ORIGINAL RESEARCH

Impact of Vitamin D Deficiency on Gingival Health Condition and Salivary Level of Prostaglandin E2 among Group of Females

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Abstract

Aim: The goal of this study was to find out the impact of vitamin D deficiency on gingival, plaque, and calculus indices (Call) and prostaglandin E2 (PGE2) levels in unstimulated saliva.

Materials and methods: This study was a case-control type, and the total sample was 80 women aged 20–30 years—the first group consisted of 40 women with vitamin D levels below 10 ng/mol, and the second group consisted of 40 women with vitamin D levels 30 ng/mol or more. The College of Dentistry/the University of Baghdad Ethical Committee provided a consent form. Oral health status assed by using the plaque index (PII) of Silness and Loe (1964), Ramfjord's Call (1959), and Loe and Silness' gingival index (GI) (1963). A collection of unstimulated saliva was done to measure salivary PGE2.

Results: Current study data revealed that the GI and PGE2 were higher in the study group, with statistically highly significant ($p < 0.01$) and significant ($p < 0.05$) differences, respectively. Both plaque and Call were found higher in the study with no significant difference ($p > 0.05$).

Conclusion: This study reported an increase in the salivary PGE2 and GI among vitamin D deficient women.

Clinical significance: To preserve oral health, healthcare providers must raise awareness. Controlling vitamin D levels for oral health is a matter that should not be ignored, particularly in early life.

Keywords: Calculus index, Case-control study, Gingivitis, Gingival index, Plaque index, Prostaglandin E2, Vitamin D deficiency.

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Introduction

Vitamin D deficiency is a widespread (pandemic) condition linked to inadequate sun exposure.[1] Women have a higher percentage of vitamin D deficiency than men due to insufficient sun exposure and poor dietary intake, sociodemographic factors, health, and cultural factors; as a result, they are at risk of a variety of vitamin D-related diseases.[2] Periodontal diseases are typically classified into two types—gingivitis and periodontitis.[3] Females had a significantly higher GI mean than males[4] Vitamin D impacts the etiology of periodontal disorders by immunomodulation, enhances bone mineral density, and lowers bone resorption.[5] It was found that individuals with sufficient vitamin D had healthier periodontal tissues; as a result, the incidence of gingivitis formation and bleeding during probing was lower in these individuals. People with low vitamin D concentration had a greater prevalence of the periodontal disease.[6] Vitamin D, a neurohormone with pleiotropic biological effects, which control the production, release, and stimulation of both pro and antiinflammatory cytokines, may change the immune system response by encouraging and preventing T helper (Th) 1 and Th2 cell proliferation.[7] PGE2 is an important physiologically active lipid that is produced by the cyclooxygenase (COX) and PGE2 synthases from arachidonic acid.[8,9] PG production differences between the sexes may be responsible for the sex-dependent incidence of numerous inflammatory diseases.[10] PGE2 is considered to play an especially crucial role in females.[11] Due to its increased expression in inflamed gingival tissues, PGE2 has been established as a marker for the onset and severity of the periodontal disease.[12] Observations suggested that diseased periodontal tissues produce a significant amount of PGE2 present locally in saliva.[13] Significant elevations in concentration were observed for PGE2 in the patients with gingivitis in comparison to the healthy participants.[14] Vitamin D may control the release of arachidonic acid and modulate the production of PGE2.[15] By increasing the expression of 15-PG dehydrogenase (15-PGDH) and suppressing COX-2, vitamin D modifies the action of PGs. PGs are degraded by the enzyme 15-PGDH, which also inhibits PGE2 receptor subtypes and subtypes of the PG-F2α receptor.[16]

As far as no previous Iraqi studies are available concerning the impact of vitamin D deficiency on oral health status and salivary PGE2 level, this study was intended to identify the impact of vitamin D deficiency on oral health status and the chemical property of saliva.

