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## ASSOCIATION BETWEEN THE DEGREE OF GINGIVAL INFLAMMATION AND 25-HYDROXY VITAMIN D STATUS IN POSTMENOPAUSAL WOMEN

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### ABSTRACT

**Objective:** To evaluate the association of the degree of gingival inflammation and 25-hydroxy Vitamin D (25(OH)D) level in postmenopausal women.

**Methods:** A cross-sectional study involved 71 postmenopausal women. Data were obtained using questionnaires, clinical periodontal examinations, and evaluations of blood samples. Serum 25(OH)D concentrations were determined using human 25-dihydroxy Vitamin D ELISA kit.

**Result:** Prevalence of Vitamin D deficiency among postmenopausal women was 74.64%. The papillary bleeding index (PBI) was lower (1.07±0.18) in postmenopausal women with normal serum 25(OH)D levels than that in postmenopausal women with Vitamin D deficiency (1.41±0.1). However, this difference was not statistically significant ( $p < 0.05$ ). This result indicated increasing trends in PBI.

**Conclusion:** There is no association between the degree of gingival inflammation and the 25(OH)D status among postmenopausal women.

**Keywords:** 25-hydroxy Vitamin D, Papillary bleeding index, Postmenopause.

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### INTRODUCTION

Menopause is defined as the cessation of menstruation for at least 1 year due to estrogen deficiency. A considerable proportion of the population is affected by estrogen deficiency; there are approximately 70 million women in the United States >50 years of age [1]. It is estimated that there will be 60 million menopausal women in Indonesia by 2025. Currently, in Indonesia, there are 14 million menopausal women, comprising 7.4% of the total population [2]. Multiple health issues affect postmenopausal women and compromise their quality of life. Postmenopausal women may experience several symptoms [3], including cardiovascular disease, osteoporosis, cancer, cognitive decline and dementia, chronic obstructive pulmonary disease, diabetes mellitus, metabolic syndrome, depression, vasomotor symptoms, sleep disturbances, and migraine [3,4].

Vitamin D and calcium are the basic requirements for bone mineralization and osteoporosis prevention [5]. Vitamin D plays an important role in calcium homeostasis, promoting calcium absorption in the intestines, and stimulating the osteoblasts to enable normal bone growth and preservation [6,7]. Low Vitamin D and calcium intake lead to a negative calcium balance and bone loss; these effects are also believed to occur in the alveolar bone in addition to that in the other bones of the body [7]. Vitamin D formed in the skin and that obtained from the diet is absorbed and accumulates in the liver where it undergoes hydroxylation and metabolization to form 25-hydroxy Vitamin D (25(OH)D), also called calcidiol, the levels of which are used to determine an individual's Vitamin D status [8].

Periodontal disease results in the destruction of the supporting structures of the teeth by an inflammatory process caused by bacterial accumulation on the hard surfaces of the teeth [9].

Vitamin D plays a significant role in bone maintenance and immunity; therefore, it can be inferred that Vitamin D influences the development of periodontal disease [9]. Vitamin D also has anti-inflammatory and antimicrobial properties that may protect against alveolar bone loss

and subsequent tooth loss [10]. Vitamin D deficiency may result in an inadequate and prolonged immune reaction to periodontal pathogens, potentially leading to more severe periodontal destruction [9]. To the best of our knowledge, few researches have been conducted on the association between the degree of gingival inflammation and Vitamin D status in postmenopausal women.

The purpose of this study was to evaluate the association between the degree of gingival inflammation and 25(OH)D status in postmenopausal women.

### METHODS

#### Study population

Approval for the study on human subjects was obtained from the Ethics Committee of the Faculty of Dentistry, Universitas Indonesia, before study initiation. The sample size calculations were performed using the sample formula for cross-sectional study design, according to which at least 59 subjects needed to be enrolled. The research procedures were explained to all patients after they had read and signed an informed consent document before any treatment.

The investigator screened the patients according to the inclusion and exclusion criteria and selected those who fulfilled the study criteria. Women, aged 50–70 years, who had entered the menopause period (calculated after 1 year of the last menstrual cycle), had no systemic diseases that could influence the outcomes of the therapy, and were willing and able to provide an informed consent were enrolled in the study. Subjects on antibiotic therapy for more than 10 days within the past 3 months of enrollment or those requiring antibiotic prophylaxis, subjects on medications affecting bone metabolism or gingiva, or those with a history of hysterectomy were excluded. Information regarding the subjects' medical histories and medications were collected using a questionnaire [11]. The questionnaire included questions about the physical activity, calcium intake, and sun exposure [11]. Total 71 patients, all of whom were citizens recruited from the Kenari District Office, Central Jakarta, were enrolled in the study.



