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Effect of vitamin D on antimicrobial peptides levels in patients with periodontitis

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Abstract---Periodontitis is a complex infectious disease that affects a wide range of people and has a lot of risk factors. Vitamin D increases innate immunity by regulating the generation of antimicrobial peptides and cytokine response, which may minimize the risk of infection through a variety of methods. Antimicrobial peptides defend the host from a variety of pathogens. This study was conducted to determine the vitamin D levels in patients and investigate how vitamin D affects the levels of antimicrobial peptides. The University of Baghdad's Department of Periodontics, College of Dentistry, and clinic recruited fifty new patients. For each patient, periodontal parameters were assessed. In order to estimate the level of vitamin D and antimicrobial peptides (cathelicidins and human beta defensins-1), blood samples from all patients were taken. The finding revealed that serum vitamin D deficiency is associated with decreased levels of cathelicidins and human beta defensins-1 in serum of patients with periodontitis. Low levels of cathelicidins and human beta defensins-1 in periodontitis patients with insufficient vitamin D, implying that vitamin D plays a role in AMPs production.

Keywords---periodontitis, antimicrobial peptides, cathelicidins, human beta defensins-1, vitamin D.

Introduction

Periodontitis is a chronic inflammation in supporting tissue of gingival with some characteristics like the inflammation in gingival supporting tissues, formation of

periodontal pockets, loss of connective tissue attachment, alveolar bone desorption, and even loose of tooth (Mulawarmanti and Parisihni, 2019). Periodontal bacteria trigger the host immune response which causes release of inflammatory mediators and cytokines in the periodontal tissues causing periodontal breakdown (Kumaresan et al., 2016). These host mediators directly or indirectly participate in periodontal tissue damage and specifically in bone resorption.(Al-Ghurabi et al., 2012; Shaker and Hashem, 2012; Al-Ghurabi and Mohssen, 2015). Vitamin D is a multifunctional hormone that is mostly produced in the skin following exposure to ultraviolet (UV) B sunlight. A negligible amount comes from exogenous sources (foods and supplements) also. Despite VD synthesis is molecularly well-regulated in a limited numbers of organs, it can reach to the distant organs through the circulatory system and act on most of the cells due to presence of VD receptors (VDR). It coordinates all the physiological functions by controlling calcium and phosphate metabolism, promotes growth, and induces necessary remodeling of the bones and teeth (Bover et al., 2015). The important role of VD in the regulation of musculoskeletal health by maintaining mineral ion homeostasis is elaborated elsewhere (Brown and Razzaque, 2016).

Studies have shown that deficiency of VD may place subjects at risk, not only for low bone mineral density, osteoporosis or osteopenia but also by promoting infectious and inflammatory diseases (Stein and Tipton, 2011). Of relevance, an association between tooth loss and alveolar bone density or osteoporosis have been documented, suggesting that low bone mass is a risk factor for the evolvement of periodontal diseases (Kuo et al., 2008). Moreover, VD may exert beneficial effects on oral health by influencing the production of antimicrobial peptides (Youssef et al., 2011). AMPs are a class of active oligopeptides that are toxic to pathogens (Gong et al., 2021). AMPs are widely distributed in nature, with examples reported from microorganisms, plants, invertebrates, fish, amphibians, birds and mammals (Zhang et al., 2021). Because they mostly have a net positive charge and also exhibit hydrophobicity, AMPs can combine with negatively charged surface due to electrostatic interactions, penetrate and destroy the membrane structure, resulting in the death of bacteria, fungi, parasites and viruses (Vineeth Kumar and Sanil, 2017). Different from the bactericidal principle of traditional antibiotics with a single target, AMPs can destroy pathogens by damaging multiple targets, which can greatly reduce the emergence of drug-resistant bacteria, and makes them one of the best alternatives for comprehensive antibiotics due to their broad-spectrum antibacterial properties (Zhang et al., 2021). There are two subfamilies of AMPs in mammals: cathelicidins and defensins. Both types of AMPs are part of the innate immune system. In humans, there are many classes of defensins, while there is only a single identified cathelicidin (Ridyard and Overhage, 2021).

Cathelicidins, encoded by the CAMP gene, are members of the AMPs family found in peroxidase-negative granules of vertebrate neutrophils. Cathelicidins consist of highly conserved N-terminal signal peptides containing a cathelin domain and a cationic AMPs, which is produced by extracellular proteolysis from the C-terminus. To date, human cationic AMP18 (hCAP-18) is the only human cathelicidin identified and can be found in neutrophils, monocytes, mast cells, dendritic cells and epithelial cells (Jin and Weinberg, 2019). Human beta

defensin-1 (HBD-1) is an important salivary component of the innate immune defense against an invasion by oral microbes. It provides signaling for the adaptive immune system and has an important role in pulling immature dendritic cells and T cells by binding chemokine receptors. It also maintains the homeostasis of microbial pathogens by preventing bacterial colonization and viral infection. Previous studies showed that patients with various oral diseases, such as lichen planus, leukoplakia, glossitis, glossodynia, and oral discomfort, have increased HBD-1 levels (Pierson et al., 2013).

