

Original Research Article

Vitamin D levels in children with autism spectrum disorders and normal children: a comparative cross-sectional study from South Kerala

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ABSTRACT

Background: Epidemiological evidence indicates that vitamin D deficiency during critical periods may increase the risk of neurodevelopmental disorders including autism spectrum disorder. The present study aimed to assess and compare the vitamin D levels of children with autism spectrum disorder (ASD) and neurotypical children and also to explore the relationship between vitamin D status and the severity of ASD symptoms.

Methods: This comparative cross-sectional study was conducted among children with ASD and children without any neuro developmental disorders. About 207 children aged two to six years were enrolled, comprising 105 participants with ASD and 102 who did not have an ASD diagnosis. ASD diagnosis was done using DSM-5 criteria and severity was assessed using CARS-2 ST scale. Serum 25-hydroxyvitamin D levels were obtained for every participant in the study. Statistical analyses included chi-square and independent t-tests, with significance set at $p < 0.05$.

Results: The majority of children in both groups were in the 24–36-month age range. Individuals with ASD showed a higher likelihood of low vitamin D levels (87.6%) relative to non-ASD cases (76.5%); while desirable vitamin D levels were observed more among normal children (23.5%) than children with ASD (12.4%). The association between Vitamin D levels and ASD status was also found to be statistically significant.

Conclusions: Our findings indicate that low vitamin D levels may be linked to ASD, highlighting a potential area for further investigation and suggest that vitamin D could have a supportive role in management.

Keywords: Autism spectrum disorder, ASD severity, Kerala, Vitamin D

INTRODUCTION

ASD is a neurodevelopmental disorder that is characterized by impairments in social interaction skills and communication, as well as restricted interests and repetitive stereotypic verbal and non-verbal behaviours and activities. (Fifth edition of the diagnostic and statistical manual of mental disorders (DSM-5)).¹ The global prevalence of autism is estimated at approximately 1 in 100 children, reflecting an average estimate, though substantial variability exists across studies.² Genetic factors are estimated to account for approximately 10–

20% of ASD cases.³ In addition, several environmental factors have been implicated, including advanced parental age, lead exposure, caesarean delivery, air pollution and maternal conditions such as obesity, hypertension and diabetes.⁴ As the risk of autism ASD is generally acknowledged to reflect both genetic and environmental factors, the interplay between these factors has become the subject of intensified research in the past several years. The developing brain is highly metabolically active and thus particularly vulnerable to nutritional deficiencies.⁵ Deficiencies in key nutrients during critical developmental periods may impair brain function by

disrupting signaling cascades essential for neuronal capacity.⁵ While multiple micronutrients are essential for optimal neurodevelopment, accumulating epidemiological evidence indicates that vitamin D deficiency during critical periods may increase the risk of neurodevelopmental disorders including autism spectrum disorder, however, definitive causal pathways have not been confirmed.^{5,6} Research has indicated that vitamin D deficiency during pregnancy and early childhood could be associated with an increased risk of ASD, making this nutrient a topic of interest for both prevention and intervention strategies.

Evidence for a possible link between vitamin D and ASD comes from a 2013 study showing that children in regions with low ultraviolet-B exposure had nearly three times the prevalence of ASD compared to those in sunnier areas.⁷ The potential mechanisms by which vitamin D may influence ASD have been extensively reviewed.^{8,9} Proposed pathways include its anti-inflammatory and neuro-protective effects, regulation of oxidative stress, enhancement of mitochondrial function, modulation of immune responses and influence on serotonin synthesis.^{5,8,9}

Autistic individuals exhibit immune dysfunctions, including elevated inflammatory cytokine levels that resemble those seen in vitamin D deficiency.^{9,10} Much of the ongoing neuro-inflammation in ASD is linked to oxidative stress, a process against which vitamin D exerts strong anti-inflammatory and antioxidant effects.¹⁰ Vitamin D enhances immune regulation by up-regulating dendritic lymphocytes and interleukin-10, thereby reducing autoimmune activity.^{11,12} Its active form, calcitriol, further protects brain tissue by lowering inflammatory cytokines, which, when elevated, are strongly associated with cognitive impairment in ASD.⁹⁻¹³ It is now recognized that the vitamin D metabolite 1,25(OH)₂D (calcitriol) functions as a neuro-steroid hormone, playing a key role in brain development and behaviour and disruptions in these vitamin D dependent processes have been implicated in autism.⁹ Current consensus suggests that ASD is primarily the result of interactions between genetic predispositions and environmental influences.

