

Evaluation of Serum Vitamin D and Calcium in Association with Caries Activity in Children

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Abstract

Background: Serum levels of Vitamin D and calcium are linked to various dental disorders, including dental caries. While Vitamin D deficiency is known to interfere with odontogenesis and mineralization, leading to defective enamel and dentine, the role of serum calcium in dental caries remains unclear.

Aim: This study aims to evaluate the association between serum Vitamin D and calcium levels with caries activity in children.

Materials and Methods: The study includes children aged 5-12, randomly divided into two groups based on

the DMFT/dmft index. Group I comprises patients with DMFT/dmft ≤ 5 (no/low to moderate caries activity), and Group II includes those with DMFT/dmft > 5 (high caries activity). Caries status is evaluated using a mouth mirror and dental explorer. Serum Vitamin D and calcium levels are measured from blood samples at an ISO 9001:2015 certified lab.

Results: The mean serum calcium levels for Group I and Group II are 9.86 mg/dl and 9.85 mg/dl, respectively, showing no significant difference ($p < 0.95$). However, serum Vitamin D levels differ significantly between the groups, with Group I having 24.18 ng/ml and Group II

15.21 ng/ml ($p = 0.00002$). This suggests that Vitamin D is a modifiable risk factor for dental caries. Other factors like oral hygiene, diet, socioeconomic status, and caregiver education should also be considered.

Conclusion: Serum Vitamin D is associated with caries activity in children, whereas the role of serum calcium is inconclusive.

Abbreviations: DMFT, decayed, missing, filled permanent teeth; dmft, decayed, missing, filled primary teeth; ISO, International Organisation for Standardisation; ALP, Alkaline Phosphatase.

Keywords: Serum Vitamin D, Serum calcium, Caries activity

Introduction

Dental caries is one of the most prevalent oral diseases in children. Multiple factors play a role in the development of dental decay, including microorganisms, diet, oral hygiene, medical conditions, and lack of important nutrients, such as vitamin D.¹ In the Indian population the prevalence of dental caries has been estimated to be as high as 52% among 3–18-year-olds and 58% in the mixed dentition.² If left untreated in young children, the disease may lead to complications like painful pulpitis, abscess, and space infection. Persistent pain reduces the quality of life by disrupting sleep, eating, speaking, and attendance in school. These factors finally take their toll on the child's general health resulting in insufficient physical development, especially in height and weight, not to mention the increased cost and time of dental rehabilitation.³

As early as 1928, Lady Mellanby along with Pattison published an article titled 'The action of Vitamin D in preventing the spread and promoting the arrest of caries in children'. They provided the first evidence that vitamin D deficiency is associated with dental caries in children and verified that oral/and/ or dietary

supplementation with vitamin D decreases the risk of caries.⁴ The role of vitamin D was explained in different studies as follows: 1. The active form of vitamin D1, 25 dihydroxy vitamin D plays a crucial role in the absorption of calcium and phosphorus from the intestine.⁵ 2. It has been observed in vitamin D-resistant rickets that there is defective mineralization of dentine. Maternal vitamin D deficiency during tooth development affects the tooth structure leading to enamel hypoplasia. These defective tooth structures are more susceptible to dental caries.⁵ 3. Vitamin D induces the formation of AMP (Anti-microbial peptide) such as cathelicidin and defensin. [Fig-1] Caries-free children had significantly higher levels of salivary cathelicidin compared to children with dental caries.⁶ 4. Furthermore, lack of this vitamin reduces salivary flow and Calcium ions in saliva thus diminishing the buffering effect of saliva, and reducing the remineralizing effect. Increases the risk of caries in primary and permanent dentition in children.⁶ Hence, optimum Vitamin D enhances innate immunity against dental caries.