In the oral cavity, VD plays a role in the immune response against infections (Cagetti et al., 2020). The deficiency of VD negatively impacts oral health and the patient's general well beings (Botelho et al., 2020). Periodontitis and gum diseases are the most common oral diseases linked to VD deficiency (Jagelavičiene et al., 2018). VD inhibits periodontal tissue damage from the excessive immune response induced by the presence of pathogenic microbes in a normal healthy person. AMPs such as cathelicidin LL-37 and beta-defensin induced by VD increases microbial killing (Schroth et al., 2016). The purpose of the study was to measure the vitamin D levels in the participants and look into how vitamin D impacts the levels of antimicrobial peptides.

2. Materials and Methods

Subjects

From November 2021 to January 2022, 50 participants in a cross-sectional study were invited to the periodontics clinic at the University of Baghdad / College of Dentistry. The patients were divided into two groups based on serum 25(OH) D3 levels: vitamin D sufficient (n=28) and vitamin D insufficient (n=22). The Institute of Medicine's (IOM) guidelines' definition of the Vitamin D status was adopted, whereby vitamin D insufficiency listed at $< 20 \text{ ng/mL}$ ($\leq 50 \text{ nmol/L}$) and levels $\geq 20 \text{ ng/mL}$ represented sufficiency status.

Inclusion and exclusion criteria

Systemically healthy, not frequently using any medications, having had no periodontal treatment in the previous six months, not smoking or drinking heavily, and having at least 20 or more natural teeth were requirements for inclusion in the study. While persons who were pregnant, receiving orthodontic treatment, or who had undergone prior periodontal therapy were not included in the study population.

Sample size

Sample size was calculate using G power 3.1.9.7 (Program written by Franz-Faul, universitatit Kiel, Germany) with power of study=95%, alpha error of probability=0.05 two sided, the statistical test is two independent sample T test and assume the effect size is 0.8 (large) between two groups with all these conditions the sample size is 84 so 85 subjects is enough and more calculated than G power (Cohen, 1988).

Oral examination

Every individual in the study had a clinical examination done by a dentist specialist. William's graduation's periodontal probe was used to evaluate the periodontal health of every tooth. The periodontal parameters included the plaque index (PI), gingival index (GI), bleeding on probing, probing pocket depth (PPD), and clinical attachment loss (CAL) (Silness and Loe, 1964; Newbrun, 1996).

Sample collection

Three mL of blood was collected from patients. Blood was transferred to sterile plain tube, and serum was separated by centrifugation at 3000 rpm for 10 minutes, then divided into small aliquots and kept at -20°C until used for analysis. **Estimation of Serum vitamin D and AMPs:** Serum vitamin D and AMPs levels (Shanghai, China) were determined by ELISA.

Statistical analysis

The T-test and chi square tests were employed to determine the statistical significance of differences between groups. The Pearson correlation coefficient test was used to determine correlations between biomarkers and clinical parameters. Significant P-values have been defined as $P < 0.05$.

3. Results and Discussion

3.1 Results

The current study found a significant increase ($P < 0.01$) in the mean value of VD among the VD sufficient group, who account for 28 (56%) of the total number of patients, compared to patients with insufficient VD, who account for 22 (44%) of the total number of patients. As shown in Table (1), the mean value of VD in the sufficient group was $(47.81 \pm 9.99 \text{ ng/ml})$, whereas in the insufficient group it was $(26.27 \pm 2.36 \text{ ng/ml})$.

Table 1: Distribution of Patients According to Vitamin D Sufficiency

Vitamin D (ng/ml)	Patients groups		
	Vitamin D sufficient group N=28	Vitamin D insufficient group N=22	T-test P-value
Minimum	31.66	17.62	0.000**
Maximum	75.01	29.8	
Mean	47.81	26.27	
SD	9.99	2.36	

The current results revealed that there was no significant differences ($P > 0.05$) in mean value of clinical periodontal parameters in patients with sufficient VD compared to patients with insufficient VD. The mean value of PLI, GI, BOP, PPD and CAL in patients with sufficient VD were $(1.80 \pm 0.15, 2.13 \pm 0.09, 36.20 \pm 4.62, 3.95 \pm 0.37$ and $4.77 \pm 0.68)$, and for patients with insufficient VD were $(1.85 \pm 0.11, 2.04 \pm 0.10, 38.18 \pm 3.79, 4.15 \pm 1.53$ and $5.44 \pm 0.62)$ respectively, as shown in Table (2).

