Salivary Vitamin D3 and Statherin as Potential Biomarkers for Caries Risk in Children 6–12 Years of Age: A Cross-Sectional Study

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Abstract

Aim: Childhood dental caries affects over 621 million children globally and poses significant challenges in early diagnosis and prevention. Salivary biomarkers such as vitamin D3 and statherin are pivotal in enamel mineralization and caries prevention, yet their diagnostic potential in children with mixed dentition remains underexplored. This study aimed to evaluate the association of salivary vitamin D3 and statherin levels with dental caries in children aged 6–12 years and explore their potential as early biomarkers of caries risk. **Materials and Methods:** This cross-sectional study included 200 children (100 caries-free and 100 with caries) aged 6–12 years recruited from Sree Balaji Dental College and Hospital, Chennai. Caries assessment was conducted using decayed, missing, and filled teeth (DMFT) scores. Analysis of vitamin D3 and statherin in the unstimulated salivary samples was done using enzyme linked immunosorbent assay. Statistical analysis of the data was done using the chi-square test, independent sample *t*-test, and Pearson's correlation. A value of $P \leq 0.05$ was considered statistically significant. **Results:** Children with caries exhibited significantly lower mean salivary statherin levels (4.34 ± 1.36 ng/mL) compared to caries-free children (5.18 ± 1.85 ng/mL; $P = 0.016$; Cohen's $d = 0.51$). Similarly, mean salivary vitamin D3 levels were lower in the caries group (2.02 ± 0.90 ng/mL) than in the caries-free group (2.57 ± 1.33 ng/mL; $P = 0.005$; Cohen's $d = 0.48$). The frequency of tooth brushing varied significantly between groups ($P = 0.013$), while DMFT scores were significantly higher in the caries group ($P < 0.001$; Cohen's $d = 2.76$). A weak negative correlation was observed between DMFT scores and both salivary statherin ($r = -0.069$; $P = 0.5$) and vitamin D3 levels ($r = -0.198$; $P = 0.06$). **Conclusion:** Salivary levels of vitamin D3 and statherin were significantly lower in children with caries, highlighting their potential as noninvasive biomarkers for early caries detection. This study emphasizes the importance of preventive measures, including optimal oral hygiene and vitamin D3 supplementation, in reducing caries risk in children.

Keywords: Dental Caries, Mixed Dentition, Saliva, Statherin Protein, Vitamin D

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Introduction

Dental caries is the most common microbial infection and a major global health issue in children and adults across the globe.[1] It is generally associated with the demineralization of the hard tissue of the tooth, followed by tooth loss if left untreated.[2]

Early stages of dental caries are symptomless and progress to a painful condition, which has a social impact on school-aged children, affecting their attendance at school, low performance, and loss of teeth. Despite improvements in prevention and treatment, caries are prevalent worldwide, especially among children and those with limited dental care access.[3]

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Globally, 2.4 billion people suffer from caries in permanent teeth and 621 million children in primary teeth.[4] Existing studies on the prevalence of dental caries in children across the globe predominantly focus on caries of the primary dentition.[5-7] In India, about 50% of the general population is affected, with 64–78% of children impacted.

The occurrence of dental caries in mixed dentition is 58%.[8] Mixed dentition is vulnerable to caries, as the oral cavity is in continuous change due to the simultaneous exfoliation of deciduous teeth and the eruption of the permanent teeth. The children follow the same dietary habits, oral hygiene, and oral microbiome profile. Furthermore, the permanent teeth are not properly aligned with others, and the eruption makes the gums tender, making it difficult to clean the teeth,[9] thus increasing the risk of caries.

In the last decade, there has been a greater interest in salivary diagnostics and salivary biomarkers for dental caries. Saliva is a protein-rich, viscoelastic fluid. As the teeth are constantly in contact with saliva, the organic and inorganic constituents of saliva play a huge role in caries development and also prevention. This highlights the role of saliva as a potential diagnostic medium for early detection of caries lesion development.[10]

Proteomics analysis of saliva from children as well as adolescents has characterized hundreds of salivary proteins and metabolites associated with dental caries.[11-13]

Among those, proteins such as lactoperoxidase, mucin 1, carbonic anhydrase, alpha-amylase, salivary α-defensins, acidic proline-rich protein, cathelicidins, statherin, and proteinase-3 are reported as candidate biomarkers in the diagnosis of caries.[14]

Amidst the aforementioned salivary proteins, salivary statherin, in particular, plays a significant role in acid resistance caused by carious microbes by forming a protective acquired enamel pellicle layer, thus preventing demineralization and enhancing remineralization of the tooth.[15] Statherin is an antimicrobial peptide, that is, acidic in nature and made up of 43 amino acids. With the potential role of statherin in the mineralization of the enamel, studies on the role of salivary statherin in early caries detection are of much significance.

Besides salivary proteins, contemporary research also focuses keenly on nutritional deficiency, specifically vitamin D deficiency, which is identified as a risk factor for dental caries. Vitamin D plays a significant role in both the mineralization of bone and tooth and, its deficiency leads to rachitic tooth, that is, highly susceptible to decay and caries.[16] Optimal vitamin D levels are reported to be associated with lower odds ratios for caries in children.[17] While the association between serum vitamin D3 levels and dental caries in children is reported in existing literature, studies correlating the effect of salivary vitamin D3 and dental caries specifically in mixed dentition are not available.

