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Original Research

Evaluation of IL-6, vitamin D level in chronic periodontitis patients after non-surgical periodontal therapy

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Abstract

Background and aims: Vitamin D is hypothesized to prevent periodontal disease progression through immune modulation and regulation of systemic calcium. A multifunctional cytokine, interleukin (IL)-6, mediates tissue injury, infection, and bone resorption in periodontitis. The goal of Phase I therapy is to halt the disease progression. Hence, this study aims at correlating the serum level of vitamin D and IL-6 in patients with chronic periodontitis before and following periodontal non-surgical therapy. **Material and method:** A total of 60 patients were divided into two groups of 30 each, comprising healthy and chronic periodontitis patients, respectively. Clinical parameters were recorded at baseline and post-treatment. Serum samples were analyzed to estimate vitamin D levels and IL-6 levels. **Results:** At baseline, the mean serum IL-6 levels were significantly higher in group B (49.13±5.65) and, the serum vitamin D level indicated an insufficiency (20.62±5.76). However, following non-surgical periodontal therapy, the vitamin D level improved (31.76±8.12) and IL-6 level decreased markedly (40.16±7.88). In addition, there was an improvement in the clinical parameters. **Conclusions:** It can be concluded that there is an association between chronic periodontitis, vitamin D and IL-6 indicating their potential role as risk indicators for periodontal disease.

Keywords: periodontitis, nutrition, cytokines, scaling and root planning, vitamin D.

Background and aims

Periodontal disease is caused by a group of microorganisms that trigger local inflammatory reactions, resulting in bleeding on probing, periodontal attachment loss, bone loss, and tooth loss [1]. Periodontitis shares common pathophysiology of a chronic inflammatory state and a compromised immune system that is seen in various systemic diseases. Hence, it has been linked to various systemic diseases such as diabetes mellitus, cardiovascular diseases, respiratory diseases, rheumatoid arthritis, etc. [2, 3].

Cytokines mediate the differentiation of immune cells in the inflammatory site [4]. Cytokines such as interleukin (IL)-1α, IL-1β, IL-18, IL-33, IL-6, IL-10, tumor necrosis factor-α are found at the site of inflammation and they have a role in many biological processes like local and systemic inflammatory responses, hematopoiesis, and repair of periodontal tissues [5]. One of the pro-inflammatory cytokines, IL-6 plays an important part in the pathogenesis of the periodontal disease. Its production is stimulated by cytokines such as IL-1β, TNF-α, and is produced by a range of immune cells, including T- cells, B-cells, macrophages and dendritic cells. IL-6 causes B-cell differentiation as well as an increase in T-cell proliferation and bone resorption. Hence it can be used as an investigative marker for periodontal diseases [6].

Periodontitis is influenced by numerous risk factors like modifiable/non-modifiable (genetic, environmental-bacterial challenge,

stress) and behavioral/ lifestyle (e.g. nutrition, smoking, exercise) [7,8]. Nutrition affects the periodontium by contributing to the growth of bacteria in gingival crevices, affecting the immunological response to antigen and repair of periodontal tissues [9]. Vitamin D is termed as the 'sunshine vitamin' [10]. The current consensus by the Vitamin D council states, serum levels of 30 ng/ml is said to be sufficient, a level of 21–29 ng/ml implies relative insufficiency of vitamin D and a level of less than 20 ng/ml is said to be vitamin D deficiency [11]. A recent survey done by the vitamin D council has concluded that the deficiency of vitamin D is the utmost underrated health problem globally [12].

Vitamin D_3 is an active metabolite, 80% of which is produced by ultraviolet irradiation which is synthesized in the skin and the rest 20% is through dietary intake in the form of oily fish, fortified foods or supplements. In the liver, hydroxylation of vitamin D occurs to form 25(OH) D. In the kidney, with the help of an enzyme 1-hydroxylase, 25(OH) D is converted to 1, 25-dihydroxy vitamin D (1, $25(OH)_2D$) which is an active metabolite of vitamin D has a crucial part in bone and calcium metabolism. Additionally, vitamin D has got anti-inflammatory effect, by releasing cathelicidin and other defensins it results in obliteration of bacterial infection; reduction in tissue assembly of damaging matrix metalloproteinase which is strongly linked with periodontal disease [13]. Clinical manifestations of deficiencies of several vitamins include alveolar bone loss, bleeding gums, defective calcification, etc. [14]. The cornerstone of periodontal therapy is non-surgical periodontal therapy, which creates a beneficial microbial shift that must be maintained with frequent scaling and root planing during supportive periodontal therapy [15].

