

**Evaluation of serum vitamin d in chronic periodontitis patients – A clinical study**

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**Abstract**

**Introduction:** Chronic periodontitis is a multifactorial disease primarily caused by plaque microorganisms, modified from the immune inflammatory response to chronic infection, which leads to the destruction of periodontal tissues in a susceptible host. It is very well known that vitamin D plays a vital role in bone homeostasis and immunity. There can be a biologic rationale to suspect that Vitamin D deficiency could affect the periodontium. The present study was conducted to

investigate any relationship between periodontitis and vitamin D.

**Material and method:** The study was carried out in 30 subjects with Chronic Periodontitis (sub classified into 10=mild periodontitis, 10=moderate periodontitis, 10=severe periodontitis) and 10 healthy subjects. Plaque Index (PI), Gingival Index (GI), Modified sulcular bleeding Index (mSBI), Probing Pocket Depth (PPD) and Clinical Attachment Level (CAL) were the clinical

parameters taken. Serum vitamin D level was estimated using Semi automated chemoluminescence immunoassay.

**Results:** Statistically significant difference was observed among the study groups (severe periodontitis and healthy group). This study revealed overall low levels of serum Vitamin D in patients with chronic periodontitis.

**Conclusion:** There is a correlation between serum Vitamin D and Severe periodontitis patients. Approximately 80% of the total screened patients of chronic periodontitis were found to be vitamin D deficient or insufficient and 50% of healthy patients were found to be vitamin D deficient or insufficient.

**Keywords:** Chronic Periodontitis, Vitamin D, Clinical attachment level.

### Introduction

Vitamin D deficiency is a worldwide public health concern and the most commonly diagnosed medical condition. Globally, it was estimated that over 1 billion of population has vitamin D deficiency or insufficiency across all the ethnicities of world as estimated in 2007.<sup>[1]</sup> Vitamin D synthesis occurs in the skin under the effect of sunlight. The Indian subcontinent is situated between 8.4°N and 36°N latitude and has ample amount of sunlight throughout the year. Even then there is a widespread prevalence of hypovitaminosis in India in all the age groups including both the sexes.<sup>[2-5]</sup> As shown in study conducted by International Osteoporosis Foundation in 2009, 96% of neonates, 91% of healthy school girls, 78% of healthy hospital staff and 84% of pregnant women in north India are diagnosed with hypovitaminosis D.<sup>[6,2]</sup>

Vitamin D has a role in metabolism of bone through maintenance of balance between calcium and phosphate. It has immunomodulatory effects which have been linked to bacterial mediated infections.<sup>[7]</sup> Vitamin D also has anti-bacterial and lipopolysaccharide neutralizing activity against periodontal pathogens by stimulating anti-

microbial peptides. Wang et al reported that the active form of vitamin D has the ability to induce expression of cathelicidine and some defensins in keratinocytes and other cell types. This improves cell to cell junction and strengthen the physical barrier present in oral cavity, thus contributing significantly to improve oral health.<sup>[8]</sup>

Recently, according to analysis of Third National Health and Nutrition Examination survey, 12000 adults were taken for an association between periodontitis and vitamin D serum level. They stated that poor periodontal health is related to low dietary intake of vitamin D in a dose dependent fashion.<sup>[9,10]</sup> The present study aims to compare and evaluate serum vitamin D levels in healthy individuals and mild, moderate and severe chronic periodontitis patients.

### Materials and Methodology

The present observational study was carried on 40 subjects in the Department of Periodontology, Himachal Institute of Dental Sciences, Paonta Sahib. The study unit consisted of patients diagnosed with chronic periodontitis.

### Inclusion Criteria

1. Male patients between the age group of 35 years to 65 years.
2. Patients diagnosed with chronic periodontitis with pocket depth  $\geq$  5mm, bleeding on probing, clinical attachment level  $\geq$  1mm and gingival index score of  $\geq$  1.
3. Healthy male patients (control) without gingival and periodontal inflammation.

### Exclusion Criteria

1. Females were excluded.
2. Patients with history of any systemic diseases.
3. Patients associated with cardiovascular disease and renal disease.
4. Patients on calcium supplements.

5. Patients with history of periodontal treatment in previous 6 months.
6. Patients who were on antibiotics or other drugs in the past 6 months that can affect periodontal status.
7. Patients with vitamin D deficiency including bone disease, malignancies and multiple sclerosis and with immune deficiency and inflammatory diseases.
8. Patients diagnosed with aggressive periodontitis.