A meta-analysis reported that children with ASD had significantly lower 25(OH) D levels compared to controls.¹⁴ Recent evidence indicates a strong association between ASD and polymorphisms in genes encoding the vitamin D receptor, which are linked to both reduced vitamin D levels and ASD risk.¹⁵ Additionally, other studies have identified polymorphisms in genes involved in vitamin D metabolism that are associated with ASD severity, with odds ratios reported up to six.¹⁶ Evidence also indicates that reduced vitamin D levels in children with ASD may have clinical implications and could be associated with atypical brain development.¹⁷ It has been suggested that, until more evidence is available, vitamin D may be considered as a potential preventive and

therapeutic option for ASD. Early identification and appropriate management are essential for mitigating the severity of symptoms in children with ASD. Vitamin D supplementation has been proposed as a potential factor influencing ASD related outcomes, however the available evidence remains limited and inconclusive. The present study aimed to assess and compare the vitamin D levels of children with ASD and neurotypical children and also to explore the relationship between vitamin D status and the severity of ASD symptoms.

METHODS

The study employed a comparative cross-sectional design. The study was conducted among children with ASD and children without any neuro developmental disorders who attended the clinics of Child Development Centre, Thiruvananthapuram Kerala between January 2022 and December 2022. Children aged 2-6 years who attended CDC clinics and diagnosed as having ASD were consecutively selected for the ASD group. For the non-ASD group, children aged 2-6 years attending CDC clinics without any neuro developmental disorders as certified by a paediatrician were consecutively selected. Children diagnosed with rickets or renal disease were excluded from both groups. Only those children whose parents gave consent to participate were included in the study.

The sample size was calculated based on previously reported serum Vitamin D levels in autistic (18.79±8.35 ng/ml) and healthy children (22.18±9.00 ng/ml), showing a mean difference of 3.39 ng/ml and a pooled standard deviation of 8.68 ng/ml.¹⁸ At a significance level of 0.05 and a statistical power of 80%, the required sample size was calculated as 103 participants per group. In the present study, a total of 207 children were included, comprising 105 ASD and 102 non-ASD children.

Children were assessed for their ASD status using DSM V diagnostic criteria by a paediatrician. CARS-2 ST was used to assess the severity of ASD by a trained developmental therapist.¹⁹ CARS rates the child's behaviour, characteristics and abilities against the expected developmental growth of a typical child and is widely used in both clinical and research settings with demonstrated reliability and validity in assessing autism severity. The 15 items scale is rated based on direct observation, producing a total score ranging from 15 to 60. Based on established scoring criteria, ASD participants were categorized based on severity into two levels namely mild-to-moderate autism (scores from 30 to 36.5) and severe autism (scores from 37 to 60).

Serum vitamin D levels were assessed for all participants. Vitamin D was measured using 25-hydroxy vitamin D (25 (OH) D) blood test. Blood collection was done by trained laboratory technicians in the genetic and metabolic unit of CDC after ensuring enough safety measures. The serum 25(OH) D levels were measured

using the electrochemiluminescence binding assay (The Elecsys Vitamin D total III assay kit). Vitamin D level is defined with the concentration of 25-hydroxy vitamin D3 in blood.²⁰ Desirable, Insufficient and Deficient Vitamin D levels were determined with 25-hydroxyvitamine D levels of ≥ 30 ng/ml, 20-29 ng/ml and < 20 ng/ml respectively.²⁰

Statistical analysis

Data were analysed using statistical software IBM SPSS version 21. Descriptive statistics were used to summarize demographic variables, Vitamin D levels and ASD severity scores. Vitamin D levels were classified into three categories: desirable, insufficient and deficient. ASD severity was categorized as mild to moderate and severe based on standardized clinical criteria. To compare Vitamin D levels between children with ASD and neurotypical children chi-square test of independence was used. An independent samples t-test was used to compare mean serum vitamin D levels between the ASD severity groups. A p-value < 0.05 was considered statistically significant.

Ethical considerations

This study adhered to the ethical principles set forth in the Declaration of Helsinki. Ethical clearance was obtained from the Institutional Ethics Committee before commencement of the study. Written informed consent was taken from parents of all study participants.