With this background, the purpose of the study was to evaluate and correlate the serum levels of IL-6, vitamin D and clinical parameters in chronic periodontitis patients at baseline and after non-surgical periodontal therapy. The null hypothesis of this study states that periodontal non-surgical therapy will not influence the serum levels of vitamin D and IL-6.

Material and method

Study design and patients

A total of 60 subjects were recruited from the routine OPD at the Department of Periodontics, Karnataka. Informed consent was obtained from all the participants after explaining the nature and purpose of the study. Institutional Ethical and Research clearance was obtained.The study comprised of sixty male and female subjects aged 30–60 years with a minimum complement of 20 teeth, gingival index less than 1.0 (Group A) and involvement of (>30% sites) clinical attachment loss (3–4mm) (Group B). They were divided into two groups i.e.

Group A (Control group) → 30 systemically healthy patients.

Group B (Study group) → 30 patients with moderate to severe chronic periodontitis.

Pregnant, lactating and menopausal women, periodontal therapy in the previous 6 months, smokers, subjects with systemic diseases, patients on medications and supplements were excluded.

Laboratory, anthropometric and clinical data collection

Clinical parameters such as full mouth gingival index (GI), plaque index (GI), clinical attachment level (CAL) and pocket probing depth (PD) and serum levels of vitamin D and IL-6 were recorded at baseline for both the groups. Non-surgical periodontal therapy was performed using ultrasonic and hand instruments. The patients were recalled for regular maintenance therapy and oral hygiene instructions were reinforced at each recall visit. The clinical parameters and serum levels of vitamin D and IL-6 were re-evaluated 3 months after completion of non-surgical therapy (Group B). The serum samples were assessed by drawing 3 ml venous blood samples from the median cubital vein between 9 and 11 am from the subjects after 20 minutes of rest to the subject. Serum vitamin D and IL-6 were estimated using ELISA KIT.

Statistical analysis

The sample size was calculated using the following formula: N=2 Sp2 (Z1-α/2+Z1-β)/-μ2. The descriptive statistics (mean and standard deviation) were calculated for continuous variables and the frequency and percentage for categorical variables. The unpaired t-test was used to compare the serum IL-6, vitamin D levels between the control and study group. The periodontal parameters between the control and study group were compared using Mann–Whitney's test as it was not following a normal distribution. Paired t-test was used to estimate the serum IL-6, vitamin D and periodontal parameters in chronic periodontitis before and after treatment.

Results

The levels of serum IL-6 and vitamin D before and following periodontal non-surgical therapy in both groups were evaluated. The mean serum IL-6 was higher (49.13±5.65) in group B before treatment compared to healthy patients (40.16±7.88). Whereas, the mean serum vitamin D (ng/ml) was lower (20.62±5.76) in group B before treatment compared to healthy patients (41.52±10.75) (Table 1). Both these values were statistically significant. The median GI, PI, PD and CAL were higher in group B before treatment compared to healthy patients (Table 2).

In group B, the mean serum IL-6 was higher (49.13±5.65) and it reduced significantly after treatment (40.16±7.88). The mean serum vitamin D was lower (20.62±5.76) in chronic periodontitis before and it increased significantly after treatment (31.76±8.12) (Table 3).

On comparing the clinical parameters (mean GI, PI, PD, and CAL) before and after treatment in group B, it was observed that the values were higher before treatment and it reduced after treatment (Graph 1).

Discussion

Nutrition has an impact on the host's immune response and also has a role in the progression and healing of periodontal tissues. The importance of nutritional factors in periodontal health was recognized a decade ago [16]. Vitamin D has an important role in physiologic bone metabolism. An increase in blood calcium levels, due to the release of calcium from the bones, including the alveolar bone due to the negative calcium

Table 1: Comparison of serum IL-6 (pg/ml) and vitamin D (ng/ml) between healthy patients and chronic periodontitis before treatment.

Group	N	Mean	S. D	95% Confidence Interval of the Difference		t	p
Serum IL-6				Lower	Upper		
Healthy	30	40.16	7.88	-12.52	-5.43	-5.068	<0.001*
Chronic Periodontitis	30	49.13	5.65				
Serum Vitamin D							
Healthy	30	41.52	10.75	16.42	23.39	9.388	<0.001*
Chronic Periodontitis	30	20.62	5.76				

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Table 2: Comparison of GI, PI, PD, CAL between healthy patients and chronic periodontitis before treatment.

