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"COMPARATIVE ANALYSIS OF SALIVARY ALKALINE PHOSPHATASE IN PRE AND POST-MENOPAUSAL WOMEN WITH AND WITHOUT PERIODONTITIS"

Original Research Paper

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ABSTRACT : Background and Objective: In the periodontium, ALP is very important enzyme as it is part of normal turnover of periodontal ligament, root cementum, and bone homeostasis. The deficiency of estrogen in women at menopause is contributing factor to osteoporosis and considered one of the risk factors for periodontal disease. It has been hypothesized that osteoporosis decreases alveolar bone density and in turn increases its susceptibility to resorption due to periodontal inflammation. Accelerated bone loss in menopause is related to increased bone turnover. This is accompanied by increased levels of biochemical markers such as Alkaline Phosphatase. Alteration in salivary Alkaline Phosphatase levels

Methodology: The study included 40 subjects, 10 in each group:

Group I (pre-menopausal women with clinically healthy periodontium);

Group II (pre-menopausal women with chronic periodontitis)

Group III (post-menopausal women with a clinically healthy periodontium); and

Group IV (post-menopausal women with chronic periodontitis.

Result: The mean ALP in saliva was found to be higher in Group IV as compared to group II and the difference was statistically significant with the p-value of 0.001 [Table:2]

Conclusion: ALP level is increased in post menopausal women with chronic periodontitis than in postmenopausal women with healthy periodontiunm. 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.

Keyword:
Alkaline Phosphatase, Menopause, Oestrogen Periodontitis

Source of Support : Nil
Conflict of Interest : Nil

Comparative analysis of salivary alkaline phosphatase in pre and post-menopausal women with and without periodontitis

INTRODUCTION: Periodontal disease is one of the common inflammatory diseases with complex etiology and is multifactorial in origin. Salivary components for periodontal diagnosis include enzymes and immunoglobulins, hormones of host origin, bacteria and bacterial products, ions, and volatile compounds. Several enzymes that are evaluated for the early diagnosis of periodontal disease are aspartate and alanine aminotransferase (AST, ALT), lactate dehydrogenase (LDH), creatine kinase (CK), alkaline phosphatase and acid phosphatase (ALP, ACP).The enzyme ALP plays a role in bone metabolism1. 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 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 2-4. In the periodontium, ALP is very important enzyme as it is part of normal turnover of periodontal ligament, root cementum and maintenance, and bone homeostasis. The deficiency of estrogen in women at menopause is contributing factor to osteoporosis and considered one of the risk factors for periodontal disease. The alteration in salivary Alkaline Phosphatase levels might be expected as an indication of periodontal disease activity1. Bhattarai T et al., also reported higher serum ALP levels in post menopausal women5. Limited studies are available to address the periodontal disease activity among post menopausal women as well as pre menuapausal women. It has been hypothesized that post menopausal women are prone for alveolar bone loss and salivary ALP levels are an indicator of periodontal disease6.

Aim: This study aims to evaluate the ALP enzyme's levels in saliva of pre and post menopausal women with and without periodontitis.

Methodology: The present case control study included 40 pre and post menopausal women with age between 25-60years.The study participants were selected from the outpatient pool of SPPGIDMS, Lucknow. The subjects were screened for periodontal and menopause status and categorized into

Group I (pre-menopausal women with clinically healthy periodontium)

Group II (pre-menopausal women with chronic periodontitis)

Group III (post-menopausal women with a clinically healthy periodontium); and

Group IV (post-menopausal women with chronic periodontitis).

Clinical examinations: The clinical evaluation of all study participants were carried out to characterize their gingival and periodontal conditions. It included the evaluation of clinical attachment loss ($\geq$ 3mm) and probing pocket depth ($\geq$ 4mm) which was recorded using a UNC 15 graduated periodontal probe. Probing was performed at 6 sites per tooth (mesiobuccal. distobuccal, mesiolingual, distolingual, midbuccal, midlingual. Plaque index and Gingival index scores were also recorded. The samples were coded before being sent for laboratory investigations.All data describing the clinical characteristics were collected by the same examiner.

Saliva collection: 1 ml of whole saliva sample in a sterile disposable plastic container, patients were instructed not to eat 1 hour before collection of sample[1].

Estimation of Alkaline Phosphatase: For analysis, each saliva sample was centrifuged at 5000 rpm for 10 minutes. Reagents were added to about

10 microlitre of supernatant sample by auto analyzer and the value of ALP estimated in U/L.

The reagents used in estimation of saliva ALP were:

Reagent 1(R1) Diethanolamine Buffer (pH 10.2) Magnesium Chloride

Reagent 2(R2) p- Nitrophenyl Phosphate 1.

Statistical analysis: The result obtained were tabulated and subjected to statistical analysis by Maan-whitney U-test. P values were considered to be statistically significant ($p < 0.05$).

