. 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 which leads to cytosolic calcium. [8]

Multivariate logistic regression analysis revealed that serum calcium, phosphate

and vitamin D levels reduced physical activity, child order, and body mass index percentile can be considered strong predictors and major factors associated with ASD.[9,10]

In autistic children, there is no standard treatment available for benefit of autistic patients. So many clinicians are moving towards biological treatments, megavitamin therapy, use of exotic dietary supplements etc. however authentic data to prove benefits of such therapies in autistic children are not available. Studies investigating nutrient intake in children with ASD have produced conflicting results.

Therefore there is a need to evaluate nutrient status (macro elements) in newly diagnosed autism spectrum disorder children.

Materials and Methods

The study was designed to evaluate serum Calcium and Phosphorus level in 75 autism spectrum disorder patients as well as age, gender, matched 25 healthy children as controls in the Department of biochemistry, S.M.S. Medical College, Jaipur (Rajasthan). Subjects were recruited from the pediatric Department of J.K Loan Hospital, attached with S.M.S. Medical College, Jaipur.

Subject selection and study design:

This comparative Case-Control type of observational study was conducted in the Department of Biochemistry, S.M.S Medical College, Jaipur. The subjects in our study were selected from J K loan Hospital Jaipur.

Distribution of subjects

Subjects	Controls	Case (Autism Spectrum Disorder Patients)		
		Mild	Moderate	Severe
Male	17	16	15	14
Female	8	9	10	11
Total	25	25	25	25

Subject selection:

Based on following inclusion and the exclusion criteria a Random selection of the subjects for the study was made on the basis of detailed history and proper clinical examination.

Inclusion criteria:

1. Age Range: - 2-15 Years.
2. Autism Group: - Prior diagnosis of Autism by Indian Scale for Assessment of Autism (ISAA Score).
3. Control Group: - In good mental and physical health and no sibling with Autism spectrum disorders and no evidence of attention deficit disorder.

Exclusion criteria:

1. Patients on vitamin/mineral supplements, use of any chelation treatment will be excluded from the study.
2. Children with epilepsy, Turner Syndrome, Down syndrome and any kind of medication were excluded from the study children with organic aciduria, dimorphic features, a diagnosis of fragile X and other serious neurological and psychiatric conditions or known medical conditions including endocrine, cardiovascular, pulmonary, liver kidney or other medical disease.

Socio-economic Status:

This Study also highlighted the relationship of Autism Spectrum Disorder Patients with their Parents education, occupation, work profile, their family income and area of residence like urban and rural background.

Clinical Criteria:

The degree of ASD severity was diagnosed by clinical psychologist by using Indian scale for assessment of autism (ISAA Score). According to ISAA, this rates the child on a scale in each of the 40 areas included.

10 ml blood samples were collected in plain vials from the subjects. After 30 minute coagulation immediate centrifugation was performed for 10 minutes at 5000 rpm. All tests were done in separated serum by centrifugation. For remaining test serum was stored at -80 degree centigrade until the time of analysis. Estimation of serum Calcium (Ca), Phosphorus (P) was done using Rendox-Imola fully automated analyzer.

Results

This comparative case control study includes 75 autism spectrum disorder patients of age Group 2-15 years as well as age, gender, matched 25 healthy children as controls.

Table 1: Distribution of subjects according to gender.

Groups studied	Gender of Subjects		
	Male N (%)	Female N (%)	Total N (%)
Healthy Control	17 (1.70%)	8 (0.8%)	25 (2.5%)
Autistic Patients	45 (4.50%)	30 (3%)	75 (7.5%)
Total	62 (6.2%)	38 (3.8%)	100 (10%)

Table 2: Distribution of subjects according age.

Group studied	Age(years) Mean \pm SD
Healthy control (25)	7.32 \pm 3.16
Autistic patients (75)	5.81 \pm 2.68

Age and gender-wise distribution of subjects is mentioned in Table 1 and Table 2 Respectively.

Table 3: Comparison of mean of Calcium and Phosphorus in the controls and Autistic subject

Parameters	Controls (n =25) Mean \pm SD	Autistic case(n= 75) Mean \pm SD	Unpaired Student t Test P value
Calcium (mg/dl)	9.32 \pm 1.14	8.67 \pm 1.32	\leq 0.05
Phosphorus(mg/dl)	4.82 \pm 0.96	3.27 \pm 1.60	0.0001

Table 4: Comparison of mean S. Calcium (Ca) (mg/dl) levels using ANOVA test.