In general, there is a paucity in the epidemiological data regarding caries prevalence in mixed dentition. A recently published systematic review on salivary markers in mixed dentition has reported on upregulation of specific salivary glycoproteins such as immunoglobulin A, antimicrobial peptide, and proteinase-3 in the caries group.[18] Also, there is not much clarity on the expression of these markers and their correlation with risk of dental caries in mixed dentition. Understanding the existing knowledge gap in the identification of salivary markers for dental caries specifically in mixed dentition, this study aimed to evaluate the salivary levels of statherin and vitamin D3 as a marker for dental caries in children, especially with mixed dentition. Our null hypothesis states that there is no relationship between dental caries and salivary levels of statherin and vitamin D3.

Materials and Methods

Ethical approval

This cross-sectional investigation was carried out at Shree Balaji Dental College and Hospital (SBDCH), Bharath Institute of Higher Education and Research, Chennai, Tamil Nadu, India. Approvals from the Institutional Review Board (IRB-PG/22-12/05) and the Institutional Ethical Committee (SBDCH/IEC/23-01/04) were obtained before the start of the study. The study protocol was carried out over a duration of between July 2023 and October 2023.

Study design and participants

A total of 200 children aged 6–12 years were recruited for this study. Children were recruited by single-stage sampling method after obtaining parental consent. The study participants were categorized into two groups: Group I comprised 100 healthy, caries-free children, while Group II included 100 children diagnosed with caries. The study population's parents/guardians provided written informed consent.

A structured self-administered questionnaire, filled by the parent/guardian, was used to collect information on sociodemographic factors such as age, gender, standard of education, annual income of the household, behavior, and tooth brushing habits of the participating children. The frequency of tooth brushing was self-reported. Tooth brushing frequency was evaluated based on the inquiry as to how often they brush their teeth; responses were recorded as: more than once a day, once a day, at least once a week but not daily, less than once a week, or never. The recorded responses were categorized as "tooth brushing at least twice a day" and "tooth brushing once a day or less."

Study criteria

Healthy, caries-free children aged 6–12 who were willing to participate in the study were categorized in Group I. Children with dental caries between the age of 6 and 12, who consented to participate were included in the caries group.

Exclusion criteria included unwillingness to participate, current vitamin D supplementation, chronic illness, and the presence of any systemic diseases.

Sample size calculation

The sample size was calculated using the formula $N = Z^2P(1 - P)/d^2$.[19] Where n is the sample size, Z is the statistic corresponding to level of confidence, P is expected prevalence, and d is precision. The value of P was considered as 65% according to a previous study.[20] With the power of 90% and alpha value of 0.05, the sample size obtained was 98 per group, rounded off to 100; therefore, 200 participants were recruited in total.

Clinical evaluation

The study participants were clinically screened by trained oral pathologists. Oral pathologists, who examined the study population, underwent a calibration process prior to data collection to ensure consistency in measurement with respect to decayed, missing, and filled teeth (DMFT) index. The inter-examiner reliability was tested using Cohen's Kappa value. The kappa score was above 0.85.

Clinical evaluations were conducted using standard dental equipment, including face shields, masks, gloves, and disposable tools. Dental caries was diagnosed according to criteria recommended by the World Health Organization (WHO).[21] Tooth-based DMFT scores of the study participants were evaluated by visual examination and recorded. The clinical behavior of the children was recorded using Frankl's behavior rating scale.[22]

Saliva collection

Unstimulated saliva (2 mL) was collected by passive drainage using sterile containers. The study participants were advised to abstain from consuming any food or beverages for a minimum of 2 h before the collection of the samples. In addition, they were asked to rinse their mouths before the collection of salivary samples. Samples were collected between 10 AM and 12 noon to avoid diurnal variation. The saliva samples were then stored under −70°C until further analysis.

Quantification of salivary vitamin D3 and statherin

Salivary vitamin D3 assay

Salivary vitamin D3 level was estimated using enzyme linked immunosorbent assay (ELISA) kit (BT Lab, China). The given standard vitamin D3 was centrifuged at 10,000 × *g* for 1 min, upon which serial dilution was performed to obtain standard solution with concentrations of 120, 60, 30, 15, and 7.5 ng/mL. In total, 50 µL of the standard solution was added to the standard well. In total, 40 µL of the salivary sample and 10 µL of anti-vitamin D3 antibody were added to 96-well micro-ELISA plate (dismountable). Then, 50 µL of streptavidin-HRP was added to sample wells and standard wells. After incubation at 37°C, 60 min, the unbound complex was removed from each well and washed with 300 µL of wash buffer.

Then, 50 µL of substrate A and substrate B were added to each well and incubated in the dark for 10 min at 37°C. To stop the reaction, 50 µL of stop solution was added to each well. The absorbance value of each well was measured at 450 nm.

Salivary statherin

Salivary statherin was estimated using an ELISA kit (BT Lab, China). The reagents were all of analytical grade. Experiments were performed in accordance with the instructions provided by the manufacturer. The given standard statherin was centrifuged at 10,000 × *g* for 1 min. Standard solutions with concentrations of 2400, 1200, 600, 300, 150, 75, and 37.5 ng/mL were obtained by serial dilution. In total, 50 µL of the standard solution was added to the standard well. In total, 40 µL of the salivary sample and 10 µL of anti-statherin antibody were added to 96-well micro-ELISA plate (dismountable). Then, 50 µL of streptavidin-HRP was added to sample wells and standard wells. After incubation at 37°C for 60 min, the liquid was removed from each well and washed with 300 µL wash buffer.

Next, 50 µL of substrate A and substrate B were added to each well and incubated in the dark for 10 min at 37°C. In total, 50 µL of stop solution was added to each well to stop the reaction, and the absorbance value of each well was measured at 450 nm.

