Research Article

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Relationship Between Alkaline Phosphatase in Saliva, Bone Mineral Density and Chronic Periodontitis in Postmenopausal Women

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ABSTRACT

Aim: To assess and compare the relationship between alkaline phosphatase in saliva, bone mineral density and chronic periodontitis in pre- menopausal and post-menopausal women.

Material and Methods: The study includes 30 women (15 premenopausal and 15 post-menopausal) based upon a defined selection criteria. All the participants were subjected to clinical and radiographic examination, Dexa scan and Alkaline phosphatase test. The data were recorded and analyzed.

Result: The interpretation of data suggested non-significant difference in both the groups in terms of Clinical examination except probing pocket depth and alkaline phosphatase Activity. However, the radiographic examination revealed significant difference in terms of bone density in both the groups ($p<0.05$)

Conclusion: within the limitations of the study it can be concluded that no co relation exist between the BMD and Alkaline phosphatase activity in pre and post-menopausal women.

Keywords: Bone Density, saliva, Biomarkers.

INTRODUCTION

Periodontitis induces the local and systemic release of proinflammatory cytokines such as tumor necrosis factor-α, interleukin 1β and interleukin-6 resulting in alveolar bone loss which is a prominent feature of periodontal diseases.[2] There are clinical parameters of disease progression which include probing pocket depth and clinical attachment loss, bleeding on probing and radiographic evidence of bone loss which are used in a semi quantitative manner to evaluate patients which suffer from periodontal diseases. The limitations of traditional diagnostic tools had confronted the researchers with the need for an innovative diagnostic test that focuses on the early recognition of the microbial challenge to the host.[1,2,3]

The deficiency of estrogen in women at menopause is a contributing factor to osteoporosis and a risk factor for periodontal diseases. The increased levels of serum, urine biochemical markers of bone turnover such as collagen cross links, alkaline phosphatase (ALP) and osteocalcin results in accelerated bone loss and are related to increased bone turn over rate.[4]

Among the salivary biomarkers, alkaline Phosphatase (ALP) plays the most important role

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from the dental point of view. The normal level of alkaline phosphate is 20-140IU/L(international unit per litre).[5] It has been shown by various authors that bone loss accelerates after menopause which was accompanied by increased level of salivary alkaline phosphatase.[5] It has been hypothesized that osteoporosis may cause decreased alveolar bone density, which may in turn be more susceptible to resorption by the effect of coexisting or subsequent periodontal infection and inflammation.[4]

Thus, the aim of the present study was to assess and compare the relationship between alkaline phosphatase in saliva, bone mineral density and chronic periodontitis in pre- menopausal and post-menopausal women.

SUBJECT POPULATION

This study includes total of 30 patients (15 from each group) based on their clinical and radiographic examination from amongst the patients reporting at the out patient Department of Periodontology, BRS Dental College and Hospital Sultanpur, Panchkula. Detailed medical and Dental histories of the selected patients were obtained. They were explained the purpose of the study and what the procedure entailed. Ethical approval for the study was obtained from the ethical committee. An informed consent was obtained from all the selected patients. Intraoral examination was carried out and the patients who were diagnosed with chronic periodontitis were selected for the study.

STUDY DESIGN INCLUSION CRITERIA

- Post-menopausal women ≥50years of age not on hormonal replacement therapy.
- Patients with moderate periodontal disease based on [Gingival Index(Loe and Silness) 1963][6] ≥ 2]
- Clinical attachment loss of 3-4mm.
- Periodontal probing depth ≥ 5mm but not exceeding 7mm.
- Subjects with good systemic health and who had not received any periodontal therapy in the past 6 months.

EXCLUSION CRITERIA

- Patients who were medically compromised or on medication.
- Patients on any drug or alcohol abuse.
- Patients with history of smoking.
- Patients who were having metabolic bone disease, parathyroid and history of hysterectomy.

The clinical examination of the patients was done and the following parameters were recorded.

1. Gingival Index (GI): Loe and Silness.(1963)[6]
2. Pocket Probing Depth (PPD).
3. Clinical Attachment loss (CAL).
4. Patient's OPG was taken to diagnose chronic periodontitis
5. Alkaline Phophatase level Test in saliva
6. Dexa Scan for bone mineral density values.