RESULTS

The sociodemographic characteristics of study participants are depicted in Table 1. The mean age of children in both the ASD (n=105) and non-ASD (n=102) groups was 22.8 (± 11.4) months. The majority of children in both groups were in the 24–36-month age range, accounting for 43.8% of the ASD group and 39.2% of the non-ASD group. The ASD group comprised 86.7% males and 13.3% females, whereas the non-ASD group included 81.4% males and 18.6% females. Most children

in both groups frequently engaged in play involving physical activity. Majority reported participating often, with 78.1% of children with ASD and 83.8% of children without ASD in this category. Engagement in outdoor activities was also found to be quite similar between children with and without ASD. Over half of the children in both groups reported frequent outdoor activity.

Children with ASD were categorized into two distinct levels based on CARS scoring criteria. About 63 children (60.0 %) were reported to have mild to moderate ASD and 42 children (40.0%) with severe ASD. The mean serum vitamin D3 level in the ASD group was 22.91 ± 6.71 ng/mL, while the non-ASD group had a mean vitamin D level of 24.28 ± 8.15 ng/ml. Among ASD cases, nearly half (47.6%) were found to have deficient vitamin D levels compared to 43.1% of non-ASD individuals. About 40.0% of ASD cases had insufficient vitamin D levels compared to 33.3% in the non-ASD group.

About 12.4% of ASD children and 23.5% of non-ASD children had desirable vitamin D levels. Thus, a considerably higher proportion of individuals in the ASD group had deficient and insufficient vitamin D levels (87.6%), compared with 76.5% among non-ASD cases; while desirable vitamin D levels were observed more frequently among normal children (23.5%) than children with ASD (12.4%) (Table 2). The association between Vitamin D levels and ASD status was also found to be statistically significant ($\chi^2(1) = 4.38$, $p = 0.036$).

Those children with mild to moderate ASD had a comparatively higher mean vitamin D level ($M = 23.55$ ng/mL, $SD = 6.37$) compared to the severe group ($M = 21.95$ ng/mL, $SD = 7.16$): though ASD severity was not significantly associated with serum Vitamin D level. ($t(103) = 1.199$, $p = 0.233$, with a mean difference of 1.60 ng/mL (95% CI: -1.05 to 4.25)) As shown in table 3, among individuals with mild to moderate ASD, 42.9% had deficient vitamin D levels, 44.4% were insufficient and 12.7% had desirable levels. In comparison, individuals with severe ASD had 54.8% in the deficient category, 33.3% in the insufficient range and 11.9% with desirable vitamin D levels.

Table 1: Sociodemographic details of study participants.

Variables	ASD (n=105)	Non ASD (n=102)
Age (in months) mean (\pmS.D.)	22.8 (± 11.4)	22.8 (± 11.4)
24-36	46 (43.8)	40(39.2)
37-48	30 (28.6)	31(30.4)
49-60	22 (21.0)	24(23.5)
61-72	7 (6.7)	7(6.9)
Gender		
Male	91 (86.7)	83 (81.4)
Female	14 (13.3)	19 (18.6)
Place of residence		
Rural	65 (61.9)	62 (60.8)
Urban	40 (38.1)	40 (39.2)

Continued.

Variables	ASD (n=105)	Non ASD (n=102)
Religion		
Hindu	69 (65.7)	62 (60.8)
Christian	23 (21.9)	26 (25.5)
Muslim	12 (11.4)	14 (13.7)
Others	1 (1.0)	-
Educational status of mother		
School education	31 (29.5)	52 (50.9)
Graduation and above	74 (70.5)	50 (49.1)
Educational status of father		
School education	42 (40.0)	69 (67.6)
Graduation and above	63 (60.0)	33 (32.4)
Socioeconomic status		
APL	73 (69.5)	50 (49.0)
BPL	32 (30.5)	52 (51.0)
Type of family		
Nuclear	51 (48.6)	40 (39.2)
Extended	39 (37.1)	48 (47.1)
Joint	15 (14.3)	14 (13.7)
Socioeconomic status		
Upper	16 (15.2)	8 (7.8)
Upper middle	33 (31.4)	15 (14.7)
Lower middle	29 (27.6)	30 (29.4)
Upper lower	27 (25.8)	48 (47.1)
Engagement in play involving physical activity		
Never	4 (3.8)	2 (2.0)
Sometimes	19 (18.1)	14 (14.1)
Often	82 (78.1)	83 (83.8)
Engagement in outdoor activities involving sunlight exposure		
Never	7 (6.7)	7 (7.1))
Sometimes	41 (39.0)	40 (40.4)
Often	57 (54.3)	52 (52.5)

Table 2: Vitamin D levels in ASD and non-ASD children.