Statistical analysis

Statistical analysis was performed using SPSS version 27.0 (IBM Corp: Armonk, NY, USA). In the case of categorical data, the percentage and number were reported. Numerical data were provided as mean ± SD. Categorical data were analyzed with the chi-square test, and continuous data were tested for normality using the Kolmogorov–Smirnov test. Parametric tests, specifically the independent sample *t*-test, were used to analyze continuous variables. Pearson's correlation coefficient was calculated to evaluate the association between salivary statherin and vitamin D3 with dental caries. Significance levels were set at $P \leq 0.05$ (significant), $P \leq 0.01$ (highly significant), and $P \leq 0.001$ (very highly significant).

Results

This study is a cross-sectional study comprising 200 participants. The total sample size of 200 in this study could be considered adequate since the power of the study calculated was >80% at a level of significance of 0.05.

The age distribution of the study population is represented in Table 1. Children aged between 6 and 12 years were chosen for this study. The mean age of the study group children was 8.73 ± 1.8 years, and the control group was 8.7 ± 1.8 years. The normal curve for age distribution is represented in Figure 1. The age distribution between caries-free and caries groups did not show any statistical significance. This is a significant feature of the study, as it did not add heterogeneity to the study population.

Among the study participants in the control group, 52 were male and 48 were female participants. In the caries group, 46 were male and 54 were female participants. The gender difference did not show any statistical significance ($P = 0.207$) in the study groups.

The behavior rating of children on the Frankl scale was found to be very positive in 178 children (89%) and positive in 18 children (18.8%). None of the study participants showed a negative behavior. The relationship was found to be statistically significant (0.077).

Table 2 shows the brushing habit of the study participants. In total, 12.5% of the participants in the control group have reported brushing their teeth twice a day, whereas it was found to be nil in the caries group, with a statistical significance of $P < 0.05$. This indicates a strong relationship between tooth brushing and prevalence of dental caries.

The DMFT scores of the study population are also mentioned in Table 2. The score values were found to be high in the caries group with a very high statistical significance ($P < 0.001$). With an effect size of $d = 2.76$, as determined by Cohen's d, a significant effect was seen.

The salivary levels of vitamin D3 and statherin in healthy controls and caries group are shown in Table 2. The mean salivary statherin levels were low in the caries group (4.34 ng/mL) when compared to healthy controls, and the values were highly statistically significant, indicating the protective role of statherin caries prevention. The effect size, $d = 0.51$, indicates a medium effect.

Table 1: Distribution of study population based on age between caries-free and caries group

Group	Age (years) (95% CI [6, 7 years and 10, 11 years])							
	6 (%)	7 (%)	8 (%)	9 (%)	10 (%)	11 (%)	12 (%)	*P* value
Caries-free	10.4	18.8	20.8	12.5	20.8	4.2	12.5	0.729[NS]
Caries group	14.6	14.6	18.8	18.8	12.5	10.4	10.4	

Values are represented as percentage (%)

[NS]*P* value statistically not significant

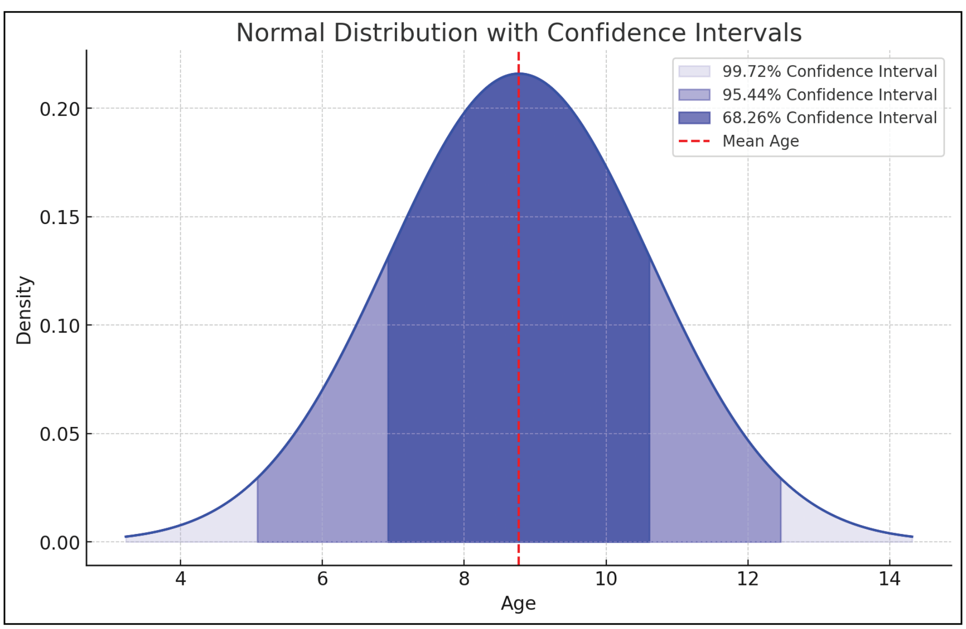

Figure 1: Normal curve with respect to the age of the study population

Table 2: Salivary vitamin D3, statherin levels, and decayed, missing, and filled teeth (DMFT) score between caries-free and caries group

		Caries-free	Caries group	*P* value	Cohen's *d* value
Vitamin D3 (ng/mL)		2.57 ± 1.33	2.02 ± 0.90	0.005**	0.48
Statherin (ng/mL)		5.18 ± 1.85	4.34 ± 1.36	0.016*	0.51
DMFT score		0.6 ± 0.3	3.4 ± 1.4	0.0001***	2.76
Tooth brushing	Once	87.5%	12.5%	0.013*	
	Twice	100%	0%		

