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A Study of Vitamins D and B_{12} Levels in Autism Spectrum Disorder Children and their Relation with Disease Severity

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ABSTRACT
Autism spectrum disorder (ASD) is a complex and mysterious neurodevelopmental disorder that appears in the early years of life. Autistic children are characterized by impaired social interaction and communication and fail to respond to certain stimuli exhibiting some restricted and repetitive behavior or action. To evaluate vitamin status [Vit. D and Vit.B12] in children with autism spectrum disorder and Its correlation with severity of Disease. This comparative study includes75 autism spectrum disorder patients of age. Group 2-15 years as well as age, 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 vitamin D, vitamin B12. The Obtained Results were analyzed statistically to calculate significance of difference among them by calculating p value. Significantly decrease levels of serum vitamin D and vitamin B12 were found in ASD patients as compared to healthy controls and Vit D and Vit. B12 deficiency were found to be strongly correlated with ASD severity. Vit D and Vit B12 deficiencies are prevalent in children with ASD and Deficiency of Vit D and B12 correlate with disease severity. Early detection and targeted supplementation may serve as adjunctive strategies in ASD management.

INTRODUCTION

Autism is a growing problem worldwide and possesses a greater healthcare and economic burden to the developing nations like India. Research is on rise in the last decade on understanding various aspects of this condition. It is estimated that there are approximately 1.7-2 million children with Autism Spectrum Disorder (ASD) in India, thus ASD is a significant public health concern[1]. A survey of the available literature suggests that nutritional and dietary interventions are considered routine treatment for development disorders including Autism spectrum disorder[2]. Developing countries lack relevant data because autism is not a common theme of research[3]. For maintaining human health vitamins and minerals are inevitable as they play a crucial role like as enzymatic cofactor in the neurotransmitter production[4]. Insufficient intake of vitamins and minerals through poor food habit has been considered as one of the main contributing factor to child health problems. The etiology of ASD is still unknown. It can be due to combination of genetic, immunological and environmental factors[5]. Interestingly, environmental risk factors disrupt the gemone-epigenome of developing neurons and trigger immune responses. Immune dysregulation may predispose to ASD by inappropriate activation of immune reactions, resulting in prolongation and persistent immune response, autoimmunity and neuroinflammation[6]. Vitamin D may be a possible environmental risk factor for ASD, as it play a role in brain homeostasis, embryo genesis, neurodevelopment immune modulation(including the brain immune system), antioxidants, anti-apoptosis, neural proliferation and gene regulation[7]. Vitamin B12 and Folate play important roles in the development, differentiation and functioning of the CNS[8]. They are involved in the methionine-homocysteine pathway, which is responsible for the methyl groups required for DNA and protein synthesis[9]. So the current study is aimed to compare the vitamins level between Autism spectrum disorder patients and healthy population. And its correlation with severity of disease.

MATERIALS AND METHODS

The study was designed to evaluate serum vitamins 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). They were recruited from the pediatric neuropsychiatric 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 child development centre (C.D.C.) of 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:

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

Exclusion Criteria:

- Patients on vitamin/mineral supplement, use of any chelation treatment will be excluded from thestudy.
- Children with epilepsy, Turner Syndrome, Down syndrome and any kind of medication were excluded from the study children of 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 was 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.

The Study was Planned to Carry Out in Two Steps:

- **Physical Examination:** It was include age, sex, history of the disease etc.
- **Biochemical Examination:** 5 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 vitamin D and vitamin B12 was performed using Chemiluminescence immunoassay method.

- Vitamin D status was classified as Deficient (<20ng/mL), Insufficient (20-29ng/mL), or Sufficient (≥30 ng/mL)
- Vitamin B^{12} deficiency was defined as level <200 pg/mL.

RESULTS AND DISCUSSIONS

This comparative study includes75 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 (N=100)

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 (N=100)

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

Table 3: Comparison of Mean of Vitamin D and Vitamin B_{12} in the Controls and Autistic Subject

Parameters	Controls (n=25) Mean±SD	Autistic case (n=75) Mean±SD	Unpaired Student t Test P value
Vitamin $D_{(ng/mL)}$	37.35±9.36	22.39±7.37	<0.001
Vitamin $B_{12\ (pg/mL)}$	312. 68±77.64	253.46±61.41	<0.001

Table 4: Comparison of Mean S. Vitamin-D (ng/mL) Levels Using ANOVA Test

Study Groups	Mean± SD	P-Value
Group-1 (Controls)	37.35±9.36	≤0.05
Group-2 (Mild ASD)	36.03±7.34	
Group-3 (Moderate ASD)	27.84±8.96	
Group-4 (Severe ASD)	21. 32±8.14	

