



Evaluation of Calcium and Alkaline Phosphatase in the Salivary Samples of Premenopausal and Postmenopausal Women

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Authors' contributions

This work was carried out in collaboration between both authors. Author KB designed the study, performed the statistical analysis, wrote the protocol, wrote the first draft of the manuscript and managed the literature searches. Author SD managed the analyses of the study. Both authors read and approved the final manuscript.

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ABSTRACT

The quality of life for women after menopause is one of the key health issues today. Rapid bone loss occurs in post-menopausal women due to hormonal factors that lead to an increased risk of fractures. The aim of the study is to evaluate the calcium and Alkaline phosphate levels (ALP) in the salivary samples of premenopausal and postmenopausal women. The study was conducted with a sample size of 30, with 15 samples from premenopausal women belonging to the age group of 40-46 years and 15 samples from postmenopausal women aged more than 50 years. Calcium and alkaline phosphatase levels were estimated using the commercial kits available which were Spinreact and Accucare alkaline phosphatase respectively. The statistical analysis was done using independent t-test. Saliva was stimulated and biochemical analysis of these salivary samples was done to evaluate the calcium and alkaline phosphatase. Mean salivary calcium in premenopausal women was found to be 7.37 ± 1.141 mg/dl and in postmenopausal women it was found to be

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5.19±1.141mg/dl,P=0.000(p<0.05). Mean salivary ALP levels in premenopausal women was found to be 10.81±3.295 mg/dl and in postmenopausal women was found to be 33.58±3.295 mg/dl, P=0.001(p <0.05), hence statistically significant. Within the limitations of the study, it was found that calcium were found to be significantly more in premenopausal women when compared to postmenopausal women, while alkaline phosphatase levels were increased in postmenopausal women when compared to premenopausal women. Hence, salivary calcium and ALP levels can be used as biomarkers for diagnosis of bone diseases as it is a non-invasive technique.

Keywords: Alkaline phosphatase; calcium; menopause; women.

1. INTRODUCTION

There is a statement that says the mouth is the mirror of health. The systemic health is reflected in the oral cavity than any other part of the body. Both quantitative and qualitative assessment of salivary components may serve as important tools for monitoring normal body function as well as diagnostic tools for underlying systemic diseases and also to monitor normal body functioning [1]. The chief mineral components of the skeletal system which are calcium and phosphorus are also found to be in saliva. Along with these alkaline phosphatase is also secreted in saliva. All these are potential markers for bone turnover [2,3].

Menopause is a physiological process that occurs due to decrease in levels of estrogens, commonly in the fourth-fifth decade of life in women. It involves permanent cessation of menstruation. This process is also accompanied by physiological and sensorial oral changes in certain individuals.

Oral symptoms have been found to be significantly greater in menopausal women (43%) than in premenopausal females (6%) [4]. Menopause causes oral conditions such as oral dryness or xerostomia and burning mouth syndrome which is of concern to the dental professionals. Xerostomia happens to be a major complaint for many elderly individuals which is found to be a subjective sensation in one-third of the cases and does not reflect a dry mouth. It is associated with an unpleasant feeling in the mouth and throat [5]. The prevalence of this complaint is more in menopausal women who are on medication and is also quite common in those without disease or drug usage, not frequently related to lowered salivary flow rates [6–8].

Other menopause-associated symptoms that are less common include bad or altered taste, viscous saliva, senile atrophic gingivitis and mucosal disorders such as lichen planus, benign

mucosal pemphigoid and Sjogren's syndrome [9]. Furthermore, menopause induced osteoporosis may lead to loss of alveolar bone height.

Calcium is an important mineral for the skeletal system. Several studies indicate that salivary calcium and phosphate concentrations show a clear increase with increasing age. It has also been studied that calcium is the only electrolyte which does not show correlation with salivary flow rate [6,10,11].

Serum alkaline phosphatase is the most commonly used biomarker of bone formation. ALP is a universal enzyme that plays an important role in osteoid formation and mineralization of bone. The serum ALP pool consists of several dimeric isoforms that originate from various tissues, such as the liver, bone, intestine, spleen, kidney, and placenta [12].

The techniques currently available for measurement of bone mass in vivo are expensive as well as invasive. They may also not be very effective for screening the general population. Salivary biomarkers have been used to assess the risk of developing oral, ovarian and breast cancers, HIV infection, Sjogren's syndrome, dental caries and periodontal diseases. Using saliva rather than serum has benefits as it is non-invasive, easy to obtain, painless and there is no need to employ specially trained personnel for sample collection. Therefore, saliva, the ultrafiltrate of plasma can serve as an efficient resource.

Our recent research portfolio slides numerous articles in reputed journals [13–17]. Based on this experience we planned to pursue the evaluation of calcium and alkaline phosphatase in the salivary samples of premenopausal and postmenopausal women.