Globally about 1 billion people are at risk of Vitamin D deficiency⁷. Vitamin D deficiency is defined as 25-hydroxy vitamin D levels less than 20ng/ml.⁸ There are many causes of vitamin D deficiency, including heritable disorders like obesity, dark skin, and acquired disorders like lack of sunlight, drugs, and malabsorption.⁹ Even though vitamin D deficiency and caries are common conditions worldwide, after an extensive literature search, few studies have been done in India and none in the state of West Bengal to determine the association between vitamin D deficiency and caries. The present study proposes to compare the levels of vitamin D in children with caries with a high caries indices score and low indices or caries-free children and to determine the

association between vitamin D deficiency and dental caries.

Material and Methods

Study design and Selection of sample population

This study was conducted on thirty (30) healthy children with caries and was approved by the institutional Ethic Committee of Dr. R. Ahmed Dental College and Hospital (registration no.-EC/NEW/INST/2023/3191 memo no.-RADCH/EC/02/2023 dated 14.12.23). The children between the age group of 5-12 years were selected randomly from the OPD. The written consent of the parent or patient's caregiver was obtained after explaining in detail the purpose and procedure of the study. After obtaining informed consent, complete data of the child's name, gender, age, oral hygiene practices, dietary habits, and socio-economic status was recorded using a structured questionnaire, and a detailed medical and dental history was taken.

The subject inclusion and exclusion criteria are presented in Table-1.

Clinical dental examination procedure

Clinical examination was done using artificial lighting by registered dentists. The isolation of teeth was done using cotton rolls for better visualisation. A mouth mirror, blunt dental probe were used for inspection and exploring the stained pit and fissure under appropriate lighting conditions. The DMFT/dmft indices were assessed according to WHO recommendations to calculate DMFT/dmft Indices. All examined children {n=30} were divided into two groups as: GROUP I: children with DMFT/dmft equal to or less than 5 (no /low to moderate caries activity) GROUP II: children with DMFT/dmft more than 5 (high caries activity).

The categories of Caries experience by the WHO - DMFT/dmft calculation criteria are: Table-2.

Blood sample collection and Estimation of serum Vitamin-D and calcium level and biochemical analysis

The blood samples were collected by some laboratory personnel from the clinical biochemistry laboratory of Probe Diagnostic Limited of Dr R Ahmed Dental College and Hospital ISO 9001:2015 Certified lab. 2ml blood samples were collected from each child with a sterile disposable plastic syringe without any anticoagulant and analyzed in a laboratory for measuring serum Vitamin D and calcium levels.

The determination of 25-hydroxyvitamin D in serum was determined using a chemiluminescent immune assay of the newer generation of Beckman Coulter immunoassay system that employs a chemiluminescent substrate named Lumi-Phos PRO, which is composed of buffered surfactant enhancer system and supports ALP-sensitive acridan. When acridan is triggered, it transforms into dioxetane, which decomposes and emits light.

The serum calcium was determined by using a colorimetric method using Arsenazo III dye which combines with calcium to form a colored complex that has an absorbance range of 640-660 nm.

Statistical analysis

The data was compiled statistically using statistical SPSS version 17.0 (Statistical software for Social Science, IBM Corporation, USA) and MS Excel. The mean, mode, and standard deviation are calculated using MS Excel. t-test and one-way ANOVA was employed to compare different variables.

Results

The statistical analysis of sociodemographic background exhibited no significant differences between the two studied groups regarding gender, parent's level of education, and income level. Gender, caregiver's level of education, and household income are not statistically significant in association with dental caries (Table-3). It

is illustrated from Table-4 that children with high DMFT/dmft scores have more dental visits than those with low to moderate caries activity. This may be attributed to their consultation with the dental professional to address their poor oral health. On the contrary, there is no association between the frequency of brushing and the use of medicated toothpaste. The dietary habit of children in Group I (low to moderate caries experience) and Group II (high caries experience) strongly indicates the association of dental caries with frequent in-between meal snacking with sticky refined carbohydrates. Interestingly, the mean vitamin D level is significantly low in Group II children with high DMFT/dmft index.

