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RESARCH ARTICALE

DECODING AUTISM:THE SCIENTIFIC LINK BETWEEN SALIVARY VITAMIN D3 LEVELS AND NEURODEVELOPMENT-A CASE-CONTROL STUDY

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ABSTRACT

Objective: Autism Spectrum Disorder (ASD) involves challenges in social communication and repetitive behaviors. Emerging evidence suggests a link between vitamin D3, vital for brain function, and ASD, though research on salivary vitamin D3 as a biomarker in ASD is limited.
Aim: To compare salivary vitamin D3 levels between ASD patients and neurotypical individuals.
Materials and Methods: This case-control study collected unstimulated saliva samples from ASD patients and neurotypical controls. Vitamin D3 levels were measured using enzyme-linked immunosorbent assay (ELISA), with paired t-tests for group comparisons
Results: ASD patients had significantly lower salivary vitamin D3 levels than controls ($p = 0.001$), with ranges of 28.9–48.4 ng/mL in ASD versus 45.2–60.9 ng/mL in neurotypical individuals.
Conclusion:ASD patients showed lower salivary vitamin D3, suggesting a potential association with vitamin D3 deficiency. Further research could explore the role of vitamin D3 in ASD and assess the benefits of monitoring and supplementation.

Keywords: Keywords: Autism Spectrum Disorder, Vitamin D3, Saliva, ELISA, Vitamin D Deficiency, Neurodevelopmental Disorders, Case-Control Study.

INTRODUCTION

Autism is a neurodevelopmental disorder characterized by repetitive patterns of interest and behavior, along with deficits in social and communication development.[1] It typically manifests within the first three years of life and is considered a serious, lifelong developmental

condition. Approximately five out of every 10,000 children are affected, with boys being four times more likely to have the disorder than girls. No psychosocial factors in a child's environment have been linked to the development of autism; instead, physical abnormalities in the brain are believed to be responsible for the symptoms.[2] Autism spectrum disorders (ASDs) are neurological conditions with a significant genetic basis, though their precise origins remain unknown.[3] The genetic complexity of ASDs, along with the broad range of observable traits, has made it challenging to pinpoint a definitive cause. These disorders are influenced by multiple genes, resulting in considerable variability in their manifestation.[4] The prevalence of autism spectrum disorder (ASD) appears to be substantial among children and adolescents worldwide, making it important to understand the most recent prevalence rates and trends.[5] Individuals with disabilities, including those with ASD, may encounter health disparities and difficulties in securing employment as adults, potentially leading to a cycle of poverty.[6,7] Vitamin D is thought to be one of the oldest hormones, with its origins dating back over 750 million years. It is crucial for bone development from birth until death and can be synthesized in phytoplankton, zooplankton, and most plants and animals exposed to sunlight. Vitamin D plays a key role in maintaining calcium balance by enhancing the gastrointestinal tract's ability to absorb dietary calcium. Its metabolism is accelerated during pregnancy and lactation. The two main forms of vitamin D are ergocalciferol (vitamin D2), which is derived from irradiating plants or food materials, and cholecalciferol (vitamin D3), which is produced in the skin after exposure to sunlight or UV light.[8] In the summer, the skin synthesizes vitamin D3, which can also be obtained through diet, particularly from fatty fish like mackerel and herring. Cholecalciferol undergoes hydroxylation in the liver to form 25-hydroxyvitamin D3, and then in the kidneys to produce 1,25-dihydroxyvitamin D3, the active metabolite that boosts calcium absorption in the intestines. Risk factors for low vitamin D levels include premature birth, pigmented skin, limited sun exposure, obesity, malabsorption of dietary fat, impaired enzymatic activation of cholesterol, and older age. Severe vitamin D deficiency can lead to rickets or osteomalacia. The active form of vitamin D, 1,25-dihydroxyvitamin D, is vital for regulating calcium homeostasis and bone mineralization. Additionally, it induces cell death, halts the cell cycle, and promotes cancer cell differentiation, which helps inhibit growth.[9] In recent years, vitamin D insufficiency has been widely observed across all age groups. In developed countries, rickets has not been entirely eradicated, particularly affecting young immigrants.[10]