Result:

	Pre Menopausal Without Periodontitis	Pre menopausal with periodontitis	Post Menopausal Without Periodontitis	Post Menopausal With Periodontitis
ALP	11.3	**46.2**	32.1	**73.3**
PI	0.4	2	0.3	2.2
GI	0.7	2.1	0.5	2.4
PD	1.3	5.6	1.8	6
CAL	0.2	3.9	0.3	4.2

Table 1: COMPARISON OF PRE AND POST WITH AND WITHOUT PERIODONTITIS

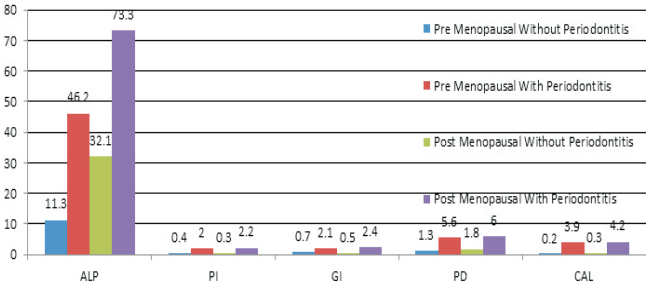

Figure 1: COMPARISON OF PRE AND POST WITH AND WITHOUTPERIODONTITIS

Parameters	Groups	N	Mean	Std. Deviation	P value	Significance
ALP	Pre Menopausal	10	46.20	13.68	0.001	Significant
	Post Menopausal	10	73.30	17.32		
PI	Pre Menopausal	10	2.00	0.81	0.548	Non-Significant
	Post Menopausal	10	2.20	0.63		
GI	Pre Menopausal	10	2.10	0.73	0.363	Non-Significant
	Post Menopausal	10	2.40	0.69		
PD	Pre Menopausal	10	5.60	0.69	0.151	Non-Significant
	Post Menopausal	10	6.00	0.47		
CAL	Pre Menopausal	10	3.90	0.99	0.517	Non-Significant
	Post Menopausal	10	4.20	1.03		

$p \leq 0.05$ is significant when analyzed using Mann Whitney U test

Table 2: Comparison Between Pre And Post Menopausal With Periodontitis

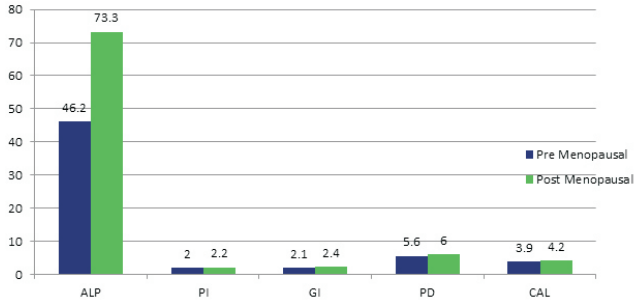

Figure 2: Comparison Between Pre And Post Menopausal With Periodontitis

DISCUSSION:

Health and menopausal problems among post-menopausal women are numerous and draws the attention of health authorities 7 .Bone turnover leading to poor health consequence is increasingly common in both developing and developed world[7,8,9]. Clinical and radiographic methods are conventional diagnostic techniques which do not determine the present activity of the periodontal disease[10].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 [11,12] . Menopause is permanent cessation of menstrual cycle after 12 consecutive months of amenorrhea and is also characterized with decreasing levels of estradiol (E2) as the principal circulating for estrogen . Estrogen deficiency enhances the rate of breakdown of the connective tissue components of the gingiva by stimulating synthesis of matrix metalloproteinases, nitrous oxide, and several cytokines implicated in bone resorption, especially in response to bacterial infection. Thus, it has been proposed that alteration in the levels of sex hormones may exacerbate periodontal tissue breakdown by altering host response[13,14]. 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[15]. The possible mechanism by which post menopausal women lead to more periodontal destruction may be the presence of less crestal 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 [16]. Oestrogen withdrawal following menopause is associated with increased osteoclast numbers due to enhanced osteoclast formation activity and reduced osteoclast apoptosis[17]. ALP is associated with osteoid formation and mineralization. ALP enzyme is considered as a potential marker of alveolar bone resorption in post menopausal women [1].

Our study results 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 in 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. Another study by Bhattarai T et al 5., and Ramesh A et al1., also reported higher serum ALP levels in post menopausal women[1]. Study done by OzlemDaltaban in postmenopausal women showed a positive statistical correlation between total ALP levels and probing depth[1].

It is a known fact fluctuations of sex hormones during menopause have been implicated as factors in inflammatory changes in the human gingiva. Therefore, dental clinicians must be aware of the effects on reduced hormones on the periodontal tissues and should take steps to prevent periodontal disease progression in such conditions.

CONCLUSION:

In this present study it was found out that Alkaline Phosphatase levels are increased in Postmenopausal women with Periodontitis. As there are various hormonal changes in postmenopausal women and numerous etiological factors causing Periodontitis. ALP aggrevates the bone loss. Hence periodontitis can progress rapidly. However ALP cannot be solely responsible for Periodontitis but it can be used as a additional aid in diagnosing Periodontitis. 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 specially among post menopausal women. However further studies with larger sample size are required

to conclude the exact role of Alkaline Phosphatase .

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