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Research Article

Influence of Vitamin D Deficiency on the Level of Salivary cathelicidin LL-37 in relation to Dental Caries Experience: A Case-Control Study

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ABSTRACT

Background: Vitamin D improves innate immunity by enhancing the expression of antimicrobial peptides. The antimicrobial action of cathelicidin is widespread and effective against cariogenic bacteria. This research aimed to investigate the effect of vitamin D deficiency on the level of salivary cathelicidin concerning dental caries experience.
Subjects and Methods: A case-control study was carried out, and the sample was composed of 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 of 30 ng/mol or more, their age range was 20-30 years. WHO 2013 index was used for the assessment of dental caries experience, and collection of unstimulated saliva was done to measure salivary cathelicidin.
Results: Data from the current study showed that the mean values of the Decayed, Missing, and Filled Teeth index and its components (Decayed Teeth, Missing Teeth) were higher in the study group than those in the control one, with a significant difference for Decayed Teeth. Salivary cathelicidin was higher in the control group with a statistically significant difference.
Conclusion: It was confirmed that serum vitamin D status and salivary cathelicidin levels are related. Vitamin D deficient patients who had higher Decayed, Missing, and Filled Teeth index values expressed a lower level of salivary cathelicidin than the control group.

Introduction

Nutrition affects oral health on a local and systemic level by influencing dental health and salivary composition (1). Malnutrition affects tooth development as well as the formation, function, and secretion of salivary glands, all of which influence susceptibility to dental caries (2). Dental caries is the most serious threat to oral health and the most common oral infection disease (3). Many people all over the world suffer from dental caries. Extensive studies have shown that dental caries is caused by bacteria (4) but is also affected by factors related to the host and the diet (5). Salivary defense mechanisms play a crucial part in preserving dental health and preventing caries (6). Saliva serves as a source of various proteins and peptides with antimicrobial action, including lactoferrin, immunoglobulin, lysozyme, and cationic peptides (7). Cathelicidin LL-37 and defensin (alpha & beta) are the two most important antimicrobial peptide (AMP) families that can be found in the oral cavity. Saliva, gingival crevicular fluid, and oral mucosa contain these peptides. Alpha defensin and cathelicidin are mostly produced by neutrophils, beta-defensins, in contrast, are produced and expressed by the oral epithelium. In the human oral cavity, these salivary AMPs serve as the initial line of innate immune defense (8).

Antimicrobial peptides were found to interact synergistically to increase their effectiveness. The primary mode of action is membrane lysis, although there are also cell-killing agents that target intracellular targets (9). Cathelicidin exhibits antimicrobial activity against a wide range of cariogenic bacteria (10). Antimicrobial peptides contribute to maintaining the normal flora in the mouth, gut, and skin niches in a stable state (11). Salivary cationic AMPs may be used as proteomic biomarkers for several oral diseases, such as dental caries (12). One of the most important functions of vitamin D in the immune system is that it has a stimulating effect on human cathelicidin. It has both antimicrobial and anti-endotoxin functions. The epithelial antimicrobial peptide cathelicidin is one of many epithelial antimicrobial peptides that have been discovered to serve key roles in maintaining oral health and have been given the nickname "guardian of the oral cavity" (13). A study has proven the relationship between vitamin D and cathelicidin levels, and it has been demonstrated that vitamin D plays a specific role in the expression of cathelicidins (14). This study was designed to detect the consequences of vitamin D deficiency on salivary LL-37 concentration concerning dental caries experience.

Subjects 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 who were matching to 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. The age was measured according to the last birthday (15). Consent had been obtained from each subject in both study and control groups before participation. The College of Dentistry/University of Baghdad, Iraq, Research Ethics Committee approved the study ref. number:484; date:19-1-2021. Sample collection period from Jan 27, 2021, to May 20, 2021. Vitamin D concentration was measured at a specialized laboratory in AL-Najaf city by using Cobas e 411 analyzers and a special kit (Elecsys Vitamin D total, Germany). For both study and control groups, the criteria for exclusion consisted of the subsequent ones:

•Patients with a medical condition or systemic diseases like diabetes, cardiovascular disease, 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(15), an intraoral examination of dental caries status was performed using Community Periodontal Index (CPI) probe. The Decayed, Missing, and Filled Teeth index (DMFT) for permanent teeth was used to assess dental caries. The examination for dental caries was carried out systematically, beginning with one tooth or space and moving on to the next in the same pattern. DMFT calculations are based on 32 teeth, or all permanent teeth, including wisdom teeth. For each woman, the saliva collection was done 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 (16). The concentration of salivary cathelicidin level was identified by an enzyme-linked immune-sorbent assay (ELISA) using a salivary cathelicidin kit (Elabscience, American) (17). Following the manufacturer's recommendations, the reagent preparation concept, technique assay, and result computation were all carried out.

Statistical analysis

The Statistical Package for Social Science was used to conduct the statistical analysis (SPSS version 22.0, Chicago, Illinois, USA). The Frequency, percentage, mean and standard deviation were calculated using descriptive analysis. The difference between the two groups was tested using inferential analysis as an independent sample t-test parametric test. For the linear correlation between two quantitative variables, the Pearson correlation parametric test was used.

Sample Size was calculated by using G power 3.1.9.7 (Program written by Franz- Faul, Universitat Kiel, Germany) With the power of study=85%, alpha error of probability=0.05, the statistical test is Two Independent Sample T-test and assuming the effect size is 0.6 (medium) between two groups with all these conditions the sample size is 40 for each group. Cohen D is Small =0.3, medium=0.5, large>=0.8 (Cohen, 1988.

Results

Table (1) illustrates the mean values of salivary cathelicidin. Salivary cathelicidin was higher in the control group than in the study group, with a statistically significant difference.