Previous studies have shown that children with autism tend to have lower vitamin D levels compared to their parents, siblings, and the general population. Low vitamin D levels at birth have been observed in children later diagnosed with ASD, while their healthy siblings did not exhibit the same deficiency. Low maternal vitamin D levels during pregnancy have been associated with adverse effects on cognitive development, early childhood development, and an increased risk of ASD diagnosis.[11] Vitamin D deficiency in mothers during pregnancy may be a risk factor for ASD in their children.[12,13] Additionally, children diagnosed with ASD have been found to have significantly lower vitamin D levels than neurotypical children, and it has been suggested that vitamin D supplementation could have beneficial effects for these children.[12,14,15,16]

There are several gaps in the existing literature on the relationship between vitamin D levels and autism spectrum disorder (ASD). Many previous studies are limited by small sample sizes, making it difficult to generalize their findings. Additionally, confounding factors such as diet, sun exposure, geographic location, and genetic differences are often not adequately controlled, which can affect the accuracy of the reported vitamin D levels. Moreover, the majority of research focuses on blood serum levels of vitamin D, with little exploration of alternative, less invasive biomarkers like salivary vitamin D. Few studies specifically examine how vitamin D3 levels correlate with neurodevelopmental milestones in children with autism. This study aims to address these gaps by focusing on salivary vitamin D3 levels, providing a non-invasive and practical measure. It also offers a direct comparison between individuals diagnosed with autism and a neurotypical population, thus contributing to a more comprehensive understanding of the potential link between vitamin D levels and ASD.

MATERIALS AND METHODS

A case-control study was conducted with a sample size of 32 students from a private educational trust for autism in Chennai, Tamil Nadu, India. The study adhered to the provisions of the declaration of

Helsinki and received approval from the scientific review board (IHEC/SDC/UG-2253/24/PHARM/097). Participants in the case group were children diagnosed with autism, aged 10 to 15 years. Exclusion criteria included those with systemic diseases, poor oral health, chronic kidney or liver conditions, individuals under vitamin D supplementation, and those who were malnourished. Unstimulated saliva (2 ml) was collected from participants between June and July 2024. The samples were centrifuged at 5000 rpm for 10 minutes and stored at 4°C for analysis. Vitamin D levels were measured using the Vitamin D Quanti Microlisa, based on delayed competitive ELISA. A diet history was also recorded, and participants consuming vitamin D supplements or fortified foods were excluded. On the day prior to and the morning of sample collection (between 8 to 9 A.M.), participants were instructed to follow their usual South Indian diet. Data analysis was performed using IBM SPSS Statistics for Windows, Version 23.0 (IBM Corp, Armonk, NY).

RESULTS

Salivary vitamin D3 levels were measured using a delayed competitive ELISA method. The mean salivary vitamin D3 levels ranged from 45.2 to 60.9 ng/mL in healthy subjects, while in patients with autism, the levels were significantly lower, ranging from 28.9 to 48.4 ng/mL. A paired sample t-test was done, yielding a highly significant p-value ($p = 0.000$, $p < 0.05$), indicating that the vitamin D3 levels in patients with autism were significantly lower compared to the healthy control group. This suggests a strong association between lower vitamin D3 levels and autism.(Table.1) (Fig.1, FIg.2)

Table.1 Table showing the statistically significant result (p<0.05) Paired-t test done using SPSS software version 23.0

Paired t test		
Study group	**Mean**	**Significance(2-tailed)**
Case group (Patients with autism)	53.05ng/ml	0.001
Control group (Healthy patients)	38.65ng/ml	