On the basis of above mentioned parameters, the patients were categorized into two groups as follows:

GROUP I: 15 Pre-menopausal women with chronic periodontitis in the age group of 35 to 45 years were taken as control group.

GROUP II: 15 Post-menopausal women with chronic periodontitis in the age group of ≥ 50 years were taken as study group.

1) SALIVA SAMPLE COLLECTION FOR ALKALINE PHOSPHATASE LEVEL

Saliva sample was collected at baseline for alkaline phosphatase level. Patient was asked to rinse with 15 ml of water to wash out the exfoliated cells and then asked to chew paraffin wax for 5 minutes. The stimulated saliva (10ml) of the patient was collected in sterile test tube (15x40mm). The saliva sample was stored in refrigerator (-2.8°C) until transported in a dry ice bag to the lab where the activity of the following salivary enzyme ie alkaline phosphatase was determined.

The Determination of ALP Id based upon the IFCC recommendations and is according the following principle:

P -nitrophenyl phosphate + water^{A} $\leftrightarrow^{LP}$phosphate+p-nitrophenol

The observed increase of absorbance at 405nm is proportional to ALP concentration in the sample.

2) DUAL ENERGY X- RAY ABSORPTIOMETRY SCAN (DEXA SCAN)

Systemic bone mineral density is determined by dual energy X- ray absorptiometery (Dexa) scan**.** A DEXA scan is a quick and painless procedure that involves lying on patient's back on an x ray table so an area of your body can be scanned. It was sometimes possible to remain fully clothed, depending on the area of your body being scanned. However, the patient was needed to remove any clothes that had metal fasteners, such as zips, hooks or buckles. In some cases, the patient was required to wear a gown. During the scan, a large scanning arm was passed over patient's body to measure bone density in the centre of the skeleton. As the scanning arm was moved slowly over the body, a narrow beam of low-dose X-rays was be passed through the part of the body being examined. Some of the X-rays that were passed through patient's body were absorbed by tissue, such as fat and bone. An X-ray detector inside the scanning arm measured the amount of X-rays that have passed through the body. This information was used to produce an image of the scanned area. A DEXA scan compares bone density with the bone density expected for a young healthy adult or a healthy adult of your own age, gender and ethnicity. The difference is calculated as a standard deviation (SD) score. This measures the difference between bone density and the expected value in terms of the natural spread of values in the healthy population.

Chronic periodontitis

The difference between patient's measurement and that of a young healthy adult is known as a T score, and the difference between patient's measurement and that of someone of the same age is known as a Z score.

3) STATISTICAL ANALYSIS

The data for the present study was entered in the Microsoft Excel 2007 and analyzed using the SPSS statistical software 19.0 Version. The descriptive statistics included mean, standard deviation .The inter group comparison was done using independent t test find the difference between the individual time intervals The level of the significance for the present study was fixed at 5%.The software used for the statistical analysis in the present study was **IBM SPSS STATISTICS (version 22.0).**

All calculations were two sided. Discrete categorical data was represented in the form of either a number or a percentage (%); Continuous data was written as in the form of its mean and standard deviation or in the form of its median and interquartile range. The normality of quantitative data was checked by measures of Kolmogorov-Smirnov tests of Normality.

RESULTS

Table 1: Comparison Between Pre And Post Menopausal Females For Periodontal Parametres.

	GPS	N	Mean	Std. Deviation	P value	Significance
Attachment Level	Pre menopausal	15	3.226	0.197	0.745	Non-Significant
	Post menopausal	15	3.180	0.514		
Gingival Index	Pre menopausal	15	1.730	0.322	0.767	Non-Significant
	Post menopausal	15	1.686	0.470		
Probing Depth	Pre menopausal	15	4.470	0.573	0.010	**Significant**
	Post menopausal	14	5.056	0.558		

P≤0.05 analyzed using independent t test

The Attachment Level, Gingival index and Probing Depth was compared between the Premenopausal and postmenopausal females using independent t test. The intergroup comparison of the Probing depth between pre and post menopausal females was significantly different whereas difference of Attachment Level and Gingival index scores was statistically non-significant between pre and post menopausal females.