Before the commencement of the study, ethical clearance was obtained from the Ethical Committee of Himachal Institute of Dental Sciences, Paonta Sahib. The participants were explained in detail about the purpose of the study, the methodology involved and the related risks and benefits, in a language well understood by them. A written consent and a thorough medical and dental history were taken from all the participants.

### Study Group

Subjects were categorized into two groups on the basis of examination.

Group I: A control group that included healthy male patients without any periodontal conditions such as visible plaque, bleeding on probing, periodontal pocket depth and clinical attachment level.

Group II: This group included male patients with chronic periodontitis having measurements for the presence of visible plaque, probing pocket depth (PPD), bleeding on probing (BOP), and clinical attachment level (CAL) on four surfaces of each tooth. On the basis of severity,<sup>[11]</sup> patients of this group are sub divided into:-

Sub group A: Mild periodontitis with 1 or 2 mm clinical attachment level.

Sub group B: Moderate periodontitis with 3 or 4 mm clinical attachment level.

Sub group C: Severe periodontitis with  $\geq 5$  mm clinical attachment level.

### Clinical Parameters

All the clinical parameters [Plaque Index<sup>[12]</sup> (PI), Gingival Index<sup>[13]</sup> (GI), Modified Sulcular Bleeding Index<sup>[14]</sup> (mSBI), Probing Pocket Depth (PD) and Clinical Attachment level (CAL)] were recorded and blood sample collections were done for vitamin D estimation.

### Collection of samples

A 5 ml blood sample was drawn by veno puncture from the antecubital vein by sterile syringe and was kept in test tube. The samples were kept for one hour at room temperature for clotting. These samples were then centrifuged for 10 minutes at approximately 3000 rpm. Using a clean pipette technique, serum was collected in a separate tube and was labelled with the patient's name, age and date. Serum samples were then kept immediately in deep refrigerator for storage purpose.

### Estimation of biochemical parameters

To determine serum vitamin D, Enzyme linked immunosorbent assay (ELISA) was performed using AccuLite 25-OH Vitamin D<sub>3</sub>/D<sub>2</sub> kit.<sup>[15]</sup>

### Statistical Analysis

The statistical analysis was done using Statistical Package for the Social Sciences (SPSS Inc., SPSS for Windows v.16, IBM, USA). Normality testing of data was done using Shapiro-Wilk test, which showed that the data were normally distributed ( $p < 0.05$ ). Descriptive statistics were calculated as mean and standard deviation. The comparison of various parameters among the groups was done using Analysis of Variance (ANOVA) followed by post-hoc Tukey's test for multiple comparisons. The level of significance for the present study was fixed at  $p < 0.05$ .

### Results

The mean plaque index scores, the mean gingival index scores, the mean Modified Sulcular Bleeding index scores and the mean clinical attachment level showed a statistically significant difference for all parameters

among the study groups ( $p < 0.001$ ) (Table 1). Multiple comparisons using post-hoc Tukey test showed that the statistical difference was observed between mild periodontitis and control group ( $p < 0.001$ ), moderate periodontitis and control group ( $p < 0.001$ ), and between

severe periodontitis and control group ( $p < 0.001$ ) for the mean plaque index scores, the mean gingival index scores, the mean Modified Sulcular Bleeding index scores and the mean clinical attachment level. Other differences were not statistically significant ( $p > 0.05$ ) (Table 2).

	N	Plaque Index		Gingival Index		Modified Sulcular bleeding index		Clinical attachment level		P value
		Mean	SD	Mean	SD	Mean	SD	Mean	SD	
Mild Periodontitis	10	1.3350	0.55580	1.4340	0.44260	0.8100	0.55015	1.9500	0.56814	<0.001*
Moderate Periodontitis	10	1.3650	0.39444	1.6040	0.61268	0.9970	0.72328	3.6130	0.48726	
Severe Periodontitis	10	1.8600	0.71946	1.9110	0.59780	1.3820	0.78900	6.5260	1.00234	
Control	10	0.3100	0.26013	0.3500	0.35355	0.1840	0.18295	0.0000	0.00000	
Total	40	1.2175	0.75392	1.3247	0.77327	0.8433	0.73086	3.0223	2.49704	

Table 1: The mean plaque index scores, the mean gingival index scores, the mean Modified Sulcular Bleeding index scores and the mean clinical attachment level among study groups.

