

Original Research Article

Reinforcement of epithelial barrier function by vitamin D induction of the antimicrobial peptide cathelicidin. A novel therapeutic approach in Chronic periodontitis.

Abstract:

Introduction: Oral epithelium forms a physical, chemical and immunological barrier against the invading microorganisms. The chemical barrier mainly comprises of anti-microbial peptides(AMPs) like cathelicidins which are endogenously produced antibiotics. Transcription of the cathelicidin anti-microbial peptide(CAMP) gene is induced by binding of the bioactive form of vitamin D to the vitamin D receptor. Thus, an increase in vitamin D should significantly improve the chemical barrier function by increasing AMPs.

Comment [MF1]: You should leave a space between the words and abbreviations almost in all the manuscript

Through a randomized controlled trial we tend to explore a well accepted molecule like vitamin D in primary prevention of chronic periodontitis by reinforcing the epithelial barrier by increased action of cathelicidin.

Aim of the study:

The aim of this study was to assess the effect of vitamin D administration on levels of cathelicidin LL-37 in gingival crevicular fluid of chronic periodontitis patients.

Comment [MF2]: Mention rote of administration 0Topical or systemic??)

Methodology: 40 vitamin D insufficient subjects with chronic periodontitis were divided into 2 groups. Gingival crevicular fluid(GCF) sampling was done a day

after the periodontal examination. Next all patients were subjected to scaling and root planning(SRP). Test group patients were prescribed oral dose of 2000IU Vitamin D daily for a period of 3months whereas control group was devoid of any medication. GCF sampling was repeated for both the groups after three months. Cathelicidin LL-37 level was estimated using Enzyme Linked Immunosorbent Assay(ELISA)method for both the groups.

Results: Vitamin D administration increases the levels of cathelicidin in periodontal diseases. Cathelicidin levels in GCF increase after Vitamin D intake.

Conclusion and Acknowledgement: Periodontal diseases might be prevented by reinforcing the epithelial barrier (chemical barrier) by vitamin D induced production of AMPs.

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Keywords: Chronic periodontitis, Cathelicidin, epithelial barrier.

Chronic periodontitis is a polymicrobial inflammatory disease of multifactorial origin. It is initiated by damage of the gingival epithelium which provides the first line of defence from the microorganisms present in dental plaque. Untreated periodontitis progressively results in tooth mortality which has significant social and economic burden on the patients.

Traditionally, only antimicrobials have been used as the chemotherapeutic modality for the treatment of periodontitis. The extent and severity of tissue destruction seen in periodontitis is determined by the host immuno-inflammatory

Comment [MF3]: Does vit D administration increases the level of cathelicidin in periodontal diseases how about patients with no periodontitis? You don't have negative control group in your study (Patients with no periodontitis)

Comment [MF4]: In conclusion section you can't predict and you only conclude the outcome of your result that's all – there should be no might either negative or positive results
Then why you mix up the conclusion section and acknowledgment section?

Comment [MF5]: Better to use the word against not from

Comment [MF6]: You should mention" as an adjunct to mechanical debridement" because antibiotics should not be used alone it is useless

response to the bacteria. The increasing awareness and knowledge of the host-microbial interaction in periodontal pathogenesis has presented the opportunity for exploring new therapeutic strategies for periodontitis by means of targeting host response in addition to conventional approaches in the management of periodontal diseases.

With shifting paradigms in healthcare facilities from cure to prevention much emphasis has been laid upon prevention of disease process at the primary level. Oral epithelium forms a physical, chemical and immunological barrier against the invading microorganisms. The chemical barrier mainly comprises of antimicrobial peptides (AMPs) which are endogenously produced antibiotics. Cathelicidins are the most potent AMPs detected in the oral cavity.¹ The link between upscaling of AMP function and vitamin D is well established.² Thus, an increase in vitamin D should significantly improve the chemical barrier function by increasing AMPs.

