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ORIGINAL ARTICALE

ASSOCIATION BETWEEN SALIVARY VITAMIN D3 LEVELS AND DENTAL CARIES IN CHILDREN: A CASE-CONTROL STUDY

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ABSTRACT

Background: Dental caries remains a significant global oral health concern, particularly among children. Vitamin D3 plays a crucial role in tooth mineralization and immune function, and its deficiency has been associated with increased caries risk. Traditional assessment of Vitamin D3 involves serum measurements, but saliva has recently emerged as a promising non-invasive alternative, especially in pediatric populations.

Aim: This study aimed to assess salivary Vitamin D3 levels in children with dental caries compared to healthy individuals.

Methods:A total of 40 children aged 10–15 years were divided into two groups: 20 children with dental caries (ICDAS II code 5) and 20 healthy controls (ICDAS II code 0). Unstimulated saliva samples were collected between 8–9 a.m., centrifuged, and stored at 4°C. Levels of 1,25-dihydroxycholecalciferol were quantified using an enzyme-linked immunosorbent assay (ELISA). Statistical analysis was performed using a paired t-test.

Results:The mean salivary Vitamin D3 level in the caries group was 30.87 ng/dL, significantly lower than the 40.125 ng/dL observed in healthy controls ($p = 0.010$). This suggests a strong association between Vitamin D3 deficiency and dental caries occurrence.

Conclusion:Children with dental caries exhibited significantly reduced salivary Vitamin D3 levels. Salivary Vitamin D3 measurement offers a practical, non-invasive approach for early identification of caries risk, particularly in young populations. These findings support further research into the diagnostic utility of saliva-based Vitamin D assessment and its potential integration into preventive pediatric dental care.

Keywords: Dental caries, Vitamin D3, salivary biomarkers

1. INTRODUCTION

Dental caries remains one of the oldest known oral diseases, yet it continues to pose a significant threat to oral health across all age groups. It's remarkable, and somewhat troubling, that a disease so well studied still prevails so widely. Defined by the World Health Organization (WHO) as a localized, post-eruptive,

pathological process of external origin that leads to the softening and eventual destruction of the hard dental tissues, caries is far from a simple problem [1]. It is not just about cavities; it's a reflection of broader issues involving biology, behavior, and environment.

At its core, dental caries results from the interaction of acid-producing bacteria with fermentable carbohydrates in the mouth [2, 3]. These bacteria metabolize sugars and release acids that erode the tooth's surface, leading to demineralization and eventual cavity formation. That part is fairly well understood. However, what often escapes casual observation is how this biological process is embedded within larger socio-economic and cultural systems. The increasing prevalence of caries in many regions—despite decades of public health efforts—suggests that microbial activity is only part of the story. Factors such as genetic changes in bacteria, declining oral hygiene practices, and the widespread availability of refined, sugar-rich foods have likely contributed to this growing burden. Interestingly, populations of lower socio-economic status tend to exhibit higher caries rates, which could be due to limited access to dental care, poor nutritional options, or a lack of public health infrastructure [4].

It's worth asking: why do we still struggle to control something that, in theory, is preventable? Public health strategies like water fluoridation, oral hygiene campaigns, and the promotion of fluoride toothpaste have certainly helped reduce caries in some areas, but their success has been uneven [5]. The fact that caries remains one of the most common chronic diseases—especially in children—points to a gap between knowledge and practice. Prevention seems to require more than just information or tools; it demands systemic change.

This leads to an emerging area of interest in the field: the role of systemic health, and in particular, Vitamin D3, in the development and progression of dental caries. Vitamin D3, or cholecalciferol, is widely recognized for its role in regulating calcium and phosphorus metabolism—both essential for the mineralization of bones and teeth [6, 7]. Yet its significance extends beyond mineral balance. Recent insights suggest that Vitamin D3 also supports the immune system and influences the activity of enamel- and dentin-forming cells, including ameloblasts and odontoblasts. These cells, which are crucial during tooth development, express receptors for Vitamin D3, implicating the vitamin in the structural integrity of dental tissues as well as their defense mechanisms [8, 9].

It seems likely, then, that adequate levels of Vitamin D3 during early development could contribute not only to stronger teeth but also to greater resistance against the microbial and acidic challenges that trigger caries. A deficiency, by contrast, might leave teeth more vulnerable. Despite this plausible link, Vitamin D's role in caries prevention hasn't received as much attention in mainstream dentistry as fluoride has. This could be due to the traditional emphasis on local, surface-level interventions rather than systemic ones.