2. MATERIALS AND METHODS

This comparative study was performed on a total of 30 subjects who were divided into an experimental group of 15 premenopausal women

(30-45 years old) and 15 postmenopausal women (>45 years old). Studied women were healthy with no systemic illness and history of bone fractures. Premenopausal women gave a history of regular menses and no contraceptive pills were taken. Postmenopausal period was defined as the absence in menses for a period of 1 year.

2.1 Saliva Collection

Salivary calcium and alkaline phosphatase levels were estimated for all the two groups. Prior to salivary sample collection, patients were instructed not to eat or drink anything for at least two hours. Saliva was collected by stimulating salivary flow. Approximately 5 ml of saliva was collected in a test tube and tested for calcium and alkaline phosphatase levels.

2.2 Estimation of Salivary Calcium and ALP Levels

Calcium was estimated by a commercially available kit (Spinreact Calcium) containing arsenazo III system which functions on the following mechanism: Calcium with arsenazo III at neutral pH yields a blue colored complex. Alkaline phosphatase was estimated by using a commercially available alkaline phosphatase kit (ACCUCARE ALKALINE PHOSPHATASE-SLR)

2.3 Statistical Analysis

Patient’s salivary calcium levels and alkaline phosphatase levels were tabulated and the values were compared using independent t-test. A prospective, comparative and observational study was carried out. A p-value of <0.05 was taken as statistically significant. The software used for statistical analysis was IBM SPSS version 20.0.

3. RESULTS AND DISCUSSION

The mean age of the female population taken in the study was found to be 40.73 years in the premenopausal group and 47.7 years in the postmenopausal group. . The mean value of calcium in the saliva in premenopausal women was found to be 7.37± 1.141 mg/d and in postmenopausal women was 5.19 ± 1.141 mg/dl, with highly significant difference, p value:0.000(p <0.05) between the two groups. (Table 1) Furthermore, there was significant difference p value: 0.001(p <0.05) in the mean values of ALP

levels in the saliva samples of premenopausal and postmenopausal women. The mean levels of ALP in premenopausal women was found to be 10.8±3.295 mg/dl and that in post menopausal women was found to be 33.58±3.295 mg/dl. (Table 2).

Table 1. Shows mean calcium levels in premenopausal and postmenopausal women. Mean calcium level in premenopausal women was found to be more than postmenopausal women. Independent T-test was done. P value is 0.000(p <0.05), hence statistically significant

Units	Mean	Standard deviation	P value
Premenopausal women	7.37	1.141	0.000
Postmenopausal women	5.19	1.141	

In our study, the salivary calcium levels were significantly higher in premenopausal patients compared to postmenopausal patients, whereas the ALP levels were significantly higher in postmenopausal women when compared to premenopausal women.

The quality of health decreases and the extent of menopausal problems among post-menopausal women are numerous and thus draws the attention of health authorities. Bone turnover that leads to various health problems is increasing in both developed and developing countries [18–20].

In relation to dentistry, the status of the periodontium is generally diagnosed clinically and using radiographs. The biochemical methods to evaluate the periodontium are done using saliva and GCF. Collection of GCF is complicated. Saliva can be easily collected and may contain both local and systemically derived markers that can be evaluated for diagnostic purposes. The use of saliva as a potential biomarker has been the subject of research activity in periodontal diagnosis in various studies [21–23]. Bone turnover rate has been studied to be higher in the alveolar bone when compared to the long bones. Therefore, systemic imbalance will be manifested in the alveolar bone than any other site [24].

The results of various case control studies in pre- and post-menopausal women have shown that

changes in the serum calcium levels in postmenopausal women are not statistically significant [25–27], however, in the present study, we found that the serum calcium levels were significantly reduced in the postmenopausal group 7.37mg/dl when compared to the premenopausal group 5.19 mg/dl. Similar results were obtained by Bhattarai et al., where in the calcium levels in the serum levels in the premenopausal women were 9.65mg/dl and in postmenopausal women were 8.73, the values being considerably higher than in the present study [28]. In a study by Ashuma et al., it was reported that aging and menopause lead to a decline in oestrogen and progesterone production, which has been implicated in the decreased calcium levels of post-menopausal women [26]. In a study conducted by Singh et al, it was found that postmenopausal women without xerostomia had higher levels of salivary calcium when compared to those with xerostomia [1].

ALP is considered as a potential marker for alveolar bone resorption in post menopausal women. It increases in the postmenopausal group due to increased bone turnover. [23] In our

study, ALP levels in postmenopausal women were significantly higher than in premenopausal women. In a study conducted by Khan et al, similar results were obtained, where in ALP levels in premenopausal women was 11.3 and that in postmenopausal women was 32.1 [23]. Bhattarai T et al., and Ramesh A et al., also reported higher serum ALP levels in post menopausal women [28,29]. Serum alkaline phosphatase in post menopausal women was higher than premenopausal women in a study conducted by Bhattarai et al. [28].