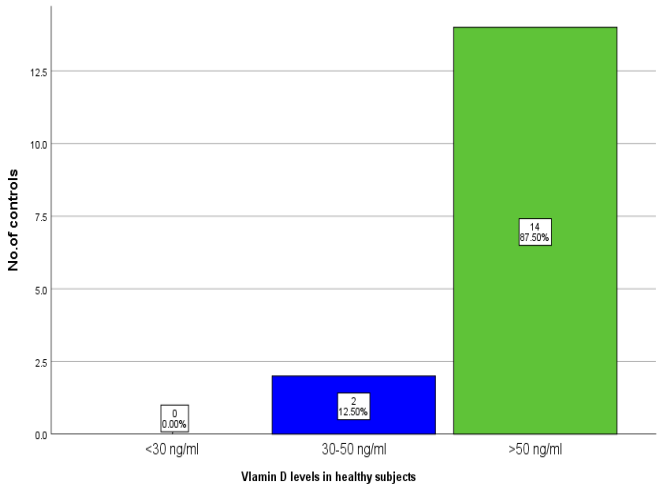

Figure1. Bar graph showing the vitamin D levels in healthy subjects

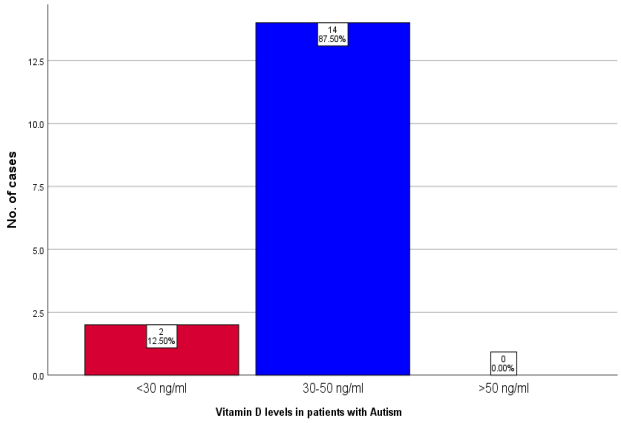

Figure 2. Bar graph showing vitamin d levels in autism patients

DISCUSSION

Vitamin D plays various biological roles, including reducing inflammation, and may influence the development of autism spectrum disorders (ASD).[17] Deficiency in vitamin D is often linked to limited sun exposure, inadequate dietary intake, and impaired liver function, which reduces the synthesis of this essential vitamin. In cases of severe obesity, lower vitamin D levels are commonly observed alongside elevated parathyroid hormone levels, which disrupt calcium metabolism and adversely affect bone health. This can also contribute to the onset of conditions like diabetes, cardiovascular disease, and hypertension.[18] Vitamin D3 is particularly crucial for brain development, and its deficiency during key periods such as pregnancy and early childhood could contribute to neurodevelopmental disorders,

including ASD.[19] Studies have indicated that children with ASD often present with vitamin D deficiency, and supplementing with vitamin D3 may improve outcomes, particularly in younger children. Vitamin D3 supplementation is a safe and cost-effective intervention that could benefit many children with ASD.[20] However, there remains limited data exploring the relationship between vitamin D deficiency and the severity of ASD symptoms.[21]

Our study found that children with autism had lower salivary vitamin D3 levels compared to their neurotypical peers. These findings align with prior research, which consistently reports lower vitamin D3 levels in children diagnosed with ASD. For example, a study by Zhang et al. (2022) found that children with ASD had significantly lower serum vitamin D levels and a higher prevalence of vitamin D deficiency (below 20 ng/ml) compared to healthy controls (67.7% vs. 34.1%).[22] Similarly, a 2022 study by Ganta Avani emphasized the need to screen children at risk of vitamin D deficiency, particularly those with ASD who may have restricted diets. The study also noted that reduced sun exposure during the COVID-19 pandemic heightened the risk of deficiency in this population.[23]

Additionally, low vitamin D levels have been reported in individuals with Down syndrome, indicating that vitamin D deficiency might be a common issue across neurodevelopmental disorders. [24] Maternal vitamin D deficiency during pregnancy, as well as early childhood deficiency, may increase the risk of developing neurodevelopmental disorders such as ASD.[25] Furthermore, research has suggested that vitamin D3 supplementation may improve autism symptoms, underscoring the importance of assessing and correcting vitamin D3 deficiencies in children with ASD.[26]