This study intends to explore an economically viable, easily available and a well accepted molecule like vitamin D in primary prevention of chronic periodontitis by reinforcing the epithelial barrier through increased action of cathelicidin.

AIM OF THE STUDY:

To assess the effect of vitamin D administration on levels of cathelicidin LL-37 in gingival crevicular fluid of chronic periodontitis patients.

OBJECTIVES OF THE STUDY:

1. To study the effect of Vitamin D on levels of Cathelicidin LL-37 in periodontal diseases.

Comment [MF7]: You should add a paragraph on GCF and its utilization for diagnosis and management of periodontal disease through the markers that are found in GCF because it is your main source of examination in this study

Comment [MF8]: You don't need this paragraph the first two points are repetitions for the aim of the study you don't have to move around besides you don't have negative control group (with no periodontitis) to make it sure is it increasing the level of the cathelicidin LL-37 in only periodontitis or even in normal patients without periodontitis?

2. To estimate the levels of cathelicidin LL-37 in GCF in chronic periodontitis patients before and after administration of Vitamin D.
3. To establish an association between Vitamin D, oral epithelium(chemical barrier) and anti microbial peptides in prevention of periodontal diseases.

Comment [MF9]: This is also not included in your methodology, neither in your study so why you put it as an objective

In objective section you can add two points related to your examination and test in this study for example

- 1-Measuring the clinical periodontal parameters
 - a) Bleeding on probing
 - b) Probing pocket depth o
 - c) Clinical attachment loss
- 2- ELISA test performed for the level of Cath. LL-37 in the GCF

Methodology

Source of Data :

Chronic periodontitis patients satisfying the inclusion criteria of the study were screened from the O.P.D of Sri Rajiv Gandhi College of Dental Sciences(SRGCDS) and hospital, Bangalore. All the patients were subjected for the estimation of Vitamin D levels in the serum. Only the patients who were found to be Vitamin D insufficient according to Vitamin D council and Indian Endocrine society were recruited for the study. The study group consisted of subjects belonging to both sexes. Written informed consent was obtained from all patients.

Inclusion Criteria :

1. Study subjects >25 years of age.
2. Patients suffering from chronic periodontitis i.e.
 - a) Bleeding on probing
 - b) Probing pocket depth of ≥ 5 mm
 - c) Clinical attachment loss of ≥ 3 mm

3. Vitamin D insufficient patients (serum levels <30ng/ml) according to vitamin D council.
4. Systemically healthy individuals with minimum 20 teeth in occlusion.

Exclusion Criteria:

1. Patients with systemic diseases that could impair immune response such as diabetes mellitus, HIV, immunological disorder and hepatitis.
2. Patients on any systemic antibiotics, NSAIDS, steroid therapy and any other drugs which are known to affect the periodontal status.
3. Smokers and former smokers were excluded from the study.
4. Patients who had received periodontal treatment in the preceding year or who had taken antibiotics within the previous 3 months.
5. Pregnant or lactating women and those taking oral contraceptive drugs.

Methods of Data collection:

The whole-mouth clinical periodontal parameters, including probing depth (PD), clinical attachment level (CAL), and bleeding on probing (BOP), were determined at six sites per tooth (mesio-buccal, mid-buccal, disto-buccal, mesio-lingual, mid-lingual, and disto-lingual), excluding third molars in all patients.

The diagnosis of periodontitis was based on clinical criteria proposed by the 1999 International Workshop for Classification of Periodontal Diseases and Conditions. The subjects selected for the study were randomized by coin toss method and allocated to two groups the test and the control .

Study Design

This was a single-center randomized controlled trial for an intervention period of three months(CONSORT flow diagram attached).

Comment [MF10]: Leave space

40 vitamin D insufficient subjects with chronic periodontitis were divided into two groups:

Group I - Test group: 20 Chronic periodontitis patients who were prescribed Vitamin D orally as an adjunct to SRP.

Group II – Control group:20 chronic periodontitis patients with SRP only.

