

Serum Tissue Non Specific Alkaline Phosphatase Isoenzyme Level in Chronic Periodontitis Patients



Medical Science

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ABSTRACT

Purpose: The purpose of this study was to estimate and compare the levels of tissue non-specific alkaline phosphatase isoenzyme in serum of healthy individual and in chronic periodontitis patients. Materials and Methods: Serum samples were obtained from 20 individuals. 10 from healthy individuals and 10 from chronic periodontitis patients. The samples were used to determine the tissue non – specific alkaline phosphatase isoenzyme (TNSALP) level. Results: Evaluation and comparison of the tissue non – specific alkaline phosphatase isoenzyme activity between the control and chronic periodontitis patient group showed a decrease in tissue non – specific alkaline phosphatase level among chronic periodontitis patients. Conclusion: The measurement of tissue non – specific alkaline phosphatase level in serum can be considered as an enzymatic assay for evaluating the severity and progression of chronic periodontitis. This might provide useful information if monitored successively over a period of time.

Introduction:

Alkaline phosphatase (ALP) is an enzyme analyzed in serum for bone related disease activity. It is a well-established serum assay to depict bone apposition. It is a membrane-bound metallo enzyme that consists of a group of true isoenzymes, all glycoproteins, encoded for by at least four different gene loci: tissue-nonspecific, intestinal, placental, and germ-cell ALP. (Van Hoof VO 1994). The tissue non specific isoenzymes (TNSALP) include bone, Liver and kidney and is widely distributed. [Mccomb et al].

In human serum, tissue-nonspecific (liver, bone, and kidney), intestinal, and placental ALP isoforms contribute to total ALP activity, making clinical interpretation difficult without fractionation of these ALP isoforms (Deftos LJ 199). Separation of the intestinal and placental ALPs are relatively easy but it is much more difficult to distinguish between B-ALP and liver ALP because these two isoforms are the products of a single gene and differ only with respect to posttranslational glycosylation (McComb RB et al 1979, Harris 1989, Fishman WH 1990). The separation of these two isoforms is necessary for a reliable clinical use related to bone involvement..

Various methods of differentiating the B-ALP from liver ALP include conventional agarose gel electrophoresis (Schreiber W 1986 & Van Hoof Vo 1998), heat and chemical inactivation (Moss DW 1975), wheat germ agglutinin precipitation (Rosalki SB 1986), and wheat germ agglutinin-high performance liquid chromatography (Gonchoroff DG 1991 & Day Ap 1992). Bone form of tissue non specific alkaline phosphatase (B-TNSALP) has been considered to be a good marker in serum for bone formation (Ohlsson.c et al 1993) Measurement of B-TNSALP in the serum provides a more specific assessment of the metabolic status of bone in normal and pathological conditions (Farley JR 1995 & Crofton P 1992).

Chronic periodontitis, an inflammatory disease of the periodontium has an underlying bone involvement. Bone destruction was evident in periodontal disease. The main purpose of this study was to evaluate and compare the levels of tissue nonspecific alkaline phosphatase in serum of healthy individuals and individuals diagnosed with chronic periodontitis.

Materials and methods:

This study was done in division of Periodontics, RMDC & H, Annamalai University after ethical committee approval. 20 individuals for this study were selected randomly from the patients who visited the division of Periodontics. 10 individuals in control group and 10 patients in experimental group (diagnosed as chronic periodontitis) of age 30 – 55 years were selected with the criteria's listed below and are grouped as

Group A: Control group – healthy individuals (CAL of 0 mm) and

Group B: Experimental groups – Chronic periodontitis patients (CAL ≥ 1mm)

Selection criteria:

Individuals included in this study are selected based on the following criteria. Smokers, malnourished, anemic, subjects taking medicines known to affect periodontal conditions or gingival secretion, any cardiac diseases, hepato - biliary diseases, diabetes, thyroid and parathyroid abnormalities, Viral, fungal or bacterial infection, history of recent trauma or tooth extractions, pregnant or lactating women, women on oral contraceptives, history of systemic antibiotic therapy within 6 months were excluded from the study.

Clinical examinations:

Brief and precise medical and dental history were recorded after informed consent followed by clinical examination. The clinical indices and parameters like Probing pocket depth level (PPD) and Clinical attachment loss (CAL) were recorded.

Sample & Evaluation:

5 ml of blood samples were collected from all participating individuals after overnight fasting and was allowed to clot in a test tube placed slantingly. After an hour the supernatant serum was extracted and sent to biochemical laboratory for assay. Total alkaline phosphatase enzyme were evaluated and then tissue non specific isoenzyme namely bone (B-TNSALP) and liver (L₁ and L₂ TNSALP) isoenzymes were separated by electrophoresis on agarose gel and visualized and quantified using the Hydragel

ISO-PAL K20 kit (Sebia, France), following the manufacturer's recommendations. The values obtained were tabulated in % for isoenzyme fractions.

Data Analysis:

Results were tabulated; Sample's mean and standard deviation for the results were determined. Between each study groups, the results were compared using Student's t - test for the determination of statistical significance. All statistical analysis were performed using standard statistical software. P <0.05 was considered as statistically significant.