Values are represented as mean ± SD and percentage (%)
***Represents $P < 0.001$,
**$P < 0.01$,
*$P < 0.05$

Table 3: Correlation analysis between decayed, missing, and filled teeth (DMFT) scores and salivary vitamin D3 and statherin

Correlation	Coefficient (*r*)	*P* value
DMFT and vitamin D3	−0.1978	0.06NS
DMFT and statherin	−0.069	0.5NS

NSThe *P* value is not significant statistically

Also, the mean salivary vitamin D3 (2.57 ng/mL) was found to be higher in healthy controls when compared to the caries group, showing a highly significant relationship statistically. Thus, indicating the protective role of vitamin D3 in caries prevention. The effect size, as measured by Cohen's *d*, was $d = 0.48$, showing a moderate effect. Pearson's correlation coefficient analysis [Table 3] showed a weak negative correlation between DMFT scores and salivary vitamin D3 levels ($r = -0.1978$; $P = 0.06$). Similarly, the relationship between statherin and DMFT scores also showed a non-significant weak negative correlation ($r = -0.1978$; $P = 0.006$).

Discussion

Dental caries, or tooth decay, is a widespread oral health issue. It results from the demineralization of tooth enamel by acids produced from the bacterial fermentation of sugars.[23] The WHO recommends the DMFT index to monitor the distribution and prevalence of caries.[24]

Early caries risk assessment is crucial because previous caries increases the risk of developing additional caries.[25] However, the DMFT score may not predict early carious lesions. Given the increased risk of caries and the limitations of early detection methods, the validation of salivary markers for dental caries in childhood could provide a novel approach to ascertain caries risk at an early stage. This would aid in preventing caries in childhood and adulthood. Despite its potential importance, the relationship between salivary proteins and dental caries remains underexplored. A better understanding of this relationship could enhance preventive strategies and improve oral health outcomes related to dental caries.

Considering the knowledge gap in the existing literature, this study aimed to explore the correlation between salivary levels of statherin and vitamin D3 and dental caries in children with mixed dentition. Dental caries often begins early in life, with age (peaking in children aged 6–12) and gender (more common in females) being crucial determinants.[26-28] In this age- and gender-matched study, these factors did not show any statistical significance. This strengthens our findings, as controlling for age and gender eliminated potential bias in the study design.

Socioeconomic status, diet, nutrition, and tooth brushing are also identified as modifiable risk factors for dental caries.[29] The link between socioeconomic status, including educational attainment, and oral health outcomes is well documented.[3] However, to avoid socioeconomic bias, participants in this study were selected from a homogenous group – middle-income households.

Brushing frequency has a direct relationship with the increase in caries and carious lesions.[30] This study's findings highlight a significant difference in brushing habits between the caries-free and caries groups, suggesting that brushing habits influence dental caries prevalence or severity. This finding underscores the importance of public health initiatives promoting regular and effective oral hygiene practices. This study primarily aimed to explore the potential of salivary vitamin D3 and statherin as early markers of dental caries in children. Vitamin D, known for its role in bone health and calcium regulation, also influences dental health. It does this by regulating immune responses, enamel remineralization, and maintaining a balanced oral microbiome, thereby reducing the risk of caries.[31] Lower vitamin D levels are identified as a potential risk factor for dental caries.[32,33] Vitamin D receptors are expressed in ameloblasts and odontoblasts, and low vitamin D levels can result in enamel hypomineralization.[34]

The relationship between caries and low vitamin D levels in children is widely reported globally.[34,35] Zhan *et al*.[36] reported an inverse relationship between serum vitamin D3 levels and tooth loss. A retrospective study in the US population also confirmed the association between low serum vitamin D3 levels and dental caries.[37] Similarly, Kim *et al*.[38] reported comparable findings in the permanent dentition of Korean children. Chhonkar and Arya[39] identified vitamin D as a risk factor for dental caries in Indian children aged 3–6 years. A study in Western Rajasthan, India, reported vitamin D deficiency in early childhood caries.[40] Furthermore, vitamin D3 deficiency is associated with caries risk in mixed dentition. Silva *et al*.[41] demonstrated low serum vitamin D3 levels in children with dental caries and mixed dentition. Pratyusha *et al*.[42] also reported similar findings in caries-affected children aged 3–11 years in the Indian population. While existing literature extensively examines serum vitamin D levels and caries risk in children, studies on salivary vitamin D3 and its correlation with caries are scarce.

In this study, we observed decreased salivary vitamin D3 levels in the caries group compared with caries-free subjects [Figure 2]. This finding aligns with previous research. Nireeksha *et al*.[43] reported that low salivary levels of vitamin D increase the severity of caries in adults. Padmanabhan *et al*.[44] also demonstrated an association between low salivary vitamin D3 levels and caries in children aged 4–12 years.

