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Vitamin D profile in autism spectrum disorder children and its relation to the disease severity

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Abstract

Background The study aimed to investigate whether vitamin D deficiency is a common finding in autism spectrum disorder (ASD) children and whether such deficiency is related to ASD severity and language age or not.

Methods A cross-sectional observational study was conducted on ASD children aged 2-6 years. The participants were 80 Egyptian children with ASD. All participants were assessed using DSM-V, the Childhood Autism Rating Scale (CARS), language assessment, and assessment of serum vitamin D using ADVIA Centaur Vit D assay.

Results About 63.8% of ASD children have vitamin D insufficiency, 28.8 % have vitamin D deficiency, and 7.4% have normal serum levels. No correlation was found between serum vitamin D and language age (r = -0.085, *P* = 0.451), DSM 5 severity levels (r = 0.015, *P*= 0.894), and CARS scores (r= 0.075, *P*= 0.511).

Conclusion ASD children have lower serum vitamin D levels, which may be one of the environmental factors contributing to ASD development in genetically susceptible individuals, and its correction may be helpful as adjuvant therapy for ASD.

Keywords Vitamin D, Autism spectrum disorder, DSM5 severity levels

Background

Autism spectrum disorder (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 behaviors. According to the "Diagnostic and Statistical Manual of Mental Disorders, Fifth Edition "(DSM-V), ASD is a new term that reflects a scientific consensus that three previously separate disorders are actually a single condition with different levels of severity. ASD now includes the previous DSM-IV (autistic disorder, Asperger's disorder, and pervasive developmental disorder not otherwise specified). The diagnosis of ASD is based on two domains, which are deficits in social communication and restricted, repetitive patterns of behavior and interests [1].

According to the Centers for Disease Control and Prevention, the prevalence of ASD is dramatically increasing; it was about 1/44. ASD exhibits a higher prevalence rate among boys compared to girls, with approximately four times as many boys being affected by the disorder. ASD has been documented to manifest across many racial, cultural, and socioeconomic backgrounds [2].

The etiology of ASD is still unknown. It can be due to a combination of genetic, immunological, and environmental factors [3]. Interestingly, environmental risk factors disrupt the genome-epigenome of developing

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neurons and trigger immune responses. Immune dysregulation may predispose to ASD by inappropriate activation of immune reactions, resulting in prolongation and persistent immune responses, autoimmunity, and neuroinflammation [4].

Modabbernia et al. [5] explain the various mechanisms underlying environmental factors' relationship with ASD. Proposed explanations include non-causal associations, gene-related effects, oxidative stress, inflammation, hypoxia/ischemia, endocrine disruption, alterations in neurotransmitter function, and signaling pathways interference. Furthermore, it is essential to note that numerous risk variables exhibit interactions during the crucial developmental period, ultimately influencing the subsequent phenotypic of individuals with autism spectrum disorder. The co-occurring impairments observed in children with autism spectrum disorder exhibit significant variability, as do their abilities [6].

Gopen & Mahmud [7] proposed that "vitamin -D may be a possible environmental risk factor for ASD, as it plays a role in brain homeostasis, embryogenesis, neurodevelopmental immune modulation (including the brain immune system), antioxidants, anti-apoptosis, neural proliferation, and gene regulation".

Our study aimed to investigate whether vitamin D deficiency is a common finding in ASD children and whether such deficiency is related to ASD severity, according to DSM-V and CARS.

Methods

This study is a cross-sectional study that was conducted on ASD children aged from 2-6 years, attending the outpatient clinic of the Phoniatric unit at XXX Hospital from October 2020 to December 2022. The IRB of the faculty of Medicine had approved the study (MD/20.07.343). The consent of the parents of the participating children in the study was obtained.

The sample size was calculated using Medclac; it was estimated to be 74 ASD children referring to a previous study [8], where the mean vitamin D among ASD patients was 32.3 ± 4, and the expected mean among the current study is 31, α error is 0.05, and the power of the study is 80%.

Children with other known neurological and psychiatric disorders, active rickets, chronic disease, history of vitamin D supplementation, and drug formulas containing vitamin D as cod liver oil were excluded from the study.

The protocol of evaluation of the studied children included history taking, language assessment using the preschool language scale 4th Arabic version [9], and Psychometric evaluation using the Stanford Binet intelligence scale "4th Arabic version" [10]. Diagnostic and Statistical Manual of Mental Disorders Fifth Edition (DSM-V) for ASD [1]: was carried out for every participant to diagnose ASD, focusing on two areas: confined, repetitive patterns of behavior and interests and difficulties in social communication. According to DSM-V, there are three levels of ASD: level I "Requiring support", level II "Requiring substantial support", and level III "Requiring very substantial support".

The authors performed the Child Autistic Rating Scale "CARS" [11]. CARS is "a diagnostic assessment method that rates children on a scale from one to four for various criteria, ranging from normal to severe, and yields a composite score ranging from non-autistic to mildly autistic, moderately autistic, or severely autistic" score range from 15 to 60, the cut-off rate for diagnosis of mild autism is 30. Scores between 37 and 60 suggest severe autism, whereas scores between 30 and 36 indicate mild to moderate autism.