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Materials and Methods

This study was a case-control type carried out in Najaf Governorate in Iraq from January to May (2021). Within this time, saliva samples were collected, the involved women were inspected, and the salivary samples were subjected to biochemical laboratory tests. Before participation, consent was obtained from each subject in the study and control groups. The College of Dentistry Ethics Committee at the University of Baghdad gave its ethical approval to project number 484322, Ref. Number—484, 19th January 2021.

Sample Size Calculation

G*Power 3.1.9.7 was used to determine the sample size (Franz Faul, Universitat Kiel, Germany, wrote the program). With a study power of 85% and an α-error of the probability of 0.05, and two independent sample *t*-tests as the statistical test, assuming the effect size was 0.6 (medium) between the two groups, the sample size for each group was 40. Cohen D is defined as small = 0.3, medium = 0.5, and large ≥0.8.

The Study Sample

The total sample was 80 women aged 20–30 years—the first group consisted of 40 women with a vitamin D level below 10 ng/mL, and the second group consisted of 40 women with a vitamin D level of 30 ng/mol or more. The age was measured according to the last birthday.[17] Vitamin D concentration was measured by using Cobas e 411 analyzers and a special kit.

Inclusion and Exclusion Criteria

Exclusion criteria comprised the following for both the study and the control groups:

- Patients were suffering from a medical problem and a systemic disease that might affect their oral health.
- Patients who took any medication.
- Smokers, lactating, and pregnant women.
- Patients who took antibiotics or antiinflammatory medication last month.
- Patients who wore an orthodontic appliance or dental prosthesis (fixed or removable).
- Patients who took vitamin D supplementation for the prior 2 months before data collection.

Inclusion criteria comprised the following for both the study and control groups:

- Around 20–30 females with vitamin D deficiency and vitamin D normal.
- Women who were free of any outward manifestations of systemic disease.
- Females who were not taking any medications or supplements.

Saliva Collecting Procedure

Saliva was collected from each woman in the morning (9–11 AM). Unstimulated salivary samples were collected consistently by Navazesh and Kumar's instructions:[18]

- The subject should be instructed to avoid food intake, chewing gum, and beverages 1 hour ago the saliva collecting procedure.
- The subject should have a seat, then rinse her mouth with distilled water and relax for at least 5 minutes.
- The subject should be asked to reduce her movement and instructed to fix her forehead above the test tube kept beneath it.
- The subject should be instructed to keep her mouth open to allow the drain of saliva into the tube for 5 minutes.

At the end of the collection time, the person was asked to get any saliva left in their mouth and spit it into the test tube as quickly as possible. Depending on the time, the actual trail should last for 5 minutes.

Salivary Centrifugation and Storage

Saliva was centrifuged by a centrifuge machine in the lab for 10 minutes at 3,000 rpm, then pipetting the supernatant and dividing into several containers (Eppendorf tubes) depending on the number and volume required for each test, placed until the time of analysis in a deep freezer set to –20°C.

Biochemical Analysis of Saliva

The concentration of salivary PGE2 level was detected by an enzyme-linked immunosorbent assay using a salivary PGE2 kit. The reagent preparation concept, technique assay, and result computation were all conducted by the guidelines provided by the manufacturer.

Oral Hygiene Status and Gingival Health Condition

Intraoral examination of Silness and Loe's (1964) PlI was used to assess dental plaque thickness. This index was scored using the dental explorer and mouth mirror (Fig. 1). The buccal surface of the teeth was examined first, proceeded by the mesial, lingual, and distal surfaces. Six permanent index teeth 16, 12, 24, 36, 32, and 44 were included in the examination to represent the entire dentition with no substitution for missing selected teeth. Gingival health condition was evaluated by Loe and Silness (1963); GI was used to assess gingival health using a mouth mirror and community periodontal index (CPI) probe (0.5 mm ball tip) (Fig. 2). The teeth included and the sequence of examination was the same as those for the (PlI). Dental calculus extent was evaluated according to the criteria of calculus component (CalI) of periodontal disease index by Ramfjord (1959) using a mouth mirror and dental explorer (Fig. 3). The facial (buccal\labial) and lingual surfaces of the selected teeth were examined. Six permanent index teeth 16, 21, 24, 36, 41, and 44 were included in the examination to represent the entire dentition with no substitution for missing selected teeth.