Comment [MF11]: Leave space

The procedures followed were in accordance with the ethical standards of the Institutional Ethical Committee(IEC) on human experimentation.

Comment [MF12]: Same leave space in between

Patients were examined through the course of the study by the same examiner to the allocated group. Measurements of PD and CAL was performed with a Williams periodontal probe. All measurements were performed by a calibrated examiner. The intra examiner reliability was high as revealed by the intraclass correlation coefficients of 0.87 and 0.85 for PD and CAL, respectively.

Check for intraexaminer reliability: Assessments were carried out using Williams periodontal probe, measuring probing depth and CAL on 6 surfaces on all teeth of the same patient(not participating in the study). Two assessments were carried out, the second 2 hours after the first, and both the sets of measurements were in excellent agreement.

Comment [MF13]: Mention the level of reliability obtained % for intra examiner calibration

GCF Sampling :

GCF sampling was performed a day after the periodontal examination. Sampling sites were selected from the buccal aspects of the mesial or distal surfaces at the interproximal sites of the teeth. Gingival crevicular fluid collection was done from

sites having probing depth ≥ 5 mm and clinical attachment loss ≥ 3 mm. Supragingival plaque was removed from the interproximal surfaces with a sterile curette; these surfaces were dried gently by an air syringe and were isolated by cotton rolls before GCF sampling. 3 μ l GCF was collected by extra crevicular method using micro-capillary pipettes. The crevicular fluid was obtained as a pooled sample from the deepest site in each quadrant, transferred into a microcentrifuge tube, frozen and stored at -70° C until analyzed.

Comment [MF14]: It is better to use recording not having

After GCF collection all patients were subjected to SRP. Test group patients were prescribed oral dose of 2000IU vitamin D daily for a period of 3 months and control group were not administered any medication. GCF sampling was repeated after three months. Cathelicidin LL-37 level was estimated using enzyme linked immunosorbent assay (ELISA) method. Values obtained from the test were recorded, computed and subjected to statistical analysis.

Data analysis and Results:

Data were expressed as means and standard deviation (SD). Full mouth probing depths, clinical attachment level, bleeding on probing, and the plaque record index were recorded to characterize participants included in this study. Furthermore, the means (\pm SD) of cathelicidin levels were calculated for those sites from which GCF samples were collected. All data were entered into the SPSS 13.0 program and were analyzed using the paired t-test. The level of significance was set at $P < 0.05$ with 95% confidence intervals.

The distribution of the total amount of GCF cathelicidin LL-37 in the study groups is shown in Figure 1. Significant differences were found among the study groups ($P < 0.05$). GCF hCAP18 concentrations strongly correlated with serum 25(OH)D concentrations in subjects with 25(OH)D levels ≤ 32 ng/ml i.e. vitamin D insufficient subjects.

In group 1, cathelicidin levels in gingival crevicular fluid was found to increase post administration of vitamin D. The change in mean Cathelicidine LL-37 value from pre to post treatment was found to be statistically significant ($P < 0.01$).

Discussion

The oral epithelial barrier i.e the gingival epithelium acts as a physical barrier to separate the biofilm from the gingival tissue, providing the first line of defense against bacterial invasion in periodontal disease. Disruption of the gingival epithelial barrier, and the subsequent penetration of exogenous pathogens into the host tissues, triggers an inflammatory response, establishing chronic infection. Research has proven that IL-31 produced by mast cells in response to *P. gingivalis* infection causes gingival epithelial barrier dysfunction, which may contribute to the chronic inflammation observed in periodontitis.³

The discovery of antimicrobial peptides in the mammalian epidermis demonstrates a mechanism by which microbial growth can be controlled in the first hours following epithelial injury, during the wound healing process, and in the face of ongoing inflammation. Thus, they provide a powerful defense system that can both protect the skin from infection and signal host cells to change their behavior in response to injury.

