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Estimation and Comparison of Levels of Alkaline Phosphatase (ALP), Acid Phosphatase (ACP), Calcium (Ca) and Potassium (K) in Serum of Subjects with and Without Periodontal Disease (PD)

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Abstract

The present study is aimed to determine the enzyme and mineral activity in serum of subjects with chronic periodontitis, namely, Alkaline Phosphatase (ALP), Acid Phosphatase (ACP), Calcium (Ca) and Potassium (K). Materials and Methods: A total of 30 subjects were selected for the study. Blood samples were taken to assess the levels of enzymes and minerals and periodontal disease was identified clinically by assessing probing depth and clinical attachment loss and bone loss confirmed by radiographs. Results: Results were presented as mean and standard deviation. The data were statistically analysed by means of the unpaired t test. Probabilities of less than 0.05 were accepted as significant. Conclusions: It was concluded that serum level of Alkaline Phosphatase was significantly elevated in patients with chronic periodontitis and can be considered as a biomarker for periodontal disease.

Keywords: Alkaline Phosphatase (ALP), Acid Phosphatase (ACP), Calcium (Ca), Potassium (K), Periodontitis, Biomarkers.

1. Introduction

Periodontal disease (PD) is an inflammatory response to the bacteria which has a complex etiology and is multifactorial in origin. Chronic periodontitis affects the supporting structures of the dentition namely the gingiva, periodontal ligament, cementum and adjacent surrounding alveolar bone. It arises from the intricate interactions between the host and around 700 bacterial taxa in the subgingival microbiota.^[1, 2, 3]

Periodontitis is often associated with a marked elevation in systemic inflammatory markers and production of several lysosomal enzymes. Inflammatory cells in the periodontal and gingival tissues can cause a discharge of lysosomal enzymes, eventually causing the destruction of the nearby tissues. The early diagnosis of tissue degradation in periodontal disease by the means of several enzymes has been revealed in the previous studies^[4, 5].

The routine methods to diagnose periodontal disease include clinical evaluation of affected site and measuring the attachment loss followed by identifying bone loss which is to be confirmed by radiographs. Traditional periodontal diagnostic parameters used clinically includes Probing pocket depth (PPD), Bleeding on probing (BOP), Clinical attachment level (CAL), Plaque index (PI) and radiographs assessing alveolar bone level^[6]. The existing clinical methods of periodontal diagnosis are outmoded as they only provide information about the presence of disease after its onset. Traditional diagnostic procedures are essentially limited in that only disease history can be assessed and not the current disease status. Advances in diagnostic research in oral and periodontal diseases are moving toward developing methods whereby periodontal risk can be justified and quantified by objective measures like biomarkers^[7].

One such is the host derived enzyme, Alkaline phosphatase (ALP) found in many cells of periodontium including neutrophils, osteoblasts and fibroblasts. ALP is released during inflammation from polymorphonuclear neutrophils, by osteoblasts during bone formation and by periodontal ligament during fibroblasts periodontal regeneration, presenting dual involvement in the process of periodontal inflammation and healing/regeneration. The sample sources through which the ALP can be identified and estimated potentially include GCF,

saliva and serum [8, 9]. Acid phosphatases (ACPs) catalyze a variety of challenging hydrolytic enzymes that occur in multiple molecular forms with lysosomes of cells from a variety of tissues [10, 11]. It could also have a bacterial origin and play a role in the formation of a pathological pocket [12]. Enzyme ACP has been associated with bone resorption and osteoclasts activities of bones in a study by Takimoto *et al.* [13]. Teeth are composed of mineralized tissue and withstand chemical as well as physical traumas to an extent. The major chemical threat for teeth is demineralization due to low pH resulting from bacterial metabolism and acidic food. The inorganic components of plaque are calcium, potassium and other minerals. Both calcium and potassium are important in the remineralization process. With the increase in the mineral content, the plaque mass become calcified to form calculus [14]. There are numerous studies available in the literature correlating the levels of these enzymes with the severity of periodontal disease. [4, 15, 16, 17, 18] However, there are inherent problems in collecting GCF and saliva in routine. The sampling technique is not easy as longer time is required for sample collection and it only reflects gingival inflammation at each specific site sampled. Thus, GCF and saliva are not suitable for sampling in larger sample size. Requirand P *et al.* and Pushparani DS have previously stated that alveolar bone loss could be reflected by the serum level. [17,19] It was of interest to investigate if an alteration of bone Alkaline phosphatase(ALP) and Acid phosphatase(ACP) as well as minerals could be detected in serum in this affection. However, the pathogenesis of periodontitis remains obscure in spite of all the research efforts that have been made. Therefore, this study is aimed to determine the enzyme and minerals activity in serum among the periodontitis subjects.