Table 2: Comparison between pre and post menopausal females for parametres of denta scan.

	GPS	N	Mean	Std. Deviation	P value	Significance
AP_spine	Pre menopausal	15	-0.673	1.395	0.014	**Significant**
	Post menopausal	15	-1.993	1.349		
Left_femur	Pre menopausal	15	-0.446	1.180	0.524	Non-Significant
	Post menopausal	15	-0.746	1.358		

Right_femur	Pre menopausal	15	-0.460	1.284	0.398	Non-Significant
	Post menopausal	15	-0.840	1.133		

P≤0.05 analyzed using independent t test

The bone mineral density for AP spine, Left Femur and Right Femur was compared between the Premenopausal and postmenopausal females was compared using independent t test. The intergroup comparison of the bone mineral density between pre and post menopausal females was significantly different in AP spine however in the right and left femur the difference was statistically non-significant.

Table 3: Comparison between pre and post menopausal females for alkaline phosphatase.

	GPS	N	Mean	Std. Deviation	P value	Significance
Alkaline Phosphotase	Pre menopausal	15	60.506	20.566	0.576	Non-Significant
	Post menopausal	15	56.573	17.394		

P≤0.05 analyzed using independent t test

The Alkaline Phosphatase level was compared between the Premenopausal and postmenopausal females using independent t test. The intergroup comparison of the Alkaline Phosphatase level between pre and post menopausal females was statistically non-significant.

Table 4: Correlation between bone mineral density and alkaline phosphatase level in premenopausal females.

Bone Mineral Density		ALKALINE PHOSPHATASE LEVEL	Significance
AP_SPINE	Pearson Correlation	.018	Non-significant
	Sig. (2-tailed)	.948	
LEFT_FEMUR	Pearson Correlation	.137	Non-significant
	Sig. (2-tailed)	.627	
RIGHT_FEMUR	Pearson Correlation	.083	Non-significant
	Sig. (2-tailed)	.770	

The pearson correlation analysis between Bone Mineral Density and Alkaline Phosphatase level was statistically non –significant at p≥0.05

Table 5: Correlation between bone mineral density and alkaline phosphatase level in postmenopausal females.

Bone Mineral Density		ALK_PHOSPHATASE_UL	
AP_SPINE	Pearson Correlation	-.482	Non-significant
	Sig. (2-tailed)	.069	
LEFT_FEMUR	Pearson Correlation	-.160	Non-significant
	Sig. (2-tailed)	.569	
RIGHT_FEMUR	Pearson Correlation	-.229	Non-significant
	Sig. (2-tailed)	.412	

The pearson correlation analysis between Bone Mineral Density and Alkaline Phosphatase level was statistically non –significant at p≥0.05

DISCUSSION

Many disease biomarkers have been investigated and it seems unlikely, that any one biomarker will provide the ideal solution to all gingival and periodontal problems. However, it is easy to envisage, a small group of chair side assays being established, each having a specific role in diagnosis and prognosis of periodontal diseases. Among the intracellular enzymes that have received considerable attention as possible biomarkers of active periodontal destruction are Aspartate aminotransferase, Alkaline Phosphatase, Gamma glutamyl transferase etc.

In the present study, alkaline phosphatase (ALP) levels in saliva was evaluated by collecting the saliva samples from periodontally healthy individuals at baseline in patients with chronic generalized periodontitis. Before taking saliva sample patient was asked to rinse with water to flush out desquamated epithelial cells which may give false high reading if taken into the sample. After rinsing, patient was asked to chew paraffin wax for 5 minutes to allow accumulation of stimulated saliva in mouth. It was then collected into sterilized vial and brought for biochemical

analysis using Hitachi's Diagnostic System Clinical Auto Analyser. Similar clinical auto analysers were used to study enzyme levels in saliva by *Cesco R de T etal 2003*[7].

