ORIGINAL RESEARCH

Assessment of the Antibacterial Effect of Vitamin D3 against Red Complex Periodontal Pathogens: A Microbiological Assay

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Abstract

Aim: The study aims is to evaluate the antibacterial effect of vitamin D3 against the *red complex bacteria*, *Porphyromonas gingivalis, Treponema denticola, Tannerella forsythia* in chronic periodontitis patients.

Materials and methods: The study comprised 98 participants with chronic periodontitis. All clinical parameters including plaque index (PI), gingival bleeding index (GBI), probing pocket depth (PPD), clinical attachment level (CAL), and a microbiological assay of *P. gingivalis, T. denticola, T. forsythia* were assessed at the baseline. All study participants who underwent scaling and root planning were divided into two groups, A and B, each with 49 patients and only group B patients were advised to take vitamin D supplementation of 60,000 IU granules, once daily for 2 months. All the patients of both the groups were recalled at the end of 2nd month and all the clinical and microbiological parameters were reassessed.

Results: After two months, there was a reduction in all the clinical markers in both groups, but the group B patients showed more improvement following non-surgical treatment vitamin D intake. There was also a statistical reduction in *P. gingivalis, T. denticola,* and *T. forsythia* following administration of vitamin D in group B patients compared to group A.

Conclusion: These discoveries proposed that vitamin D has a superb antimicrobial impact against red complex periodontal microbes and might be considered a promising compound in the counteraction of periodontal disease.

Clinical significance: Vitamin D is considered to possess anti-inflammatory and antimicrobial activity, which may help to delay the progression of periodontitis. So, vitamin D3 can be used as a potential supplement that could be employed to stop the advancement of periodontal disease.

Keywords: Chronic periodontitis, Polymerase chain reaction, Red complex, Vitamin D.

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Introduction

An inflammatory condition known as periodontitis damages the tissues that support the teeth. It is brought on by specific microorganisms and affects the periodontal ligament and alveolar bone, causing pockets to form, gingival bleeding, pathological tooth movement, abscesses, and ultimately tooth loss.[1–2]

Periodontitis is brought on by a dysbiosis of the oral microbiota and an inappropriate immune response in periodontal tissues.[3] According to Socransky et al., the red complex, comprising *Porphyromonas gingivalis, Tannerella forsythia,* and *Treponema denticola complex*, constitutes the majority of the periodontal microbes.[4] Due to their ability to create a variety of trypsin-like enzymes that destroy the intercellular matrix of periodontal tissues, these *red-complex bacteria* have become the most significant periodontal pathogens in the progression of periodontal disease.[5]

The identification of bacteria in oral biofilm is a difficult task due to the large number of resident species. Identification methods include microscopy which can only distinguish morphotype and cultural technique, which identifies specific species that can be grown. Molecular techniques such as polymerase chain reaction (PCR) can identify a wide range of species even in a low number of samples. However, PCR only provides the presence or absence of data while real-time PCR is quantitative. Micro assay and checkerboard DNA-DNA hybridization are the two other techniques that come closest to the ideal attributes of a bacterial identification technique.[6]

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Periodontitis is generally treated by periodontal therapy without surgery, like scaling and root planning aiming to eliminate

pathogens associated with the disease and attain periodontal health.

The potential to investigate the effects of adjunctive usage of host modulation treatment together with mechanical periodontal therapy has arisen as a result of the understanding of the significant role of the host in the etiology of periodontitis and its response to therapy.[7]

The vitamin D active metabolite 1, 25 dihydroxy suppresses cytokine production by simultaneously boosting the innate immune system and reducing the over activation of the adaptive immune system in response to increased viral load. Vitamin D also affects the pathogenesis of periodontal diseases (PD) via immunomodulation, increases bone mineral density (BMD), reduces bone resorption, and is important in fighting against agents that cause PD.

A fat-soluble steroid hormone called vitamin D interacts with the vitamin D receptor (VDR), a member of the transcriptional regulatory factor family, to control a variety of biological processes, including immune response control and bone metabolism.

Variations in the gene for the VDR alter the subgingival microbiota's makeup, influencing the persistence of pathogenic bacteria and raising the risk of periodontitis. Adequate vitamin D levels enhance jawbone bone density, alveolar bone resorption prevention, and periodontal health.[8]

Periodontal disease is in danger from a vitamin D deficit, which satisfies Hill's requirements for a biological system's causation. Additionally, the possibility of causality is supported by the fact that hypovitaminosis D has been shown to bypass host defense mechanisms after bacterial infection and to accelerate tissue degradation, bone loss, and bone loss through known pathways.[9]

The evidence is unclear as to how serum vitamin D3 levels affect periodontal health.[10–12] At this time, the evidence showing a link between low vitamin D3 levels and periodontitis is either "inconclusive" or does not show a direct link between vitamin D3 insufficiency and an eventual higher risk of developing periodontitis.[13,14] Therefore, clinical trials are required to assess the effect of the blood levels of vitamin D3 on periodontal pathogens in patients with chronic PD.