2. Material and methods

A comparative study was done on 30 patients in the Department of Periodontology and Oral Implantology at Rajasthan Dental College and Hospital, Jaipur. The inclusion criteria were: Healthy subjects, aged over 18 years, devoid of any systemic or psychological condition and who had signed informed consent. Patients with known medical conditions and patients who were not willing to participate in the study were excluded. The study protocol was reviewed and approved by the institutional ethical committee.

All the subjects underwent a detailed clinical examination. Periodontal disease was identified by assessing probing depth, clincal attachment loss and bone loss, which was confirmed by radiographs. Blood samples were collected after an overnight fast for each subject. Serum was obtained by centrifuging the blood at 1500 rpm for 10 minutes and was used for further estimations.

2.1 Technique used for measurements

2.1.1. Alkaline Phosphatase: pNPP Kinetic Method

ALP at an alkaline pH hydrolyses p-Nitrophenylphosphate to form p-Nitrophenol and Phosphate. The rate of formation of p-Nitrophenol is measured as an increase in absorbance which is proportional to the ALP activity in the sample. Normal reference values in serum for adults: 80 - 290 U/L at 37 C.

2.1.2. Acid Phosphatase: α Naphthylphosphate Kinetic Method

ACP at an acidic pH hydrolyses α Naphthylphosphate to form α Naphthol and Inorganic Phosphate. The α Naphthol formed is coupled with Fast Red TR salt to form a diazo dye complex. The rate of formation of this complex is measured as an increase in absorbance which is proportional to the ACP

activity in the sample. Tartarate inhibits prostatic ACP and the testing in its presence is done to find the non-prostatic ACP. The difference between the activities of the total and non-prostatic ACP gives the activity of the prostatic ACP.

2.1.3. Calcium: Arsenazo III Method

Calcium combines specifically with Arsenazo III at a neutral pH to form a blue purple coloured complex. Intensity of the colour formed is directly proportional to the amount of calcium present in the sample.

Potassium: Elyte - 2 Kit Colorimetric Method

Potassium reacts with sodium tetraphenyl boron in a specially prepared buffer to form a colloidal suspension. The amount of the turbidity produced is directly proportional to the concentration of potassium to the sample

3. Statistical analysis

Data was presented as mean and standard deviation. The data was statistically analysed using SPSS (ver 19) by means of the unpaired t test. Probabilities of less than 0.05 were accepted as significant.

4. Results

Table 1: Descriptive data of the study subjects

Age (years)	Sex N (%)		Total
	Male	Female	
20-30	7 (23.3)	0	7 (23.3)
30-40	3 (10)	0	3 (10)
40-50	7 (23.3)	9 (30)	16 (53.3)
50-60	4 (13.3)	0	4 (13.3)
Total	21 (70)	9 (30)	30 (100)

Table 1 showed half of the subjects were from 40-50 year age groups (53.3) and females (30%). Male subjects (70%) were more than female subjects in gender wise distribution.

Table 2: Descriptive data of the study variables

	N	Minimum	Maximum	Mean	Std. Deviation
Alkaline Phosphatase	30	53	410	205.03	101.4
Acid Phosphatase	30	0.60	11	4.32	2.39
Calcium	30	3.5	30.2	13.29	7.03
Potassium	30	3	7.3	5.02	1.24

Table 2 revealed descriptive data of the study variables. Alkaline phosphatase (205.03) and calcium (13.29) minerals showed highest mean value

Table 3: Comparison between periodontitis patients and study variable (phosphatase)

		N	Mean	Std. Deviation	P value
Alkaline Phosphatase	No periodontal disease	12	159.66	78.92	0.04 (S)
	Periodontitis	18	235.27	105.3	
Acid phosphatase	No periodontal disease	12	4.34	2.71	0.97
	Periodontitis	18	4.31	2.23	

Test applied: Unpaired t test.