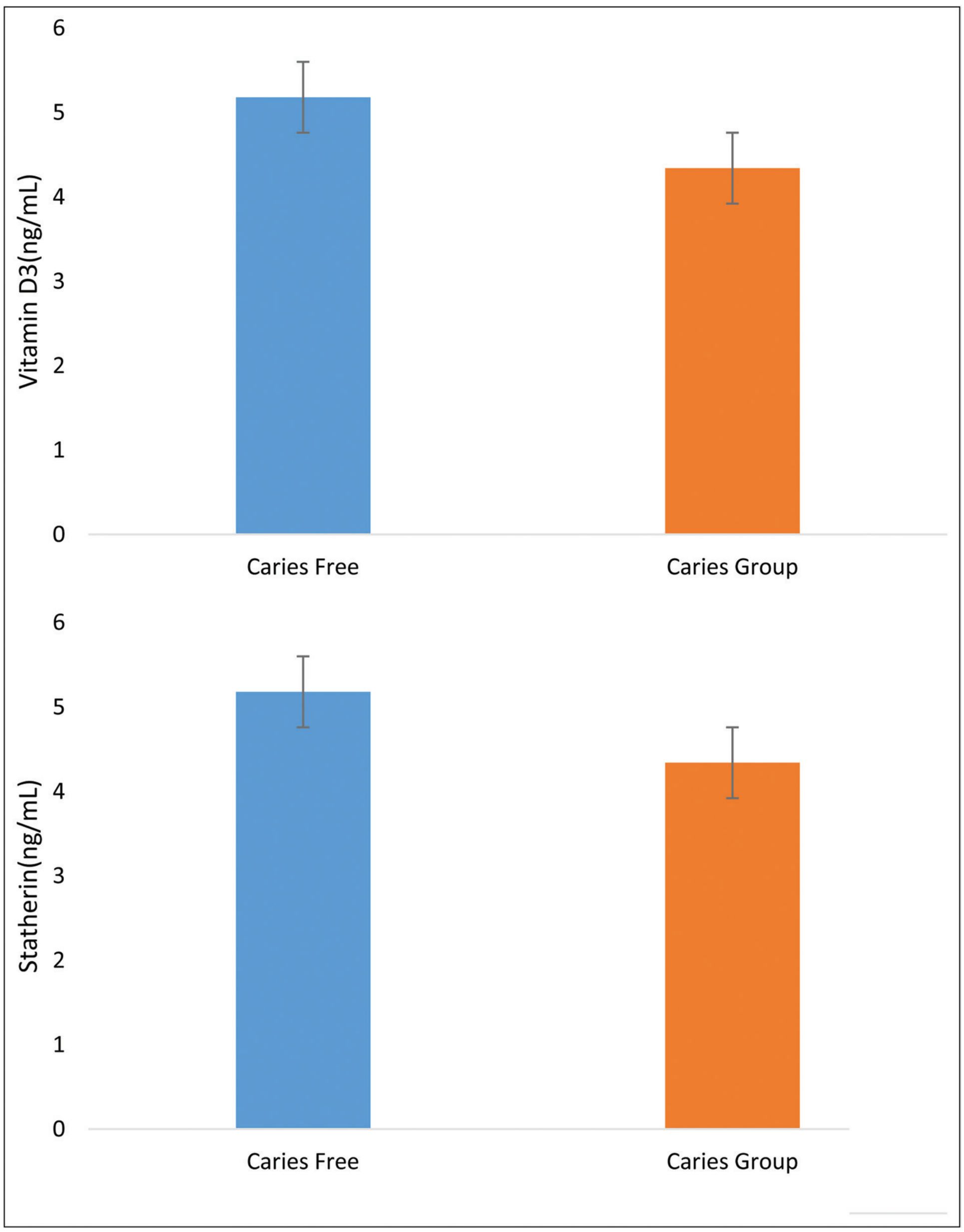

Figure 2: Mean salivary levels of vitamin D3 and statherin among the study population

Consistent with these findings, our study revealed a weak negative correlation between salivary vitamin D3 levels and DMFT scores, suggesting that salivary vitamin D3 plays a role in preventing dental caries. The inverse relationship between low vitamin D3 and caries risk in both primary and permanent dentition is supported by a recent systematic review and meta-analysis by Durá-Travé *et al*.[32]

The decreased vitamin D levels observed in this study could be attributed to the widespread prevalence of vitamin D deficiency and insufficiency in the general population. Notably, both vitamin D deficiency and severe early childhood caries are common in India, where the prevalence of vitamin D deficiency ranges from 50% to 90%.[45] The literature extensively discusses oral vitamin D supplementation and its preventive role in reducing the risk of dental caries.[46,47]

However, the precise molecular mechanism underlying the relationship between low vitamin D3 levels and caries remains unclear and requires further exploration. This study also investigated the relationship between salivary statherin and dental caries. Statherin, a salivary protein, stabilizes calcium phosphate in enamel and prevents its premature precipitation, which is essential for enamel protection.[48] Wang *et al*.[49] reported the anti-caries application of statherin-derived peptide. In this study, salivary statherin levels were significantly lower in the caries group compared with caries-free participants [Figure 2], supporting our hypothesis. The reduced statherin levels in the caries group might be due to decreased effective delivery of statherin in saliva to eroded tooth surfaces compared with non-eroded surfaces.[50]

Interestingly, our key findings are consistent with those of Pateel *et al*.[51] and Angarita-Díaz *et al*.[52] Both studies reported a decline in statherin levels in the caries group, although results were not statistically significant in the former study. Nussrat *et al*.[53] also reported decreased salivary statherin levels in children with caries. Furthermore, systematic reviews on salivary biomarkers in dental caries by Alamoudi *et al*.[54] and Ahmad *et al*.[14] have highlighted the significant relationship between statherin and dental caries.

In our study, we also found a weak, statistically non-significant, negative correlation between DMFT scores and statherin [Figure 3]. This finding does not strongly support a direct clinical implication of low salivary statherin and vitamin D3 levels with the DMFT score. This could be due to the small sample size.

To our knowledge, this research represents the first attempt to investigate the importance of both salivary vitamin D3 and statherin in children aged 6–12 years with mixed dentition and dental caries, using a noninvasive saliva sampling method.

