



Original Article

Effect of Vitamin D Deficiency on Dental Caries and Salivary Parameters

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ABSTRACT

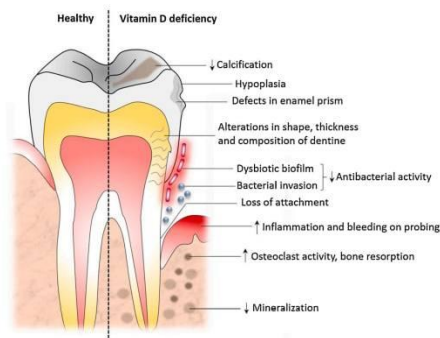
Background: Vitamin D deficiency is widespread and a major global health problem. Dental caries is associated with vitamin D deficiency and its pathophysiologic processes. This research aimed to investigate the effect of vitamin D deficiency on dental caries experience concerning selected physicochemical characteristics (pH, flow rate, calcium ion, and phosphorus ion).

Materials and Methods: A case-control study was carried out. The sample comprised 80 females; the study group involved 40 females with a serum vitamin D concentration of less than 10 ng/ml. In addition to the control group involving 40 females who matched the case in number and age but serum vitamin D concentration 30 ng/mol or more, their age range was 20-30 years old.

Results: Data from the current study showed that the mean values of the Decayed, Missing, and Filled Surface index and its components (Decayed Surface, Missing Surface) were higher in the study group than those in the control one, highly significant for Decayed Surface. The salivary pH and flow rate were less in the study group than those in the control with statistically significant differences. Both salivary calcium and phosphorus were lower in the study group than in the control with significant differences for phosphorus ions and highly significant for calcium ions.

Conclusion: The results of the current study revealed that vitamin D deficiency has a significant effect on dental caries experience in addition to changes in salivary level to selected physicochemical characteristics (pH, flow rate, calcium ion, and phosphorus ion). There is a need for adequate awareness regarding oral hygiene; specifically, the effective preventive measures could help reduce the effect of vitamin D deficiency on oral health.

GRAPHICAL ABSTRACT



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Introduction

Vitamin D is a fat-soluble secosteroid with a structure very similar to cholesterol. It is essential in maintaining bone mass and mineral metabolism [1]. The vitamin D receptor is a receptor molecule that binds to the active form of vitamin D [2] and controls different processes, such as differentiation, inflammation, calcium, and phosphate absorption [3]. Both good overall health and oral health require a healthy and well-balanced diet. Phosphorus, magnesium, and calcium absorption from the digestive tract requires vitamin D for healthy tooth development and bone mineralization [4]. Various possible mechanisms have been put up to clarify the function of vitamin D in reducing caries risk. One of these mechanisms involves the control of parathyroid hormone, serum phosphate, and calcium, which are essential for tooth protection, mineralization, and calcification. Homeostasis of phosphate and calcium is required for the creation, mineralization, calcification, and preservation of oral teeth, bone, and hard tissues. Hypoplasia of the enamel and dentin has been linked to hypocalcemia and hypophosphatemia [5]. Vitamin D sufficiency may support saliva flow rates leading to a more effective anti-caries action from the saliva. Vitamin D deficiency has been associated with the diminished parotid gland function and decreased saliva production [6]. In rats deficient in vitamin D, therapy with vitamin D3 increased salivary flow rates [7]. He *et al.* showed that salivary flow rates considerably increased with time in the vitamin D3 group following supplementation, and it was further noted that vitamin D receptors were found in the parotid, submandibular, and sublingual salivary glands, suggesting a potential involvement for vitamin D in the control of salivary secretion [8]. Salivary buffer energy and pH affect saliva's capacity to neutralize acids produced by oral microorganisms [9]. A significant feature of salivary buffering systems is the control of oral pH, including a protein-based buffer, urea, bicarbonate, and phosphate [10]. Thus, no previous Iraqi studies are available concerning the effects of vitamin D deficiency on the oral health

status and selected salivary constituents, and because of the rising worldwide prevalence of vitamin D deficiency and its detrimental consequences on oral health, for all of the above, this study was designed to detect the consequences of vitamin D deficiency on oral health status and physicochemical properties of saliva.