Table 3 showed peridontitis parients (235.27) have high level of alkaline phosphatase which indicate statistically significant results

(0.04) as compared to Acid phosphatase which has non-significant results.

Table 4: Comparison between periodontitis patients and study variable (minerals)

		N	Mean	Std. Deviation	P value
Calcium	Absent	12	15.77	8.73	0.11
	Present	18	11.64	5.27	
Potassium	Absent	12	5.08	1.28	0.84
	Present	18	4.98	1.25	

Test applied: Unpaired t test.

Table 4 depict mineral level and periodontal diseases among study subjects. There was non-significant difference in Calcium and Potassium level between the two groups.

5. Discussion

Periodontitis is one of the major threats to oral as well as overall health. It is a slowly progressing disease and could affect 70% of the global population [19]. The processes responsible for destruction of human periodontium are highly complex and vast ranges of biological substances are involved. Several enzymes are evaluated for the early diagnosis of periodontal disease [20]. The enzyme ALP plays a role in bone metabolism. In the periodontium, ALP is of utmost importance as it is a part of normal turnover of periodontal ligament, root cementum, and bone homeostasis.

This comparative study was done among 30 subjects in the Department of Periodontology and Oral Implantology at Rajasthan Dental College and Hospital, Jaipur. In this study males had higher prevalence of periodontitis than females which was in accordance with study conducted by Desvarieux M *et al.* (2004) where males had higher percentage of tooth loss due to periodontitis compared to females [21]. The gender differences reported here might be attributable to treatment bias, practice differences, or sociocultural determinants or adverse habits like smoking, alcohol which are more common in men than females [21].

ALP and ACP are intracellular enzymes present in most of tissues and organs, particularly in bones. Some studies have shown a remarkably increased activity of ALP in the acute phase of periodontal disease, and after the periodontal therapy, the activity of these enzymes restored to the value as found with the healthy persons [22]. Table 3 showed periodontitis patients had significantly higher level of alkaline phosphatase which was in agreement with study conducted by Todorovic T *et al.* (2006). Increased levels of ACP and ALP, especially ALP, indicates that the pathological destructive process has affected the alveolar bone and periodontal disease has significantly advanced and thus the prognosis is much worse.

Chronic periodontitis is associated with elevated systemic inflammatory markers and production of several lysosomal enzymes. Acid phosphatase is an intracellular enzyme present in most of the tissues and is an indicator of increased cellular damage in the soft tissues of the periodontium and inflamed gingival tissues. Clinical and microbiological studies have identified the increased activity of ACP might be a consequence of destructive processes in alveolar bone and are associated with the advanced stages of development of periodontal disease. The increased levels of ACP in periodontitis was more likely to be associated with the metabolic disorder [23]. In the present study Acid phosphatase had non-significant difference between periodontitis and non-periodontitis patients. This result is in contrast with study

conducted by Pushparani DS (2015) where the level of ACP enzyme in the gingival tissue correlates with the severity of periodontitis [18].

A paucity of literature exists on the possible role that mineral content may play in periodontal diseases. The deficiency of minerals may not initiate periodontal disease but perpetuate it, however, marginal intakes of dietary calcium have been implicated as an etiological factor in previous studies [24]. In the present study serum calcium and potassium levels did not differ much between the two groups. These results are in contrast with study done by Freeland JH *et al.* (1976) where significant relationship was found between calcium, potassium and periodontal diseases [24]. This contrast may be due to small sample size in this study which does not reflect the whole population. The loss of alveolar bone is one of the most important hallmarks of periodontal disease. Calcium and potassium intake influences bone mineral density. A cross-sectional study among young Japanese women showed a positive association between calcium intake and bone mineral density [25].

The present study has certain limitations, such as the data collection was confined to small sample size. This sample is only a very small proportion of the entire population.

6. Conclusion

From the present study it was concluded that serum level of Alkaline Phosphatase was significantly elevated in patients with periodontitis and can be considered as a biomarker for periodontal disease, whereas, the levels of Acid Phosphatase, Calcium and Potassium had no significant alterations in the same patients. The research activity must be performed with larger sample size to ensure appropriate findings of the study.

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