The standard way to measure Vitamin D status is through serum levels of 25-hydroxyvitamin D3[10]. While accurate, this method is invasive, requiring a blood draw, which is particularly problematic when working with children. Understandably, many parents—and children themselves—are hesitant about routine blood testing for something perceived as non-urgent. This hesitancy poses a practical challenge for researchers and clinicians trying to explore Vitamin D status in pediatric populations.

Interestingly, saliva has recently been proposed as a viable, non-invasive alternative for Vitamin D assessment. Though salivary diagnostics are still gaining traction in mainstream clinical settings, several studies have shown that salivary levels of Vitamin D metabolites correlate with serum levels, offering a gentler approach to data collection. Techniques like ELISA (enzyme-linked immunosorbent assay) have made it possible to measure these levels with reasonable sensitivity and specificity, potentially transforming how we screen for nutrient deficiencies in children.

Nonetheless, there are still questions to be addressed. How reliable is salivary Vitamin D measurement across diverse populations? Are the cut-off values for deficiency the same as those in serum? What confounding factors—such as hydration, circadian rhythms, or oral health status—might influence salivary readings? These are not trivial concerns. But the potential benefits of a child-friendly, needle-free test for a vitamin so critical to oral and general health make this area worth investigating further.

Given the concerning rates of dental caries in children and the emerging evidence of Vitamin D's involvement in tooth integrity and immune defense, this study aims to explore the association between salivary Vitamin D3 levels and dental caries. Using an ELISA-based quantification method, it seeks to evaluate whether saliva can serve as a reliable medium for screening Vitamin D status and predicting caries risk. While it may not offer immediate solutions, such research could pave the way for more holistic, child-friendly approaches to oral health—approaches that consider not just brushing and fluoride, but also the role of nutrition, immunity, and systemic wellness.

2. MATERIALS AND METHODS

A case control was conducted among n = 40 patients at a private dental hospital. The sample size calculation was done using G-power analysis by keeping the alpha error probability as 0.05, allocation ratio as 1 and the power of the study (1-beta error probability) as 0.99, the sample size was calculated as n = 40. The protocol of the study was approved by the Scientific Review Board and it conforms to the provisions of the declaration of Helsinki. This study included forty participants, with twenty healthy individuals showing no signs of caries and twenty diagnosed with dental caries. The subjects were between 10 and 15 years old, within the mixed dentition period. Inclusion criteria restricted participants to those without chronic medical conditions. Healthy participants met the ICDAS II code 0 criteria for dental caries, while those with dental caries were classified under ICDAS code 5 (Table 1). Exclusion criteria included malnourished subjects, individuals with systemic diseases, periodontitis, known chronic/ liver diseases, known bone disorders, and those taking Vitamin D3 supplements.

2 ml of unstimulated saliva sample was collected from each of the forty participants after taking a proper diet history from the patients between 8 a.m. and 9 a.m. Patients taking any form of Vitamin D supplementation or Vitamin D fortified foods were excluded from the study. Patients were instructed to have their routine South Indian diet the previous night and morning of sample collection. These collected samples were centrifuged at 5000 RPM for 10 minutes using Eppendorf vials, stored at 4 °C and then analysed for Vitamin D3 levels using an ELISA kit. The assay involved buffering the samples with a Vitamin D buffer, which resisted pH fluctuations and prevented non-specific binding. Afterward, the samples were treated with biotinylated Vitamin D analog specific for human VDR, followed by the addition of ELISA HRP (horseradish peroxidase) to produce color compounds indicating Vitamin D3 presence. The reaction was halted using a TMB (tetramethylbenzidine) solution when the OD values reached around 0.7 units, and the Vitamin D3 levels were quantified using an ELISA plate analyser. (Fig.1)

The collected primary results data were analysed with IBM SPSS Statistics for Windows, Version 23.0. Armonk, NY: IBM Corp. A paired t-test was done to compare the salivary Vitamin D3 levels between the two groups.

Table 1 Table showing the ICDAS II criteria for caries detection on the pit and fissures.