Discussion

According to Fejerskov and Kidd dental caries is a chronic multifactorial disease (Fejerskov and Kidd, 2008).¹⁰ The main factors are the tooth surface, salivary factors, the dietary substrate, and aciduric bacteria.¹¹ Besides several variables could significantly influence caries experience. They are social, cultural, and socioeconomic factors such as the pattern of feeding or frequency of sugary and sticky snack consumption, oral hygiene practices with fluoridated toothpaste and frequency of brushing, and parent's education level, including their socioeconomic status.¹² The present study aimed to find out the association between the above-mentioned factors and dental caries and also to determine whether serum Vitamin D level is associated with caries activity.

A study was conducted among the 5–15-year-old children of Ghaziabad to investigate the prevalence of dental caries and its associated risk factors. They detected a statistically significant increased caries prevalence in females (58.2%) when compared with males (51%).¹³ However, we did not obtain any statistically significant

difference between the children's gender, the caregiver's level of education, occupation, or even the income level of the caregiver and dental caries assessed as Group 1 with low DMFT/dmft index and Group 2 with high DMFT/dmft index. There are several studies conducted by Dhar et al.,¹⁴ Dash et al.,¹⁵ Moses et al.,¹⁶ who mentioned a higher caries prevalence rate in males, which have similar observations that of our study. Studies performed in other parts of India by Bhatia et al.,¹⁷ Dutta and Dutta,¹⁸ Patloth et al.,¹⁹ Moses et al.,¹⁶, concluded that low socioeconomic status significantly correlated with dental caries.

In a study designed by Hala Nadhim Khadim²⁰ to establish any existing relationship between Vitamin D level and dental caries among Iraqi children no significant relationship could be noted between caries incidence and age, socioeconomic status like family income or residency had no bearing on the occurrence of dental caries. However, she had observed a low level of Vitamin D in children is associated with a high prevalence of caries, like the results produced by this study. Research on Socio-behavioural risk factors of dental caries among adolescents in Ibadan, Nigeria proved that in developing countries subjects of higher SES are more likely to suffer from dental caries.²¹

The current study utilizes a 24-hour diet recall chart to record the form of sugar intake (solid form or liquid form), and frequency of sugar consumption (at meals and between meals). In this study, a strong association was found between the caries prevalence and frequency of sugar exposure in -between- meals for both Gr-I and Gr-II. The children who consumed more sugar between meals were more likely to have dental caries. The results coincided with the research done by Gustafsson et al. (Vipeholm study).²² The association of dental caries with excessive sugar intake has also been stated by an expert

panel of the WHO.²³ The panel outlined an increased risk of caries related to frequent and total intake of free, simple sugars. Various research studies imply the frequency of eating sugars to be the main etiological factor for caries rather than the total consumption of sugars.

The present study did not find any significant difference in tooth brushing frequency between the two studied groups. This fact concurred with the systemic review on toothbrushing frequency and dental caries by Kumar et al.,¹³ who found no difference between caries increment estimates of twice per day and less than twice per day among toothbrush yielders for oral hygiene practices. However, in contrast, Brusius et al.,²⁴ concluded from a population-based cohort study conducted among adolescent Brazilians that they benefitted from a thrice daily brushing practice to prevent increment of caries score.

This study showed that children with high caries index were taken to the dental personnel only after they experienced lingering pain or gross destruction due to advanced caries and hence a greater frequency of dental visits was seen in these children belonging to Gr-II in this study. This suggests an increased need for dental health awareness among the public and the propagation of the necessity of a semiannual dental visit so that dentists can play a role in the prevention of dental caries.