Statistical Analysis

Statistical Package for the Social Sciences (SPSS) was used to perform the statistical analysis (SPSS version 22.0, Chicago, Illinois,

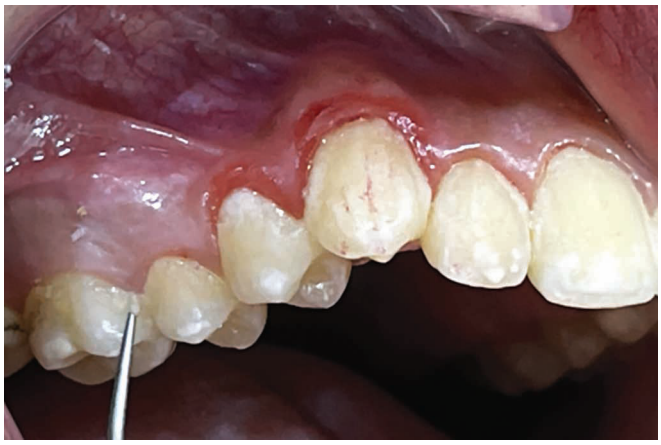

Fig. 1: Examination of dental plaque thickness was assessed by using a mouth mirror and dental explorer

United States of America). Utilizing descriptive analysis, we were able to determine the frequencies, percentages, means, and standard error (SE). Inferential analysis, specifically the independent sample *t*-test parametric test, was used to examine the difference between the two groups. Pearson's correlation is a parametric test used to examine the linear relationship between two quantitative variables.

Results

According to the findings of this study, the mean value of the GI was higher among the study group (0.890) compared to the control group (0.261); these differences were highly significant ($p < 0.01$), as observed in Table 1. Healthy gingiva was detected only in the control group (15%). Mild gingivitis was present in both the study and control groups, with a higher percentage in the control group (85%) in comparison to the study group (60%), while moderate gingivitis was found only in the study group (40%), as observed in Table 2. The PII's mean value was higher in the study group (0.574) than in the control group (0.486). However, the difference was statistically not significant *p*-value was 0.067, while the mean value of the CaII was higher in the study group (0.069) than in the control group (0.052). However, the difference was statistically not significant *p*-value was 0.244, as shown in Table 3. Salivary PGE2 mean value was found to be higher in the study group (1.008 pg/mL) than those in the control group (0.943 pg/mL), with a significant difference ($p < 0.05$). The correlation coefficients between serum vitamin D concentration and oral health indices are in Table 4. Vitamin D with PII was negative in the study group ($r = -0.207$ and $p = 0.200$) but positive in the control group ($r = 0.396$ and $p = 0.011$), but both were nonsignificant. Vitamin D with calculus was negative and nonsignificant in both study ($r = -0.217$ and $p = 0.179$) and the control group ($r = -0.213$ and $p = 0.187$), as observed in Table 5.

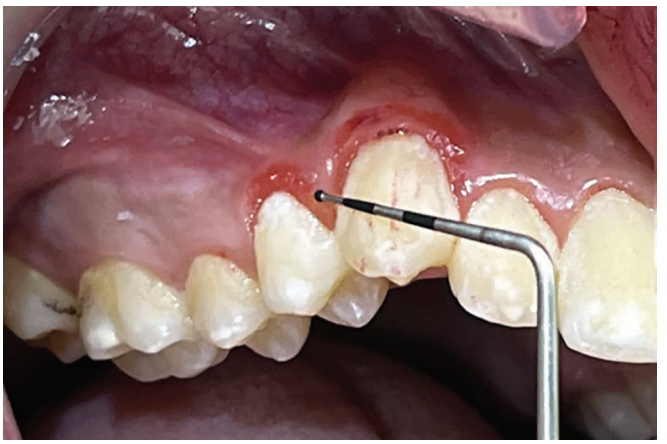

Fig. 2: Examination gingival health condition was assessed by using a mouth mirror and CPI probe

