Original Research Paper

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A Comparative Evaluation of Salivary Alkaline Phosphatase Level in Pre-Menopausal and Post-Menopausal Women with and without Periodontitis: A Biochemical Study.

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: This study includes 48 samples of pre and post menopausal females. All the subjects were selected from the Department of Periodontology, Peoples College of Dental Science and Research Centre, Bhopal. The study participants were divided into 4 groups, Group A: 12 pre- menopausal women without periodontitis with systemically healthy, Group B : 12 post-menopausal women without periodontitis with systemically healthy, Group C: 12 pre- menopausal women with periodontitis and systemically healthy, Group D: 12 post- menopausal women with periodontitis and systemically healthy. 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.

Result: The result of the present study showed considerably higher salivary ALP levels in post menopausal women with chronic periodontitis compared to other groups.

Conclusion : The present study suggest that the presence of ALP levels is not simply a reflection of the local inflammatory state in females but their estrogen levels may possibly influence bone destruction in periodontitis.

Key words: Alkaline phosphatase; Menopause; Saliva; Chronic periodontitis

Introduction:

Periodontitis is the inflammation that effects the tissues supporting the teeth and periodontium, caused by micro-organisms leading to periodontal defects, such as pockets, gingival recession, mobility, and bone loss. Periodontitis can also be seen in patients who have multiple endocrine disorders, often caused by hormonal changes in the body during puberty, pregnancy, premenopause, postmenopause and stress. There are many conventional methods for assessing periodontal tissue condition, proper diagnosis and treatment plan, but each method has its own limitations. As a consequence, genetic susceptibility, microbiological analysis and biochemical analysis[2] are used in many other methods.

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Enzymes released from different tissues can be useful for diagnosis and can also serve as a potential biomarker to detect the condition of the tissue.[2] Various enzymes involved in both the intracellular and extracellular pathways of tissue degradation have been studied as a potential diagnostic marker for periodontitis. Lactate Dehydrogenase (LDH), AlkalinePhosphatase (ALP), Aspartate and Alanines

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Aminotransferases (AST),Creatine Kinase (CK), and Acid Phosphatase (ACP) are numerous enzymes tested for the early detection of periodontal tissue destruction.

Alkaline phosphatase is among the intracellular degradation enzymes that have received the most interest as a possible marker of periodontal destruction. The enzyme alkaline phosphatase (ALP) is found in saliva, as it is released from the damaged and the dead cells of the periodontal tissues. The most commonly used biomarker of bone formation is serum alkaline phosphatase [3].ALP is a universal enzyme that plays a significant role in osteoid formation and mineralization in the bones. Many dimeric isoforms from various tissues, including the bone, liver, spleen, kidneys and intestines, are part of the ALP serum [3]. In addition to serum, ALP is also present in saliva which can be used as a periodontitis biomarker. It is a membrane bound enzyme that hydrolyzes the monophosphate ester bonds and raises the concentration of local phosphate ions. ALP is one of the most important enzyme in periodontium, as it participates in the natural turnover of periodontal ligament, alveolar bone and root cement development . ALP is also found in certain cells, such as osteoclasts, osteoblasts, neutrophils and fibroblasts. According to some studies, since ALP can be seen at tissue destruction sites, higher values have been detected in the gingival crevicular fluid in the area of periodontal tissue destruction. [3]. Menopause is a hormonal phenomenon that occurs in women due to reduction in estrogen levels, typically in the fourth and fifth decades of life. It requires irreversible menstrual cessation. Estrogen deficiency may inflammation, osteoporosis - decreases alveolar bone density and in turn increases its susceptibility to resorption. This is followed by elevated levels of biochemical markers that are consistent with bone metabolism, such as alkaline phosphatase. As the result of this process, we conducted a study on premenopausal women and post menopausal women checking the periodontal condition, by using saliva as a biomarker, as it is the potential diagnostic aid of Periodontitis.

Material and Method:

This study includes 48 samples of pre and post menopausal females. All the subjects were selected from the Department of Periodontology, Peoples College of Dental Science and Research Centre, Bhopal. The study participants were divided into 4 groups, 12 participants of pre - menopausal women without periodontitis, 12 participants of post - menopausal women without periodontitis, 12 participants of pre menopausal women with periodontitis, 12 participants of post - menopausal women without periodontitis. Clinical parameters like GI, PI, PPD, CAL and unstimulated whole saliva was obtained from all the participants and statisticaly analyzed .

Method of collection of saliva:

Whole saliva (unstipulated) sample will be collected in a disposable

Plastic container; patient will be instructed not to eat 1 hour prior collection of sample. The saliva sample will be coded before transporting it to the laboratory. The saliva sample will be stored at 2-40Celsius until it will be transported to the laboratory for analysis of ALP.