### Clinical periodontal examination

The periodontal status was assessed by measuring the papillary bleeding index (PBI) (Muhlemann, 1977) [12]. Clinical measurements were recorded by the calibrated investigator using a periodontal probe with 1 mm markings (Osung, Korea). Calibration was conducted by performing double measurements of a randomly selected patient not involved in the study. Third molars were not included in the clinical assessment because of the substantial variation in their anatomy and position in the oral cavity.

### Laboratory analysis

A venous blood sample was drawn from each subject on the day of the examination. Serum samples were separated after centrifugation at 3000 rpm for 10 min; thereafter, they were stored at  $-4^{\circ}\text{C}$  until further assays. These samples were removed from the freezer and defrosted at room temperature before the assays.

All assays were performed in the Integrated Laboratory Faculty of Medicine Universitas Indonesia. Serum 25(OH)D was analyzed using a commercially available enzyme-linked immunoassay (human 25(OH) D ELISA Kit, Qayeebio, Shanghai) according to the manufacturer's instructions. The results are expressed as nanogram per milliliter (range: 7.8–500 ng/mL). For this study, Vitamin D levels were categorized as deficient if the levels were  $<30$  ng/mL and adequate if the levels were  $\geq 30$  ng/mL, as per the guidelines of the Institute of Medicine [13-15].

### Statistical analyses

All statistical analyses were performed using SPSS. Univariate analyses were performed to obtain the mean and standard deviation values of all parameters. Descriptive statistics were obtained, and data were tested for normality using the Kolmogorov-Smirnov test for distribution. Variables that conformed to a normal distribution were analyzed using independent T-tests, while those that showed considerable deviation from the normal distribution were analyzed using the Mann-Whitney U test and Kruskal-Wallis test to evaluate the statistical significance of the differences between the two groups.  $p < 0.05$  was considered statistically significant.

## RESULTS

The distribution of the baseline characteristics of the study population is shown in Table 1.

The tests for normality of the clinical parameters of the postmenopausal women were performed using Shapiro-Wilk test. These tests showed that the distribution of the serum 25(OH)D levels across age, use of veil, and menopause duration was abnormal ( $p=0.02$ ,  $p=0.00$ , and  $p=0.00$ , respectively).

Table 2 summarizes that there were no significant differences in the serum 25(OH)D levels across age ( $p=0.61$ ); use of veil ( $p=0.71$ ); and menopause duration ( $p=0.17$ ) among postmenopausal women.

The Shapiro-Wilk test was used to test the normality of the clinical parameters of the postmenopausal women. The result showed that the distribution of serum 25(OH)D levels as per sun exposure was abnormal ( $p=0.01$ ). The normality test showed that the distribution of PBI was normal ( $p=0.33$ ).

This table summarizes that there were no significant differences in the 25(OH)D status of the postmenopausal women, depending on their sun exposure ( $p=0.68$ ) and PBI ( $p=0.12$ ) (Table 3).

## DISCUSSION

The supporting tissue of the periodontium is vulnerable to the physiological variations in the levels of circulating hormones in women. Menopause triggers numerous changes in women's bodies, and the oral cavity is also affected [16]. Postmenopausal women are at risk of

**Table 1: Demographic, clinical, and laboratory characteristics of postmenopausal women**

Postmenopausal women	n (%)	Mean (SD)
Age (year)		
50–54	18 (25.35)	
55–59	16 (22.53)	
60–64	15 (21.12)	
$\geq 65$	22 (27.16)	
Serum 25 (OH) D (ng/ml)		
Normal ( $\geq 30$ ng/ml)		18 (25.35)
Deficiency ( $< 30$ ng/ml)		53 (74.64)
Use of veil		
Yes	43 (60.6)	
No	28 (39.4)	
Menopause duration (years)		
$\leq 5$	51 (71.8)	
$> 5$	20 (28.2)	
Sun exposure (min/week)*		15.52 (2.42)
PBI*		1.33 (0.09)

\*Data are presented as mean and standard deviation. SD: Standard deviation, PBI: Papillary bleeding index

**Table 2: Comparative analysis of the serum 25(OH) D levels across age, use of veil, and menopause duration among postmenopausal women**