Several epidemiological studies have claimed that there might be a possible link between vitamin D levels in the general population and the development of dental caries. Schroth et al pointed out that caries was associated with 25(OH)D levels less than 75nmol/l and < 50nmol/l, lower household education, not brushing twice daily, and a yearly visit to the dentist.²⁵ Kim et al. suggested that the findings from their study on Korean children reveal that Vitamin D insufficiency might be a risk factor for the

increased incidence of dental caries.²⁶ In a study on Iraqi Kurdish children by Mahmood et al., it was proved that having a serum Vitamin level greater than or equal to 15ng/ml is significantly associated with lower caries index in the studied groups.²⁷ In a prospective study by Johanna Gill et al., where caries status was evaluated after 2 years of administering Vitamin D supplements, whereas some were put on a placebo, for 3 months, it was seen that Vitamin D was associated with caries.²⁸ Kühnisch et al. observed a correlation between an increase in serum 25(OH)D levels and a decrease in dental caries incidence.²⁹ Hujoel PP. also claimed that vitamin D exposures in childhood may play a crucial role in caries prevention and suggested that vitamin D supplementation could reduce the risk of dental caries by 47%.³⁰

The results obtained in the present study are in accordance with those derived from studies conducted among different populations of the world. This study being conducted in a tertiary care hospital may not be representative of the entire population. Considering, that dental caries has become a menacing burden on public health, there is a need for similar studies in the state of West Bengal and other states of India with much larger sample sizes and controlled trials or prospective cohort studies.

Conclusion

In conclusion, despite the limited sample size, dental caries risk was considerably increased by low serum vitamin D levels. The risk of dental caries decreased linearly with increased vitamin D concentration. The study population is relatively small, which is the main drawback of this study due to several patient constraints.

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Legend Tables and Figures

Table 1: Inclusion and exclusion criteria

Inclusion criteria	Exclusion criteria
1. Patients with dental caries	1. Children who are medically compromised.
2. Patients whose parents consented to participate in the study.	2. Special children
3. Children who readily co-operated with the clinical examination process.	3. Syndromic children
4. Children who were between 5-12 years of age.	4. Children with metabolic disease
5. Children who were healthy with no systemic disease or disability.	5. Patients under long term medication, radiotherapy, or chemotherapy.

Table 2: Categories of Caries experience by the WHO - DMFT/dmft calculation criteria

Categories	DMFT/deft Score
Very low	0.0-1.1
Low	1.2-2.6
Moderate	2.7-4.4

High	4.5-6.5
Very high	Above 6.6

Table 3: Sociodemographic background of children in Group I and Group II

Variable		GROUP I	Group II	p-value
Gender	Male n(%)	12	9	0.25
	Female n(%)	3	6	
caregivers' level of education	Illiterate	1	5	0.5
	Primary education	5	7	
	Secondary education	7	3	
	Higher education	2	0	
Occupation of the caregiver	unemployed	0	1	0.5
	unskilled	6	8	
	Semi-skilled	7	5	
	Skilled	2	1	
Income level	Low income	3	8	0.5
	Medium income	10	7	
	High income	2	0	

Table 4: Oral health practices in children with low to moderate DMFT/dmft Index and High DMFT/dmft index

Variable		Group I	Group II	P- value
Dental visit every 6 months	yes	5	9	0.005*
	no	10	6	
Frequency of brushing	Once daily	10	13	0.67
	Twice daily	5	2	
Use of fluoridated toothpaste	yes	3	5	0.36
	no	12	10	

Table 5: Dietary habit of children in Group I (low to moderate caries experience) and Group II (high caries experience)

Variables		Group I	Group II	p-value
In-between the meals sticky refined sugary snack-intake	1-2 times	11	9	0.003*
	More than 2 times	4	6	

Table 6: Comparison of serum 25(OH)D, Calcium levels of children with high level of caries and low to moderate level of caries

Variable	Group I (mean)	Group II (mean)	P-value
25(OH)D(ng/ml)	24.18	15.21	0.00002*
Total Ca ²⁺ (mg/dl)	9.86	9.76	0.95

