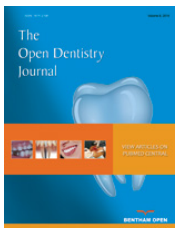

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RESEARCH ARTICLE

Serum Alkaline Phosphatase and Calcium in Relation to Periodontal Status among Perimenopausal and Postmenopausal Women

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Abstract:

Background:

Menopause is a physiological phenomenon that occurs in aging women. Periodontal disease is associated with menopausal status. Alkaline phosphatase (ALP) plays a role in general and periodontal bone turnover. Calcium is essential for the maintenance of bone and teeth, and serum ALP and calcium are specific bone markers related to the acceleration of bone mass loss in elderly women and periodontitis.

Objectives:

The aim of this study was to correlate the levels of serum ALP and calcium with periodontal status in perimenopausal and postmenopausal women with periodontitis.

Methods:

A total of 22 perimenopausal and 49 postmenopausal women underwent a full periodontal examination assessing the pocket depth, number of teeth lost, clinical attachment loss, plaque index, calculus index, and papillary bleeding index. Using these measurements, the subjects were divided according to periodontal severity. Serum ALP and calcium were measured using the Enzyme-Linked Immunosorbent Assay (ELISA) method. A correlation between serum ALP and calcium to periodontal status was investigated.

Results:

Serum ALP was significantly correlated with the severity of periodontitis, clinical attachment loss, and the number of teeth lost among perimenopausal and postmenopausal women ($p < 0.05$). Serum calcium levels were not correlated with periodontal status.

Conclusion:

Postmenopausal women tended to have more periodontal breakdown, and the level of serum ALP was increased in severe periodontitis.

Keywords: Alkaline phosphatase, Calcium, Periodontitis, Menopausal women, Aging, Serum.

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1. INTRODUCTION

Aging is a life process that begins from birth and continues throughout life. This process affects the biological systems, which reach their peak in early adulthood and decline in the elderly phase [1]. Aging is a complex multifactorial process that increases susceptibility to chronic inflammatory diseases and microbial infections, such as periodontitis. Tissue destruction is caused by the immune response of the host. Variations in the host response may increase or decrease the susceptibility of different individuals to destructive periodontal disease [2].

Women's aging is a process that involves an imbalance of estrogen hormones; this phenomenon impacts individuals and their quality of life. This natural process in the female life cycle is often of concern to women in their perimenopausal and menopausal periods [3]. The perimenopausal period is a physiological phenomenon in women's lives in which changes occur in the reproduction system, and it also represents a transition from the reproductive phase to old age [4]. The

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prevalence of menopausal women in Indonesia is 7.4% of the population, or approximately 14 million [5].

The aging population of the developed world will have important consequences for oral health and disease. Oral diseases that are more common with aging include oral cancers, dental caries, and periodontal diseases [6]. Periodontitis is a destructive inflammatory disease of periodontal tissue and is a major cause of tooth loss caused by dental plaque and calculus. Hormonal changes occur in older women as a result of menopause. Hormonal changes occur during perimenopause, resulting in significant changes in periodontal tissue [7]. Periodontal tissue damage in older women is caused by various changes in biochemical, immunological, and physiological processes during aging [8].

The prevalence of periodontitis is still high, and most cases affect elderly patients. Periodontitis is one of the biggest oral and dental problems in Indonesia. The prevalence of periodontitis in Indonesia is 74.1% in the 55–64 age group and 75.9% in those older than 65 [9 - 12]. Tadjoedin stated that periodontitis was the most prevalent periodontal disease in the elderly. The periodontal disease tends to relate to age [13 - 16].

Alkaline phosphatase (ALP) is a plasma membrane-bound glycoprotein that is particularly concentrated in the liver, kidney, and bone. It plays a role in the normal turnover of the periodontal ligament, cementum formation, and bone homeostasis in periodontal tissues [17, 18]. Strong ALP activity has been found in periodontal ligaments for the apposition of the acellular cementum mechanism [19]. Serum ALP has been found to be related to bone diseases. This suggests that serum ALP levels also reflect alterations in the alveolar bone due to periodontal disease [17]. ALP is involved in periodontal inflammation and regeneration because it is capable of allowing bone mineralization by organic phosphate and releasing and hydrolyzing inorganic pyrophosphatase [15]. Some studies have found that ALP is correlated with periodontal pocket depth and periodontal inflammation. This protein may act as a predictive indicator for future periodontal tissue damage [20, 21].