In this study, salivary samples were used to measure vitamin D3 levels. Saliva is increasingly recognized as a valuable tool in translational research and diagnostics, providing molecular biomarkers for both oral and systemic conditions.[27] The collection process was simple, involving participants spitting saliva into a container. This non-invasive method is particularly advantageous for children with ASD, who may have sensory sensitivities and behavioral challenges that make traditional blood sampling difficult.[28,29] Saliva sampling offers a cost-effective, painless, and less anxiety-inducing alternative to blood tests, while also providing abundant biological material for analysis, including DNA, proteins, hormones, and microRNA.[29] Despite these advantages, saliva sampling does have limitations, such as potential contamination from food or oral conditions, and some biomarkers may be present at lower concentrations compared to blood or urine. However, these limitations can be addressed with highly sensitive detection methods.[30-34]

Previous studies have also highlighted the risks associated with vitamin D deficiency during pregnancy, which can lead to various adverse outcomes. Maternal vitamin D3 levels during pregnancy significantly influence newborns' vitamin D3 levels, potentially affecting early brain development and increasing the risk of ASD.[35,36] This emphasizes the importance of monitoring vitamin D levels in expectant mothers to reduce the risk of neurodevelopmental disorders in their offspring.[32]

This study stands out due to its focus on salivary vitamin D3 levels in autism patients, particularly in the Indian population, where such research is limited. While the study contributes valuable insights, it has some limitations. Since only salivary vitamin D3 levels were assessed, the study lacks a comprehensive analysis that includes other potential biomarkers. Despite these limitations, this research is significant as it highlights the importance of monitoring vitamin D3 levels in children with autism. By identifying and addressing vitamin D3 deficiencies in this population, we could improve their overall health and potentially alleviate some autism symptoms, offering a better quality of life for individuals with ASD and their families. Future studies should expand the sample size to improve the generalizability of the findings and consider longitudinal research to explore how vitamin D3 levels fluctuate over time in children with autism. Incorporating other biomarkers and evaluating neurodevelopmental milestones in parallel with vitamin D3 levels could offer a more comprehensive understanding of the relationship between vitamin D3 deficiency and autism severity. Additionally, exploring the effects of vitamin D3 supplementation in different age groups and varying levels of autism severity would help determine its therapeutic potential. Finally, broader population-based studies, especially in diverse geographic regions, can further elucidate the role of environmental factors like sun exposure and dietary habits in contributing to vitamin D3 deficiency in individuals with ASD.

CONCLUSION

This study highlights the significant difference in salivary vitamin D3 levels between children with autism and their neurotypical peers, with autism patients exhibiting notably lower levels. The findings support previous research suggesting that vitamin D3 deficiency may play a role in the development and progression of autism spectrum disorders (ASD). By utilizing a non-invasive and practical method such as saliva sampling, this study offers a novel approach for monitoring vitamin D3 levels in children with ASD, especially those with sensory sensitivities. Despite the small sample size and limitations in assessing other biomarkers, the study underscores the potential value of vitamin D3 supplementation as an adjunct treatment for improving symptoms in children with autism.

DECLARATIONS

Author contributions

DD and SS wrote the manuscript. PR and GJ reviewed the manuscript.

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Data availability

All data and materials of the study are available and can be provided on request. The corresponding author can be contacted anytime to get the data of the study.

Conflict of interest

No conflict of interest is declared by all the authors.

Ethics approval and consent to participate

Ethical Committee Clearance Number: (IHEC/SDC/UG-2253/24/PHARM/097). “The study was approved by the institutional human ethical committee board of Saveetha Dental College and Hospitals. (IHEC/SDC/UG-2253/24/PHARM/097). The protocol of the study was approved by the Scientific Review Board (IHEC/SDC/UG-2253/24/PHARM/097) and it conforms to the provisions of the declaration of Helsinki. Informed consent was obtained from all the patients and their legal guardians by informing and clearly explaining the details of the study. All the methods in the study were performed in accordance with the relevant regulations and guidelines.

Consent to participate

Informed consent was obtained from all the patients and their legal guardians by informing and clearly explaining the details of the study.

Consent for publication

Informed consent was obtained from all the patients and their legal guardians by informing and clearly explaining the details of the study.