The study aims to evaluate the antibacterial effect of vitamin D3 against the *red complex bacteria, P. gingivalis, T. denticola, T. forsythia* in chronic periodontitis patients.

Materials and Methods

The present study was conducted in the Department of Periodontology, Rajah Muthiah Dental College and Hospital, Annamalai University between 2020 and 2021. Systemically healthy chronic periodontitis patients between the age of 30 and 55 years with serum vitamin D levels <30 ng/mL were selected for the study. Female subjects and any patients with adverse habits, drugs, systemic factors that would influence the outcome of the study, and patients who had undergone periodontal treatment 6 months before the onset of the study were excluded.

Considering the inclusion and exclusion standards, 98 patients with chronic periodontitis were included in the clinical investigation. The study was approved by the Institute Ethical Committee before the start of the study. The nature and design of the clinical study were explained and informed consent was obtained from all the participants.

A single investigator measured the clinical parameters including plaque index (PI), gingival bleeding index (GBI), probing pocket depth (PPD), clinical attachment level (CAL), and sub-gingival plaque samples was collected from a deepest periodontal site with the help of a curette (Hu-Friedy). The plaque samples were then immediately transferred into a sterile Eppendorf tube containing phosphate buffer saline and stored at –40°C for further molecular analysis.

Table 1: Baseline inter-group comparison at different time points of evaluation

	N	*Mean*	*Standard deviation*	*p-value*
Plaque index				
Group A	49	3.595	0.396	0.001
Group B	49	3.617	0.375	
Gingival bleeding index				
Group A	49	3.000	0.738	0.001
Group B	49	4.044	0.705	
Probing pocket depth				
Group A	49	6.666	0.603	0.001
Group B	49	6.711	0.588	
Clinical attachment level				
Group A	49	5.822	2.249	0.001
Group B	49	6.666	2.354	

Plaque samples were analyzed for red complex microorganisms (*P. gingivalis, T. forsythia, T. denticola*) using real time-polymerization chain reaction (RT-PCR). Gene-specific oligonucleotide primers were commercially obtained from Synergy Scientific Services for *P. gingivalis, T. denticola*, and *T. forsythia*. The primers used were selected by the method of Becerik et al.[15]

Following the initial periodontal clinical examination, SRP was completed for all the patients enrolled in the study and divided into two groups of 49 each, group A and group B. Vitamin D supplementation of 60,000 IU granules, Cipcal D3, which is a commercial product available in the form of sachets, was supplied to only group B patients and asked to take once a week for 2 months. All the patients were present for the follow-up and at the conclusion of the second month following interventions, all the clinical and microbiological parameters were reviewed for patients in both groups.

Statistical Analysis

Data were analyzed using the Statistical Package for the Social Sciences software version 20.0 (IBM, Armonk, NY). Baseline parameters were compared using a *t*-test and 2 × 2 repeated measures ANOVA test were used to find the effectiveness of vitamin D supplementation.

Results

In the present study, 98 chronic periodontitis patients with a mean age of 41.74 were included in the study sample. At the conclusion of the second month, group A and group B's mean plaque indices decreased from their initial values of 3.59 and 3.61 to 1.42 and 1.43, respectively. The gingival index was also reduced in both groups after 2 months, from 3.00 to 1.39 in group A and from 4.04 to 1.94 in group B. The results also showed a mean decrease in both the PPD and CAL in both groups with a significant *p*-value of less than 0.001 (Tables 1 and 2).