Vitamin D levels	ASD (n=105)	Non-ASD (n=102)
	N (%)	N (%)
Deficient and insufficient	92 (87.6)	78 (76.5)
Desirable	13 (12.4)	24 (23.5)

Table 3: Association between ASD severity and vitamin D levels.

ASD severity	Vitamin D levels			
	Deficient	Insufficient	Desirable	Total
Mild to moderate	27 (42.9%)	28 (44.4%)	8 (12.7%)	63 (60.0%)
Severe	23 (54.8%)	14 (33.3%)	5 (11.9%)	42 (40.0%)
Total	50 (47.6%)	42 (40.0%)	13 (12.4%)	105 (100%)

DISCUSSION

This study investigated the Vitamin D levels in ASD children and compared it with neurotypical children as well as the relationship between Vitamin D status and severity of ASD symptoms. Our findings revealed that children with ASD had significantly lower mean serum

vitamin D levels compared to their neurotypical counterparts. The mean serum vitamin D level in children with ASD in our study (22.91 ng/ml) was lower than that of the non-ASD group (24.28 ng/ml), consistent with numerous earlier reports suggesting a link between low vitamin D levels and autism.⁵ Previous studies have similarly demonstrated significantly reduced vitamin D concentrations in children with ASD compared to normal

children.²¹⁻²³ A meta-analysis of 24 case-control studies also revealed that children and adolescents with ASD exhibited significantly lower serum vitamin D concentrations compared to controls (mean difference=7.46 ng/ml, 95% CI: 4.66–10.26 ng/ml).²⁴

Research suggests that ASD may stem from a combination of organ-specific physiological issues and broader systemic abnormalities, including genetic mutations, oxidative stress, impaired detoxification, inflammation, immune dysregulation and disrupted neurotrophic or neurotransmitter signalling.²⁴ Emerging evidence links low vitamin D levels to many of these abnormalities and deficiency during early development may promote excessive neuronal proliferation and early brain overgrowth, potentially contributing to ASD in some individuals.²⁴ In the present study a higher proportion of children in the ASD group exhibited vitamin D deficiency and insufficiency, whereas desirable vitamin D levels were more frequently observed among neurotypical children. Studies investigating the vitamin D status of children and adolescents with ASD from different countries and races have also reported that ASD children and adolescents had lower vitamin D levels.²⁵⁻²⁷

In the present study, nearly 88% of children with ASD exhibited either deficient or insufficient vitamin D levels, compared to 76.5% among neurotypical children, indicating a generally poor vitamin D status in both groups. This widespread insufficiency may reflect common environmental and lifestyle factors such as limited sun exposure, dietary insufficiency or skin pigmentation patterns in the study population. Although engagement in outdoor and physical activities was reported similarly across both groups, children with ASD may have less effective sunlight exposure due to behavioural patterns, sensory sensitivities or reduced time spent outdoors.

Despite the lower mean vitamin D levels observed in ASD, our data did not reveal a significant association between vitamin D concentration and ASD severity as measured by CARS scores. This aligns with findings from some previous studies which also reported that while vitamin D deficiency was common among children with ASD, its severity did not consistently correlate with CARS scores.^{27,28} Conversely, other studies by Cannell & Grant, 2013 and Jia et al, 2018 have suggested a dose-dependent relationship, where higher vitamin D levels were associated with milder autism symptoms.^{9,29} The absence of such an association in our study may be due to the small sample size, cross-sectional design and the influence of unmeasured confounders such as dietary intake, seasonal variation, other nutritional deficiencies or genetic polymorphisms affecting vitamin D metabolism. Since ASD is a heterogeneous condition, it is possible that the relationship between vitamin D and severity may vary between individuals. However, inclusion of age-matched controls, standardized diagnostic criteria for

ASD and biochemical assessment of vitamin D adds to the strength of the study. Our findings indicate that low vitamin D levels may be linked to ASD, highlighting a potential area for further investigation and suggest that vitamin D could have a supportive role in management.

CONCLUSION

Our study found that children with ASD exhibited significantly lower vitamin D levels than neurotypical children. While vitamin D status did not correlate significantly with ASD severity, the findings suggest that hypovitaminosis D is common among children irrespective of diagnosis and warrants attention. Routine screening and appropriate supplementation of vitamin D may be considered as part of comprehensive paediatric care, especially in populations with limited sunlight exposure. Future longitudinal and interventional studies are recommended to further clarify the role of vitamin D in the aetiology and management of ASD.

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Conflict of interest: None declared

Ethical approval: The study was approved by the Institutional Ethics Committee

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