A venous sample was collected to measure vitamin D concentration using ADVIA Centaur Vit D assay, which is an eighteen-minute antibody competitive immunoassay. The latter makes use of a vitamin D analog tagged with fluorescein, an anti-vitamin D monoclonal mouse antibody labeled with acridinium ester (AE), and an antifluoresce in monoclonal mouse antibody covalently bonded to paramagnetic particles (PMP). The amount of vitamin D in the patient sample is inversely related to the amount of relative light units (RLU) detected by the system.

The reference values for levels of 25-(OH) Vitamin D are as follows: deficiency is defined as less than 20 ng/ml, insufficiency ranges from 20-29 ng/ml, normal levels are between 30-100 ng/ml, and levels beyond 100 ng/ml are considered hazardous [12].

Version 25 of the SPSS program (SPSS Inc., PASW Statistics for Windows) was used to analyze the data: the SPSS Inc., Chicago. Numbers and percentages were used to describe the qualitative data. When describing quantitative data that were regularly distributed, the mean± Standard deviation was used, and the Kolmogrov-Smirnov test was used to confirm that the data were normal. The results were evaluated for significance at the (≤0.05) level. Monte Carlo test, One Way ANOVA test, and Spearman's rank-order correlation are used.

Results

This study was conducted on 80 children with ASD (67 males and 13 females). Their chronological age ranged from 2 to 6 years (mean 3.62±1.01). About 56.2% of ASD children are from urban areas, while 43.8% are from rural areas (Table 1).

According to the DSM-V severity level, participants were divided into (level I, level II, and level III).

Table 1 Demographic characteristics of studied cases

	Number	%
➢ ***Age (years)***	3.62±1.01 (2-6)	
➢ ***Family history***		
• ***-VE***	80	100.0
• ***+VE***	0	0.0
➢ ***Sex***		
• ***Male***	67	83.8
• ***Female***	13	16.2
➢ ***Residence***		
• ***Rural***	35	43.8
• ***Urban***	45	56.2

Data expressed as mean ± SD or number (%)

Table 2 Phoniatric assessment (IQ, CARS, serum vitamin D, DSM-V level of severity, and Language age) among studied cases

	Number	%
➢ ***IQ***	66.76±12.63 (43-93)	
➢ ***CARS***	34.35±2.57 (23-43)	
➢ ***DSM-V level of severity***		
➢ ***Level 1***	20	25.0
➢ ***Level 2***	36	45.0
➢ ***Level 3***	24	30.0
➢ ***Language age (months)***	11.75±3.29 (6-30)	

Data expressed as mean ± SD or number (%)

Table 3 Vitamin D level among studied cases

Vitamin D level	*N*	%
➢***Deficient <20 ng/ml***	**23**	28.8
➢***Insufficiency 20-30 ng/ml***	51	63.8
➢***Normal >30 -100 ng/ml***	6	7.4
➢***Mean ± SD of serum vitamin D*** (min–max).	22.93±6.56 (10.4-55.5)	

Data expressed as mean ± SD or number (%)

Level I accounted for about 25% of total cases, level II accounted for 45%, and level III accounted for 30%. Their IQs ranged from 43-93 (mean 66.76±12.63), CARS ranged from 23-43 (34.35±2.57) while their total language age ranged from 6-30 months (mean (11.75±3.29) (Table 2).

Assessment of serum vitamin D revealed that about 63.8% of autistic children have vitamin- D insufficiency, and about 28.8% % of autistic children have vitamin- D deficiency. However, children with ASD with normal serum vitamin D levels accounted for 7.4% (Table 3).

Table 4 Correlation between serum vitamin D and IQ, CARS, DSM-V levels of severity, and Language age among studied cases

	Serum vitamin D (ng/ml)	*Test of significance*
IQ	*r*= -0.085	*P*= 0.451
CARS	*r*= 0.075	*P*= 0.511
DSM-V levels of severity	*r*= 0.015	*P*= 0.894
language age (months)	*r* = 0.085	*p* = 0.451

r: Spearman correlation coefficient

Table 4 shows no statistically significant correlation between serum vitamin D and IQ, CARS, language age, and DSM-V levels of severity.

Table 5 shows no statistically significant difference between different serum vitamin D levels and CARS, language age, and DSM-V levels of severity.

Discussion

ASD is known as a multifactorial disorder that can result from an interplay between genetic and environmental factors [13, 14]. Environmental risk factors for (ASD) refer to non-genetic factors that have the potential to impact disorder development in individuals who are genetically predisposed. The individual vulnerability to environmental risk factors for (ASD) is limited to the early stages of life, mainly during the embryonic and fetal developmental periods, when the developing brain exhibits heightened sensitivity to these factors. The potential link between immunological dysregulation and (ASD) may be attributed to the improper activation of immune responses, as well as the protracted and persistent nature of these reactions, potentially leading to autoimmunity [4]. Possible environmental ASD risk factors comprise folic acid deficiency, neonatal hypoxia, maternal obesity, and gestational diabetes mellitus [14]. Furthermore, several studies proposed that alteration of vitamin D levels, whether deficiency or insufficiency, might be an unfavorable factor for ASD [15].