	Plaque index		Gingival Index		Modified Sulcular bleeding index		Clinical attachment level	
	Mean Difference	p-value	Mean Difference	p-value	Mean Difference	p-value	Mean Difference	p-value
Mild Periodontitis Moderate	-0.03000	0.999	-0.17000	0.880	-0.18700	0.901	-1.66300*	<0.001*
Mild Periodontitis Severe	-0.52500	0.119	-0.47700	0.180	-0.57200	0.172	-4.57600*	<0.001*
Mild Periodontitis Control	1.02500*	<0.001*	1.08400*	<0.001*	0.62600	0.117	1.95000*	<0.001*
Moderate Periodontitis Severe	-0.49500	0.154	-0.30700	0.546	-0.38500	0.499	-2.91300*	<0.001*
Moderate Periodontitis Control	1.05500*	<0.001*	1.25400*	<0.001*	0.81300*	0.025*	3.61300*	<0.001*
Severe Periodontitis Control	1.55000*	<0.001*	1.56100*	<0.001*	1.19800*	0.001*	6.52600*	<0.001*

Table 2: Multiple comparisons for plaque index scores, gingival index scores, Modified Sulcular Bleeding index scores and clinical attachment level among study groups.

Table 3: shows the mean serum vitamin D levels in various groups. Analysis of variance showed that probability value obtained was  $p = 0.05$ . Multiple comparisons using post-hoc Tukey test (Table-4) showed that the statistical difference was observed between severe periodontitis and control group ( $p = 0.03$ ). Other differences were not statistically significant ( $p > 0.05$ ).

	N	Mean	Std. Deviation	95% Confidence Interval for Mean		p-value
				Lower Bound	Upper Bound	
Mild Periodontitis	10	22.9298	11.13361	14.9653	30.8943	0.050
Moderate Periodontitis	10	24.4084	16.73158	12.4394	36.3774	
Severe Periodontitis	10	12.4439	8.13912	6.6215	18.2663	
Control	10	31.8372	20.53757	17.1455	46.5289	
Total	40	22.9048	15.96652	17.7985	28.0112	

Table 3: Mean serum vitamin D levels

		Mean Difference	p-value	95% Confidence Interval	
				Lower Bound	Upper Bound
Mild Periodontitis	Moderate Periodontitis	-1.47860	0.996	-19.4642	16.5070
Mild Periodontitis	Severe Periodontitis	10.48590	0.408	-7.4997	28.4715
Mild Periodontitis	Control	-8.90740	0.548	-26.8930	9.0782
Moderate Periodontitis	Severe Periodontitis	11.96450	0.294	-6.0211	29.9501
Moderate Periodontitis	Control	-7.42880	0.684	-25.4144	10.5568
Severe Periodontitis	Control	-19.39330*	0.030*	-37.3789	-1.4077

\*Statistically significant ( $p < 0.05$ ), Post-hoc Tukey test

Table 4: Multiple comparisons of serum vitamin-D levels

### Discussion

According to Nordic Nutrition Recommendation, US National Institute of Health and Endocrine Society's Practice Guidelines, Vitamin D deficiency is defined as concentration  $< 20$  ng/ml, insufficiency as 21-29 ng/ml and sufficiency as 30 ng/ml.<sup>[16,17,18]</sup> Vitamin D deficiency prevails in epidemic proportion all over the Indian sub-continent, with a prevalence of 50%-90% in the general population.<sup>[6]</sup> According to Arya V et al. (2004), 78.3% of healthy hospital staffs were found to be vitamin D deficient.<sup>[2]</sup> Kouchupillai N et al. (2008) concluded that

even doctors and nurses from the northern latitude of India have Vitamin D deficiency.<sup>[19]</sup> These observations were confirmed by other authors of the southern part of the country.<sup>[5,20]</sup> These reports thus scientifically establish evidence for prevalence of vitamin D deficiency in the subcontinent despite being a tropical country with abundant sunlight.

Male patients were enrolled into the study as females usually have hormonal imbalances that may affect the bone metabolism. All subjects were systemically healthy and all had been enrolled in periodontal maintenance

programs and received regular treatments and were well motivated for maintaining good dental health.