Table 2. Table shows mean ALP levels in premenopausal and postmenopausal women. Mean ALP levels were less in premenopausal women than postmenopausal women. Independent T-test was done. P value is 0.001(p <0.05), hence statistically significant

Units	Mean	Standard deviation	P value
Premenopausal women	10.81	3.295	0.001
Postmenopausal women	33.58	3.295	

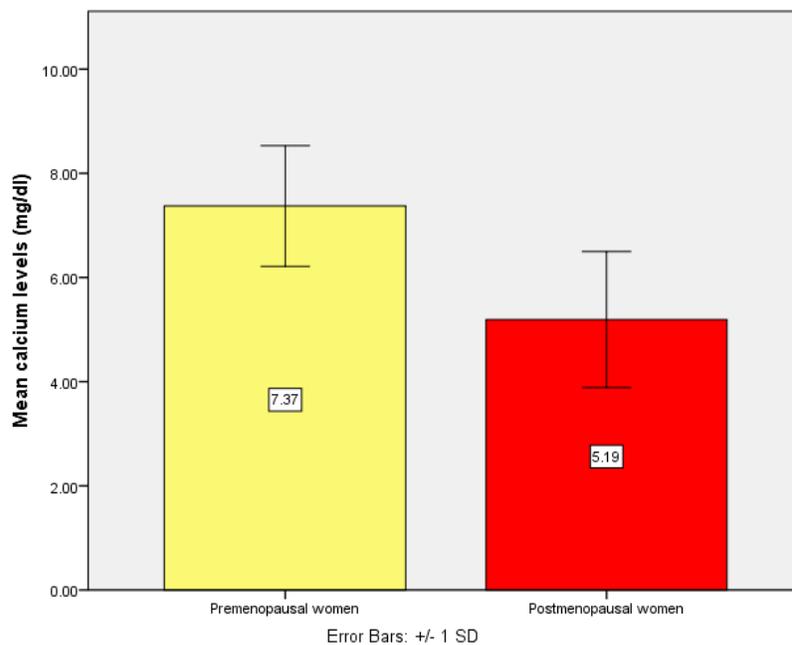


Fig. 1. Bar graph shows mean calcium levels in premenopausal and postmenopausal women. X-axis denotes conditions of the patient and Y-axis denotes mean calcium levels. Mean calcium levels in premenopausal women (yellow)7.37±1.141mg/dl was more than post menopausal women (red). P value is 0.000(p <0.05), hence statistically significant

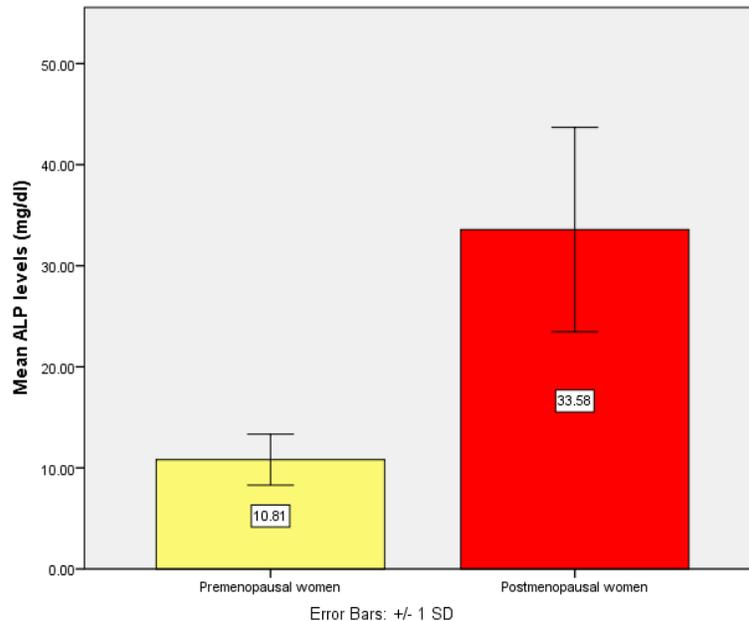


Fig. 2. Bar graph shows mean ALP levels in premenopausal and postmenopausal women. X-axis denotes conditions of the patient and Y-axis denotes mean ALP levels. Mean ALP levels in postmenopausal women (red) 33.58 ± 3.295 mg/dl was more than premenopausal women (yellow). P value is 0.001 ($p < 0.05$), hence statistically significant

Since salivary calcium and ALP is associated with altered bone metabolism, it clearly shows that in post menopausal women, the balance between bone formation and resorption is lost and hence they are susceptible to alveolar bone resorption.

4. CONCLUSION

Within the limitations of the study, it was found that calcium levels were found to be significantly higher in premenopausal women when compared to postmenopausal women while Alkaline Phosphatase levels were increased in postmenopausal women compared to premenopausal women. Hence salivary calcium and ALP can be taken as an additional biomarker for early diagnosis of development and progression of bone diseases especially among post menopausal women.

CONSENT AND ETHICAL APPROVAL

Written informed consent was obtained from all the participants before the salivary samples were taken for the study. Ethical Clearance was obtained from the Institutional ethical committee of Saveetha Dental College and Hospitals.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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