Antimicrobial peptides (AMPs) are considered a rapid and first-line response of the innate immune system to microbial pathogens. Together with their antimicrobial effects, AMPs also exert immunomodulatory effects by inducing cell migration, proliferation, and differentiation, regulating cytokine/chemokine production, improving angiogenesis and wound healing, and sustaining the barrier function of the skin.^{4,5} Interestingly, various studies have demonstrated that AMPs, such as

cathelicidin LL-37, human-defensin (hBD)-1, hBD-3, and S100A7 protein, increase the levels of TJ-related proteins and promote epidermal barrier function.⁶⁻⁹

Comment [MF15]: All these paragraphs should be removed from discussion section or should be moved to introduction section
The discussion section should be for discussion of your results not an introduction section

In this study, we highlight the association between oral epithelial barrier dysfunction in patients with chronic periodontitis and vitamin D derived AMPs to consider employing these peptides in barrier repair strategies as an additional therapeutic approach for CP.

It is estimated that 1 billion people worldwide have vitamin D deficiency or insufficiency.¹⁰ Although the beneficial effects of calcium and vitamin D supplementation on bone health have been well recognized,^{11,12} their potential role in periodontal disease has not been fully determined. A number of studies¹³⁻¹⁶ suggested that vitamin D and/or calcium intake results in reduced alveolar bone loss, gingival inflammation, and/or attachment loss.

Vitamin D plays an important role in calcium homeostasis, promoting calcium absorption in the intestine and stimulating osteoblasts to enable normal bone growth and preservation. Besides its role in bone and calcium homeostasis, the biologically active form of vitamin D, 1 α ,25-dihydroxyvitamin, has been demonstrated to function as an immunomodulator because of its anti-inflammatory effect through inhibition of cytokine production by immune cells and stimulation of monocytes and macrophages to secrete peptides with potent antibiotic activity.¹⁷

This effect of vitamin D has been linked to susceptibility to bacterial-mediated infections, with low levels of vitamin D being associated with increased risk of infectious diseases.¹⁸ Therefore, vitamin D may be beneficial for the treatment of periodontal disease.

Although most epidemiologic studies have found beneficial effects of vitamin D and calcium supplementation, the use of oral supplementation remains low and

varies greatly. With the discovery that bio-active forms of vitamin D induce the expression of the CAMP gene, it has been hypothesized that vitamin D status may affect the levels of circulating hCAP18.^{19,20}

Bhan and colleagues discovered a positive correlation in healthy individuals (mean age 39) at 25(OH)D levels <32 ng/ml, but not when levels were higher.²¹

In a study by Alvarez-Rodriguez and colleagues of 71 healthy individuals with about two-thirds of participants below 32 ng/ml serum 25(OH)D, a positive correlation between serum 25(OH) D and LL-37 levels was observed.²² On the other hand, no relationship between maternal 25(OH) D serum levels and LL-37 were detected in cord-blood samples²³ or in patients with active pulmonary tuberculosis.²⁴

Given the nature of the relationship between serum 25(OH)D and hCAP18/LL-37 levels, it is possible that vitamin D supplementation or exposure to sunlight to synthesize vitamin D may provide a means to raise systemic levels of hCAP18/LL-37, thus enhancing protection against infection and/or sepsis. In support of this, in vivo supplementation of individuals with serum 25(OH)D levels <32 ng/ml resulted in an increase in hCAP18 levels in those individuals showing the greatest increase in serum 25(OH)D.²⁵ In another study, supplementation of normal and atopic dermatitis (AD) patients with 4,000 IU oral vitamin D3 (cholecalciferol) for 21 days resulted in a statistically significant increase in cathelicidin expression in the AD lesions.²⁶

The present study demonstrated that GCF levels of cathelicidin LL-37 were significantly elevated in patients with chronic periodontitis who were administered vitamin D. The presence of cathelicidin LL-37 in GCF suggests an important role for this antimicrobial peptide in the pathogenesis of chronic periodontitis. A limited

number of studies^{27,28,29} have investigated cathelicidin LL-37 levels in the GCF or gingival tissues of patients with periodontal disease. In this study we have tried to elicit the GCF cathelicidin levels in chronic periodontitis patients after administration of vitamin D supplements .