Enzyme Assay for Saliva:

The level of ALP was estimated with an auto analyzer by using

International Federation of Clinical Chemistry method using Erba

Mannheim kit for analysis, each saliva sample was centrifuged at 5000 rpm for 10 minutes. Reagents were added to about 10□l of unstimulated saliva and determined spectrometrically by the auto analyzer and the value of ALP estimated was expressed in units per litre of saliva.

Observations and Results:

This study was conducted in the Department of Periodontology, Peoples College of Dental Science and Research Centre, Bhopal, to evaluate the level of salivary alkaline phosphatase in pre and post menopausal women in healthy and patients suffering from chronic periodontitis. A total of 48 study participants were divided into 4 groups,

Group A: 12 premenopausal women without periodontitis with systemically healthy,

Group B: 12 post- menopausal women without periodontitis with Systemically healthy,

Group C: 12 pre- menopausal women with periodontitis and systemically healthy & Group D: 12 post- menopausal women with periodontitis and systemically healthy. Clinical

measurements and un stimulated whole saliva was obtained and analysed at baseline. Statistical evaluation of clinical observations was carried out. Evaluation of the level of salivary alkaline phosphatase and clinical observations Gingival Index(GI), plaque index(PI), Probing Depth(PPD), clinical attachment level (CAL) in GroupA,B,C,D are represented in the following tables-

Overall comparison of all parameters in all the groups evaluated

	Group	N	Mean	Standard deviation	P value
Age	1	12	31.00	3.97	<0.001**
	2	12	49.25	4.75	
	3	12	39	31.08	
	4	12	58	51.50	
BMI	1	12	25.77	4.91	0.025*
	2	12	28.50	23.57	
	3	12	26.10	21.58	
	4	12	32.10	25.80	
GI	1	12	1.17	0.40	<0.001**
	2	12	1.26	0.41	
	3	12	1.43	0.37	
	4	12	2.27	0.32	
PI	1	12	1.21	0.46	0.005*
	2	12	1.39	0.51	
	3	12	1.13	0.34	
	4	12	1.81	0.50	
BOP	1	12	1.33	.77	0.010*
	2	12	1.08	.79	
	3	12	1.33	0.65	
	4	12	2.08	0.79	
PPD	1	12	1.25	0.58	<0.001**
	2	12	2.02	.91	
	3	12	1.27	0.53	
	4	12	4.69	1.31	
CAL	1	12	1.46	.36	<0.001**
	2	12	1.57	0.54	
	3	12	1.46	0.36	
	4	12	4.04	0.60	
Salivary alkaline phosphatase	1	12	30.40	7.15	<0.001**
	2	12	50.01	8.30	
	3	12	31.96	10.29	
	4	12	68.68	13.46	

** = Highly significant ; * = Significant

Data analysis:

Data collected was entered into spreadsheets and analysed using SPSS version 20.0 (IBM; Chicago). Descriptive analysis was presented in forms of mean and standard deviation.. One way Analysis of Variance (ANOVA) was used to determine any difference in mean of clinical parameters and salivary biomarker between the groups. $P < 0.05$ was considered to be statistically significant.

Discussion:

In the 21st century, saliva is used as a diagnostic tool of choice. The use of saliva as a biomarker in periodontal diagnosis has been the focus of considerable research activity[11,12]. Menopause is a permanent termination of the menstrual cycle and is also characterized as the main circulating estrogen with declining amounts of estradiol (E2). By stimulating the synthesis of matrix metalloproteinases, nitrous oxide, and several cytokines involved in bone resorption, especially in response to bacterial infection. In the present study considering that menopause as a risk factor for periodontitis, we compared the clinical parameter values and salivary ALP levels between pre and post menopausal women with and without periodontitis.The subjects were divided into 4 groups; Group A: 12 premenopausal women without periodontitis with systemically healthy, Group B: 12 post-menopausal women without periodontitis with systemically healthy, Group C: 12 pre- menopausal women with periodontitis and systemically healthy, Group D: 12 post-menopausal women with periodontitis and systemically healthy. All the participants are systemically healthy. Clinical and radiographic approaches are standard diagnostic methods but they do not determine the current periodontal disease activity [10]. Saliva sample can be used as a biochemical method for periodontal diagnosis. One of the most common diseases leading to alveolar bone resorption is periodontitis. It has been suggested that various salivary markers are linked to periodontal diseases such as CK (creatine kinase), LDH (lactate dehydrogenase), AST (aspartate aminotrasferase), GGT (gamma-glutamyl-transferase), ALP (alkaline phosphatase) and ACP (alkaline phosphatase) (acid phosphotase). Their activities can be found beyond the normal limits in saliva of diseased patients, as these enzymes are even present in blood and saliva of healthy individuals. [2] Alkaline phosphatase (ALP) was one of the first to be identified among the many host enzymes suggested as diagnostic bone resorption markers for periodontal disease. [3].

Menopause is considered a permanent cessation of menstruation that occurs physiologically/hysterectomy resulting in decreased secretion of ovarian hormone.