Fig. 3: Examination of dental calculus extent was assessed by using a mouth mirror and dental explorer

Table 1: GI (mean ± SE) and statistical difference in the study and control groups

Variable	*Groups*	*Mean*	*± SE*	*t-test*	*p-value*
GI	Study	0.890	0.076	7.321	**0.000**[a]
	Control	0.261	0.040		

[a] Highly significant at $p < 0.01$

Discussion

The importance of vitamin D as a hormone has been shown in various physiological and pathological processes involving multiple organs and systems of the human body.[19] Middle Eastern countries had the highest prevalence of vitamin D deficiency and insufficiency; because of the hot climates in Iraq and the Middle East, people tended to avoid the hot sun whenever possible. Women in Iraq, particularly in Najaf city, when they go outside, cover their whole bodies with black clothes, wear veils, and sometimes black gloves for social and religious reasons. This is a good reason to prevent cutaneous vitamin D production in those women, even if they live in a sunny climate. Furthermore, the women in this study were between 20 and 30 years old. This age was selected to avoid the potential consequences of hormonal changes and age on the results; vitamin D deficiency was more common in women of reproductive age and is of particular concern because it can have adverse consequences for the mother, fetus, infant, and child.[20] Bone mass peaks at 30 years, so the diagnosis of vitamin D deficiency and interventions to increase peak bone mass are more effective than later interventions in preventing osteoporosis.[21] They were free of any systemic diseases as well as any local diseases to exclude the effect of these diseases on oral health.

Poor oral hygiene causes the accumulation of dental plaque, which is responsible for the etiology of periodontal disease.[22] Dental calculus is regarded as the most significant plaque-retentive component due to its capacity to retain and harbor plaque bacteria internally and on its rough outside.[23] The findings of this study can be justified by the fact that the salivary flow rate was affected by vitamin D deficiency.[24] When the salivary flow rate is reduced, this will decrease the irrigation action of saliva, favoring the accumulation of dental plaque and mucosal inflammations.[25,26] The

Table 2: Distribution of the study and control groups according to a gingival health condition

Gingival health condition	*Groups*			
	Study		*Control*	
	N	*%*	*N*	*%*
Healthy (0)	0	0.00	6	15.00
Mild (0.1–1)	24	60.00	34	85.00
Moderate (1.1–2)	16	40.00	0	0.00
Total	40	100.00	40	100.00

Table 3: Dental plaque and CalI (mean ± SE) and statistical difference in the study and control groups

	Groups					
	Study		*Control*			
Variable	*Mean*	*± SE*	*Mean*	*± SE*	*t-test*	*p-value*
PII	0.574	0.031	0.486	0.036	1.855	0.067
CalI	0.069	0.011	0.052	0.010	1.174	0.244

Table 4: Concentration of salivary PGE2 (mean ± SE) and statistical difference in the study and control groups

Variable	*Groups*	*Mean*	*± SE*	*t-test*	*p-value*
PGE2 (pg/mL)	Study	1.008	0.024	2.308	**0.024**[a]
	Control	0.943	0.015		

[a] Significant at $p < 0.05$

Table 5: Correlation coefficients between serum vitamin D concentration and (plaque and CalI)

		PII		*CalI*	
Groups		*r*	*p*	*r*	*p*
Study	Vitamin D concentration	−0.207	0.200	−0.217	0.179
Control	Vitamin D concentration	0.396	0.011	−0.213	0.187

results of the present study showed negative nonsignificant correlations between vitamin D and PII for the study group. This result agrees with Abdul-Hafidh's salivary flow rate, [27] who found negative nonsignificant correlations between vitamin D and PII and disagreement with Perayil et al.,[28] who found there were highly significant differences for vitamin D in correlation to PII.

In this study, the CalI was found to be higher in the study compared to the control group, with no statistically significant difference. It showed negative nonsignificant correlations between vitamin D and CalI in both groups. It is worth noting that no published clinical studies examined the effect of vitamin D deficiency on calculus formation, limiting the ability to compare the finding of this study to those of other similar studies.