Study Groups	Mean \pm SD	P-Value*
Group-1 (Controls)	9.32 \pm 1.14	\leq 0.05
Group-2 (Mild ASD)	8.81 \pm 1.11	
Group-3 (Moderate ASD)	8.35 \pm 1.35	
Group-4 (Severe ASD)	8.22 \pm 1.50	

* ANOVA Test

Table 5: Comparison of mean S. Phosphorus (P) (mg/dl) levels using ANOVA test

Study Groups	Mean \pm SD	P-Value
Group-1 (Controls)	4.82 \pm 0.96	<0.001**
Group-2 (Mild ASD)	3.48 \pm 1.45	
Group-3 (Moderate ASD)	3.22 \pm 1.92	
Group-4 (Severe ASD)	3.12 \pm 1.43	

**High significant value (P value<0.001)

Comparison of different groups of autistic with control group shows highly significant difference in serum phosphorus levels. (p value :<0.001) (Table 3)

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 was assessed by estimating many nutrients like calcium, phosphorus and vitamins. All these variables were also studied in apparently healthy age, height, weight and BMI matched children for

comparison. Children with autism also exhibit greater difficulties with feeding and at meal times compared with non-autistic children. Parents of autistic children find it difficult to feed their children, probably because of pica, a disorder in which children tend to be picky eaters, prefer to eat non-edible objects, and resist consuming solid food. [11]

Seventy five(75) clinically diagnosed patients with Autism (male 45 and female 30) were included in the study, and results were compared with age and sex matched 25 normal healthy control subjects(17 male and 8 female)(table no. 1, 2)

In our study, serum calcium levels were found significantly decreased in autistic patients as compared to control group(mean \pm SD, 9.32 ± 1.14 vs 9.32 ± 1.14 mg/dl, $p \leq 0.05$). Whereas serum phosphorus levels were statistically highly significantly decrease in patients with autism as compared to healthy control subjects (mean \pm SD, 4.82 ± 0.96 vs 3.27 ± 1.60 mg/dl, $p \leq 0.0001$) (Table no. 3).

ASD subjects (n=75) were further segregated into three groups according to severity of ASD as assessed by the ISAA score as Group 1 (mild n=25), Group 2 (moderate n=25),and Group 3 (severe n=25). Macro nutrients serum calcium and phosphorus were assessed for severity of ASD. Comparison of parameters among the three groups (mild, moderate, severe) and control group was performed by using one-way analysis of variance (ANOVA) test. Serum Calcium level was slightly decreased with an increase in severity of disease ($p=0.03$)` (Table no. 4) moreover phosphorus showed very significantly decrease with advancement ASD ($p=0.0001$) (mean \pm SD, mild autism 3.48 ± 1.45 , moderate 3.22 ± 1.92 , in severe autism group was 3.12 ± 1.43 and in control group was 4.82 ± 0.96 with P-value < 0.001 (Table no. 5)

Because of Phosphate plays important role in several biological processes such as bone formation, cell signaling, energy metabolism, biochemical pathway and nucleic acid synthesis. Again, the results of serum phosphate in ASD were similar to other investigators. [11, 16]

On the contrary, one group of researchers found significantly lower serum phosphate levels in ASD children compared to healthy control.16 Dietary history of ASD children reveals that 48% of them (data not shown) avoid all kinds of dairy foods (which are the rich sources of calcium and phosphate19). Moreover, they also have restrictive type of food habits and did not take enough vegetables. Therefore, their regular diet may fail to meet the demand for calcium and phosphate in developing age.

Krey and Dolmetsch [16] reported that calcium is an important mineral that plays an important role in the regulation of synaptic transmission and also in the regulation of neuronal excitability. Laumonier et al. suggested that increased intracellular calcium in ASD children compared to control was due to inactivation of ionized Ca^{2+} -activated K^+ channel (BKCa) which leads to neuronal hyperexcitability [17].

However some researchers found no significant difference in serum calcium levels between autism and control groups. [18] one group of researchers found significantly lower serum phosphorus levels in ASD children as compared to healthy children. [19] Herdon et al. reported that ASD children have dietary selectivity which results in less consumption of calcium and other mineral. [20]

Several authors have suggested that hypocalcemia during early brain development could be a risk factor for the altered neurobehavioral outcome. Moreover, low plasma calcium may

increase the possibility of blood lead and arsenic accumulation and toxicity, which were associated with pathophysiology of ASD. [21] Again, phosphate depletion may result in a reduction in the ATP content of cells. Low ATP impedes the function of the Ca-ATPase, which leads to elevated cytosolic calcium and impairs mitochondrial function. [22-26]

Conclusion

From this study, we would like to conclude that Hypophosphatemia and hypocalcemia are common in autism spectrum disorder children. These macronutrients need to be taken care of in Autistic children.

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