Statistically significant at P<0.05

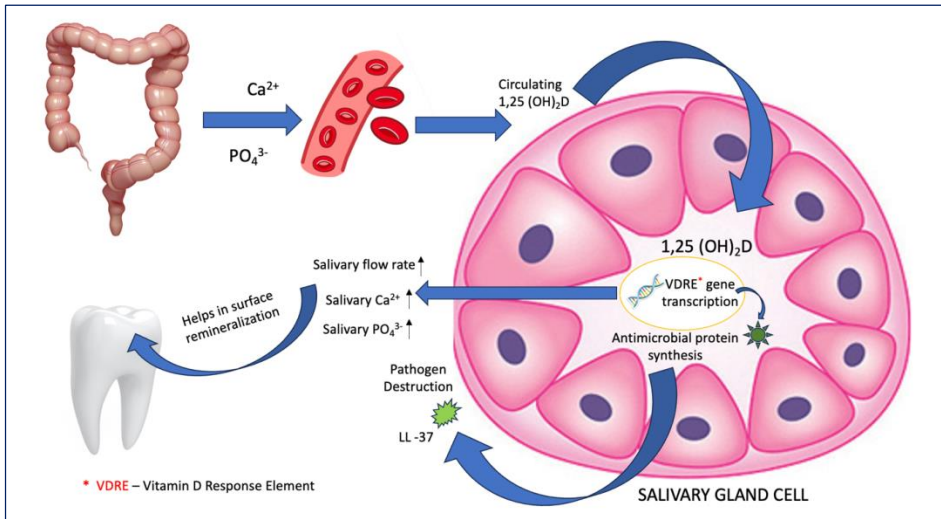


Figure 1: Possible biological mechanism of Vitamin D in caries prevention

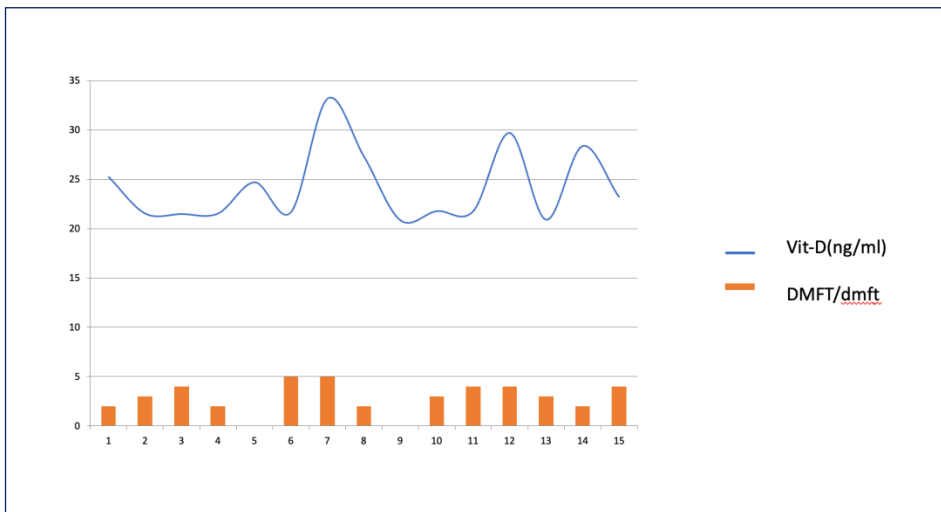


Figure 2: Vitamin-D levels and DMFT/dmft score in group-I

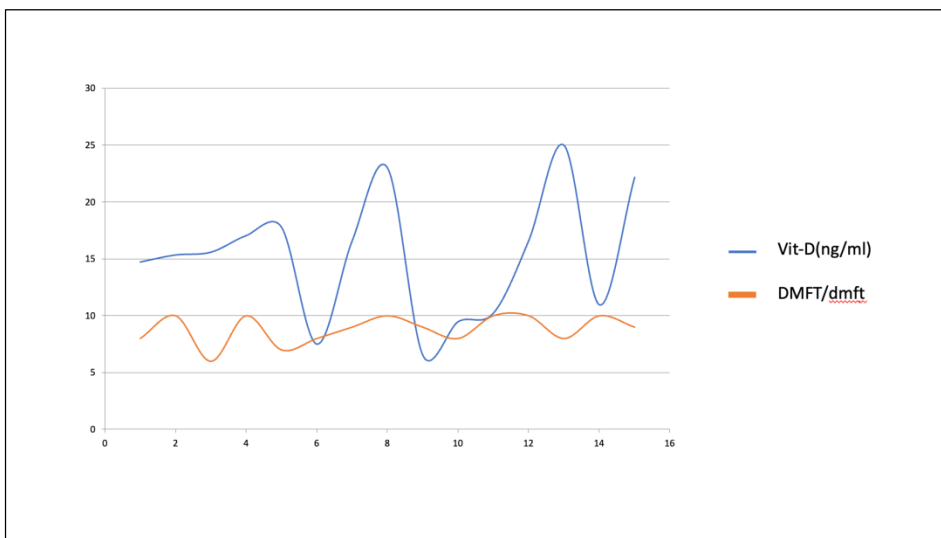