Vitamin D deficiency has been linked to long-term negative consequences on oral health, including an increased risk of gingival inflammation.[29] Dietrich et al., Hiremath et al., and Millen et al.[30–32] found that inverse association between serum vitamin D concentrations and gingivitis. Moreover, Hiremath et al.[33] investigation showed that gingivitis susceptibility was reduced by optimal blood vitamin D levels due to its dose-dependent antiinflammatory effect.

The higher GI in the study group, which was recorded in the present study, can be justified by:

- Plaque accumulation among the study group was higher than in the control group. It has been reported that dental plaque is the primary cause of gingivitis.[34] There is a significant association between increased dental plaque accumulation, high prevalence, and severity of periodontal disease.[35] Iraqi studies also reported a positive correlation between the amount of dental plaque and gingival inflammation.[36,37] Masood in 2010,[36] also detected, in addition to the previous result, a positive, highly significant correlation between calculus accumulation with gingival inflammation.
- Vitamin D's immunomodulatory actions can modulate innate and adaptive immune responses.[38] Vitamin D affects the immune system by activating the synthesis of proteins necessary in epithelial cell tight, gap, and desmosome junctions.[5] As a result of the junctional epithelium's loose connections with the tooth, which allow bacteria from dental plaque to invade, the periodontal tissue becomes inflamed at first.[39]

Further, several studies have looked into and confirmed the link between plasma levels of vitamin D and the presence of periodontal diseases.[32,40,41] Other research has not demonstrated this connection and recommended more research.[42,43] It is worth mentioning that the study group had a higher percentage of moderated gingivitis (40%), while it was absent in the control group. On the contrary, healthy gingiva was present only in the control group.

In this study, the salivary PGE2 was higher in the study group with a significant difference. This can be explained by higher GI among the study group, and PGE2 level raises with increased periodontal inflammation. The same result was reported by Miller et al., Rathnayake et al., and Syndergaard et al.[14,44,45] Patients with periodontal disease have higher levels of COX-2 and PGE2 in their gingival tissues and gingival crevicular fluid than do subjects without periodontal disease,[46] and directly related to all features of periodontal disease from the beginning of the inflammation to the periodontal tissue destruction.[47] The small sample size was the main limitation of this study; therefore, additional studies with larger sample sizes are recommended. Detection of the general health status of the participants only by taking information from them.

So, due to the detrimental effects of vitamin D deficiency on oral health that concluded in the current study and supported by previous ones, because obesity is linked to vitamin D deficiency, it is critical to encourage individuals, particularly women, to engage in regular physical activity to maintain a healthy weight. Get moderate exposure to sunlight and eat a healthy diet that includes foods high in vitamin D, including fatty fish and small amounts of fortified, low-fat dairy.

For further research, the following topics are recommended:

- Studying the effect of obesity on vitamin D deficiency in relation to oral health status.
- Further investigations are needed to assess salivary oxidative status among vitamin D deficiency patients in relation to oral health.

- Bacteriological studies are needed concerning the oral microflora of vitamin D deficient individuals compared to normal vitamin D individuals to identify the effect of vitamin D deficiency on the oral microflora in relation to oral health status.
- Studying another biomarker in saliva, like salivary magnesium. Other salivary immunological parameters can be investigated as lactoferrin, interleukin 6, and tumor necrosis factor alpha, in relation to oral health in vitamin D deficient individuals.
- Studying the effect of vitamin D deficiency in a male group on oral health and salivary parameters.

Conclusion

The current research revealed that vitamin D deficiency significantly affects gingival health conditions; healthy gingiva was detected only in the control group, while moderate gingivitis was found only in the study group. This study also reported increased salivary PGE2 and GI among vitamin D deficient women.

Clinical Significance

The majority of women with vitamin D deficiency have a greater GI, and this difference is clinically significant. Gingivitis is thought to be prevented by vitamin D, which is a promising oral health protective agent. Raising public awareness about the impact of vitamin D deficiency and applying education, preventive, and treatment programs to reduce this impact.

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