Calcium is required for the normal functioning of muscles and body systems and is essential for the maintenance and formation of calcified tissues, such as bones and teeth [22]. It is also required for blood cells to function. A study conducted on elderly Danish patients indicated that a higher intake of dairy products decreased the severity of periodontitis in later life [23, 24]. Calcium levels have been associated with low levels of estrogen in postmenopausal women because estrogen induces calcium absorption in the intestine and sends it to the bone. Estrogen deficiency in postmenopausal women might suppress calcium absorption in the intestine so that calcium levels are reduced [25].

Thus, the present study aims to correlate the levels of two important bone biomarkers in serum (calcium and ALP levels) and periodontal status between two groups of perimenopausal and postmenopausal women with periodontitis.

2. MATIERALS AND METHODS

2.1. Study Population

Inclusion criteria for all subjects were subjects ≥ 50 years of age with periodontitis who had at least 20 natural teeth, excluding the third molar both in the upper and lower jaw. The exclusion criteria were former smokers and alcoholics, subjects with current or past systemic diseases (diabetes mellitus, hyperthyroidism, cardiovascular diseases, renal diseases, and liver diseases), and subjects who had received hormone therapy within the past five years. The Ethical Committee of the Faculty of Dentistry Universitas Indonesia approved this study. All subjects signed an informed consent form before being recruited.

The subjects were divided into two groups based on menopausal status: perimenopausal and postmenopausal. Women who still had a menstruation cycle were classified as perimenopausal, while those who did not have a menstrual cycle for an entire year were classified as postmenopausal. To assess periodontal status, full-mouth periodontal examinations included periodontal probing depth (PPD), clinical attachment loss (CAL), and a number of teeth lost. PPD and CAL were performed on all teeth, six sites per tooth, with a standardized UNC-15 periodontal probe on a millimeter scale.

2.2. Procedure

Five milliliters of blood were drawn from the median cubitus vein to collect the sample. The blood was then centrifuged to get the serum. Serum ALP and calcium levels were measured using an Enzyme-Linked Immunosorbent Assay (ELISA).

PPD was defined as the distance from the gingival margin to the base of the sulcus or pocket, while CAL was measured from the cemento-enamel junction (CEJ) to the bottom of the sulcus or pocket. Age, plaque index (PlI), calculus index (CI), and papillary bleeding index (PBI) were recorded since these are potential determinants of periodontal diseases. The severity of periodontitis was classified into three groups based on the 2017 World Workshop of Periodontal dan Peri-Implant Diseases and Conditions: (1) mild: interdental CAL 1–2 mm; (2) moderate: interdental CAL 3–4 mm; and (3) severe: interdental CAL ≥5 mm [26].

2.3. Analysis of Data

The data were analyzed using SPSS from IBM. A normality test was performed before bivariate analysis. Age differences, serum calcium levels, and PD between perimenopausal and menopausal women were analyzed using an independent T-test, while differences in PlI, CI, PBI, serum ALP level, CAL, and tooth loss were analyzed using a nonparametric test (Mann-Whitney). A Spearman correlation test was performed to measure the correlation of periodontitis severity, PD, CAL, and tooth loss with serum ALP and calcium in all subjects.

3. RESULTS

Table **1** shows that of the 71 subjects, 69% (n = 49) were classified in the postmenopausal group. The results of this study showed that age (p = 0.001), serum ALP levels (p = 0.042), and CAL (p = 0.006) were significantly higher in postmenopausal subjects. Other variables, such as PlI, CI, PBI, tooth loss, and PPD, were higher in menopausal women, although they did not show any statistical significance.

Table 1. Characteristic of subjects based on menopausal status.