Following the administration of vitamin D supplements, the mean *P. gingivalis* score was reduced more in group B from 25.4 to 16.7 than in group A (Table 3). The mean Treponema Denticola

Table 2: Two months-inter-group comparison at different time points of evaluation

	N	*Mean*	*Standard deviation*	*p-value*
Plaque index				
Group A	49	1.421	0.345	0.001
Group B	49	1.436	0.285	
Gingival bleeding index				
Group A	49	1.391	0.566	0.001
Group B	49	1.945	0.766	
Probing pocket depth				
Group A	49	4.457	0.897	0.001
Group B	49	4.511	0.293	
Clinical attachment level				
Group A	49	4.765	2.594	0.001
Group B	49	4.564	2.198	

Table 3: Inter-group comparison of the prevalence of *Porphyromonas gingivalis* by assessment wise and groupwise

	Group A			*Group B*		
Assessment	*Mean*	*SD*	*p-value*	*Mean*	*SD*	*p-value*
Base line	24.2	1.5	0.001	25.4	1.17	0.001
2nd month	19.5	1.4		16.7	1.2	

Table 4: Inter-group comparison of the prevalence of *Treponema denticola* by assessment wise and groupwise

	Group A			*Group B*		
Assessment	*Mean*	*SD*	*p-value*	*Mean*	*SD*	*p-value*
Base line	23.3	1.2	0.001	22.1	1.2	0.001
2nd month	20.08	1.1		18.7	1.0	

Table 5: Inter-group comparison of the prevalence of *Tannerella forsythia* by assessment wise and groupwise

	Group A			*Group B*		
Assessment	*Mean*	*SD*	*p-value*	*Mean*	*SD*	*p-value*
Base line	23.3	1.5	0.001	20.2	1.7	0.001
2nd month	19.2	1.3		17.5	1.2	

score was reduced more in group B from 22.1 to 18.7 than in group A (Table 4). The mean score reduction of *Tannerella forsythia* was more in group B from 20.2 to 17.5, following the administration of vitamin D supplements (Table 5).

Group B patients demonstrated a reduction in microbiological components and an improvement in all clinical parameters after receiving vitamin D.

Discussion

In the current study, an interventional periodontal therapy in the form of SRP was carried out for all the patients enrolled in the study as SRP remains the gold standard form of periodontal management. A decrease in the plaque score, which is statistically significant, percentage of gingival bleeding sites, PPD, and significant gain in CAL at the end of 2nd month following SRP were observed.

The present findings were consistent with research by Mohan et al.[16] that evaluated the individuals with persistent periodontitis who may benefit from scaling and root planning and showed that SRP improved periodontal clinical indicators in a statistically meaningful way. Similar studies done by Cugini et al.,[17] Haffajee et al.,[18] and Praveen et al.[19] also found clinical markers for periodontal health have significantly improved following SRP.

Real time-polymerization chain reaction was used in the present investigation to determine the prevalence and distribution of red complex in plaque samples. Polymerase chain reaction has rapidly become one of the most widely used techniques in molecular biology and for good reason: It is a rapid, inexpensive, and simple means of producing large numbers of copies of DNA molecules from minute quantities of source DNA material. Real time-polymerization chain reaction has added advantages which include ease of quantification, greater sensitivity, reproducibility and precision, rapid analysis, better control of quality in the process, and a lower risk of contamination.

A statistically significant reduction in the prevalence of species of the red complex was noted following SRP in both the groups', thus establishing SRP was effective in reducing the prevalence of *P. gingivalis, T. forsythia*, and *T. denticola* levels and bacterial bio load. According to a study by Cugini et al.,[17] scaling and root planing's effects on the microbiome resulted in a considerable decrease in *P. gingivalis, T. forsythia*, and *T. denticola* following SRP. Similarly, studies done by Haffajee et al.,[18] Zijnge et al.[20] also stated a significant reduction in microbial load following SRP.

A drastic and statistically significant narrowing of the distribution of prevalence of red complex was observed following vitamin D supplementation in group B patients. This finding was well supported by the studies by Tang et al.[21] and McMahon et al.[22]

The findings of this investigation demonstrated that red-complex bacteria's typical cell shape may be changed by the vitamin D3 cholecalciferol and that this modification could also prevent the bacteria from growing normally. As a result, it implies that the vitamin D3 cholecalciferol, which is completely independent of its hormonal effects, directly combats these microbes. Studies have shown that the vitamin D3 prevents the recolonization of red complex pathogens thus preventing the destruction of the periodontal structures.[23,24]

To date, the antibacterial effects of cholecalciferol vitamin D3 against *P. gingivalis, T. denticola*, and *T. forsythia* have not yet been studied. To assess vitamin D3's impact on the ultrastructure of bacterial membranes, more research is required.

Limitations

One of the limitations is that longitudinal studies with various vitamin D regimens should also be attempted to validate the results of the current study.

Conclusion

The result of the study concludes that vitamin D3 has antibacterial effects and can be used as an adjunctive supplement to stop the progression of periodontal disease. Further studies should be conducted to clarify the mechanism underlying vitamin D3's antibacterial action on red complex oral bacteria.

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