Vitamin D reduces the risk of infection through multiple mechanisms, boosts innate immunity by modulating production of anti-microbial peptides (AMPs). Antibiotics remain an expensive option and misuse of these agents results in significant antibiotic resistance. Vitamin D constitutes an inexpensive prophylactic option and possibly therapeutic product either by itself or as a synergistic agent to traditional antimicrobial agents.

Calcium and vitamin D are important adjuncts to standard treatments for preventing and treating periodontal disease. Vitamin D supplementation (1,000 IU/day) had a modest positive effect on periodontal health.³⁰ In subjects receiving periodontal maintenance therapy, there was a trend for better periodontal health with vitamin D and calcium supplementation.³¹

Our results suggest that Vitamin D administration increases the levels of cathelicidin in periodontal diseases. Cathelicidin levels in GCF increase after Vitamin D intake and periodontal diseases might be prevented by reinforcing the epithelial barrier (chemical barrier) by vitamin D induced production of AMPs. It was not determined if supplementation improved immunologic outcomes in these studies; therefore, future randomized controlled trials are needed to establish an immune enhancing role for vitamin D supplementation and to determine optimal circulating levels. Future work will determine the validity of such speculation and establish the utility of these peptides in treatment of skin disease.

Conclusion

Progressive research on cathelicidin molecule with emphasis on the functional properties and its role in host defense will help the investigators to reveal its multifunctional nature that may mediate various host responses, and thus represents an essential component of the innate immune system in humans.

The cathelicidin gene carries a vitamin D response element and the vitamin D pathway could therefore be targeted for cathelicidin regulation. As the development and course of periodontal diseases might be influenced by vitamin D signaling these pathomechanisms could explain the growing evidence connecting vitamin D to periodontal diseases.

Although historically vitamin D has been associated with the regulation of musculoskeletal health recent research has indicated its cardiovascular, anti-cancer, immunomodulatory, and various systemic effects through VDR activation. The 1, 25(OH)₂D₃-VDR system plays a significant role in oral homeostasis and its dysfunction leads to periodontal disease.

Hence, Vitamin D research should make important contributions to the understanding of periodontal diseases and may benefit in the treatment due to its direct effect on bone metabolism and its anti-inflammatory properties. This research project is a step towards realizing the potential of Vitamin D as a therapeutic modality in management of periodontal diseases.

Limitations of the study

- Shorter duration of the study.
- Inability of some patients to turn up after treatment.
- Keeping a check on utilization of vitamin D tablets provided to patients.

Comment [MF16]: Limitations or critical appraisal of the study should be first paragraph of discussion section not here

