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# Association between serum concentration of 25-hydroxyvitamin D [25(OH)D] and chronic periodontitis

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**Abstract.** Serum concentration of [25(OH)D] in Indonesia is at low level although Indonesia is one of Asia country which has a tropical climate. Vitamin D as an immunomodulatory agent invitro and in animal studies. However, data from clinical studies involvement vitamin D to chronic periodontitis are still controversy. The aim of this study was to evaluate the association between serum concentration of [25(OH)D] and chronic periodontitis. Totally 45 chronic periodontitis patients were analyzed. Spearman correlation models adjusted for subject and parameter included periodontal pocket depth (PPD), clinical attachment loss (CAL), and bleeding on probing (BOP) are analyzed. Totally 51.11% person have serum concentration of [25(OH)D] 21-50ng/mL were called insufficiency, and 15.56% is deficiency. Mean values of PPD >3mm indicating moderate criteria, CAL >4mm meaning severe criteria, and mean grade of BOP >2 degrees means the interdental papilla appears to be filled with little or much blood. Spearman correlation test results showed an inverse correlation between serum concentration of [25(OH)D] to the value of PPD, CAL, and BOP, however, was not statistically significant ( $r$  PPD -0.09;  $r$  CAL -0.118;  $r$  BOP 0.001,  $p>0.05$ ). Insufficiency serum concentration of 25-hydroxyvitamin D [25(OH)D] was not associated significantly with PPD, CAL, and BOP of chronic periodontitis.

## 1. Introduction

Chronic periodontitis is an inflammatory disease in the dental support tissue that can cause tooth loss if not treated<sup>1</sup>. Periodontal tissue destruction that occurs due to the presence of pathogenic bacteria may trigger the body response and also the response of inflammation, causing periodontitis to be severe<sup>2</sup>. In addition to the invasion of bacteria, another factor that can aggravate chronic periodontitis conditions was vitamin D concentration deficiency<sup>3</sup>. The prevalence of vitamin D deficiency in developing countries is about 30%-80% of the entire population worldwide<sup>4</sup>.

Vitamin D deficiency occurs in almost entire Asian countries especially in both South Asia and in Southeast Asian countries<sup>5</sup>. This means that vitamin D deficiency can also be found in tropical countries. Most of the Indonesian people who live in big cities move before sunrise, come home after sunset and all day in the closed rooms that use the air conditioner. Also, vitamin D coverage has not been the part of the basic household health research program, so it escapes from national attention. Therefore, it is undeniable that Indonesian population did not escape from the problem of vitamin D deficiency<sup>6</sup>.



Several studies have been conducted to determine the association of vitamin D status to chronic periodontitis events and to know the effect of vitamin D supplementation in chronic periodontitis patients, but the results still show controversy.

## 2. Methods

### 2.1 Study population

This type of research was a descriptive analytic study with a cross-sectional design. The population is adults (men and women) who visited the periodontal department, Faculty of Dentistry, University of North Sumatra (FKG USU) in June-September 2016. 45 subjects were obtained and received scaling and root planning treatment to measure the parameters clearly (Clinical Attachment Loss / CAL), (Periodontal Pocket Depth / PPD), and Bleeding On Probing (BOP). Inclusion criteria were chronic periodontitis patients aged 31-55 years who were first diagnosed with moderate or severe, willing to do periodontal tissue examination and taken blood samples, as evidenced by signing the informed consent sheet. Exclusion criteria include periodontal surgical treatment during the last 6 months, undergoing orthodontic treatment, having a history of the systemic disease (Diabetes, Hypertension, Cardiac, Kidney, Liver) or other systemic conditions that may have an impact on the therapy given or on the subject's compliance, exposed sunlight directly more than 3 hours a day, do not consume nutritional supplements such as Estrogen, Biphosphonate, other fat-soluble vitamins, more than 6 months, not taking antibiotics, anti-inflammatory drugs, steroid medications, and anticonvulsants, have smoking habits.

### 2.2 Assessment of Serum concentrations of 25-hydroxyvitamin D

Serum concentrations of 25(OH) D assayed with Direct ELISA kit Vit D (Immunotek; brand DBC, Canada). As much 2ml of subjects blood was taken and centrifuged at 3000rpm for 15 min to obtain serum sample and then stored in freezer by -80°C.

