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Research Article

Analysis of Serum Vitamin D levels in Pediatric Patients with Autism Spectrum Disorder

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Abstract

Background

Autism spectrum disorder also called ASD is identified as issues with communication and show repetitive behavior. Causes of ASD are complex and are not fully understood, however, they are thought to be multifactorial with genetic components and some environmental factors. One such environmental factor may be Vitamin D, which is a neurosteroid hormone. ASD and other neurodevelopmental disorders have a growing amount of evidence for the involvement of neurosteroids and neuroimmunomodulation. Cortical and synaptic plasticity has been cited in the literature when speaking of the involvement of neurosteroids in neurodevelopmental disorders, thus, it is of interest to look at the relation of ASD and neurodevelopmental disorders with Vitamin D, which is shown to be less in many pediatric populations.

Objectives

The goal of this study is to compare serum vitamin D levels of pediatric ASD patients to those children in typically developing, age-matched peer groups. The vitamin D deficiency and sufficiency distribution will be assessed for children
possessing ASD and classically developing children.

Study design

Case-control study

Place of study and Duration of Study: This study was conducted at Indus Medical College/Hospital Tando Muhammad Khan from March 2025 to March 2026

Methodology

A case control method was used for children with ASD aged between three and ten. The sample consisted of 50 children each one diagnosed with ASD and other non-diagnosed developing children with similar age and gender.

In the standard physical examination, the recording of vitamin D levels of all the patients in both the groups was done. The comparison of Vitamin D levels in control samples of ASD was done using Chi-square tests. The comparison of other control samples was done using independent t-tests.

Results

Both populations in the study demonstrated overall low levels of Vitamin D. On average, children diagnosed with ASD had lower serum 25(OH)D levels than the control group. Breaking down the data, 40% of the ASD group was classified as deficient and 38% as insufficient, compared to 32% and 36% in the control group, respectively. While 32% of the control group had "sufficient" Vitamin D levels compared to only 22% of the ASD group, this gap was not considered distinct. Ultimately, despite the ASD group showing a higher overall percentage of deficient and insufficient participants, statistical analysis revealed no significant connection between Vitamin D status or deficiency and an ASD diagnosis between the two populations.

Conclusion

The deficiency of Vitamin D is more of a public health issue rather than an ASD-related issue, as all children with or without ASD potentially suffer the consequences. Vitamin D levels are lower among children with ASD. However, if there aren't any significant differences, vitamin D status may not help in differentiating between the two groups. Although better nutrition and increased exposure of children to the sun may help with overall physical and minor improvements to the mental and neurological development of children, the differences may not be very significant.

Keywords: Serum 25-hydroxyvitamin D; Vitamin D deficiency; Neurodevelopment; Case–control study, autism spectrum disorder

Introduction

ASD is a multifaceted neurodevelopmental condition that can be characterized by tenacious difficulties in social communication, reciprocity, and the presence of restricted as well as repetitive behaviors. It is a lifelong disorder affecting children across all ethnic, cultural, and socio-economic groups and is associated with significant functional challenges. Reported prevalence has increased over recent decades,likely due to heightened awareness, broader diagnostic criteria, and improved screening practices [1]. Despite extensive research about the aetiology of ASD is still unclear. Although it is widely accepted that a combination of genetic predispositio n and environmental influences contributes to its development [2,3].

When looking at environmental factors, vitamin D has drawn a lot of attention because its biological roles go way beyond just regulating calcium. It actually functions as a neurosteroid hormone that is crucial for brain development, playing a key role in neuronal differentiation, synaptic plasticity, and managing immune and inflammatory responses [4,5]. In fact, experimental studies show that vitamin D receptors are located in numerous areas of the brain that control behavior, cognition, and emotional regulation. This points to a likely connection between a person's vitamin D levels and their overall neurodevelopmental outcomes [6]. Furthermore, maternal vitamin D deficiency during child bearing has been linked to altered brain development in fetuses [7].

Several epidemiological research has observed lower serum vitamin D levels in pediatric ASD patients, suggesting that hypovitaminosis D might be associated with the disorder [8,9]. Researchers believe this connection could be driven by mechanisms such as impaired neuroimmune regulation, increased oxidative stress, and the dysregulation of both neurotransmitter synthesis and calcium signaling—all of which have the potential to negatively impact neurodevelopmental trajectories [10,11]. But, it's important to note that the conclusions regarding the connection between vitamin D levels and fetal neurodevelopment remain somewhat inconsistent across the literature [12,13].