In our study, we measured enzyme levels in stimulated saliva which was in accordance with the study done by *Ramesh A, Bhandary R et al (2013)*[8] we measured enzyme level in stimulated saliva who also analysed levels of salivary ALP as a diagnostic marker of periodontitis in postmenopausal women with chronic periodontitis. 1ml of whole saliva sample was collected in a sterile container. Collection of saliva was easier, less time consuming, non invasive procedure. Assessing enzyme levels in saliva was cost effective.

Subjects with altered physiology (with systemic diseases, on medications, pregnancy) were excluded from the study since these changes are manifested in the oral cavity. Pregnant females showed exacerbated inflammatory response to dental plaque (*Raber-Durlacher JE et al (1994)*[9]. Patients on medications (antibiotics, anti inflammatory, steroids, anxiolytcs) may present with alteration in oral microbiota, inflammatory status, salivary composition and salivary flow rate. Smokers were also excluded as they are known to have altered conditions of oral mucosa, salivation and host responses (*Ah MKB etal 1994)*[10].

In our study, we could not see a significant difference in Alkaline phosphatase when compared between the premenopausal (60.50+_20.56) and postmenopausal (56.57+_17.39) females. The potential value of Alkaline phosphatase as a biomarker of periodontal disease activity was identified by Ishikawa and Cimasoni [11].

Systemic bone mineral density is determined by dual energy X- ray absorptiometery (Dexa) scan**.** A DEXA scan is a quick and painless procedure that involves lying on patient's back on an x ray table so an area of your body can be scanned. It was sometimes possible to remain fully clothed, depending on the area of your body being scanned. However, the patient was needed to remove any clothes that had metal fasteners, such as zips, hooks or buckles. In some cases, the patient was required to wear a gown.

In the present study, BMD estimation was done using Dexa scan (Lunar Prodigy Advance Bone Densitometer. Each radiograh covers AP spine, left femur and right femur. Due to the availability and efficacy as good as conventional and frequently used aids to measure BMD, *Lunar Prodigy Advance Bone Densitometer* was used in the present study to assess Systemic BMD.

The comparison of Bone Mineral density between pre (-0.673+_1.395) and post menopausal (-1.993+_1.349) females was significantly different in AP spine. However in the right and left femur it was statistically non-significant.

In the present study bone mineral density and alkaline phosphatase level was correlated in Pre Menopausal and postmenopausal women and the results were found to be non-significant for all three sites of Dexa scan (p value> 0.05).

Further studies are needed to evaluate the relation between bone mineral density and alkaline phosphatase levels. Other clinical parameters that were evaluated are as follows:

Gingival Index (Loe and Silness, 1963)[6] was used. Gingival scores were recorded in premenopausal and postmenopausal patients. The mean difference in group A was 1.730 ±0.32 and group B was 1.68±0.470 and were statistically not significant.

Clinical attachment Level was recorded using the UNC-15 PROBE at baseline in both the groups. Mean difference in clinical attachment level was 3.226±0.197 in group A and 3.180±0.514 in group B which was again not statistically significant.

Probing Pocket Depth The results between premenopausal (4.470±0.573) versus postmenopausal women (5.056±0.558) were found to be **significant (p value = 0.010).**

The intergroup comparison of the Probing depth between pre (4.470±0.573) and post menopausal (5.056±0.558) females was significantly different whereas difference of Attachment Level and Gingival index scores was statistically non-significant between pre and post menopausal females.

It was concluded that:

- There was no significant difference between CAL and GI of Premenopausal and Postmenopausal women.
- There was significant difference in Pocket Probing Depth of Premenopausal and Postmenopausal women.
- There was significant difference in Pocket Probing Depth of Premenopausal and Postmenopausal women i.e P value = 0.010
- BMD of AP spine showed significant difference between Premenopausal and Postmenopausal women whereas left and right femur had no significant difference.
- Alkaline phosphatase levels showed insignificance difference between Premenopausal and Postmenopausal women.
- No correlation was seen between BMD and alkaline Phosphates values.

Within limits of this study, it can be concluded that BMD of AP spine can be used as marker for evaluating the final condition of PD tissue in Pre and Post menopausal women. But Alkaline Phosphatase and Bone mineral Density show no significance correlation. Further studies are needed to reach to better conclusion.

CONFLICTS OF INTEREST

The authors declare they have no potential conflict of interests regarding this article.

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