Code	Description
0	There should be no evidence of caries after prolonged air drying (5 seconds). Surfaces with developmental defects (enamel hypoplasia, fluorosis, attrition, abrasion and erosion) and intrinsic and extrinsic stains will be considered as sound.
1 – First visual change in enamel	When seen wet, there is no evidence of any change in color attributable to the carious activity, but after prolonged air drying a carious opacity or discoloration (White or brown lesion) is visible that is not consistent with the clinical appearance of sound enamel.
2 – Distinct visual change in enamel	The tooth must be viewed wet. When wet there is a (a) carious opacity (white spot lesion) and/or (b) brown carious discoloration which is wider than the natural fissure⁄fossa that is not consistent with the clinical appearance of sound enamel.
3 – Localized enamel breakdown due to caries with no visible dentin or underlying shadow	The tooth viewed wet may have a clear carious opacity (white spot lesion) and/or brown carious discoloration which is wider than the natural fissure ⁄fossa that is not consistent with the clinical appearance of sound enamel. Once dried for approximately 5 seconds there is carious loss of tooth structure at the entrance to, or within, the pit or fissure⁄fossa. If in doubt, or to confirm the visual assessment, the CPI probe was used gently across a tooth surface to confirm the presence of a cavity apparently confined to the enamel.
4 – Underlying dark shadow from dentin with or without localized enamel breakdown	This lesion appears as a shadow of discolored dentin visible through an apparently intact enamel surface which may or may not show signs of localized breakdown (loss of continuity of the surface that is not showing the dentin).
5 – Distinct cavity with visible dentin	Cavitation in opaque or discolored enamel exposing the dentin beneath.
6 – Extensive distinct cavity with visible dentin	Obvious loss of tooth structure, the cavity is both deep and wide and dentin is clearly visible on the walls and at the base. An extensive cavity involves at least half of a tooth surface or possibly reaching the pulp.

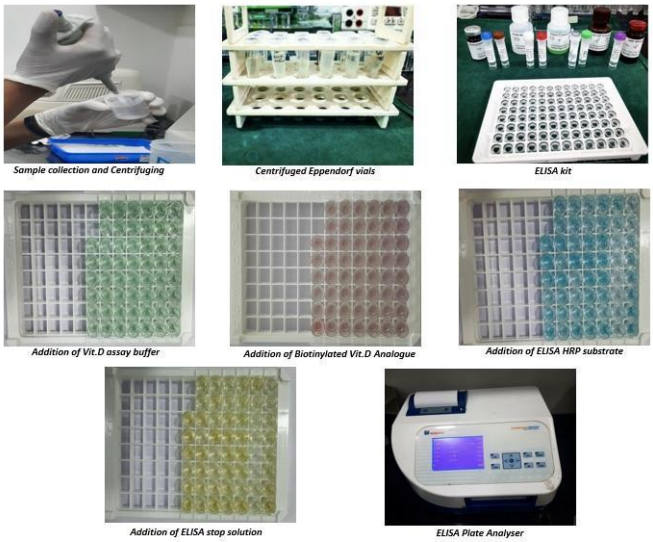

Figure1. Image showing the various steps involved in the evaluation of salivary samples for Vitamin D using ELISA.

RESULTS

Salivary Vitamin D3 levels in patients were analysed using delayed competitive ELISA. There was a mean salivary Vitamin D3 level of 40.125ng/dl in healthy individuals whereas the mean salivary vitamin D3 level of the patients with dental caries was found to be about 30.87 ng/dl. A paired t-test was done to compare the salivary vitamin D3 levels between the two groups. The results revealed a significant difference in salivary Vitamin D3 levels between healthy individuals (mean 40.125 ng/dl) and those with dental caries (mean 30.87 ng/dl), with a p-value of 0.010. (Fig.2) The salivary Vitamin D3 levels in children with dental caries were notably less than those of healthy subjects (Table 2).

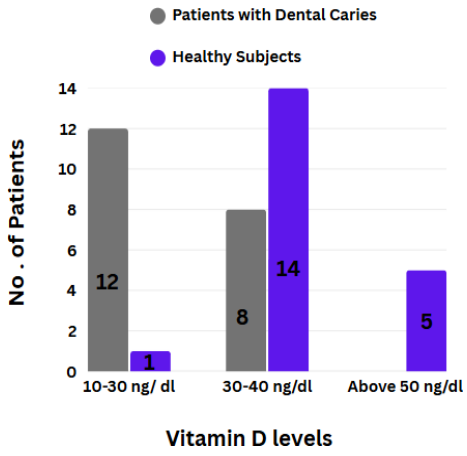

Fig.2 Graph showing Salivary Vit. D levels in patients with dental caries and in healthy subjects.