In the present study on comparison of plaque index scores, gingival index scores, Modified Sulcular Bleeding index scores and clinical attachment level there was a statistically significant difference for all parameters among the study groups ( $p < 0.001$ ) similar results were seen in a study by Bhavya B et al where there was statistical significant differences between the two groups (chronic periodontitis with type 2 diabetes and chronic periodontitis without type 2 diabetes) in postmenopausal women for all periodontal indices such as periodontal probing depth (PPD), clinical attachment level (CAL), and gingival index (GI).<sup>[21]</sup> The inter-group comparison showed that the statistical difference was observed between mild periodontitis and control group ( $p < 0.001$ ), moderate periodontitis and control group ( $p < 0.001$ ), and between severe periodontitis and control group ( $p < 0.001$ ) for the plaque index scores, gingival index scores, Modified Sulcular Bleeding index scores and clinical attachment level. A study by Joseph R et al study showed significant differences for plaque index between control and chronic periodontitis group but was not statistically significant between chronic periodontitis and chronic periodontitis with type 2 diabetes mellitus groups.<sup>[22]</sup>

Serum Vitamin-D Levels observation showed that there was a significant difference among study groups ( $p = 0.05$ ). A statistical difference was observed between severe periodontitis and control group ( $p = 0.03$ ). Other differences (mild and moderate periodontitis, mild and severe periodontitis, mild periodontitis and control, moderate and severe periodontitis and moderate periodontitis and control) were not statistically significant ( $p > 0.05$ ). In a case-control study conducted by Laky M et al (2016) patients with periodontal disease presented a significantly higher proportion of deficient 25(OH)D

levels compared to health controls.<sup>[23]</sup> In another study by Antonglou et al (2013) showed a positive correlation between serum level of 1,25(OH)D and periodontal condition in diabetic subjects. However, they found that the elimination of periodontal infection that would increase the serum level of 1,25(OH)D.<sup>[24]</sup>

As a result, 24 out of 30 patients with periodontitis were diagnosed with Vitamin D deficiency or insufficiency which comes out to be 80% approximately whereas in the control group comprising healthy individuals, 5 out of 10 people exhibited vitamin D deficiency or insufficiency which comes out to be 50%.

In mild periodontitis cases, 8 out of 10 subjects, that is 80% had vitamin D deficiency or insufficiency. In moderate periodontitis cases, 6 out of 10 subjects, that is 60% had vitamin D deficiency or insufficiency. In severe periodontitis cases, all 10 subjects had vitamin D deficiency which comes out to be 100%.

A total of 40 subjects taken for the study, 29 subjects had vitamin D deficiency or insufficiency which comes out to be 72.5%.

Studies have suggested that patients with chronic inflammatory diseases are deficient in 25(OH)D. The Vitamin D nuclear receptors (VDR) are located in the nucleus of variety of cells including immune cells. The body controls the activity of VDR through recognition of Vitamin D metabolite 25(OH)D, which antagonize or inactivate the receptor, while 1,25(OH)D agonize or activates the receptor. When exposed to infection, the body begins to convert the inactive form of 25(OH)D into the active form, 1,25(OH)D. As cellular concentrations of 1,25(OH)D increase, 1,25(OH)D activates the VDR. However, bacteria create ligands, which like 25(OH)D, inactivates the VDR and in turn, the innate immune response. An intra-phagocytic bacterial microbiota is proposed to be the primary cause of VDR dysfunction



which allows the microbes to proliferate. In response, the body increases the production of 1,25(OH)D from 25(OH)D, leading to low 25(OH)D one of the hallmarks of chronic inflammatory disease. A low level of serum 25(OH)D is seen as the result of down regulation.<sup>[25,26]</sup>

The insufficient Vitamin D level in control subject may be due to inadequate intake of Vitamin D containing food substances coupled with inadequate sunlight exposure. With modernization the number of hours spent on outdoor activity is decreased.

### Conclusion

The following conclusions were drawn according to observed results:-

1. There is a correlation between serum Vitamin D and Severe periodontitis patients.
2. Approximately 80% of the total screened patients of chronic periodontitis were found to be vitamin D deficient or insufficient and 50% of healthy patients were found to be vitamin D deficient or insufficient.
3. Serum Vitamin D can be used as a clinical parameter for diagnosing severe periodontitis.

This study concludes that low level of serum vitamin D could be a risk factor for chronic periodontitis. The results of this study suggests that the levels of serum vitamin D in patients with chronic periodontitis should be measured and if needed supplements of vitamin D could be used as an adjunctive treatment.

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