### 2.3 Dental assessments

Periodontal measurements are probing around the gingival sulcus using a University of North Carolina (UNC) probe # 15. Clinical measurements were recorded on 6 sides per tooth (mesiobuccal / labial, mid-buccal / labial, buccal / labial, mesiolingual / palatal, mid-lingual / palatal, distolingual / palatal) of each Ramfort tooth gear 16, 21, 24, 36, 41, 44 (use the worst teeth next to it if index teeth have been dated). Also, the examiner recorded probing pocket depth (in mm), clinical attachment level (in mm), and bleeding on probing grade. For assessment of chronic periodontitis, a periodontal probe was inserted  $\geq 2$ mm in gingival sulcus and then gently moved into the mesial interproximal area.

### 2.4 Data Analysis

Parameters CAL, PPD, BOP, and serum concentration of 25(OH)D were analyzed using Pearson correlation test or nonparametric correlation Spearman correlation with 95% confidence degree ( $\alpha=0.05$ ).

## 3. Results

Characteristics are grouped by sex, age group, occupation, education level, number of teeth remaining in the oral cavity, and serum concentration of 25(OH)D as in table 1 below. Women are overrepresented in this sample.

**Table 1.** Baseline characteristic subjects.

Parameter	n=45
<b>Sex n(%)</b>	
Male	4(8.89%)
Female	41(91.11)

<b>Age (years)</b>	
30-40	11(24.44%)
41-50	27(60.00%)
>50	7(15.56%)
<b>Occupation</b>	
Housewife	28(62.22%)
Entrepreneur	10(22.22%)
Office worker	7(15.56%)
<b>Total teeth</b>	
20-25	12(26.67%)
26-30	25(55.56%)
>30	8(17.77%)

All subjects with chronic Periodontitis in this study were severe criteria because there were >2 interproximal surfaces of teeth that had a tooth attachment loss >5mm (not on the same tooth) and or at least one interproximal tooth surface with a pocket diameter >6mm (on the same tooth). The average of each parameter is shown in table 2 below.

**Table 2.** Average value of PPD, CAL, BOP, and serum concentration 25(OH)D subject.

Parameter	Mean± SD
PPD (mm)	3.13±0.41
CAL (mm)	4.17±0.69
BOP	2.67±0.33
25(OH)D3	40.12±19.35

Based on the above table the mean value of PPD >3mm indicating moderate criteria, mean CAL value of the three groups >4mm which means severe criteria, and mean grade of BOP > degree 2 which means the interdental papilla appears to be filled with little or much blood.

Analysis to see the status of vitamin D concentrations can be seen in table 3 below.

**Table 3.** Status of serum concentration 25(OH)D of subjects.

Parameter	n=45
<b>25(OH)D (ng/mL)</b>	<b>n(%)</b>
< 20 (Deficiency)	7(15.56)
21-50 (Insufficiency)	23(51.11)
51-100 (Optimal)	15(33.33)

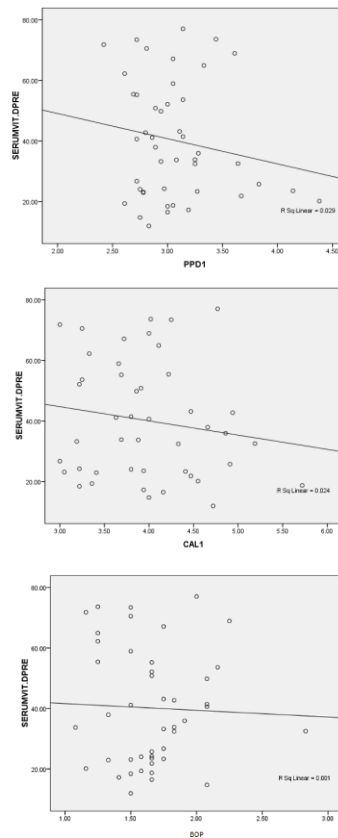
Status of serum vitamin D in most subjects are Insufficiency which means experiencing an inadequate amount of vitamin D in the body (<50ng/mL). The result of normality test of data with Saphiro wilk test shows homogenous data but not normally distributed, then data are tested using Spearman Rho correlation test with interpretation value if  $r = 0.000 - 0.199$  (very weak correlation),  $r = 0.200 - 0.399$  (weak correlation),  $r = 0.400 - 0.599$  (medium correlation),  $r = 0.600 - 0.799$  (strong correlation) and  $r = 0.800 - 1.000$  (very strong correlation). The association of vitamin D levels with periodontal severity of PPD, CAL, and BOP is seen in the following table.