Results:

Table 1: Tissue nonspecific alkaline phosphatase isoenzyme (TNSALP):

	GROUP	N	Mean	Std. Deviation	Std. Error Mean	t-value	P-value
Bone (%)	Health individuals	10	44.200	1.378	0.436	5.47	0.001(S)
	Chronic periodontitis	10	32.970	6.337	2.004		
Liver 1 (%)	Health individuals	10	49.520	1.339	0.423	1.50	0.15(NS)
	Chronic periodontitis	10	46.810	5.552	1.756		
Liver 2 (%)	Health individuals	10	6.270	0.157	4.955	3.80	0.001(S)
	Chronic periodontitis	10	20.220	11.597	3.667		

Figure 1: TNSALP in Healthy and chronic periodontitis patients:

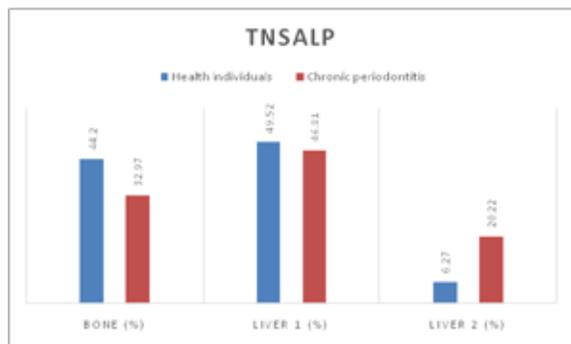


Table 1 compares the bone type of tissue non specific alkaline phosphatase enzyme level with liver type of tissue non specific-isoenzyme level in serum of healthy and chronic periodontitis patients. Average mean B-TNSALP for control (group A) was found to be 44.2 with a standard deviation of 1.38. Similarly for the chronic periodontitis group (group B), the B-TNSALP level was found to be 32.97 with a standard deviation of 6.337. Similarly, the mean value for Liver type of TNSALP was found to be 49.520 for L₁ with SD of 1.339 and 6.270 for L₂ with SD of 0.157 in a healthy individual. For patients with chronic periodontitis, the mean for L₁ and L₂ are 46.810 and 20.220 with a SD of 5.552 and 11.597 respectively.

Comparison of the above mean values were done statistically using student's "t" test. It was found to be significant at 1% ("P" value ≤ 0.001). These values show that there is a significant-decrease in the bone type of tissue nonspecific alkaline phosphatase level among chronic periodontitis patients as compared to healthy individuals.

Table 2: Probing pocket depth (PPD) and Clinical attachment loss (CAL):

	GROUP	N	Mean	Std. Deviation	Std. Error Mean	t-value	P-value
PPD (mm)	Healthy individuals	10	1.60	0.52	0.16	4.30	0.001(S)
	Chronic periodontitis	10	4.34	1.95	0.62		
CAL (mm)	Healthy individuals	10	0.00	0.00	0.00	4.70	0.001(S)
	Chronic periodontitis	10	3.25	2.19	0.69		

Tables 2 shows the mean probing pocket depth and the clinical attachment loss values measured in healthy and chronic periodontitis patients.

Discussion:

In chronic periodontitis, there is always an inflammatory changes in the underlying connective tissue and bone. High alkaline phosphatase activity (ALP) was shown in the periodontal ligament due to the constant renewal of periodontal ligament tissue or pathological circumstances. Requirand et al in 2000 shown that the activity level of this enzyme could be reflected at the serum level. The present study was focused on the tissue non specific isoenzyme of alkaline phosphatase level especially the bone type as it was presumed to be linked with alveolar bone loss that occurs in chronic periodontitis. Many studies were done on ALP level in either gingival crevicular fluid or in saliva based on the fact that the ALP is secreted in the local environment.

This study was done based on the assumption made by Gibert P in 2003, where they believed about existence of some kind of interaction between periodontal ligament space and serum to reflect the levels of TNSALP especially the bone type that is involved in periodontal disease.

It was noticed that there is a general decrease in the bone type TNSALP activity in the periodontal ligament space. Similar results could be obtained in the serum if there is some kind of interaction between the local environment and at serum level. (Gibert P 2003)

Gibert P (2003) in their study has noticed a significant decrease in the bone type of TNSALP in chronic periodontitis patients. Similar results were obtained in this study. There was a marked decrease in bone type of TNSALP in chronic periodontitis patients compared with healthy individuals. This decreased level of B-TNSALP could be attribute to various factors such as reduced passage of B-TNSALP into general circulation, reduced synthesis of TNSALP due to decreased osteoblastic activity, also may be due to very less bone apposition nature during the active phase of the disease and or may be related to the severity of disease leading to increased bone destruction with reduction in bone formation.

Not much studies has been attempted to establish the relationship between TNSALP and chronic periodontitis because of technique sensitivity and quantity of sample availability in gingival crevicular fluid for TNSALP assay. The results of this study done using serum sample might serve as a gateway leading to further investigations to establish the role of bone type TNSALP as a definitive diagnostic assay.

Conclusion:

TNSALP assay will be a more reliable method compared to total alkaline phosphatase estimation in terms of determining the stage, severity and progression of periodontal disease provided

the sample has to be obtained from local environment for analysis.

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