Materials and Methods

The sample used in the study consisted of two groups: study group including 40 females with vitamin D deficiency, serum vitamin D concentration less than 10 ng/mL. In addition to the control group involving 40 females matching the case in number and age, but without vitamin D deficiency, serum vitamin D concentration 30 ng/mol or more, their age range was 20-30 years old. The age was measured according to the last birthday [11]. Before participation, consent was obtained from each subject in both the study and control groups. The College of the Dentistry/University of Baghdad, Iraq, Research Ethics Committee approved the study ref. no:484; date:19-1-2021. The sample collection period from January 27, 2021, to May 20, 2021. The duration of the study was five months. Vitamin D concentration was measured at AL-Amal specialized laboratory in AL-Najaf city using Cobas e 411 analyzer and special kit (Elecsys Vitamin D total, Germany).

For both study and control groups, the criteria for exclusion consisted of the subsequent ones:

- Patients with medical conditions or systemic diseases like diabetes, cardiovascular disease (Coronary artery disease, heart arrhythmias, heart failure, heart valve disease, pericardial disease, congenital heart disease, and cardiomyopathy) and hypertension that may affect oral health.
- Patients who were taking any medications.
- Smoker, pregnant, and lactating women.
- Taking anti-inflammatory or antibiotics drug last month.
- Wearing of a dental prosthesis or orthodontic appliance (fixed or removable).
- Taking vitamin D supplements last two months previous to data collection.

According to WHO (2013) [9] an intraoral examination of dental caries status was performed using a Community Periodontal Index (CPI) probe. For permanent teeth, dental caries was measured by Decayed, Missing, and Filled Teeth/Surface (DMFT/S). A systemic approach to the examination for dental caries was performed in an orderly manner from one tooth or space to the adjacent tooth or tooth space. The basis for Decayed, Missing, and Filled Teeth (DMFT) calculations is 32 teeth, i.e. all permanent teeth, including wisdom teeth. For each woman, saliva was collected in the morning (9-11A.M). Salivary Unstimulated samples were collected under uniform conditions; it was conducted according to the instructions constructed by Navazesh and Kumar [12]. Saliva pH was tested by using a pH meter (Hanna Instruments, USA) [13]. Calcium and phosphorous ion concentrations were determined in the salivary supernatant samples. This was done through different biochemical tests. Calcium was determined using a flame atomic absorption spectrophotometer (Buck scientific, 210 VGP, USA). Phosphorous was measured by phosphorus colorimetric assay kit (Elabsience, American) (Phospho Molybdate Method) [14].

Statistical analysis

The Statistical Package for Social Science was used to conduct the statistical analysis (SPSS version 22, Chicago, Illinois, USA). The Frequency, percentage, mean, and standard deviation were calculated using descriptive analysis. The difference between both groups was tested using inferential analysis as an independent sample t-

test parametric test. For the linear correlation between two quantitative variables, Pearson correlation parametric test was used. Receiver Operating Characteristic Curve (ROC) for the optimal cutoff point for differentiation between the two groups.

Results and Discussion

Results showed the descriptive statistic and statistical difference of dental caries experience by surface Decayed Surface, Missing Surface, and Filled Surface and Decayed, Missing, and Filled Surface (DS, MS, FS, and DMFS) among control and study groups, as presented in Table 1. Findings in this table demonstrated that DMFS and its components were higher in the study group than those in the control one except in FS. Its result was found to be greater in control than in the study, with a significant difference for FS and highly significant for DS. At the same time, it was not significant for MS and DMFS.

Table 2 illustrates the mean values of salivary flow rate and pH among control and study groups. Findings in this table demonstrated that the salivary pH and flow rate was less in the study group than those in the control with a statically significant difference.

Table 3 indicates the mean values of the salivary calcium and phosphorous ions among control and study groups. The findings represented in this table demonstrated that salivary calcium and phosphorous were lower in the study group than in the control with a significant difference for phosphorus ions and a highly significant for calcium ions.