Vitamin D deficiency is widely known public health concern, particularly in children, it often results from limited sunlight exposure, dietary habits, and lifestyle patterns that reduce time spent outdoors. ASD patient may be at an

even greater risk due to characteristic behaviours such as sensory sensitivities, selective eating, and reduced outdoor activity, all of which can influence their vitamin D levels [14]. Addressing deficiency in vitamin D in this population is therefore important not only for understanding potential aetiological mechanisms but also for informing nutritional and lifestyle strategies aimed at improving overall health. Although interest in the connection between vitamin D levels and occurrence of ASD has grown, the evidence remains inconclusive. Some studies propose that deficiency in vitamin D can be a conducive factor in the biological pathways underlying ASD, while some studies argue that low vitamin D readings are a result of behavioural and lifestyle differences rather than a causal factor. Given these uncertainties, further research is needed for comparing the vitamin D readings in children with ASD and the ones who do not have it.

This study is designed to examine the vitamin D levels in children with ASD and compare them with age-matched healthy control children. It also aims to provide more information on the distribution of vitamin D deficiency, insufficiency, and sufficiency in both groups.

Methodology

This case-control study involved a total of 100 participants: a study group of 50 diagnosed children with ASD, and a control group of 50 typically developing children. All children in the study group were between the ages of 3 and 10. To ensure diagnostic validity, registered clinicians confirmed the ASD diagnoses only after conducting comprehensive clinical assessments that strictly met established diagnostic criteria. Additionally, participants in the case group were purposefully selected not just to fulfill these specific ASD inclusion criteria, but also to ensure they were medically stable and clinically asymptomatic at the time of the study.

Children with the listed disorders were also excluded from the study: cerebral palsy, Down syndrome, chronic malabsorption syndrome, seizures, renal insufficiency, and any other neurological and/or metabolic disorders. Also, excluded were participants using multivitamins and other combined preparatory calcium and vitamin D, as well as other medications that would affect the vitamin D in serum or other pharmaceuticals that would affect the vitamin D. The study consisted of 50 participants with ASD. To lessen the effects of environmental and dietary factors, a control group of 50 typically developing participants was included. Members in the control group were paired to the study participants in age, gender, BMI, and socioeconomic status, and were free from any history of neurodevelopmental, chronic and/or metabolic disorders.

For the assessment, parents were instructed to fast their children and to refrain from breakfast. During the assessment, 5 ml venous blood samples were drawn and sent to an accredited clinical laboratory. To measure serum levels of 25-hydroxyvitamin D, an automated fully automated Chemiluminescent Microparticle Assay was used following the process of centrifugation. High creatinine levels can indicate chronic kidney disease, which can cause problems with vitamin D metabolism. For each step, standard operating procedures for laboratories were adopted.

Established clinical cutoff points were most often used to determine the vitamin D levels. When vitamin D levels were < 20 ng/mL, it was classified as deficiency. When 25(OH)D levels were from 20 to 29 ng/mL, it was classified as insufficiency. It was classified as sufficiency when 25(OH)D levels were > 30 ng/mL. Additionally, the ratios of deficits, insufficiencies, and sufficiencies were analyzed in the ASD and control groups.

Record databases were designed to perform statistical analyses with the PSPP software, version 26. The vitamin D 25(OH) data were described with the mean, SE, and percentage of vitamin D status. In this study, the Independent t-test was used for data analysis, and the association of vitamin D deficiency and ASD was analyzed with the Chi-square method. Statistically significant differences were reported when $p < 0.05$. In this way, the subjects and the biochemical parameters to compare vitamin D status of diagnosed children and healthy control children were determined.

Results

Demographic data for the ASD and control groups from the semi-structured questionnaire is found in Table I. No meaningful differences in age, sex, or BMI were present. Other baseline data and matching confirmed that no biochemicals will be impacted by demographic or anthropometric changes. Nutrition and lifestyle differences were also minimized, as the groups came from similar socioeconomic backgrounds.

Vitamin D in addition to its metabolites were the primary biochemicals of interest in this study. Children who were a part of placebo group had higher levels of Vitamin D than children with ASD. The control group's recorded a mean of 25(OH)D level was 16.25 ng/mL. The ASD group's mean 25(OH)D level was 15.54 ng/mL. Although the mean for the ASD group was lower, this difference was not significant statistically. It is also worth mentioning that the mean Vitamin D levels for the control and ASD groups were both lower than the recommended levels. Therefore, the children in both groups were considered to be Vitamin D deficient. This is also indicated in Table II.

As per the reported clinical cut-off levels, the participants were designated to one of the categories as "Vitamin D Deficient," "Vitamin D Deficient," and "Vitamin D Sufficient." In the ASD group, 10 (20%) participants were classified as "Vitamin D Sufficient," 21 (42%) as "Vitamin D Insufficient," and 19 (38%) as "Vitamin D Deficient."

In the control group, out of the 50 patients 18(36%) recorded to have Vitamin D insufficiency, 16 (32%) recorded to have Vitamin D deficiency and 16 (32%) recorded to have Vitamin D Insufficiency.

