Original Article

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Dentistry Section

Comparative Analysis of Salivary Alkaline Phosphatase in Post menopausal Women with and without Periodontitis

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ABSTRACT

Introduction: Alkaline phosphatase is an intracellular destruction enzyme in the periodontium, and it takes part in the normal turnover of the periodontal ligament, alveolar bone, and root cementum formation and maintenance.

Aim: The aim of this case control study was to evaluate the enzyme Alkaline Phosphatase (ALP) level in saliva of post menopausal women with and without chronic periodontitis.

Materials and Methods: In this study, 40 individuals, satisfying the study inclusion and exclusion criteria, were recruited. They were categorically divided, on the basis of gingival index, probing pocket depth and clinical attachment level, into two groups: Group I (post menopausal women with a clinically healthy periodontium, n=20); and Group II (post menopausal women with generalized chronic periodontitis, n=20). Clinical parameters assessed were Plaque Index (PI), Gingival Index (GI), Clinical Attachment Level (CAL) and Probing Pocket Depth (PPD). Unstimulated salivary samples were obtained in which the ALP concentration was measured using p-Nitrophenylphosphate, and 2-amino-2-methyl-1-propanol reagents in Beckman and Coulter, AU 480 auto analyser. Mann-Whitney U test was used to find statistical difference with respect to all clinical parameters such as PI, GI, CAL, PPD and salivary ALP levels.

Results: The mean ALP in saliva was found to be higher in Group II compared to Group I and the difference was statistically significant with the p-value of 0.008.

Conclusion: A noteworthy increase in the ALP concentration was seen in saliva in our study (Group II) may be due to increased periodontal inflammation in post menopausal women. Thus salivary ALP can be taken as an additional biomarker to early diagnosis, development and progression of periodontitis especially among post menopausal women.

Keywords: Alveolar bone loss, Biomarker, Menopause, Oestrogen, Saliva

INTRODUCTION

Periodontitis is inflammation of the periodontium that extends beyond the gingiva and produces destruction of connective tissue attachment of teeth [1]. In periodontal disease, the balance between synthesis and degradation is disrupted and the collagen fibrils of periodontal ligament are broken down together with the supporting alveolar bone. Different enzymes involved in both the intracellular and the extracellular pathway of tissue destruction have been investigated as potential diagnostic markers of periodontitis [2]. Among the intracellular destruction enzymes that have received the most attention as possible marker of active periodontal destruction is ALP. This enzyme is released from dead and dying cells of the periodontium, mostly from polymorpho-nuclear leukocytes. ALP is a membrane bound enzyme, which hydrolyzes monophosphate ester bonds and increases the local concentration of phosphate ions. ALP is a very important enzyme in the periodontium, as it takes part in the normal turnover of the periodontal ligament and alveolar bone and root cementum formation and maintenance. ALP is produced by many cells like neutrophils, fibroblasts, osteoblasts and osteoclasts. Few longitudinal studies reported higher Gingival Crevicular Fluid (GCF) ALP levels in active periodontal disease sites [3-5].

In women, menopause is associated with oestrogen deficient state. In menopause, the balance between bone formation and bone resorption is altered, resulting in excessive bone resorption. Bhattarai T et al., also reported higher serum ALP levels in post menopausal women [6]. Reduced oestrogen leads to alveolar bone resorption, clinical attachment loss and tooth loss [7]. Oestrogen deficiency is considered as a risk indicator for periodontal disease. ALP levels in saliva and GCF are considered as a potential diagnostic marker for periodontitis [8]. Limited studies are available to address the periodontal disease activity among post menopausal women. It has been hypothesized that post menopausal women are prone for alveolar bone loss and salivary ALP levels are an indicator of periodontal disease. This study was aimed to evaluate the enzyme ALP level in saliva of post menopausal women with and without chronic periodontitis.

MATERIALS AND METHODS

The present case control study included 40 post menopausal women with age between 45-60 years. The study participants were selected from the outpatient pool of Thaimoogambigai Dental College, Chennai. The study was conducted from January 2016 to May 2016. The ethical clearance was obtained from Dr. MGR University Ethical Committee, Chennai, India. Based on the study of Daltaban O et al., the sample size was estimated to be 20 per group [9]. The protocol was explained and written informed consent was obtained from all the participants. The subjects were screened for periodontal and menopause status and categorized into two groups as 20 post menopausal women and periodontally healthy (Group I) and 20 post menopausal women with generalized chronic periodontitis (Group II).

The participants were enrolled according to inclusion and exclusion criteria. The inclusion criteria for both the groups were that the post menopausal women should be aged between 45-60 years, with minimum number of 20 teeth. Group I subjects had GI score of 0 and Group II subjects had CAL of 3-5 mm in more than 30% of sites. The patients having less than 20 numbers of teeth, patients who had taken anti-inflammatory drugs or antibiotics within previous three months and who underwent periodontal treatment in the past six month were excluded. Clinical parameters assessed were PI, GI, CAL and PPD.

PI was recorded at four sites (mesiobuccal, midbuccal, distobuccal and mid palatal sites) around each tooth [10]. Four gingival areas of the tooth (facial, mesial, distal and lingual) around each tooth were

examined according to GI scores given by Loe and Silness [11]. Subjects with healthy periodontium had GI score of 0. The selection criteria for generalized chronic periodontitis were patients having 3-5 mm of CAL in more than 30% of the sites [12]. The CAL was recorded using a William's graduated periodontal probe at six sites per tooth and measured in millimetres. CAL was measured as the distance from the cemento-enamel junction to the bottom of the periodontal pocket. Periodontal Probing was performed at six sites per tooth (mesiobuccal, distobuccal, mesiolingual, distolingual, midbuccal, midlingual).

