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Evaluation of aspartate aminotransferase enzyme levels in saliva and gingival crevicular fluid with periodontal disease progression - A pilot study

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Abstract:

Periodontal disease is one of the most common inflammatory disease of the oral cavity which is characterized by the progressive destruction of the alveolar bone and soft tissues surrounding the teeth. It occurs in several forms, of varying etiology, pathogenesis and clinical presentation. Rates of tissue destruction differ widely between these forms of the diseases. The field of periodontal diseases, the variability in the rate of destruction across sites and times and the elusiveness of clinical measurements combine to make identification of currently active sites difficult. New methods are expected to facilitate the diagnosis of active periodontal lesion and help to determine the risk of an inactive site from becoming active. The purpose of this study was to evaluate the relationship between Aspartate aminotransferase (AST) levels in saliva and gingival crevicular fluid with periodontal disease progression. A total number of 18 patients were equally divided into 3 groups, Group 0 (clinically healthy), Group 1 (gingivitis), Group 2 (chronic periodontitis). Clinical parameters were recorded using the Russell's Periodontal Index, Turesky-Gilmore-Glickman modification of the Quigley Hein Plaque index. Biochemical assay of saliva and gingival crevicular fluid Aspartate aminotransferase was done

by Reagent strip[3]. It was observed that the chronic periodontitis patients showed elevated levels of Aspartate aminotransferase than the gingivitis and clinically healthy. In comparison between clinically healthy and gingivitis group there was no significant difference. When compared between the Aspartate aminotransferase levels in saliva and gingival crevicular fluid, gingival crevicular fluid showed to have a higher level of Aspartate aminotransferase enzyme in comparison to saliva. Saliva and gingival crevicular fluid Aspartate aminotransferase (AST) enzyme could serve as a potential biochemical marker for periodontal disease progression.

Keywords: Aspartate aminotransferase, Saliva, Gingival crevicular fluid.

Introduction:

Periodontal diseases are multifactorial conditions that are affected by both genetic and environmental factors. The natural history of periodontitis follows a discontinuous pattern of exacerbation and remission characterized by disease active and inactive periods. Although clinical parameters like bleeding on probing, probing depth, clinical attachment level and radiographic assessment of alveolar bone loss provide information on the severity of periodontitis, they do not measure the disease activity. Clinical and radiographic assessment of periodontal disease remains the basis for patient evaluation. This is true despite the fact that clinical monitoring is time consuming, subject to considerable measurement error and is often poorly tolerated by patients.

A rapid, simple diagnostic test can provide a reliable evaluation of periodontal disease and identify patients at risk. Development and application of rapid and simple diagnostic tests based on host salivary and immune factors may facilitate early detection of patients at risk for periodontal diseases, which allows appropriate intervention, decrease the need for more aggressive treatment and improve the response to periodontal therapy.

Saliva has been used as a diagnostic fluid in medicine and dentistry. Recent studies on salivary compounds show co-relations between some of the biochemical markers like Alkaline phosphatase, Glucoronidase, Immunoglobulins (IgA, IgG), hormones and their relation to the severity of periodontitis[1].

The enzyme Aspartate aminotransferase, formerly called glutamic oxalotransferase (GOT), in medicine, is a useful marker for the cell death that occurs in cardiac muscle after a myocardial infarction or in the liver during hepatic disease. Following tissue damage, Aspartate aminotransferase is released from injured and dead cells into extracellular fluid and can be readily assayed in serum, tears and in oral cavity (Gingival crevicular fluid and Saliva).

Chambers et al (1984)[2], evaluated the changes in the Aspartate aminotransferase level in Gingival crevicular fluid during the development of experimental periodontitis in beagle dogs.

Cesco R De T et al (2003)[3] in a study of 60 patients divided into 4 groups concluded that Aspartate aminotransferase levels in saliva from patients presenting code 4 were higher than from patients coded lower. Periodontal destruction was seen to be related to higher Aspartate aminotransferase levels in saliva.