Figure 3: Vitamin-D levels and DMFT/dmft score in group-II

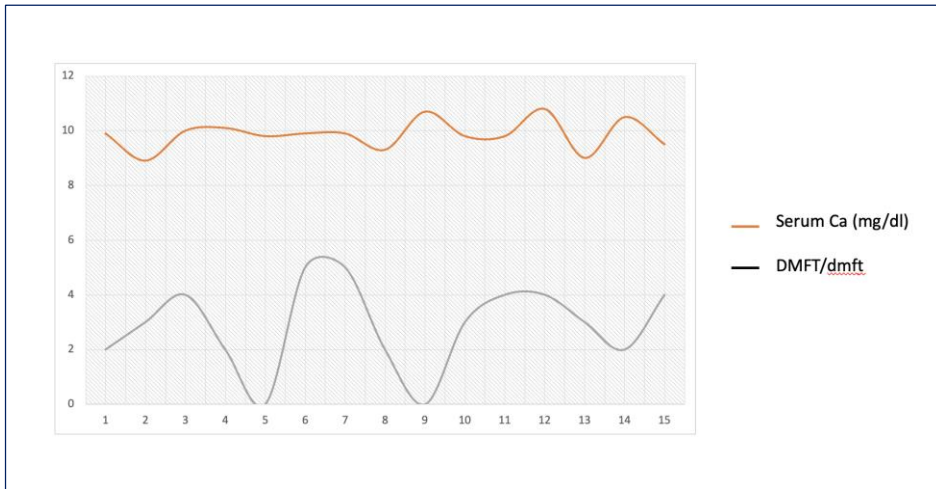


Figure 4: Serum calcium level and DMFT/dmft scores in group-I

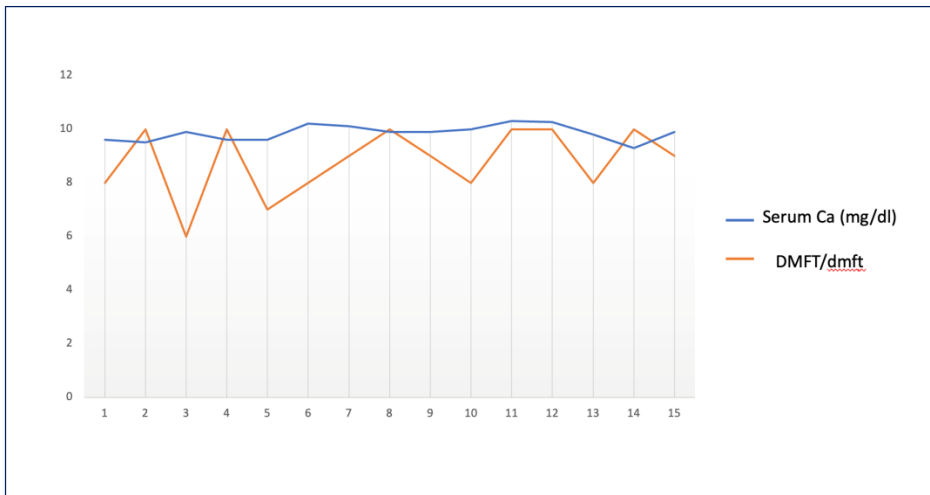


Figure 5: Serum calcium and DMFT/dmft scores in group-II