Table 2: The distribution of clinical periodontal parameters in two patient groups

Periodontal parameters	Patients groups		
	Vitamin D sufficient group N=28	Vitamin D insufficient group N=22	T-test P-value
PLI	1.80±0.15	1.85±0.11	0.394 ^{NS}
GI	2.13±0.09	2.04±0.10	0.279 ^{NS}
BOP	36.20±4.62	38.18±3.79	0.371 ^{NS}
PD	3.95±0.37	4.15±1.53	0.304 ^{NS}
CAL	4.77± 0.68	5.44± 0.62	0.235 ^{NS}

Another important result in this study that there was significant difference ($P<0.01$) in serum level of LL-37 between two groups of patients, the mean value of LL-37 in sufficient group and in insufficient group was (48.53 ± 7.58 ng/ml and 42.70 ± 13.09 ng/ml) respectively, as shown in Table (3). Similarly, the results showed that there was significant difference ($P<0.01$) in serum level of HBD-1 between two patients groups. The mean value of HBD-1 in sufficient group was (612.07 ± 202.88 pg/ml), while in insufficient group was (486.19 ± 190.56 pg/ml) , as observed in Table (4).

Table 3: Comparison of the levels of serum LL-37 (ng/ml) in patient groups according to Vitamin D Sufficiency

Serum LL-37 (ng/ml))	Patients groups		
	Vitamin D sufficient group N=28	Vitamin D insufficient group N=22	T-test P-value
Minimum	32.97	28.74	0.033*
Maximum	57.90	89.34	
Mean	48.53	42.70	
SD	7.58	13.09	

Table 4: Comparison of the levels of serum HBD-1 (pg/ml) in patient groups according to vitamin D sufficiency

Serum HBD-1 (pg/ml))	Patients groups		
	Vitamin D sufficient group N=28	Vitamin D insufficient group N=22	T-test P-value
Minimum	110.10	82.17	0.014*
Maximum	971.92	841.46	
Mean	612.07	486.19	
SD	202.88	190.56	

3.2 Discussion

This study was revealed that the mean value of VD decreased in the insufficient VD patients as compared to the sufficient VD patients. Low serum concentrations of 25(OH)D are substantially related with periodontal disease, according to Laky and colleagues (2017), suggesting that insufficient VD levels may be implicated in periodontal disease progression. 1,25(OH)₂D has been demonstrated to potently down-regulate expression of monocyte TLR2 and TLR4, inhibiting inflammatory responses that are normally activated by these receptors, suggesting that VD may provide feedback modulation of immune activation pathways. VD may help to stimulate proper innate immune responses while reducing over-shooting and tissue damage, both of which are usually linked with this (Hewison, 2010).

Also the current data revealed that there was no significant difference in mean value of clinical periodontal parameters in patients with sufficient VD compared to patients with insufficient VD. This finding was in partial agreement with Bhargava and colleagues (2019) who noted no relationship between VD and PLI levels. Moreover, Finnish adults were shown to have no association between the severity of periodontitis (CAL and PPD) and VD levels (Antonoglou et al., 2015). This lack of association might be attributed to variations in the population characteristics, including the population's genetic profile, smoking, and the overall levels of VD (Deng et al., 2011; Lee et al., 2015). However this result was in disagreement with other recent study reported by Isola and colleagues (2021) who found that serum VD was negatively correlated with the levels of PLI, BOP, PPD and CAL. This study found that there was significant decrease in serum level of LL-37 and HBD-1 in periodontitis patients with insufficient VD as compared to sufficient group. This finding supports the idea that VD plays a role in the control of these peptides. Wang et al. (2004) found that VD induces gene expression of cathelicidin and defensin that play a role in primary defense against infections (Wang et al., 2004). These proteins are also expressed by epithelial cells in the gingiva (Dale and Fredericks, 2005; Greer, et al., 2013) and play a role in the first defense against bacteria by reducing the proportion of bacteria in the mouth. The presence VD response element on promoter regions of cathelicidin and defensin supports the contribution of VD in the regulation of these peptides (Wang et al., 2004). Furthermore, Bayirli et al. found that serum 25(OH)D deficiency is associated with decreased HBD-2 and LL-37 expression of GCF and gingival tissues in both gingivitis and periodontitis patients and suggested that the response to bacterial challenge through AMPs in periodontal tissues may be hampered by low VD levels. The lower level of AMPs in insufficient group may cause the failure to respond properly to invading pathogens in periodontal diseases (Bayirli et al., 2020). Besides the role of AMPs as antibiotic peptides, they provide a link between innate and adaptive immunity, act as modulators of inflammation and have a role in LPS neutralization (Lee et al., 2010; Walters et al., 2010). The lower expression may hamper the LPS' neutralization capacity, which in turn may aggravate the extent of inflammation (Auvynet and Rosenstein, 2009).

Conclusions

Vitamin D deficiency significantly associated with periodontitis. The assessment of VD levels in patients with periodontal disease seems advisable, as VD deficiency might be involved in the disease onset and progression. Moreover, low levels of LL-37, and HBD-1 in periodontitis patients with insufficient VD, implying that vitamin D plays a role in AMPs production.

Ethical Approval

This study was approved by the Ethical Committee of the College of Dentistry/ University of Baghdad.

Conflict of interest: None

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