The purpose of this study was to evaluate the relationship between Aspartate aminotransferase (AST) levels in saliva and gingival crevicular fluid with periodontal disease progression as hypothesized by Yoshiaki Nomura et al[4], Cohen RL et al[5] and Richard J. Oringer et al[6].

Material and methods:

The study was conducted on 18 subjects (10 females and 8 males), in the age group of 30-60 years. Subjects were selected from those attending the outpatient in the Department of Periodontics.

The study was approved by the ethical committee of the college.

Study design:

The selected subjects were divided into 3 equal groups of 6 patients each based on Community Periodontal Index (CPI).

Group 0 - Clinically healthy (Subjects free of any signs of gingival inflammation).

Group 1- Gingivitis (Subjects showing signs of gingival inflammation, without any attachment loss).

Group 2- Chronic Periodontitis (Subjects with gingival inflammation and loss of attachment).

Exclusion criteria: includes patients with systemic diseases like diabetes mellitus, renal failure, hypertension etc, patients who had undergone periodontal therapy within 6 months prior to the study, patients who had taken antibiotics for any reasons within 6 months prior to the study, pregnant and lactating women.

Clinical parameters were recorded using the Russell's Periodontal Index, Turesky-Gilmore-Glickman modification of the Quigley Hein Plaque index.

Study Design:

The study was made clear to the patients and a written consent was obtained from those who agreed to participate in this study.

sample collection

Saliva:

About 1ml of non-stimulated saliva was collected from the patients in a sterile test tube immediately after making them rinse with 15ml of water (20 seconds) in order to wash out exfoliated cells.

Gingival crevicular fluid (GCF) :

The gingival crevicular fluid was collected from the patients by using a micro pipette. The subjects were seated in an upright position. Test sites were chosen from the teeth in the maxillary arch to avoid contamination with saliva. After isolation and drying of the site, micro pipettes were placed at the entrance of the gingival crevice for the collection (till approximately 32 micro liters of GCF is obtained). The micro pipettes were placed at the distobuccal surface of the lateral incisor.

Biochemical assay of saliva and gingival crevicular fluid samples for Aspartate aminotransferase was done by reagent strips for specific testing of important clinical-chemistry parameters directly from whole blood, plasma or serum and saliva[3].

For analysis each saliva and gingival crevicular fluid sample was stirred for 10 seconds. An aliquot of 32µl was placed on specific Reagent strip and immediately processed in the Reflotron® photometer for Aspartate aminotransferase levels by the following test principle:

Ketoglutarate + alanine sulfinate $\xrightarrow{AST}$ glutamate + pyruvate $+SO_3^{2-}$

Pyruvate + PO_4^{3-} + O_2 + H_2O $\xrightarrow{PyOD}$ acetyl phosphate + H_2O + CO_2

Indicator + H_2O_2 $\xrightarrow{POD}$ blue dye + H_2O.

Indicator:4-(4-dimethylaminophenyl)-5-methyl-2-(3,5-di-tert-butyl-4hydroxyphenyl) imidazole dihydrochloride. The instrument takes charge of all functions such as heating, automatic calibration, test execution and evaluation, and calculation of results. All data obtained were sent for statistical analysis.

Results:

In the present study, association between Aspartate aminotransferase (AST) levels and Community Periodontal Index groups (Clinically healthy, gingivitis and chronic periodontitis) was done using Post Hoc Tests and T-test.

Post Hoc Tests (Multiple Comparisons) LSD

Dependent Variable	(I) GROUP	(J) GROUP	Mean Difference (I-J)	Std. Error	Sig.
SALIVA	**0**	**1**	**-25.8900**	**14.9594**	**0.105**
		2	**-86.5200(*)**	**14.2632**	**0.000**
	1	**2**	**-60.6300(*)**	**14.9594**	**0.001**
GCF	**0**	**1**	**-31.048**	**15.976**	**0.072**
		2	**-115.672(*)**	**15.232**	**0.000**
	1	**2**	**-84.623(*)**	**15.976**	**0.000**

* The mean difference is significant at the 0.05 level.