Subject Characteristics	Mean (SD)		*p* value
	Perimenopausal (n=22)	Postmenopausal (n=49)	
Age (years)	53.36 (4.22)	62.61 (7.71)	0.001*[i]
Plaque index	1.285 (0.54)	1.52 (0.66)	0.157[ii]
Calculus index	1.52 (0.97)	1.79 (0.80)	0.221[ii]
PBI	1.06 (0.78)	1.37 (0.92)	0.176[ii]
Serum ALP (IU/l)	79.32 (23.27)	90.12 (24.52)	0.042*[ii]
Serum calcium (mg/dL)	9.06 (0.38)	8.94 (0.33)	0.181[i]
Tooth loss	6.41 (5.84)	9.2 (6.88)	0.122[ii]
Pocket depth (mm)	2.17 (0.74)	2.47 (0.79)	0.126[i]
CAL (mm)	2.67 (1.01)	3.32 (1.10)	0.006*[ii]

*Statistically significant differences ($p < 0.05$).

[i] Independent T-test; [ii] Mann-Whitney non parametric test.

To investigate the relationship between serum ALP and calcium levels with periodontal parameters, the data were analyzed with the *Spearman* correlation test. A significant but moderate relationship was found between serum ALP levels and severity of periodontitis ($r = 0.252$; $p = 0.034$), number of teeth lost ($r = 0.291$; $p = 0.014$), and CAL ($r = 0.242$; $p = 0.042$).

Accordingly, the variables with $p < 0.05$, namely severity of periodontal disease, tooth loss, and CAL, were correlated to serum ALP levels in perimenopausal and postmenopausal women with periodontitis (Table **2**).

Table 2. Correlation between serum ALP and periodontal status.

Periodontal Status	Serum ALP Level		
	N	*r*	*p*
Severity	71	0.252	0.034*
Tooth loss		0.291	0.014*
Pocket depth		0.279	0.18
CAL		0.242	0.042*

*Statistically significant differences ($p < 0.05$).

In contrast to the relationship between serum ALP and periodontal status, this study did not find any relationship between serum calcium levels and periodontal parameters (Table **3**).

Table 3. Correlation between serum calcium and periodontal status.

Periodontal Status	Serum Calcium Level		
	N	*r*	*p*
Severity	71	-0.016	0.893
Tooth loss		-0.04	0.713
Pocket depth		0.093	0.442
CAL		-0.035	0.770

*Statistically significant differences ($p < 0.05$).

4. DISCUSSION

Studies have suggested that the aging process contributes to the incidence and severity of periodontal diseases [27 - 29]. Menopause is a physiological phenomenon in older women. At its transition, perimenopause is a turning point for physical, emotional, and psychological changes [16]. A review of aging and periodontal tissues emphasized the observation of tooth loss in elderly adults due to periodontitis and that this reflected a biological process of aging changes related to altered osteoblasts and osteoclasts, dysregulated responses of periodontal tissue cells to the oral microbiota, resulting in inflammatory changes, and more general biological changes in aging that can alter bone and tissue homeostasis [30].

In the current study, CAL was found to be significantly ($p < 0.05$) higher in postmenopausal subjects than perimenopausal subjects. Other periodontal parameters (PlI, CI, PBI, PD, and the number of teeth lost) were not significantly higher, but there was a higher tendency in postmenopausal women. CAL is considered a better parameter for determining the amount of damage in periodontal tissue because it shows cumulative disease over time [31]. Hormonal imbalances occur due to menopause, causing lower estrogen levels that may affect the oral environment. This impacts decreased physical function due to increasing proinflammatory cytokine production, such as IL-1b, TNF-a, and IL-8, which are involved in periodontal bone loss [32].

A study conducted by Pitu *et al*. (2017) showed that there were no differences in periodontal status among perimenopausal and postmenopausal women, but PD, CAL, and the number of teeth lost means were higher in the postmenopausal subjects [16]. Dodd *et al*. stated that there was a clear relationship between postmenopausal women with low bone mineral density tending to have more CAL and alveolar bone loss [33]. According to Choi *et al*., women with periodontitis were likely to go on to develop osteoporosis, and those aged above 50 with periodontitis would likely have a higher rate of osteoporosis than the younger group [34].