REFERENCES

1.Landa R (2007) Early communication development and intervention for children with autism. Ment Retard Dev Disabil Res Rev 13:16–25

2. Ritvo ER, Freeman BJ (1977) National Society for Autistic Children Definition of the Syndrome of Autism. J Pediatr Psychol 2:146–148

3. Bailey A, Phillips W, Rutter M (1996) Autism: towards an integration of clinical, genetic, neuropsychological, and neurobiological perspectives. J Child Psychol Psychiatry 37:89–126

4. Risch N, Spiker D, Lotspeich L, et al (1999) A genomic screen of autism: evidence for a multilocus etiology. Am J Hum Genet 65:493–507

5. Yan X, Li Y, Li Q, Li Q, Xu G, Lu J, Yang W (2024) Prevalence of Autism Spectrum Disorder Among Children and Adolescents in the United States from 2021 to 2022. J Autism Dev Disord. https://doi.org/10.1007/s10803-024-06390-7

6. Qiu N, Jiang Y, Sun Z, Du M (2023) The impact of disability-related deprivation on employment opportunity at the neighborhood level: does family socioeconomic status matter? Front Public Health 11:1232829

7. Lavigne É, Abdulaziz KE, Murphy MS, Stanescu

C, Dingwall-Harvey AL, Stieb DM, Walker MC, Wen SW, Shin HH (2024) Associations of neighborhood greenspace, and active living environments with autism spectrum disorders: A matched case-control study in Ontario, Canada. Environ Res 252:118828(2006) Vitamin D physiology. Prog Biophys Mol Biol 92:4–8

9. Sagar S, Raman P, Gheena S, Abilasha R, Krishnan RP, Selvaraj J (2022) Salivary vitamin D levels among OSCC and normal Indian patients. Bioinformation 18:884–887

10. Kimball S, Fuleihan GE-H, Vieth R (2008) Vitamin D: A Growing Perspective. Crit Rev Clin Lab Sci. https://doi.org/10.1080/10408360802165295

11. Kočovská E, Gaughran F, Krivoy A, Meier U-C (2017) Vitamin-D Deficiency As a Potential Environmental Risk Factor in Multiple Sclerosis, Schizophrenia, and Autism. Front Psychiatry 8:47

12. Bolognesi E, Guerini FR, Sotgiu S, Chiappedi M, Carta A, Mensi MM, Agliardi C, Zanzottera M, Clerici M (2022) GC1f Vitamin D Binding Protein Isoform as a Marker of Severity in Autism Spectrum Disorders. Nutrients. https://doi.org/10.3390/nu14235153

13. Uçar N, Grant WB, Peraita-Costa I, Morales Suárez-Varela M (2020) How 25(OH)D Levels during Pregnancy Affect Prevalence of Autism in Children: Systematic Review. Nutrients. https://doi.org/10.3390/nu12082311

14. Şengenç E, Kıykım E, Saltik S (2020) Vitamin D levels in children and adolescents with autism. J Int Med Res 48:300060520934638

15.Petruzzelli MG, Marzulli L, Margari F, De Giacomo A, Gabellone A, Giannico OV, Margari L (2020) Vitamin D Deficiency in Autism Spectrum Disorder: A Cross-Sectional Study. Dis Markers 2020:9292560

16. Wang T, Shan L, Du L, Feng J, Xu Z, Staal WG, Jia F (2016) Serum concentration of 25-hydroxyvitamin D in autism spectrum disorder: a systematic review and meta-analysis. Eur Child Adolesc Psychiatry 25:341–350

17. Guerini FR, Bolognesi E, Chiappedi M, et al (2020) Vitamin D Receptor Polymorphisms Associated with Autism Spectrum Disorder. Autism Res 13:680–690

18. Haghighat N, Sohrabi Z, Bagheri R, et al (2023) A Systematic Review and Meta-Analysis of Vitamin D Status of Patients with Severe Obesity in Various Regions Worldwide. Obes Facts 16:519–539

19. Rodgers MD, Mead MJ, McWhorter CA, Ebeling MD, Shary JR, Newton DA, Baatz JE, Gregoski MJ, Hollis BW, Wagner CL (2023) Vitamin D and Child Neurodevelopment-A Post Hoc Analysis. Nutrients. https://doi.org/10.3390/nu15194250

20. Feng J, Shan L, Du L, et al (2017) Clinical improvement following vitamin D3 supplementation in Autism Spectrum Disorder. Nutr Neurosci 20:284–290