T-Test

Paired Samples Statistics

	Mean	N	Std. Deviation	Std. Error Mean
SALIVA	**50.2812**	**18**	**44.7678**	**10.8578**
GCF	**66.869**	**18**	**57.237**	**13.882**

P value is significant for gingival crevicular fluid at 0.011 level

The analysis showed that in comparison between clinically healthy and gingivitis group there was no statistical significant difference but in comparison between clinically healthy and chronic periodontitis, gingivitis and chronic periodontitis the results were statistical significant.

The obtained results have shown that when compared between the Aspartate aminotransferase levels in saliva and gingival crevicular fluid, gingival crevicular fluid showed to have a higher level of Aspartate aminotransferase enzyme in comparison to saliva.

Discussion:

Serum analysis is routinely done as a diagnostic marker for many systemic disorders. In contrast, periodontal disease diagnosis relies primarily on clinical parameters (Gingival status, Bleeding status, Pocket depth measure etc) and radiographic parameters. These measures are useful in detecting evidence of past disease or verifying periodontal health, but provide only limited information about patients at risk for future periodontal breakdown. Saliva and gingival crevicular fluid have been researched extensively for the presence of reliable biochemical markers. Several enzymes including lactate dehydogenase, β-glucoronidase, alkaline phosphatase, aspartate aminotransferase (AST) etc have been investigated. Aspartate aminotransferase (AST) is an intracellular enzyme which upon cell death is released extracellularly[7].

The result of the present study demonstrated that the activity of Aspartate aminotransferase enzyme levels in saliva was increased in periodontitis as compared to other groups (healthy and gingivitis). The results obtained in the present study were in coordination with the study done by Cesco R de T et al (2003)[3], Tatjana Todorovic et al (2006)[8] and Totan A et al (2006)[9]

Similarly the levels of Aspartate aminotransferase enzyme in gingival crevicular fluid was increased in periodontitis patients to healthy and gingivitis patients similar to Magnusson I et al (1996)[10] and Man–Ying Wong et al (1999)[11]**.**

No comparative study was done between saliva and gingival crevicular fluid for Aspartate aminotransferase enzyme levels.

The results in this study showed that Aspartate aminotransferase enzyme levels in gingival crevicular fluid are higher than that of saliva, making gingival crevicular fluid a better diagnostic marker than saliva.

In between healthy and gingivitis groups, the levels of Aspartate aminotransferase enzyme was not significant, in contradiction to the study done by Yoshiaki Nomura et al (2006)[4] but in coordination with the study done by Cesco R de T et al (2003)[3]. Between gingivitis and periodontitis groups, the levels of Aspartate aminotransferase enzyme was found to be significantly different similar to Yoshiaki Nomura et al (2006)[4].

Similarly between healthy and periodontitis groups, the levels of Aspartate aminotransferase enzyme was found to be significantly different in coordination with Kuru B et al (1999)[12] and Shimada et al (2000)[13].

It is universally accepted that periodontal disease is a response of the tissues to invading microbes present in dental plaque. The first line of defence against any invasion is by the polymorphonuclear leukocytes which are chemotactically attracted to the affected sites. The result of interaction between the polymorphonuclear leukocytes and bacteria release

Fig. 1- Collection of Saliva

Fig. 2- Collection of Gingival crevicular fluid through micropipette

various enzymes from both microbes and host cells. These enzymes are indicators of a higher level of cellular damage and their increased activity in gingival crevicular fluid and saliva is a consequence of their increased release from the damaged cells of soft tissues of periodontium and a reflection of metabolic changes in the inflamed gingiva.

The results of the present study indicate a significant correlation between the severity of periodontal disease with salivary and gingival crevicular fluid enzyme activity of aspartate aminotransferase.

Conclusion:

In this study it can be concluded that there is a significant difference in the Aspartate aminotransferase enzyme levels between saliva and gingival crevicular fluid favoring gingival crevicular fluid to be a better marker for periodontal disease progression than saliva. The Aspartate aminotransferase enzyme levels were increased in chronic periodontitis as compared to clinically healthy and gingivitis group. Therefore, the Aspartate aminotransferase (AST) enzyme levels could serve as a potential biochemical marker for periodontal disease progression.

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