In our study, we observed a significant increase in serum ALP in postmenopausal women. A similar result was also found in a study performed by Pardhe *et al* [35]. The concentration of ALP tended to be higher in elderly patients; the biggest significance was found in women in their 60s and 80s [36]. Generalized bone atrophy often occurs after the 60s. Osteoid formation and bone mineralization involve the ALP. Estrogen deficiencies in postmenopausal women induce the synthesis of cytokines with the help of osteoblasts. Therefore, some stimulation in bone mineralization occurs, which results in bone resorption by increasing osteoclast activity [37].

This investigation found that serum ALP levels were significantly higher in the postmenopausal group compared to the perimenopausal group. This finding is in accordance with the other studies [38, 39]. The finding that serum ALP levels had a significant correlation with periodontal disease severity, number of teeth lost, and CAL is in accordance with other studies, and this phenomenon occurred more frequently in women than men [19]. ALP is released from dead cells of periodontal tissue, leukocytes, and inflammatory, epithelial, and connective tissue cells from affected sites during the progression of the disease [40].

The presence of high levels of serum ALP might be caused by an increase in periodontal inflammation and an imbalance between bone formation and resorption in elderly women due to aging and menopausal processes. Elevation of serum ALP in periodontium activity might cause the release of phosphate ions that can lead to more calculus accumulation, resulting in worsened periodontitis [41]. Another study stated that ALP was not only produced by cells from the periodontium but also from the neutrophil granule and pathogen bacteria present in dental plaque [42]. It makes periodontal tissue more susceptible to alveolar bone resorption; therefore periodontal severity, tooth loss, and CAL are increased [43].

In contrast to serum ALP levels, this study did not find any correlation between serum calcium levels and periodontal parameters in perimenopausal and postmenopausal women with periodontitis. This finding was similar to a study by Tanaka *et al*. (2014), which stated that serum calcium did not correlate with periodontal status, but calcium intake might have a beneficial effect on periodontal tissue [40]. In contrast, Amarasena *et al*. found that serum calcium had a significantly negative correlation with the progression of periodontal disease [41].

In normal conditions, the adult human body contains about 1,200 grams of calcium. Only 1% is found in blood, muscle, and extracellular tissues, while the other 99% is stored in bones and teeth [42]. Serum calcium might have low sensitivity because its concentration is measured after the intake of its high doses. The result reflects the moment of its absorption. The serum concentration of calcium is measured after the intake of a high dosage of the nutrient, so the results reflect its instantaneous absorption. The homeostasis of calcium prevents great changes in serum calcium levels after its absorption, due to which the increments tend to be small [43].

It should be noted that this study is limited by the cross-sectional method that was used and cannot determine cause and effect nor analyze behavior over time. More subjects must be recruited, and longitudinal studies are needed based on this study. It is recommended that future researchers conduct similar studies with a larger sample and more biomarkers, with longitudinal studies for further confirmation and evaluation of the relationship between periodontal diseases and menopause.

CONCLUSION

This study showed that postmenopausal women had higher serum ALP levels and clinical attachment loss than a group of perimenopausal women. Furthermore, Serum ALP level had a positive correlation with the severity of periodontitis, CAL, and the number of teeth lost in this study. Serum calcium levels were not correlated with all periodontal parameters in our investigation. These findings may contribute to better periodontal health among perimenopausal and postmenopausal women.

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

This study was approved by the Dental Research Ethics Commission (KEPKG), Faculty of Dentistry, Universitas Indonesia, Indonesia (No: 14//Ethical Approval/FKGUI/III/2017)

HUMAN AND ANIMAL RIGHTS

No animals were used in this research. All human research procedures were followed in accordance with the ethical standards of the committee responsible for human experimentation (institutional and national), and with the Helsinki Declaration of 1975, as revised in 2013.

CONSENT FOR PUBLICATION

All subjects signed an informed consent form before being recruited.

AVAILABILITY OF DATA AND MATERIALS

The data supporting the finding of the article is available in the Zenodo Repository at zenodo.org, reference number https://doi.org/10.5281/zenodo.5746429.

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CONFLICT OF INTEREST

The authors declare no conflict of interest, financial or otherwise.

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Declared none.

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