	Median	Interquartile range [Q I, Q3]	P
GI Healthy	0.10	[0.10.0.20]	**<0.001***
Chronic Periodontitis	2.43	[2.22.2.70]	
PI Healthy	0.20	[0.10.0.33]	**<0.001***
Chronic Periodontitis	1.54	[1.32.2.10]	
PD Healthy	2.36	[2. 18,2.51]	**<0.001***
Chronic Periodontitis	4,90	[4,32.5 .24]	
CAL Healthy	2.36	[2.18,2 .51]	**<0.001***
Chronic Periodontitis	5.82	[5.20.6.12]	

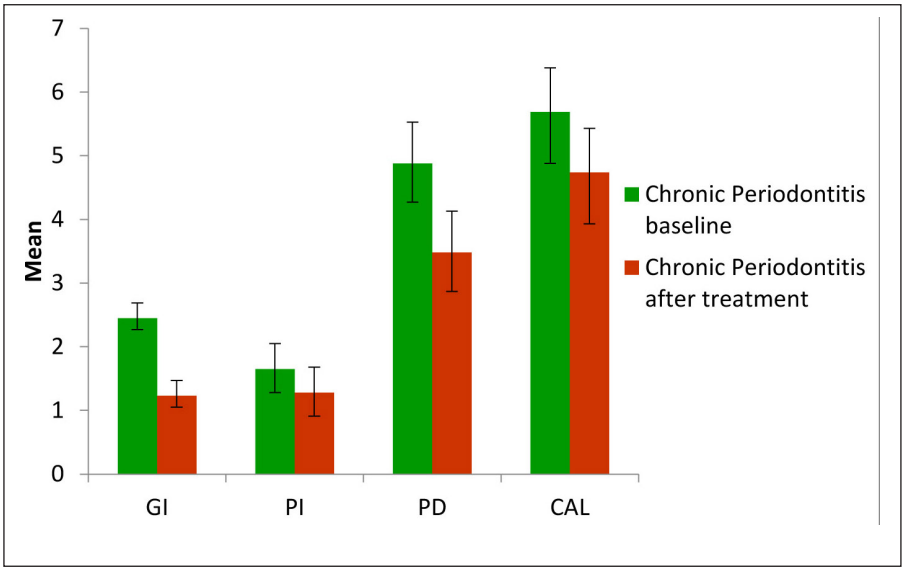
Table 3: Comparison of serum IL-6 and vitamin D in chronic periodontitis before and after treatment.

					95% Confidence Interval of the Difference			
	Group	N	Mean	S. D	Lower	Upper	t	P
Serum IL-6	Before Treatment	30	49. 13	5.65	7.59	12:33	8.599	**<0.001***
	After Treatment	30	39.18	7.00				
Serum Vitamin D	Before Treatment	30	20.62	5.76	-13.28	-8.99	–10.603	**<0.001***
	After Treatment	30	31.76	8.12				

balance is caused due to inadequate ingestion of vitamin D and calcium. This resultant bone loss can contribute to the loss of tooth-supporting structures [17]. Additionally, vitamin D, inhibits cytokine production and inflammatory cell proliferation, thus playing a crucial role in various chronic inflammatory disease conditions. Therefore, vitamin D affects periodontal disease both by affecting bone density and through its immune-modulatory effects [18]. A majority of the general population are vitamin D deficient or insufficient. Hence, vitamin D levels could be a risk indicator of periodontal disease susceptibility through its regulation of calcium homeostasis [19].

A well-known relationship exists between bacteria and periodontitis. In periodontitis, the inflammatory process that occurs results in the intrusion of leukocytes, which inhibit the invasion of bacteria. Leukocyte recruitment is mediated through factors like bacterial toxins, cytokines and involvement of innate and adaptive immune responses. The stimulation of the host response to bacterial challenge results in tissue and bone destruction [20]. Cytokines are protein in nature that helps in cell-to-cell communication. These alter the cell behavior by binding to the cell surface receptors resulting in an increase in the secretion of cytokines ultimately leading to inflammation. Various cytokines that have been identified so far are interferon, interleukins, chemokines, and growth factors. At the commencement of acute inflammation, an acute phase response is initiated which is mediated by IL-6. When its action as a pro-inflammatory cytokine continues, it results in chronic inflammation that includes immune responses [21].

This study consisted of sixty patients in total equally allocated into two groups. Group A comprised of systemically healthy patients and group B comprised of chronic periodontitis



Graph 1: Comparison of GI, PI, PD and CAL in chronic periodontitis before and after treatment.

patients. This study was designed to measure the clinical parameters and compare IL-6 and vitamin D levels in the serum at baseline and 3 months following non-surgical periodontal therapy in patients with chronic periodontitis.