While this study provides valuable insights into the association between salivary vitamin D3 and statherin with dental caries in children, it has some limitations. The cross-sectional design limits our ability to establish a causal relationship between biomarker levels and caries progression. We did not account for key factors that influence caries development, such as fluoride exposure, dietary habits, and sugar consumption. To avoid unnecessary radiation exposure, we also did not perform radiographic evaluation of caries, which may have resulted in an underestimation of caries prevalence.

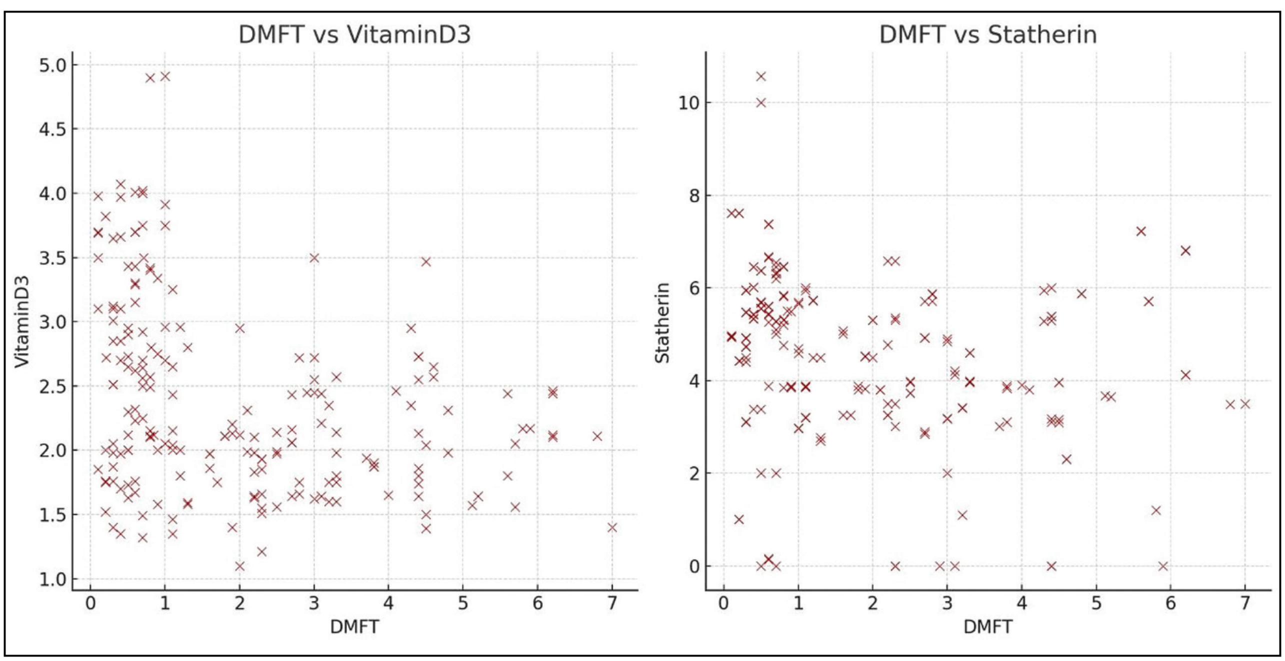

Figure 3: Scatter plot showing the correlation between decayed, missing, and filled teeth scores and vitamin D3 and statherin levels

Future studies should employ a longitudinal design to assess changes in salivary biomarker levels over time and their predictive value for caries risk. Incorporating detailed dietary data, fluoride usage information, and oral health-related behaviors could strengthen the findings. Expanding the sample size and including participants from diverse socioeconomic and geographic backgrounds would improve the generalizability of the results. Further molecular research is also needed to elucidate the mechanistic roles of salivary vitamin D3 and statherin in enamel mineralization and caries development.

Conclusion

This study establishes that salivary levels of vitamin D3 and statherin are significantly lower in children with dental caries compared to caries-free children. These findings underscore the potential of salivary biomarkers as noninvasive tools for the early detection of caries risk in children with mixed dentition. The study further emphasizes the importance of preventive measures such as regular tooth brushing and optimal vitamin D3 levels in reducing caries risk. Investigating these biomarkers on a larger scale and overtime could pave the way for personalized preventive strategies and targeted interventions designed to enhance oral health outcomes in children.

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Conflicts of interest

No conflict of interest declared by the authors.

Authors contribution

RM, AB: conceptualization, methodology, investigation, writing – original draft, KMKM: conceptualization, writing – review and editing. ND: writing reviews and editing Dr. JA – writing, data collection.

Ethical policy and Institutional Review Board statement

The study was conducted in accordance with the Declaration of Helsinki, and the protocol was approved by the Institutional Review Board of Sree Balaji Dental College and Hospital, BIHER(SBDCH/IEC/23-01/04). All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee.

Patient declaration of consent

The "patient declaration consent" has been properly signed by the parents of the research subjects.

Data availability statement

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

Authors contributions

Not applicable.