Future Perspective

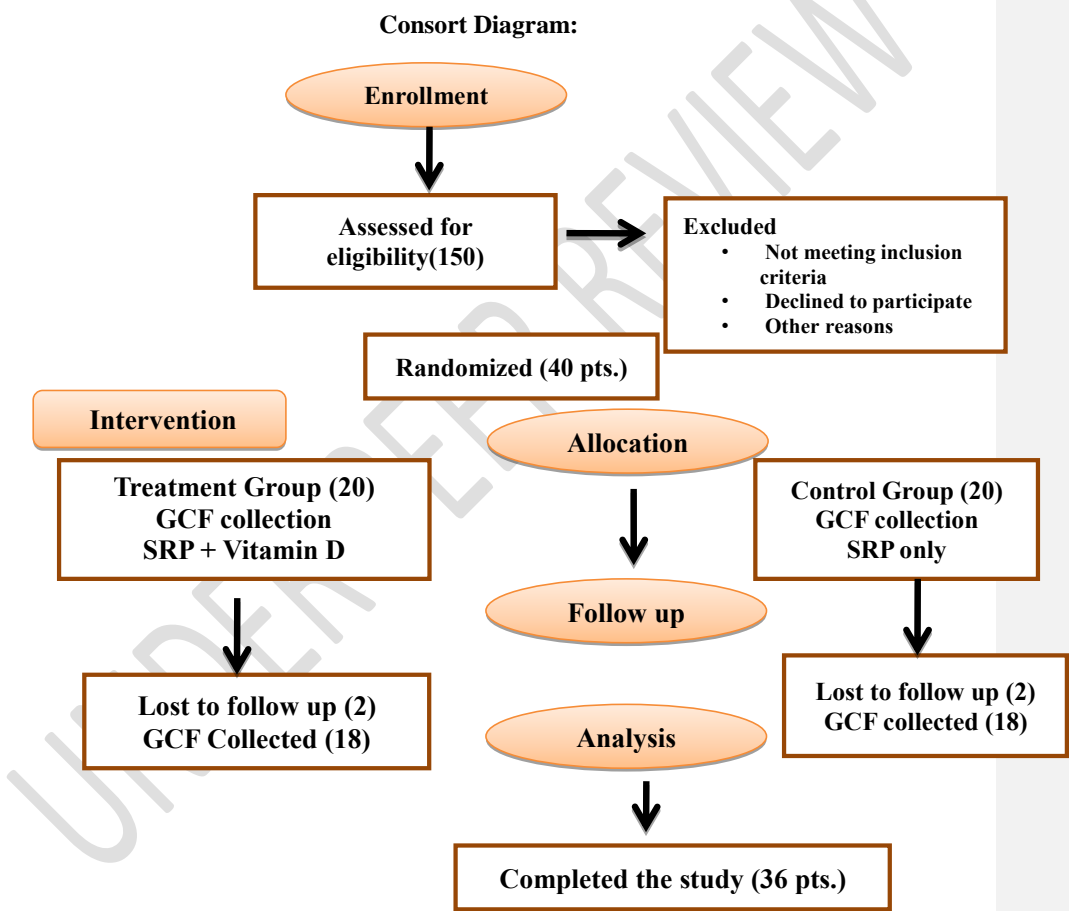
The future for the sunshine vitamin looks bright. Considering that most people have insufficient levels of vitamin D and that nearly 1 billion people worldwide are deficient, properly designed supplementation studies in humans will be important for determining the benefits from raising serum levels of vitamin D on immune system function.

It will be particularly interesting to determine if sufficient vitamin D levels will aid in treating patients with TB and HIV infection. These are classically deficient populations and vitamin D supplementation could be a potentially cheap adjuvant therapy for these conditions and is particularly attractive for impoverished countries where these diseases are rampant.

Vitamin D research could help in identifying new targets of the VDR through creative approaches involving animal models, human studies and genomic approaches. Many epithelial tissues such as the oral mucosa, intestinal tract, skin, urinary tract and reproductive organs are constantly exposed to the environment. Additional studies focusing on vitamin D–cathelicidin pathway should reveal just how important vitamin D is in barrier defense of the body.

Comment [MF17]: This should be the last part of the discussion section

Fig 1: Consort Diagram



References:

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Table 1: Mean Cathelicidine LL-37 Value Recorded in Group I Test Group: (PAIRED T-TEST)

Time Interval	Treatment	Mean	Std Dev	SE of Mean	Mean Difference	t	P-Value
Baseline	Pre Treatment	22.75	2.01	0.47	-4.112	-3.567	0.002*
3 months	Post Treatment	26.86	5.04	1.19			

Table 2 Mean Cathelicidine LL-37 Value Recorded in Group II Control Group: (PAIRED T-TEST)

Time Interval	Treatment	Mean	Std Dev	SE of Mean	Mean Difference	t	P-Value
Baseline	Pre Treatment	25.33	2.59	0.61	3.643	4.872	<0.001*
3 months	Post Treatment	21.69	2.32	0.55			