Table 1: Dental caries experience (DS, MS, FS, and DMFS) (mean ±SD) and statistical difference in the control and study groups

Variable	Groups				T-test	P-value
	Control		Study			
	Mean	±SD	Mean	±SD		
DS	5.750	3.966	8.375	4.062	2.924	0.005 **
MS	1.250	3.712	1.500	3.789	0.298	0.766
FS	2.825	3.594	1.425	2.581	2.001	0.049 *
DMFS	9.825	5.777	11.300	6.186	10.102	0.274

* =significant at P<0.05.

** =Highly significant at P<0.01.

Table 2: Salivary pH and flow rate (mean ±SD) and statistical difference in the control and study groups

Variable	Groups				T-test	P-value
	Control		Study			
	Mean	±SD	Mean	±SD		
pH	6.318	0.392	6.135	0.288	2.373	0.020 *
Flow rate (ml\min)	0.345	0.193	0.254	0.136	2.450	0.017 *

*=significant at P<0.05.

Table 3: Concentration of salivary calcium and phosphorous ions and statistical difference in the control and study groups

Variable	Groups	Mean	±SD	T-test	P value
Calcium (mmol/L)	Control	1.700	0.703	3.263	0.002 **
	Study	1.273	0.438		
Phosphorous (mmol/L)	Control	1.627	0.796	2.497	0.015 *
	Study	1.244	0.558		

*=significant at P<0.05.

** = Highly significant at P<0.01.

The receiver operative characteristics curve was used to determine the best salivary biomarkers for disease detection and their sensitivity (the rate of true positives) and 1-specificity (the rate of false positives), as presented in Table 4. The results showed that the best salivary test for differentiation between both groups is the calcium level followed by phosphorous.

Table 5 indicates the correlation coefficients between dental caries experience (DS, MS, FS, and DMFS) and flow rate. In the study group, the salivary flow rate had no significant negative correlation with DS, MS, and DMFS, but it was positively correlated with FS. In the control group, the salivary flow rate had no significant positive correlation with MS, FS, and DMFS, but it was negatively correlated with DS.

Table 4: Specificity and sensitivity test of salivary biomarkers calcium ion and phosphorous ion

Test Result Variable(s)	Area		P value	Optimal cutoff point	%Sensitivity	%specificity
Calcium ¹	0.678	Sufficient	0.006**	0.52	100	0
Phosphorous ²	0.665	Sufficient	0.011*	0.61	95.1	10.3

*=Significant at P<0.05.

** = Highly significant at P<0.01.

Table 5: Correlation coefficients between dental caries experience (the DMFS index) and flow rate in the control and study groups

Groups		Flow rate	
		R	P
Control	DS	-0.027	0.868
	MS	0.234	0.146
	FS	0.075	0.645
	DMFS	0.178	0.271
Study	DS	-0.148	0.361
	MS	-0.143	0.380
	FS	0.142	0.381
	DMFS	-0.125	0.441

*=Significant at P<0.05.

Table 6 indicates the correlation coefficients between vitamin D concentration and salivary flow rate. In the study group, a significant positive

correlation was found between serum vitamin D concentration and salivary flow rate.

Table 7 demonstrates the correlation coefficients between serum vitamin D concentration and selected salivary chemical parameters. A non-significant positive correlation was found

between serum vitamin D concentration and selected salivary biomarkers in the study group. Concerning the control group, the relation between serum vitamin D concentration and each of the calcium and phosphorous ions was positively significant.

Table 6: Correlation coefficients between serum vitamin D concentration and flow rate in the control and study groups

Groups		Salivary flow rate	
		R	p
Control	Vitamin D concentration	-0.190	0.239
Study	Vitamin D concentration	0.403	0.010*

*=Significant at P<0.05.

Table 7: Correlation coefficients between serum vitamin D concentration and selected salivary chemical parameters (calcium ion and phosphorus ion) in the control and study groups

Groups		Calcium		Phosphorous	
		r	p	r	p
Control	Vitamin D concentration	0.376	0.017*	0.331	0.037*
Study	Vitamin D concentration	0.149	0.357	0.070	0.667

*=Significant at P<0.05.

** = Highly significant at P<0.01.

The DS component of DMFS increased as vitamin D decreased. The lower D component was observed when vitamin D >30 ng/mL. A sufficient vitamin D level has been reported to stop tooth decay initiation and development, reduce caries formation, and prevent enamel loss [4].