21. Saad K, Abdel-Rahman AA, Elserogy YM, et al (2016) Vitamin D status in autism spectrum disorders and the efficacy of vitamin D supplementation in autistic children. Nutr Neurosci 19:346–351

22. Zhang Z, Liu J, Jiang G, Yu H (2022) Vitamin D receptor gene variants and serum vitamin D in childhood autism spectrum disorder. Mol Biol Rep 49:9481–9488

23. Ganta A, Pillai SS, Fredette ME, Topor LS (2022) Severe Vitamin D Deficiency in Youth with Autism Spectrum Disorder During the COVID-19 Pandemic. J Dev Behav Pediatr 43:461–464

24. Boyd NK, Nguyen J, Khoshnood MM, Jiang T, Nguyen L, Mendez L, Spinazzi NA, Manning MA, Rafii MS, Santoro JD (2023) Hypovitaminosis D in persons with Down syndrome and autism spectrum disorder. J Neurodev Disord 15:35

25. Wang J, Huang H, Liu C, et al (2022) Research Progress on the Role of Vitamin D

in Autism Spectrum Disorder. Front Behav Neurosci 16:859151

26. Jia F, Wang B, Shan L, Xu Z, Staal WG, Du L (2015) Core symptoms of autism improved after vitamin D supplementation. Pediatrics 135:e196–8

27. Henson BS, Wong DT (2010) Collection, storage, and processing of saliva samples for downstream molecular applications. Methods Mol Biol 666:21–30

28. Pasomsub E, Watcharananan SP, Boonyawat K, Janchompoo P, Wongtabtim G, Suksuwan W, Sungkanuparph S, Phuphuakrat A (2021) Saliva sample as a non-invasive specimen for the diagnosis of coronavirus disease 2019: a cross-sectional study. Clin Microbiol Infect 27:285.e1–285.e4

29. Galiana-Simal A, Muñoz-Martinez V, Calero-Bueno P, Vela-Romero M, Beato-Fernandez L (2018) Towards a future molecular diagnosis of autism: Recent advances in biomarkers research from saliva samples. Int J Dev Neurosci 67:1–5

30. DuBois D, Lymer E, Gibson BE, Desarkar P, Nalder E (2017) Assessing Sensory Processing Dysfunction in Adults and Adolescents with Autism Spectrum Disorder: A Scoping Review. Brain Sci. https://doi.org/10.3390/brainsci7080108

31. Sandra Sagar, Pratibha Ramani, Rajeshkumar.S Gheena.S. “Evaluation Of Antioxidant and Anti-Inflammatory Effects Of 1,25 Dihydroxycholecalciferol Formulation- An Invitro Study”, Journal of Population Therapeutics and Clinical Pharmacology, 30(16), pp. 247–253. doi: 10.47750/jptcp.2023.30.16.032.

32. Sagar S, Ramani P, Moses S, Gheena S, Selvaraj J. Correlation of salivary cytokine IL-17A and 1,25 dihydroxycholecalciferol in patients undergoing orthodontic treatment. Odontology. 2024 Jul;112(3):966-975. doi: 10.1007/s10266-023-00890-1. Epub 2024 Feb 6. PMID: 38319548.

33. Sandra Sagar, Pratibha Ramani, Monal Yuwanati, Sagar Moses, Karthikeyan Ramalingam. Role Of 1, 25 Dihydroxycholecalciferol On The Acceleration Of Orthodontic Tooth Movement - A Systematic Review. Int J Orthod Rehabil 2023; 14 (4) 19-32. Doi: 10.56501/intjorthodrehabil.v14i4.877.

34. Wong DT (2006) Salivary diagnostics powered by nanotechnologies, proteomics and genomics. J Am Dent Assoc 137:313–321

35. Principi N, Esposito S (2019) Vitamin D Deficiency During Pregnancy and Autism Spectrum Disorders Development. Front Psychiatry 10:987

36. Zhang H, Wang S, Tuo L, Zhai Q, Cui J, Chen D, Xu D (2022) Relationship between Maternal Vitamin D Levels and Adverse Outcomes. Nutrients. https://doi.org/10.3390/nu14204230