Table.2 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)**
Vitamin D levels in patients with dental caries	**30.85 ng/dl**	**0.01**
Vitamin D levels in healthy subjects	**40.125 ng/dl**	

DISCUSSION

Dental caries remains one of the most widespread chronic conditions globally, and it continues to affect individuals of all age groups despite decades of public health efforts. According to WHO estimates, its prevalence ranges from 49% to as high as 83% across various populations [11]. Such staggering numbers point not only to the biological tenacity of the disease but also to the broader socio-environmental conditions that allow it to thrive. In many ways, the modern lifestyle—with its emphasis on processed foods, sugary diets, and often inadequate oral hygiene—has created the perfect storm for dental caries to persist. Although fluoride programs and oral health awareness campaigns have brought measurable improvements in some areas, the disease disproportionately impacts children from disadvantaged socio-economic backgrounds[12, 13]. Early Childhood Caries (ECC), in particular, underscores the urgency of this issue. It does not merely affect teeth; it interferes with a child's speech, eating, sleep, and self-esteem, exerting long-term effects on quality of life.

Vitamin D, long recognized for its importance in bone health, has garnered attention in recent years for its potential role in oral health. The relationship is complex, spanning mineral metabolism, immune modulation, and gene expression. Notably, Vitamin D deficiency has been linked with defective enamel and dentin formation, making teeth more susceptible to demineralization and caries [14, 15]. While Vitamin D2 and D3 are both available in the diet, it is cholecalciferol (Vitamin D3)—primarily synthesized through skin exposure to sunlight—that serves as the biologically active form. This dependency on sun exposure is problematic in regions where cultural practices, climate, or urban lifestyles reduce sunlight exposure, particularly among children. In these contexts, Vitamin D deficiency may be both widespread and underdiagnosed.

The physiological mechanisms by which Vitamin D influences oral health are multifaceted. Severe deficiency,

defined as serum levels below 10 ng/mL, leads to disruptions in calcium and phosphate metabolism. This not only triggers secondary hyperparathyroidism but also alters the functionality of ameloblasts and odontoblasts—the key cells involved in enamel and dentin development [15]. Inadequate Vitamin D signaling in these cells results in hypomineralized tooth structures, which are more prone to decay. These cellular mechanisms are further mediated by the presence of Vitamin D receptors (VDRs), which regulate gene expression critical for dental tissue development [16]. Therefore, Vitamin D's role extends beyond nutrient status and into the realm of molecular regulation of tooth integrity.

Importantly, the effects of Vitamin D deficiency are not confined to the postnatal period. Emerging evidence supports the notion that maternal Vitamin D levels during pregnancy can significantly influence the dental health of the offspring. Several studies have found that inadequate maternal Vitamin D is linked to developmental dental defects in children, particularly when deficiency occurs during the first or second trimester—key periods of odontogenesis [17-19]. This prenatal link highlights how early and subtle disruptions in nutrient status can have structural consequences for primary teeth, often unnoticed until the child begins to show signs of ECC. As such, a life-course approach to Vitamin D sufficiency may be warranted when thinking about oral health promotion.
Beyond its structural roles, Vitamin D is also involved in immunomodulation. Numerous studies advocate for maintaining serum Vitamin D levels above 75 nmol/L to optimize immune responses [20-22]. This becomes particularly relevant in the context of oral health, where the mouth is constantly exposed to a complex and dynamic microbiome. Vitamin D has been shown to upregulate antimicrobial peptides such as cathelicidin and defensins, which are part of the innate immune system and play a protective role in the oral cavity. Therefore, children with adequate Vitamin D levels may be better equipped to mount immune defenses against cariogenic bacteria, adding another layer to its preventive potential.
Meta-analytical evidence further reinforces the association between Vitamin D and dental caries. A landmark review by Hujoel et al. reported that Vitamin D supplementation could reduce caries incidence by as much as 47% [23]. Schroth and colleagues echoed these findings, noting that children with sufficient serum Vitamin D levels consistently had fewer carious lesions [23]. These associations, while not always implying causation, make a compelling case for the protective role of Vitamin D in oral health, particularly among pediatric populations.
Our study builds upon this foundation by demonstrating a statistically significant relationship between lower salivary Vitamin D3 levels and the presence of dental caries in children. While prior investigations have largely relied on serum Vitamin D levels, our findings suggest that salivary assessments can offer comparable diagnostic value. This aligns with reports by Adegboye et al., who found higher caries rates among children with Vitamin D deficiency, and with studies by Hujoel and Schroth that confirmed this inverse relationship between Vitamin D status and caries prevalence [23- 28]. Notably, our study reinforces that these findings hold true even when Vitamin D3 is measured in saliva rather than serum.