The salivary flow rate was less in the study group than those in the control with a statically significant difference. Vitamin D sufficiency may support saliva flow rates leading to a more effective anti-caries action from the saliva, vitamin D deficiency has been associated with reduced parotid gland function, and reduced volume of saliva produced [6]. This fact is supported in this study by a significant positive correlation between serum vitamin D concentration and salivary flow rate in the study group and agreed upon by other studies by Stumpf and He *et al.* [7, 8]. In contrast, an opposite finding was reported by another study by Gholizadeh *et al.* [15]. They found no significant association between salivary flow rate and vitamin D levels in the serum and saliva. Reduced salivary flow rates have been linked to the dental caries [16]. This study supports this fact by a negative correlation between DS and DMFS with salivary flow rate. The other studies reported the

same result Hick *et al.* [17], while the other studies reported an opposite finding, Karnik *et al.* stated that no relation was found between salivary flow rate and caries prevalence [18]. Cunha-Cruz *et al.*, in their study, reported that salivary characteristics like salivary flow rate were associated weakly with the recent dental caries experience [19].

Regarding salivary pH, the decrease in salivary flow rate is linked to the decrease in buffer capacity, and salivary pH negatively impacts the oral sugar clearance and provides bacteria with acidic pH food, enabling them to survive and reproduce, potentially increasing the severity of dental caries [20]. Studies concerned with the relationship between salivary pH and dental caries, demonstrated by Cunha-Cruz *et al.* and Seethalakshmi *et al.* have reported the decreased salivary pH associated with increased dental caries [21]. In this study, the salivary pH was lower in the vitamin D deficiency women, as compared with the control group, with a significant difference. Another study reported the same result. Muhammed found that after taking vitamin D supplements for two months, the average salivary pH increased significantly, rising

from 6.7 to 7.1 [22]. This is probably because of a decrease in the salivary flow rate. The low salivary flow rate causes hyposalivation and increases saliva's acidity [23]. At low salivary flow rates (the unstimulated saliva), the phosphate buffer system plays a significant but minor role in the total capacity of the salivary buffer [24]. Lower phosphate ion in the study group, these findings may provide another explanation of lower pH value in the study group. Lower pH values reflect that acidic pH is favorable for enamel demineralization.

It is well-established that salivary calcium and phosphate levels are influenced by their serum concentration. Hence, serum vitamin D indirectly affects salivary calcium and phosphate levels. Calcium concentration in the saliva increased in correlation with the serum vitamin D concentration. As serum vitamin D levels increase, there is a rise in salivary calcium levels [25]. This study supports this fact by the significant positive correlation between serum vitamin D and salivary calcium and phosphorus in the control group. The current study revealed that salivary calcium and phosphate levels increased as serum vitamin D levels increased. This can be attributed to the role of vitamin D in the absorption and transportation of serum calcium and phosphate [26]. In the present study, both calcium and phosphate concentrations were recorded to be higher in the control than study group. However, the difference was significant. This may give another explanation for the higher values of the DMFS/DS in the study group as these ions are found to be decreased in the saliva of patients with active carious lesions [27, 28]. This result agrees with other studies by Jawed *et al.* and Rajesh *et al.*, which found an adequate level of salivary calcium ion and phosphorus ion greatly reduces the caries development [29, 30] and disagrees with Al-Obaidi which found a positive correlation between calcium ion and phosphorus ion concentrations and dental caries [31]. Calcium and phosphate found in saliva control the rate of demineralization and remineralization [27]. Pratyusha *et al.* reported that the decline in serum vitamin D is associated with a significant increase in the number of decayed teeth; as there is a

decrease in salivary calcium and phosphate levels, there is a significant increase in the number of decayed teeth [32].

Conclusion

The results of the current study revealed that vitamin D deficiency has a significant effect on dental caries experience in addition to changes in salivary level to the selected physicochemical characteristics (pH, flow rate, calcium ion, and phosphorus ion). There is a need for adequate awareness regarding oral hygiene. Specifically, the effective preventive measures could help reduce the effect of vitamin D deficiency on oral health.

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Authors' contributions

All authors contributed to data analysis, drafting, and revising of the paper and agreed to be responsible for all the aspects of this work.

Conflict of Interest

We have no conflicts of interest to disclose.

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