No notable associations was deteremined between vitamin D levels and the ASD group. In the control group vitamin D deficiency and insufficiency was recorded to be more common. There was no noteworthy difference in vitamin D levels between the ASD and control groups. See Table III for results.

There seemed to be no obvious difference in the experience of vitamin D deficiency and insufficiency among the ASD and control groups. There was no obvious difference in classification of vitamin D in the groups. The high occurrence of vitamin D insufficiency in both groups may indicate that the reason for the low status of vitamin D in both groups may be environmental and/or due to lifestyle factors and may be due to factors other than ASD. The significant prevalence of low vitamin D in both groups may suggest that when considering children's health, vitamin D should be included.

Table I. General characteristics of ASD and control children

Variable	**ASD ($n = 50$)**	**Control ($n = 50$)**
Age (years)	7.5 ± 3.42	7.63 ± 3.91
Sex Male Female	 50% 50%	 50% 50%
BMI (kg/m²)	18.69 ± 3.21	18.97 ± 4.21

Table II. Comparison of serum vitamin D levels between groups

Group	Mean serum 25(OH)D (ng/mL) ± SE	p-value
ASD (n=50)	15.54 ± 0.74	0.50
Control (n=50)	16.25 ± 0.74	

Table III. Distribution of vitamin D status in ASD and control groups

Vitamin D Status	ASD ($n = 50$)	Control ($n = 50$)
Deficient	19 (38%)	18 (36%)
Insufficient	21 (42%)	16 (32%)
Sufficient	10 (20%)	16 (32%)

Discussion

Children who are documented to have ASD were compared with typical children to assess for any link between ASD and recorded vitamin D level. The mean of serum 25(OH)D was recorded to be lower in the ASD group when compared to the control group, but not statistically significant. Similarly, categorizing children as having a vitamin D deficiency, insufficient or sufficient vitamin D status did not reveal any noteworthy differences between the two research groups. Overall, both ASD and non-ASD children displayed similar patterns of vitamin D deficiency, suggesting that vitamin D cannot be used as a distinguishing biomarker for ASD [15, 16].

Children who have ASD are recorded to have recorded low levels of vitamin D as compared to typical children, but the variance was not significant statistically, according to Basheer et al. Likewise, Kocovska et al. reported high deficiency rates of vitamin D in both ASD and control groups, suggesting that the low vitamin D levels may be related to other social or environmental factors, not specific to ASD [17]. The present study thus confirms the findings of previous studies that there are not clear relationships between a vitamin D deficiency and ASD.

There are, however, some studies that have yielded conflicting results. Bener et al. observed children who had ASD had significantly lower levels of vitamin D and speculated that there may be a link between vitamin D deficiency and ASD [18]. Other factors that could contribute to discrepancies include differences in the sample size, geographic location, dietary habits, exposure to sunlight or methodological differences among the studies. Differences in geographic location,

sample size, dietary habits, sunlight exposure and methodological differences among studies could contribute to discrepancies.

Meguid et al. recorded children with ASD to have low levels of vitamin D in Egypt, and proposed that ASD could be linked to immune dysfunction and physiological stress [19]. However, it was found that vitamin D wasbeneficial for behavioural outcomes in children with ASD and some researchers suggested vitamin D may be a therapeutic option. However, it is important to note that while supplementation may help with overall health, it may not affect the core symptoms of ASD and may have adverse effects [20].

However, Kerley et al. did not observe any improvements in behaviour after normalising levels of vitamin D in children who have ASD, which casts doubt on the potential causal relationship. They proposed that the low recorded levels of vitamin D were a proxy for behavioral attributes of ASD, but were not causative factors [21]. In a like manner, Arastoo et al. determined that there was no association between vitamin D levels and severity of ASD but put forward several uncertainties in this regard [22].

The current study found a slight decrease in vitamin D levels for children with ASD, but this difference was not statistically significant, considering the mixed findings and lack of clear mechanism of explanation. The recorded deficiency in vitamin D is prevalent in both groups and the pattern seen is more likely to be related to shared environmental or lifestyle factors than to ASD.

Conclusion

According to this case-control study, there was a slight decrease in Vitamin D status for children who had ASD as compared to typically developing children, although the difference was not statistically that significant. Furthermore, both groups were found to have a deficiency and/or insufficient volume of Vitamin D. This indicates that low recording of vitamin D is a larger public health concern, rather than particular to those diagnosed with an ASD. Until such time as suitable evidence is produced regarding the linking between Vitamin D deficiency and ASD, it is likely that the low Vitamin D records of children diagnosed with an ASD reflects the same socio-economic, biological and/or lifestyle circumstances present in the general population. Good Vitamin D status should be maintained in all children.

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