Estimation of ALP: A 10 ml of unstimulated saliva sample was collected and centrifuged at 500 rpm. Using Beckman and Coulter, AU 480 auto analyser, ALP was estimated and the value of ALP was expressed in U/L. The reagents used were p-Nitrophenylphospate, and 2-amino-2-methyl-1-propanol. The salivary ALP levels were found to be 20.70±7.18 U/L in healthy periodontium, 33.06±5.49 U/L in gingivitis and 49.62±16.46 U/L in Periodontitis in the study conducted by Patel RM [13]. Mann-Whitney U test was used to find statistical differences with respect to clinical parameters like PI, GI, PPD, CAL, salivary ALP levels.

RESULTS

The mean scores of periodontal parameters like PI, GI, PPD, CAL of group I and group II has been mentioned in [Table/Fig-1] and difference was statistically significant with p-value of 0.008. The mean salivary ALP level was found to be higher in group II compared to group I and difference was statistically significant with p-value of 0.008 [Table/Fig-1].

	Group	N	Mean	SD.	p-value
Age	I	20	51.6000	6.26897	.420
	II	20	54.8000	5.21536	
PI	I	20	.6260	.21138	.008
	II	20	1.4880	.25636	
GI	I	20	0.340	.01140	.008
	II	20	.8840	.34041	
PPD	I	20	1.4920	.18606	.008
	II	20	5.1140	.79321	
CAL	I	20	1.1460	.10877	.008
	II	20	4.6480	1.05365	
ALP	I	20	37.2600	9.91806	.008
	II	20	70.4320	14.36427	

[Table/Fig-1]: Mann-Whitney U Test for comparison of clinical parameters between the two groups.

DISCUSSION

Conventional diagnostic techniques like clinical and radiographic methods do not determine the present activity of the periodontal disease [14]. The biochemical methods for periodontal diagnosis use saliva and GCF samples. GCF collection is cumbersome and complicated. Saliva is the diagnostic fluid of choice in the 21st century. Saliva can be collected easily and may contain both locally and systemically derived markers of periodontal disease, which can be evaluated for diagnostic purposes. The use of saliva as biomarker has been the subject of considerable research activity in periodontal diagnosis [15,16].

Menopause is termed as permanent cessation of menses that occurs naturally or is induced by surgery resulting from reduced ovarian hormone secretion. Both aging and menopause are associated with accelerated bone mass loss. Bone turnover rate is higher in alveolar bone compared to long bones. Therefore, the systemic imbalance in bone resorption and deposition might be manifested initially in the alveolar process than in other sites [17]. The possible mechanism by which post menopausal women lead to more periodontal destruction may be the presence of less cresta alveolar bone per unit volume, this bone of lesser density may be more easily resorbed. Oestrogen acts by blocking the production of cytokines that promote osteoclast differentiation and osteoclast apoptosis [18]. Oestrogen withdrawal following menopause is associated with increased osteoclast numbers due to enhanced osteoclast formation activity and reduced osteoclast apoptosis [19]. ALP is associated with osteoid formation and mineralization. ALP enzyme is considered as a potential marker of alveolar bone resorption in post menopausal women [20]. Our study was carried out to evaluate the enzyme ALP level in saliva of post menopausal women with and without chronic periodontitis.

On comparison of clinical parameters like PI, GI, PPD and CAL, group II had higher mean scores compared to group I, which is similar to the study by Amitha et al., [20].

The present study results showed an increase in salivary ALP levels in post menopausal women with chronic periodontitis compared to post menopausal women with healthy periodontium which is comparable to previous studies [21,22]. The presence of high levels of ALP in saliva in post menopausal women with periodontitis may be due to increased periodontal inflammation and rapid bone turnover rates as ALP is produced not only by the cells of the periodontium, but secreted mostly by neutrophils and bacterial sources present in supra and sub gingival plaque. ALP is normally stored in specific granules of neutrophils and is released on migration to the site of periodontal infection. As this enzyme is associated with periodontal inflammation, ALP levels can also be used as an indicator of efficacy of periodontal therapy [23].

The salivary ALP level in periodontally healthy subject was found to be 20.70±7.18 U/L in a previous study [13] but in our study, Group I subjects had slightly higher mean salivary ALP levels compared to mean ALP levels of periodontally healthy subjects in the previous study, in spite of minimal inflammatory status in the periodontal tissues. Bhattarai T et al., and Ramesh A et al., also reported higher serum ALP levels in post menopausal women [6,20]. Since salivary 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, CAL and tooth loss. Since salivary ALP levels are an early indicator of periodontal disease, prompt attempt can be carried out in the form of periodontal therapy or systemic therapy to arrest the progression of periodontal disease in post menopausal women. This post menopausal women cohort represents a large number of patients that present in clinical practices. Therefore dental clinicians must be aware of the effects of reduced hormones on the periodontal tissues and can take steps to prevent periodontal disease progression. Further longitudinal interventional trials are needed to confirm this association.

LIMITATION

Our study is an observational study with small sample size and has proved the use of salivary ALP levels as a periodontal disease marker in post menopausal women. Further longitudinal studies involving more participants from similar population with intervention trials can overcome these limitations.

CONCLUSION

ALP level is increased in post menopausal women with chronic periodontitis than in postmenopausal women with healthy periodontium. In our study, ALP was found to be high in both the groups. Post menopausal women are more prone to periodontal infections therefore they should be motivated to maintain proper oral hygiene. Hence salivary ALP can be taken as an additional biomarker to early diagnosis of development and progression of periodontitis especially among post menopausal women.

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