Accelerated bone mass loss is associated with both aging and menopause. It has thus been suggested that changes in sex hormone levels can worsen the breakdown of periodontal tissue by altering the host response [13,14]. Compared to long bones, the bone turnover rate in the alveolar bone is greater. The systemic difference in bone resorption and deposition could therefore initially be manifested in the alveolar process rather than in other locations [15]. The presence of less crestal alveolar bone per unit volume may be the potential mechanism by which post menopausal women contribute to further periodontal damage; this lowers bone density that may be more readily resorbed. Oestrogen works by blocking the development of cytokines that encourage the differentiation of osteoclasts and apoptosis of osteoclasts[16]. Increased osteoclast numbers due to increased osteoclast formation activity and decreased osteoclast apoptosis[17] are correlated with oestrogen withdrawal after menopause. ALP is concerned with the formation and mineralization of osteoids. In postmenopausal women, the ALP enzyme is considered a possible marker of alveolar bone resorption [1].

The result of the present study showed considerably higher salivary ALP levels in post menopausal women with chronic periodontitis compared to other groups. The presence of high levels of ALP in saliva of post menopausal women with periodontitis may be due to increased periodontal inflammation and rapid bone turnover rate. 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.

Along with these findings the Bhattarai T et al[5], measured the serum calcium levels of the participants and found decreased levels of calcium in the postmenopausal women, which showed no such correlation with the serum ALP levels of the participants, they also stated that higher levels of calcium and ALP were shown in early stages of menopause compared to late menopause. In our study no such significance was noticed as the age of postmenopausal women in the group left undifferentiated, the findings were noticed on the whole. Ramesh A et al[1] conducted a study on postmenopausal women with and without chronic periodontitis, through the results showed higher ALP levels in post menopausal women with chronic periodontitis, similar as the results showed in this study. When the intergroup comparison was done among all the clinical parameters and the alkaline phosphatase levels, there were significantly high levels in the Group IV participants, which can be seen in results and the statistical analysis of the data. A similar study was conducted by Shashikant Hegde et al [27], showing results of Mean ALP level of the Group 1(pre menopausal,periodontitis) as 92.761 ± 19.139, Mean ALP level of the Group 2(premenopausal, healthy) as 23.451 ± 11.462, Mean ALP level of the Group 3 (post menopausal, periodontitis) as 194.903 ± 22.138, Mean ALP level of the Group 4 (post menopausal, healthy) as 56.377 ± 8.284. Which is at par with our study the values of Mean ALP level of the Group A is 30.40 ± 7.15, Mean ALP level of the Group B is 50.01 ± 8.30, Mean ALP level of the Group C is 31.96 ± 10.29, Mean ALP level of the Group D is 68.68 ± 13.46. According to the values noticed ALP level was significantly high in the Group D participants in our study and Group 3 in Shashikant et al study, i.e post menopausal women with chronic periodontitis. Our Study when compared with the similar studies done, a comparison is made between the Group B and D of post menopausal women healthy and with periodontitis respectively, it was observed that the ALP values in our study are 50.01 ± 8.30 in post menopausal healthy and 68.68 ± 13.46 in post menopausal chronic periodontitis patients. When compared with Santosh Shenoy et al [24] study, the value shows 32.04 ± 5.35 in healthy and 46.79 ± 7.93 in chronic periodontitis patients. In both the studies the values were on the higher side with postmenopausal women with chronic periodontitis.

When the comparision is done between the Group B and D of post menopausal women healthy and with periodontitis respectively, it was observed that the PI of the Group B is 1.39 ± 0.51, and Group D is 1.81 ± 0.50. A similar study was done by Khumukcham Sophia et al shows similar scores of .6260 ± 0.21138 in healthy post menopausal women and of 1.4880 ± 0.25636 in postmenopausal women with chronic periodontitis. Similar results were found between ALP, in our study the values stay 50.01 ± 8.30 in healthy and 68.68 ± 13.46 in chronic periodontitis patients. When compared with Khumukcham Sophia et al[32] study, the value shows 37.2600 ± 9.91806 in healthy and 70.4320 ± 14.36427 in chronic periodontitis patients.

Conclusion

Periodontal disease being multifactorial, a myriad of enzymes and proteins are involved in the disease causation and progression. Alkaline phosphatase is among the vast array of enzymes in the diagnostic panel used as a potential biomarker of periodontal diseases The present study suggest that the presence of ALP levels is not simply a reflection of the local

inflammatory state in females but their estrogen levels may possibly influence bone destruction in periodontitis. It is important that postmenopausal women should be adviced to have a regular periodontal evaluation and receive preventive periodontal treatment to maintain plaque control to reduce risk of further periodontal damage. There is plethora of possibilities for the future use of oral fluids in the periodontal diagnosis and hence offering earlier, less invasive and more cost effective treatment therapies. To prevent such condition, women in the stage of menopause should take particular measures to maintain periodontal health, oral hygiene should be maintained regular follow-ups of dentists, supportive periodontal therapy, daily dietary supplementation of Calcium and Vitamin D.

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