Postmenopausal women	Serum 25 (OH) D Level Mean (SD) (ng/mL)	p
Age (y)		0.61*
50-54	27.89 (2.1)	
55-59	29.26 (3.09)	
60-64	35.32 (5.03)	
$> 65$	28.66 (2.38)	
Use of veil		0.71**
Yes	28.6 (2.06)	
No	30.9 (2.21)	
Menopause duration (years)		0.17**
$\leq 5$	28.7 (3.01)	
$> 5$	30.52 (1.84)	

\*Kruskal-Wallis test, \*\*Mann-Whitney test;  $p < 0.05$  significant. SD: Standard deviation

**Table 3: Comparative analysis of the association of sun exposure and the papillary bleeding index with serum 25(OH) D status among postmenopausal women**

Postmenopausal women	Serum 25(OH) D status		p
	Mean (SD)		
	Normal ( $\geq 30$ ng/mL) (n=18)	Deficiency ( $< 30$ ng/mL) (n=53)	
Sun exposure (min/week)	16.06 (3.95)	15.33 (2.97)	0.68*
PBI	1.07 (0.18)	1.41 (0.10)	0.12**

\*Mann-Whitney test, \*\*independent sample t-test,  $p < 0.05$  significant. SD: Standard deviation, PBI: Papillary bleeding index

Vitamin D deficiency. The findings of this study showed that 74.64% of postmenopausal women had Vitamin D deficiency (Table 1). Ragab *et al.* also reported similar results; 68.6% of the healthy postmenopausal women in their study had Vitamin D deficiency [17]. Yared *et al.*'s study on postmenopausal Lebanese women with osteoporosis showed a prevalence of 84.9% for Vitamin D deficiency [18].

The high prevalence of Vitamin D deficiency among the women in our study may be because many women cover most of their bodies, considering that most of them were Muslim and that most of them wear a veil. In the present study, 60.6% of the postmenopausal women reported wearing a veil (Table 1), and those who wear veils had lower serum 25(OH)D levels ( $28.6 \pm 2.06$  ng/dL) than those who do not wear veils ( $30.9 \pm 2.21$  ng/dL). However, this difference was not statistically significant (Table 2).

This study showed that the women with normal 25(OH)D levels ( $16.06 \pm 3.95$  min/week) tended to have longer sun exposure than those with Vitamin D deficiency ( $15.33 \pm 2.97$  min/week); however, this difference was not statistically significant (Table 3). The major source of Vitamin D is exposure to natural sunlight. Vitamin D produced in the skin lasts at least twice as long in the blood as the ingested Vitamin D [19]. However, topical sunscreen application, increased skin pigmentation, and obesity can substantially reduce Vitamin D synthesis [19].

This study evaluated the association between the degree of gingival inflammation and the 25(OH)D levels in postmenopausal women. The findings from this study demonstrated that the PBI was lower in the women with normal or adequate serum 25(OH)D levels ( $1.07 \pm 0.18$  ng/mL) than in those with Vitamin D deficiency ( $1.41 \pm 0.1$  ng/mL). However, this difference was not statistically significant (Table 3). Vitamin D deficiency plays a role in dental and oral bone pathologies (altered formation, periodontal disease, and jaw osteonecrosis) [13].

Another study has reported that optimal serum Vitamin D levels may reduce an individual's susceptibility to gingival inflammation and that gingivitis may be a useful clinical indicator to evaluate the anti-inflammatory effects of Vitamin D [20]. This finding was supported by the results of a more recent randomized clinical trial that showed a dose-dependent anti-inflammatory effect of Vitamin D on gingivitis [21].

Further, Vitamin D may also reduce periodontal disease through its general anti-inflammatory and immunomodulatory effects [22]. For example, the Vitamin D receptor is expressed on several human immune cells, decreases the proliferation of the T and B lymphocytes, and inhibits the T-helper (Th1) and Th17 (proinflammatory) cell response, while promoting a Th2 (anti-inflammatory) cell response [23]. Vitamin D may help suppress local inflammation at the site of an oral infection.

This study has some potential limitations that need to be considered. First, the lack of an association between the Vitamin D status and the periodontal disease is also dependent on the correct answers being given by the respondents in the questionnaire rather than only on the clinical evaluations and the medical history. The use of questionnaires has been validated in several studies [11].

## CONCLUSION

There was no association between gingival inflammation and 25(OH)D status among the postmenopausal women in this research.

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