References

1. Chen J, Chen W, Lin L, Ma H, Huang F. The prevalence of dental caries and its associated factors among preschool children in Huizhou, China: A cross-sectional study. Front Oral Health 2024;5:1461959.
2. Machiulskiene V, Campus G, Carvalho J, Dige I, Ekstrand K, Jablonski-Momeni A, *et al*. Terminology of dental caries and dental caries management: Consensus report of a workshop organized by ORCA and Cariology Research Group of IADR. Caries Res 2019;54:1-8.
3. Kassebaum NJ, Smith AGC, Bernabé E, Fleming TD, Reynolds AE, Vos T, *et al*.; GBD 2015 Oral Health Collaborators. Global, regional, and national prevalence, incidence, and disability-adjusted life years for oral conditions for 195 countries, 1990–2015: A systematic analysis for the Global Burden of Diseases, Injuries, and Risk Factors. J Dent Res 2017;96:380-7.
4. Kassebaum NJ, Bernabé E, Dahiya M, Bhandari B, Murray CJL, Marcenes W. Global burden of untreated caries: A systematic review and metaregression. J Dent Res 2015;94:650-8.
5. Myint ZCK, Zaitsu T, Oshiro A, Ueno M, Soe KK, Kawaguchi Y. Risk indicators of dental caries and gingivitis among 10–11-year-old students in Yangon, Myanmar. Int Dent J 2020;70:167-75.
6. Youssefi MA, Afroughi S. Prevalence and associated factors of dental caries in primary schoolchildren: An Iranian setting. Int J Dent 2020;21:8731486.
7. Aalemi AK, Yaqubi B. Prevalence of dental carries among school-going children aged 7–13 years in Kabul city. BMC Oral Health 2024;24:1092.
8. Pandey P, Nandkeoliar T, Tikku AP, Singh D, Singh MK. Prevalence of dental caries in the Indian population: A systematic review and meta-analysis. J Int Soc Prev Community Dent 2021;11:256-65.
9. Lynch RJ. The primary and mixed dentition, post-eruptive enamel maturation and dental caries: A review. Int Dent J 2013;63:3-13.
10. Paqué PN, Herz C, Wiedemeier DB, Mitsakakis K, Attin T, Bao K, *et al*. Salivary biomarkers for dental caries detection and personalized monitoring. J Pers Med 2021;11:235.
11. Havsed K, Carda-Diéguez M, Isaksson H, Stensson M, Carlsson E, Jansson H, *et al*. Salivary proteins and metabolites as caries biomarkers in adolescents. Caries Res 2024;58:573-88.
12. Fidalgo TKS, Freitas-Fernandes LB, Almeida FCL, Valente AP, Souza IPR. Longitudinal evaluation of salivary profile from children with dental caries before and after treatment. Metabolomics 2015;11:583-93.
13. Kim S, Song Y, Kim S, Kim S, Na H, Lee S, *et al*. Identification of a biomarker panel for diagnosis of early childhood caries using salivary metabolic profile. Metabolites 2023;13:356.
14. Ahmad P, Hussain A, Carrasco-Labra A, Siqueira WL. Salivary proteins as dental caries biomarkers: A systematic review. Caries Res 2022;56:385-98.
15. Chawhuaveang DD, Yu OY, Yin IX, Lam WY, Mei ML, Chu CH. Acquired salivary pellicle and oral diseases: A literature review. J Dent Sci 2021;16:523-9.
16. Dortenzio L, Kahlon B, Peacock T, Salahuddin H, Brickley M. The rachitic tooth: Refining the use of interglobular dentine in diagnosing vitamin D deficiency. Int J Paleopathol 2018;22:101-8.
17. Wójcik D, Krzewska A, Szalewski L, Pietryka-Michałowska E, Szalewska M, Krzewski S, *et al*. Dental caries and vitamin D3 in children with growth hormone deficiency. Medicine (Baltimore) 2018;97:e9811.