The present study was conducted on 80 ASD children; we observed male predominance among studied children (67 male,13 female), which consisted of other studies, e.g. [16–18].

Assessment of serum vitamin D among ASD children revealed that about 63.8% of autistic children have vitamin D insufficiency, and about 28.8% of autistic children have vitamin D deficiency. These results are consistent with other studies, e.g., [19–25]. Serum vitamin D deficiency and insufficiency can result from insufficient sunlight exposure, inadequacy of vitamins in diet, impaired conversion into active forms, and usage of antiepileptic drugs. Wang et al. [26] assume that these reasons

Table 5 Relation between serum vitamin D and CARS, language age and DSM-V levels of severity

	Serum vitamin D			Test of significance
	Deficient <20 ng/ml	Insufficiency 20-30 ng/ml	Normal >30-100 ng/ml	
	N=23	*N*=51	*N*=6	
➢***CARS***	34.26±2.45	34.16±2.57	36.33±2.58	*F*=1.99 *P*=0.143
➢***language age (months)***	11.56±2.37	12.0±3.68	10.33±2.88	*F*=0.733 *P*=0.484
➢***DSM-V levels of severity n(%)***				
· level1	6 (26.1)	14 (27.5)	0	*MC*=3.21
· level2	9 (39.1)	24 (47.1)	3 (50.0)	*P*=0.523
· level3	8 (34.8)	13 (25.5)	3 (50.0)	

F One Way ANOVA test, *MC* Monte Carlo test

Similar superscripted letters denote significant differences between different groups in the same row

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.

According to Cui and Eyles [27], "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 processes 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".

The presence of VDR and enzymes in brain neurons and glial cells suggests that vitamin D may have a function in prenatal neurodevelopment [28]. Additionally, Magnusson et al. [29] provided evidence suggesting that "vitamin- D might provide therapeutic advantages in mitigating autism symptomatology in those diagnosed with the condition".

Several studies proposed that vitamin D considerably affects neurodevelopment [25]. Vitamin D plays a role in regulating synaptic plasticity and the dopaminergic system. Additionally, it helps reduce the oxidative burden [30]. Mak [31] states that "vitamin D can facilitate the maturation of regulatory T cells and hinder immune response hyperactivity and autoimmunity". Furthermore, vitamin D assumes a crucial function in the modulation of gene expression. According to Trifonova et al. [32], "about 223 autism spectrum disorder (ASD) risk genes listed in the SFARI database exhibited sensitivity to vitamin- D. This proposed that vitamin -D may have a regulatory role in these genes associated with ASD".

However, no correlation was observed between serum vitamin D and IQ, CARS, language age, and DSM-V levels of severity, so vitamin D may be related to the pathophysiology of ASD. Still, it is not associated with these aspects of ASD. These results were in contrast with other studies, e.g. [8, 33], which showed a negative correlation between serum vitamin-D levels and the severity of ASD based on CARS scores. Moreover, these results were consistent with Basheer et al. [21], who did not observe a correlation between serum vitamin D levels and the severity of ASD.

We recommend that the assessment of serum vitamin D be mandatory for ASD children. In addition, we recommend vitamin D supplementation for ASD children to show its effects on the symptoms and severity of ASD.

Conclusion

ASD children have lower levels of serum vitamin D, which may be one of the contributing environmental factors of developing autism in genetically susceptible children but is not correlated with ASD severity according to CARS and DSM-V.

Acknowledgments
Not applicable.

Code availability (software application or custom code)
Not applicable.

Study design
An observational cross-sectional with an analytic component study was carried out to ascertain whether vitamin D deficiency is a common finding in ASD children and whether such deficiency is related to ASD severity and language age.

Authors' contributions
All authors contributed to the study's conception and design. Material preparation and data collection were performed by A.M. Running the lab tests and interpreting the lab results were performed by A.S. and M. Z.. Data analysis was performed by A. M., A. A., and T.A. The first draft of the manuscript was written by A.M. and T.A., and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

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Availability of data and materials
All data generated or analyzed during this study are included in this article. Further inquiries can be directed to the corresponding author.

Declarations

Ethics approval and consent to participate
The research procedures were conducted in accordance with the principles of the Declaration of Helsinki. The IRB of the faculty of Medicine had approved the study (MD/20.07.343). The informed verbal consent of the parents of the participating children in the study was obtained, and their data was anonymous and confidential.

Consent for publication
Not applicable.

Competing interests
We (all the authors) declare no conflicts of interest. The authors have no relevant financial or non-financial interests to disclose.

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