The statistically significant difference in mean serum IL-6 and clinical parameters such as GI, PI, PD, CAL was detected in Group B as compared to group A at baseline ($p<0.001$) [Tables 1 and 2]. IL-6 is considered a multifunctional cytokine as it has numerous biological activities which include differentiation and proliferation of B and T lymphocytes, respectively, and also the regulation of the function of immune cells [22]. In chronic inflammation, IL-6 has an undesirable role that results in an increase in the number of mononuclear cells at the site of injury. This may raise serum levels of IL-6 and offers the basis for the strengthening step of chronic inflammatory proliferation [21]. Bone resorption may be induced by IL-6 alone or with other bone-resorbing agents. Thus, in periodontal destruction, there's an increase in IL-6 levels indicating the extent of periodontal disease in group B.

An immunohistochemical study done by Bartold et al. revealed higher IL-6 levels in inflamed tissues samples of gingiva [23]. Noh et al. in their study also concluded that elevated levels of IL-6 in patients with periodontitis can have diagnostic and prognostic potential for assessing the disease and therapeutic decision [22]. However, contrary to the above-mentioned studies, no correlation was detected in studies done by Chen et al. and Takashi et al. [24, 25].

The mean serum vitamin D (ng/ml) was lower (20.62±5.76) indicating insufficiency in group B before treatment in comparison to group A (41.52±10.75) [Table 1]. A number of studies show an inverse association between serum vitamin D and inflammatory and infectious diseases, which like periodontitis may induce an increased systemic inflammatory burden [26, 27].

The three possible explanations for low serum vitamin D in chronic periodontitis could be:

1) Impairment of 1-α-hydroxylation of 25(OH)D.

2) Increased binding of 1,25(OH)2D on immune-competent cells.

3) Increase in degradation of 1,25(OH)2D [28].

Several interventional studies were conducted to investigate the impact of periodontal therapy on proinflammatory and anti-inflammatory mediators, with substantial variation in the results [1]. In this study, a reduction in the mean serum IL-6 levels following nonsurgical periodontal therapy with the significant improvement in the clinical parameters were observed in group B [Table 3, Graph 1]. Reduction in the serum levels of IL-6 following nonsurgical therapy may be due to the alleviation of inflammation [1].

Lower serum IL-6 in chronic periodontitis patients following nonsurgical periodontal

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therapy was observed by several authors [29–31]. However, no alterations in serum levels of IL-6 were noticed by Ide et al. and Yamazaki et al. [32].

Following nonsurgical periodontal therapy in group B a significant increase in mean serum vitamin D could be attributed to the reduction of inflammation and improvement in clinical parameters [Table 2, Graph 1].

The following mechanism could explain the reduction of inflammation i.e. vitamin D has anti-inflammatory and immune-modulatory effects by its ability to downregulate the proliferation of T cells and production of cytokines. It also produces cathelicidins and defensins from gingival epithelium against infection [33]. There are few studies in the literature where contrary results have been obtained [1, 34]. A report by Pinto et al. stated that the data to support or refute the link between vitamin D levels and periodontal disease is currently inconclusive [35].

Dietrich et al. reported that individuals with high serum vitamin D were presented with significantly lower pocket depth and clinical attachment level [36]. In a similar study, women with 25(OH)D >50 nmol/l were 33% at a lower risk of periodontal diseases when compared to women with an inadequate levels of 25(OH)D [37, 38]. The above results were in accordance with studies done by Antonoglou et al. [39]. A recent systematic review supported the link between 25(OH)D serum levels and chronic periodontitis. It was observed that the patients with chronic periodontitis had low levels of 25(OH)D serum levels than periodontally healthy subjects [40].

Within the limitations of this study mainly the cross-sectional design, non-consideration regarding exposure to sunlight, dietary charts and radiographs, it can be concluded that non-surgical periodontal therapy led to an improvement of clinical parameters, a reduction in IL-6 and an increase in vitamin D in chronic periodontitis patients. Hence, the null hypothesis stated earlier was disproved.

Conclusions

The results of this study can be summarized as follows:

1. At baseline, compared to healthy subjects, the chronic periodontitis group had a low level of serum vitamin D and high levels of serum IL-6.

2. Post-non-surgical periodontal therapy, it was observed that the serum level of vitamin D increased to an optimal level and serum IL-6 level was reduced in patients with chronic periodontitis.

Hence, to conclude, non-surgical therapy improved periodontal clinical parameters, decreased plasma levels of IL-6, and increased serum vitamin D levels in chronic periodontitis patients. Furthermore, as IL-6 is related to disease severity it could be used as a reliable diagnostic marker and deficiency of vitamin D could be used as a risk factor for periodontal disease. Vitamin D is essential for bone health and immunity, and it can also have a significant impact on periodontal health and systemic disorders. Despite a close link of vitamin D deficiency with poor general health, it is not taken seriously by patients' dentists and medical practitioners. Hence initiation of more awareness programs and longitudinal studies to clearly define the role of vitamin D is the need of the future.

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