18. Raja M, Nazzal H, Cyprian FS, Matoug-Elwerfelli M, Duggal M. Association of salivary proteins with dental caries in children with mixed dentition: A systematic review. Eur Arch Paediatr Dent 2025. https://doi.org/10.1007/s40368-024-00994-4.
19. Pourhoseingholi MA, Vahedi M, Rahimzadeh M. Sample size calculation in medical studies. Gastroenterol Hepatol Bed Bench 2013;6:14-7.
20. Sivanandam S, Balaji SS, Vikash P, Rajendran K, Jeevithan S. Prevalence of oral health conditions among children in the mixed dentition stage attending a tertiary care hospital in Western Tamil Nadu, India: A cross-sectional study. Eur J Cardiovasc Med 2023;13:1290-96.
21. World Health Organization. Oral Health Surveys: Basic Methods. Geneva, Switzerland: World Health Organization; 2013.
22. Frankl SN, Shiere FR, Fogels HR. Should the parent remain with the child in the dental operatory? J Dent Child 1962;29:150-63.
23. Touger-Decker R, van Loveren C. Sugars and dental caries. Am J Clin Nutr 2003;78:881S-92S.
24. Radić M, Benjak T, Vukres VD, Rotim Z, Zore IF. Presentation of DMFT/DMFT index in Croatia and Europe. Acta Stomatol Croat 2015;49:275-84.
25. Fan CC, Wang WH, Xu T, Zheng SG. Risk factors of early childhood caries (ECC) among children in Beijing – A prospective cohort study. BMC Oral Health 2019;19:34.
26. Wu C, Yuan Y, Liu H, Li S, Zhang B, Chen W, *et al*. Epidemiologic relationship between periodontitis and type 2 diabetes mellitus. BMC Oral Health 2020;20:204.
27. Shaffer JR, Leslie EJ, Feingold E, Govil M, McNeil DW, Crout RJ, *et al*. Caries experience differs between females and males across age groups in northern appalachia. Int J Dent 2015;2015:938213.
28. Karuveettil V, Krishna K, Ramanarayanan V. Is gender a risk factor for oral diseases in India? A metadata exploration. Public Health Toxicol 2022;2:1-14.
29. Boustedt K, Dahlgren J, Twetman S, Roswall J. Tooth brushing habits and prevalence of early childhood caries: A prospective cohort study. Eur Arch Paediatr Dent 2020;21:155-9.
30. Holmes R. Tooth brushing frequency and risk of new carious lesions. Evid Based Dent 2016;17:98-9.
31. Hung M, Mohajeri A, Sadri M, Khodabandeh E, Zeitoun I, Lipsky MS. The association of vitamin D levels and dental caries in older adults: A cross-sectional study. Nutrients 2024;16:2307.
32. Durá-Travé T, Gallinas-Victoriano F. Dental caries in children and vitamin D deficiency: A narrative review. Eur J Pediatr 2024;183:523-8.
33. Li Z, Wei X, Shao Z, Liu H, Bai S. Correlation between vitamin D levels in serum and the risk of dental caries in children: A systematic review and meta-analysis. BMC Oral Health 2023;23:768.
34. Botelho J, Machado V, Proença L, Delgado AS, Mendes JJ. Vitamin D deficiency and oral health: A comprehensive review. Nutrients 2020;12:1471.
35. Gyll J, Ridell K, Öhlund I, Åkeson PK, Johansson I, Holgerson PL. Vitamin D status and dental caries in healthy Swedish children. Nutr J 2018;17:11.
36. Zhan Y, Samietz S, Holtfreter B, Hannemann A, Meisel P, Nauck M, *et al*. Prospective study of serum 25-hydroxy vitamin D and tooth loss. J Dent Res 2014;93:639-44.
37. Hung M, Patel H, Lee S, Nguyen J, Mohajeri A. The influence of vitamin D levels on dental caries: A retrospective study of the United States population. Nutrients 2024;16:1572.
38. Kim IJ, Lee HS, Ju HJ, Na JY, Oh HW. A cross-sectional study on the association between vitamin D levels and caries in the permanent dentition of Korean children. BMC Oral Health 2018;18:43.
39. Chhonkar A, Arya V. Comparison of vitamin D level of children with severe early childhood caries and children with no caries. Int J Clin Pediatr Dent 2018;11:199-204.
40. Aijaz A, Chaudhary P, Malawat K, Almalki SA, Sharma T, Jameel AHA, *et al*. Clinical correlation of vitamin D deficiency and early childhood caries: A cross-sectional study in Western Rajasthan. J Pharm Bioallied Sci 2024;16:S672-4.
41. Silva CC, Gavinha S, Manso MC, Rodrigues R, Martins S, Guimarães JT, *et al*. Serum levels of vitamin D and dental caries in 7-year-old children in porto metropolitan area. Nutrients 2021;13:166.
42. Pratyusha N, Vinay C, Uloopi KS, RojaRamya KS, Ahalya P, Devi C. Association of serum vitamin D and salivary calcium and phosphorus levels in 3-11-year-old schoolchildren with dental caries. J Indian Soc Pedod Prev Dent 2021;39:240-5.
43. Nireeksha, Hegde MN, Kumari NS. Potential role of salivary vitamin D antimicrobial peptide LL-37 and interleukins in severity of dental caries: An exvivo study. BMC Oral Health 2024;24:79.
44. Padmanabhan V, Islam MS, Goud M, Rahman MM, Matar R, Aleskafi F, *et al*. Association of salivary vitamin D and vitamin C levels with dental caries in children: A cross-sectional study. Int J Clin Pediatr Dent 2024;17:773-6.
45. Harinarayan CV, Joshi SR. Vitamin D status in India – Its implications and remedial measures. J Assoc Physicians India 2009;57:40-8.
46. Al-Jubori SH, AL-Murad MA, Al-Mashhadane FA. Effect of oral vitamin D3 on dental caries: An in-vivo and in-vitro study. Cureus 2022;14:e25360.
47. Hujoel PP. Vitamin D and dental caries in controlled clinical trials: Systematic review and meta-analysis. Nutr Rev 2013;71:88-97.
48. Hannig M, Fiebiger M, Güntzer M, Döbert A, Zimehl R, Nekrashevych Y. Protective effect of the in situ formed short-term salivary pellicle. Arch Oral Biol 2004;49:903-10.
49. Wang K, Wang X, Li H, Zheng S, Ren Q, Wang Y, *et al*. A statherin-derived peptide promotes hydroxyapatite crystallization and in situ remineralization of artificial enamel caries. RSC Adv 2018;8:1647-55.
50. Mutahar M, O'Toole S, Carpenter G, Bartlett D, Andiappan M, Moazzez R. Reduced statherin in acquired enamel pellicle on eroded teeth compared to healthy teeth in the same subjects: An in-vivo study. PLoS One 2017;12:e0183660.
51. Pateel DGS, Gunjal S, Dutta S. Association of salivary statherin, calcium, and proline-rich proteins: A potential predictive marker of dental caries. Contemp Clin Dent 2022;13:84-9.
52. Angarita-Díaz MP, Simon-Soro A, Forero D, Balcázar F, Sarmiento L, Romero E, *et al*. Evaluation of possible biomarkers for caries risk in children 6 to 12 years of age. J Oral Microbiol 2021;13:1956219.
53. Nussrat RL, Aldhaher ZA. Evaluation of statherin and ß-Defensin-3 in saliva of children with dental caries: Case control study. J Emerg Med Trauma Acute Care 2024;2024:7.
54. Alamoudi A, Alamoudi R, Gazzaz Y, Alqahtani AM. Role of salivary biomarkers in diagnosis and detection of dental caries: A systematic review. Diagnostics (Basel, Switzerland) 2022;12: 3080.