Table 5: Comparison of Mean S. Vitamin-B 12(pg/ mL) Levels Using ANOVA Test

Study Groups	Mean±SD	P-Value*
Group-1 (Controls)	312. 68±77.64	≤0.05
Group-2 (Mild ASD)	301.58±74.66	
Group-3 (Moderate ASD)	257.46±60.51	
Group-4 (Severe ASD)	241.56±61.54	

Autism spectrum disorder (ASD) is a developmental disability that can cause significant social, Communication and behavioral challenges. There is often nothing about how people with ASD look that sets them apart from other people, but people with ASD may communicate, interact, behave and learn in ways that are different from most other people. The learning, thinking and problem-solving abilities of people with ASD can range from gifted to severely challenged. Some people with ASD need a lot of help in their daily lives. In the present study, Vitamins status has been examined in Autistic and healthy children. The data in this study are expressed as Mean±SD. Unpaired student t test was used for comparison of Autistic patients with healthy controls. Various groups of autism patients which was divided in three groups (Mild, Moderate, Severe) was compare with healthy control group. The association between variables was assessed. The statistical analysis was performed using ANOVA test. P-values was considered as high significant (P<0.001) and significant (P<0.05) respectively. Seventy five (75) clinically diagnosed patients of Autism (male 45 and female 30) were included in the study and result were compared with age and sex matched 25 normal healthy control subjects (17 male and 8 female) (Table 1, 2). In our study, Vitamin D levels were found very significantly decreased in autistic patient as compared to control group (mean±SD, 37.35±9.36 vs 22.39±7.37(ng/ml), p=0.001). Whereas serum Vitamin B12levels were also found highly significantly decrease in patient with autism as compared to healthy control subjects (mean±SD, 312.68±77.64 vs 253.46±61.41 (pg/ml), p=0.001) (Table 3). ASD subjects (n=75) were further segregated into three groups according to severity ASD as assessed by the ISAA score (Indian scale for assessment of autism) as Group 1 (mild n=25), Group 2 (moderate n=25) and Group 3 (severe n=25). To evaluate Vitamin Status [Vitamin D and Vitamin B 12] were assessed for severity of ASD. Comparison of parameters among the three groups (mild, moderate, sever) and control group was performed by using one way analysis of variance (ANOVA) test. Serum Vitamin D level was significantly decrease with increased in severity of disease ($p \leq 0.05$) (Table 4) moreover Vitamin B_{12} also showed significantly decrease with advancement Autism Spectrum Disorder ($p \leq 0.05$) (Table 5). Serum Vitamin D deficiency and insufficiency can result from insufficient sun light exposure, inadequacy of vitamin in diet, impaired conversion into active forms and usage of anti epileptic drugs. Wang *et al.* reported that these reasons are responsible for lowering serum vitamin D levels in Autistic children. Also genetic factors such as vitamin D receptor (VDR) gene variants influence vitamin D levels[10]. According to Cui and Eyles the distribution of VDR is extensive in various parts of the brain. For instance, the expression of VDR increases in the prefrontal cortex and hippocampus, which are areas that are closely associated with cognitive process such as learning, memory and executive functioning. Furthermore, the presence of VDR was observed in regions characterized by a high concentration of dopaminergic neurons, suggesting a possible connection between vitamin D and the transmission of dopamine in the brain[11]. Vitamin D is physiological converted to its active form, 1,25 (OH) D (calcitriol), through two consecutive hydroxylation processes in the liver and kidney[12]. Calcitriol is a neuro active hormone, that is responsible

for different aspects of brain development and early cognitive development, vitamin D assists in neural cell proliferation and neurotransmission functions, thus theoretically affecting the neurodevelopmental processes[13]. Folate and Vitamin B12 are crucial for normal neuron function and serious deficiencies of these vitamins might directly affect brain function by disrupting myelination and synapse formation during neural development in the brain[14]. Notably, a higher vitamin B12 level at age >3 years was associated with higher adaptive behavior DQ and language DQ in children with ASD. James et al also reported that vitamin B12 supplementation in children with ASD aged >3 years is effective in improving autism symptoms[15]. However, Larger studies in further are needed to verify these association. This finding suggest that serum folate and vitamin B12 concentration are closely related to cognitive functioning. Some studies reported that decrease serum vitamin B12 levels were correlated with neurodevelopment disorders[16].

CONCLUSION

Vitamin D and Vit. B_{12} deficiencies are more prevalent in children with autism spectrum disorder and potentially linked to disease severity, the evidence remains mixed and more research is needed to clarify this relationship and determine effective interventions.

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