The decision to utilize saliva as the diagnostic medium in this study is not merely a methodological choice but also a practical and ethical one. Blood draws in children often pose significant barriers—ranging from fear and discomfort to logistical difficulties in remote or under-resourced settings. Saliva, in contrast, is non-invasive, easy to collect, and does not require specialized equipment or personnel. This makes it an ideal medium for pediatric populations and for use in school-based or community health initiatives. Prior studies, including those by Sari et al., have validated saliva as a feasible and reliable medium for Vitamin D estimation [29-32].

That said, the adoption of salivary diagnostics in clinical practice remains limited. Part of the hesitation stems from a lack of standardized protocols. Although early assays, such as those by Fairney & Saphier and later by Tatsuya Higashi using LC-ESI-MS/MS, have demonstrated promising correlations between serum and salivary Vitamin D levels, broader consensus is lacking [33–38]. Our study contributes to this growing evidence base, affirming that salivary Vitamin D3 assessment—particularly using ELISA techniques—can be both sensitive and practical. As technology advances, it is plausible that saliva-based diagnostics could become standard tools in pediatric oral healthcare.

Nonetheless, no study is without limitations. Ours was conducted with a relatively small cohort, which constrains the generalizability of the findings. The cross-sectional nature of the study also limits causal inference; it is unclear whether low Vitamin D3 precedes caries development or results from it. Seasonal variation—affecting both sun exposure and Vitamin D synthesis—was not rigorously controlled, and this may have influenced salivary Vitamin D levels. Furthermore, while saliva collection offers practical benefits, factors such as time of day, hydration status, and oral hygiene at the time of sampling could impact the concentration of Vitamin D metabolites. These confounding variables warrant attention in future research.

Another consideration is the lack of comprehensive data on confounding lifestyle and genetic factors. For instance,

children's dietary intake, frequency of outdoor activities, skin pigmentation, and even VDR polymorphisms may all modulate the relationship between Vitamin D and caries risk. Future studies should aim to control for or stratify based on these variables to refine our understanding of causality. Longitudinal studies, in particular, could help unravel the directionality of this relationship by tracking children over time as their Vitamin D status and caries risk evolve.

In terms of public health implications, our findings raise important questions about how best to incorporate Vitamin D screening and supplementation into pediatric dental care. If salivary testing can be standardized and validated across diverse populations, it could be integrated into routine dental checkups, much like fluoride varnish or oral hygiene instruction. Such integration would allow early identification of at-risk children, potentially before caries develops. Moreover, targeted interventions—ranging from dietary guidance to supervised sun exposure or supplementation—could be deployed more efficiently.[39-40]

Interestingly, while Vitamin D is often discussed in terms of deficiency, optimal levels may vary between individuals. The conventional thresholds for sufficiency may not reflect ideal levels for dental health, especially when considering the complex interplay of immune response, microbial ecology, and tissue mineralization. Future research might investigate whether personalized Vitamin D targets—based on genetic, environmental, and dietary factors—could yield better preventive outcomes.

This study adds to a growing body of literature that highlights the critical role of Vitamin D3 in oral health, particularly in children. By demonstrating that salivary Vitamin D3 levels correlate with caries status, we offer support for non-invasive, scalable diagnostic strategies that could be transformative for pediatric oral healthcare. While further research is essential to validate these findings and overcome current limitations, the integration of salivary Vitamin D3 screening into preventive dental programs holds considerable promise.

CONCLUSION

Our study reveals a significant association between Vitamin D deficiency and dental caries in children. Given the non-invasive nature of saliva-based assessments, salivary Vitamin D3 levels could serve as a reliable marker for identifying children at risk of developing dental caries. Early detection and Vitamin D supplementation, particularly during prenatal and postnatal periods, may help prevent dental caries during the critical stages of dentition development. Future research should focus on further validating saliva as a diagnostic medium for Vitamin D and exploring its potential in broader public health applications.

DECLARATIONS

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Author contributions

SS and SM wrote the manuscript. GJ and PR 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 Dr. Genickson Jeyaraj 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/FACULTY/23/OPATH/109). “The study was approved by the institutional human ethical committee board of Saveetha Dental College and Hospitals. (IHEC/SDC/FACULTY/23/OPATH/109). The study protocol was approved by the Scientific Review Board (IHEC/SDC/FACULTY/23/OPATH/109) and 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.

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