**Table 4.** Results of the correlation test of vitamin D 25(OH)D content and clinical parameters PPD, CAL, and BOP.

	PPD		CAL		BOP	
vitamin D 25(OH) <sub>3</sub> D	<i>r</i>	<i>p</i>	<i>r</i>	<i>P</i>	<i>r</i>	<i>p</i>
	-0.09	0.559	-0.118	0.442	0.001	0.995

(- : Inverse correlation)

Spearman correlation test results showed the association between serum concentration of 25(OH)D to the value of PPD, CAL, and BOP ( $r$  PPD = -0.09;  $r$  CAL = -0.118,  $r$  BOP = 0.001) was not statistically significant ( $p > 0.05$ ). Based on the scatter image to plot the three parameters are known the parameters that are stronger related to vitamin D concentration is the value of PPD.



#### 4. Discussion

In the present study, we found an inverse association between serum concentration of 25(OH)D and chronic gingivitis as measured by bleeding on probing among participants who never smoked aged 13 to >90 years. Based on the description of vitamin D status of subjects, 51.11% had insufficient vitamin D in the body, 15.56% had a deficiency or vitamin D deficiency, and 33.33% had optimal vitamin D concentration. According to Setiati (2008)<sup>6</sup>, vitamin D deficiency is estimated rare in Indonesia because Indonesia is a country exposed to the sun, throughout the year. But it is realized that the lifestyle of Indonesian people today tend to avoid exposure to the sun, and vitamin D intake is still low. The condition of vitamin D deficiency is also considered to be associated with the innate and adaptive immune system. According to some studies, it is reported that vitamin D has an immunomodulatory role in controlling the upregulation and downregulation of innate and adaptive immune system<sup>7</sup>.

In the innate immune system, Vitamin D reduces inflammation by inducing the release of LL-37 Cathelicidin and Defensin proteins. Cathelicidin and Defensin can perform repair function for damage in chronic periodontitis through vitamin D signaling to repair epithelial cells in periodontal tissues that have been damaged by inflammation. Besides that, Vitamin D can reduce symptoms of chronic periodontitis by suppressing the producing pro-inflammatory cytokines ((Interleukin-1 / IL-1 and IL-6) and doing proliferation of immune cells by induces the production of anti-inflammatory cytokines immune cells like Treg cells to secrete the cytokine IL-10. Vitamin D also can inhibit the release of molecules that trigger the degradation of gingival tissue (ROS and MMPs) and alveolar bone

resorption (RANKL) that resulted in periodontal pockets and loss of attachment of the tooth to the periodontal tissues<sup>8</sup>.

The average periodontal pocket depth (PPD) of the subject is >4mm. In the study of Stoltenberg et al. (2005), the mean pocket depth >3.5mm has described severe chronic periodontitis, but according to Jacob<sup>9</sup>, the pocket depth >3mm indicates moderate/moderate periodontitis. In the study Torrungruang<sup>10</sup> classifies the average CAL value >4mm is included in the category of severe, but in the study preliminary<sup>11</sup> criteria for the average loss of attachment (attachment loss/AL) are grouped into five categories: very mild to very severe, i.e., 0 - 1mm, 1.1 - 1.5mm, 1.6 - 2.0mm, 2.1 - 2.5mm, and >2.6mm.

BOP values do not necessarily indicate the occurrence of active periodontitis because active periodontitis marked by the presence of marginal bleeding from gingival margin. Checchi et al.<sup>12</sup> in his study reported that BOP was directly related to the number of subgingival tartar deposits. That is, when the deposit of tartar removed then the BOP will gradually disappear. BOP absence indicates stable periodontal tissue stability.

Some factors that can cause periodontal disease factors like oral hygiene, bad habits (smoking, wrong toothbrushing, toothpick use), age, gender and systemic disease and nutritional deficiencies, include there is a causal relationship between the function of 1.25 (OH)<sub>2</sub>D-Receptor vitamin D (RVD) complex with the body's immunity to infection. Changes to Receptor vitamin D function due to mutations resulting in the entry of mycobacteria infection or viral infection into the body<sup>13</sup>.

## 5. Conclusion

The conclusion is insufficiency serum concentration of 25-hydroxyvitamin D [25(OH)D] in the body of is not significantly related to pocket events and loss of dental clinical attachment, and bleeding on probing.

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