Table 1: Concentration of salivary cathelicidin and statistical difference in the study and control groups

Variable	Groups	Mean	±SE	T-test	P value
Cathelicidin (ng/ml)	Study	14.416	2.593	2.670	**0.010** *
	Control	29.350	4.955		

*=significant at P less than 0.05

Table (2) shows the descriptive statistic and statistical differences in dental caries experience by surface (Decayed Teeth, Missing Teeth, Filled Teeth, and Decayed, Missing, and Filled Teeth) among the study and control groups. Findings in this table demonstrated that DMFT and its components were higher in the study group than those in the control one except in FT, its result was found to be greater in the control than in the study, with a significant difference for FT and DT, while it was not significant for MT and DMFT.

Table 2: Dental caries experience (DT, MT, FT, and DMFT) (mean ±SE) and statistical difference in the study and control groups

Variable	Groups					
	Study		Control			
	Mean	±SE	Mean	±SE	T-test	P value
DT	6.450	0.345	4.675	0.470	3.043	**0.003** *
MT	0.300	0.120	0.250	0.117	0.298	0.766
FT	1.125	0.306	2.400	0.479	2.243	**0.028** *
DMFT	7.875	0.327	7.325	0.453	0.984	0.328

*=significant at P less than 0.05

Table (3) shows the descriptive statistic and statistical differences in vitamin D concentration among groups. Results from that table showed that vitamin D concentration was lower in the study group than in the control with a statistically highly significant difference.

Table 3: Serum vitamin D concentration (ng/mL) (mean ±SE) and statistical difference in the study and control groups

Groups	Mean	±SE	T-test	P value
Study	7.1155	0.3085	19.680	**0.000****
Control	37.7605	1.5263		

**Highly significant at p less than 0.01

Table (4) shows the correlation coefficients between serum vitamin D concentration and salivary cathelicidin. In the study group, the relation between serum vitamin D concentration and cathelicidin was negative and not significant. Regarding the control group, a highly significant positive relation was illustrated for serum vitamin D concentration with cathelicidin.

Table4: Correlation coefficients between serum vitamin D concentration and salivary cathelicidin and DMFT in the study and control groups

Groups		Cathelicidin		DMFT	
		r	p	r	p
Study	Vitamin D concentration	-0.148	0.363	0.299	0.061
Control	Vitamin D concentration	0.537	**0.000****	0.228	0.157

**= Highly significant at P less than 0.01

Table (5) shows the correlation coefficients between dental caries experience (DT, MT, FT, and DMFT) and salivary cathelicidin. Regarding the study, group cathelicidin was not had a significant positive correlation with DT, MT, and DMFT, but it was negatively correlated with FT. In the control group, cathelicidin was a highly significant negative correlation with DT, but it was a significant positive correlation with FT, was a negative, not significant correlation with MT and DMFT.

Table 5: Correlation coefficients between dental caries experience (DMFT index) and salivary cathelicidin in the study and control group

Groups		Cathelicidin	
		r	p
Study	DT	0.223	0.166
	MT	0.047	0.774
	FT	-0.329	**0.038***
	DMFT	-0.056	0.733
Control	DT	-0.585	**0.000****
	MT	-0.137	0.400
	FT	0.376	**0.017***
	DMFT	-0.245	0.128

*=Significant at P less than 0.05
** = Highly significant at P less than 0.01

Discussion

The current study's findings revealed that the mean value of DT, MT, and DMFT were higher in the study group than control and the difference was significant concerning the DT can be explained by: individuals who have a high caries index have lower levels of cathelicidin expression in their unstimulated saliva as compared to patients of comparable age who have an average level of caries activity. In most cases, the concentration of cathelicidin is much higher in patients who do not have caries activity or who have minimal caries activity (18). This fact is supported in this study by a highly significant negative correlation between the DT component of DMFT and salivary cathelicidin in the control group. Another study reported the same result by Al-Ali et al. (19), while an opposite finding was reported by Malcolm et al. and Colombo et al. (20,21). It is worth noting that cathelicidin has an anti-biofilm effect by limiting bacterial adhesion to the tooth surface, as well as affecting biofilm thickness (22). Almoudi et al. showed that Individuals with low salivary cathelicidin levels and higher S. mutans and S. sobrinus counts supported salivary cathelicidin's preventive effect against dental caries (23). In this study, the level of salivary cathelicidin was lower in the study group than in the control with a significant difference. Vitamin D presents immunomodulatory effects that lead to increased production of antimicrobial peptides. Altogether, vitamin D has the potential to protect against bacterial infections (24,25). Antimicrobial peptide cathelicidin can fight a variety of infections; it has antibacterial and immunomodulatory characteristics (26). Cathelicidin plays a role in chemotaxis, the production of cytokines and chemokines, cellular reproduction, vascular permeability, wound healing, and the neutralization of bacterial endotoxins (27). Studies were conducted by Wang et al.; Hertting et al.; Stein and Tipton, and He et al. (28,29,30,31); according to studies, vitamin D has a good effect on dental health due to its anti-inflammatory properties and its ability to enhance the production of antimicrobial peptides such as cathelicidin. Furthermore, according to the Protein Abundance Database, cathelicidin is the third most prevalent AMP in saliva and the fourth most abundant AMP in the body as a whole (32). In the present study, a highly significant positive correlation was found between serum vitamin D and cathelicidin in the control group. The same result was also reported by Raftery et al. and Gyll et al. (33,34). This study reported that salivary LL-37 levels positively correlate with serum vitamin D levels in females with concentrations of vitamin D 30ng/ml or more, the results support the idea of vitamin D directly induces the expression of LL-37. As the level of salivary LL-37 increases, there's a decrease in caries activity.

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Conflict of Interest
No conflict of interest

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