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SALIVARY PROTEOME IN PERIODONTAL DIAGNOSIS

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ABSTRACT

Periodontitis is a chronic bacterial infection to gingival tissue leading to inflammation, degeneration of connective tissue or increased bone turn over. Apart from clinical examination, analysis of GCF is a good diagnostic tool but, has several limitations. Components of salivary proteome, enzymes, are gaining importance in clinical diagnosis because of non-invasive, simple and less demanding procedures.

Aim of our present study is to estimate LDH, AST, ALP and CPK enzymes in unstimulated saliva for valuing their importance as routine screening tool in diagnosis of Periodontitis.

In our study we conclude that increased activity of enzymes like LDH, AST, and ALP in whole unstimulated saliva when compared with normal control provides good insight of Periodontitis. Estimations are simple, convenient and do not need any expert examiner, it can be adapted as test for screening Periodontitis in public health.

KEY WORDS

Periodontitis, Saliva, Enzymes, diagnostic tool

INTRODUCTION

Periodontitis is a chronic inflammatory state owing to bacterial infection of gingival tissue. It is characterized by persistent inflammation, annihilation of connective tissue matrix and ruining of alveolar bone [1]. It affects large population of middle age adult group and one of the major causes of tooth mobility or tooth loss, [2] causing great distress for person's health. Traditionally Periodontitis is diagnosed by measuring probing depths of the gingival crevice, bleeding on probing, clinical attachment levels, plaque index, gingival index and radiographic analysis.[3] Necessity of expensive equipment, skilled clinician and time factor imposes restraint to this diagnostic approach. Secondly these diagnostic parameters are excellent to assess when significant level of damage has occurred and determines only a past history of disease but they fall short in assessment of ongoing disease. [4] From last two decades Gingival Crevicular Fluid (GCF), inflammatory exudates, has been investigated as an alternative diagnostic approach. Again a technically demanding, time consuming procedure with limitation in sample volume and having site specific variation [5] has made scientific community to look for an alternative simple, non-invasive, less demanding diagnostic technique.

Salivary diagnostics is an emerging field in Proteomics. It uses salivary proteome for the prognosis, diagnosis and management of periodontal diseases. Saliva composed of water, electrolyte and organic molecules like amino acids, peptides, proteins, glycoprotein and glycolipid is derived from local vasculature originating from carotid arteries.[6] Saliva contains biomarkers derived from serum, gingival crevicular fluid and mucosal transudate. Many analytes associated with periodontal diseases have been detected in this bio-fluid. [7,8,9] Periodontal disease divulge any one or more of their three phase of damage i.e. inflammatory phase, connective tissue degradation phase or bone turnover phase. Each of these phases is supposed to reflect in oral cavity. Several biomarkers are identified in saliva [10] but, robust clinical exploration is required to substantiate their validation in disease diagnosis.

In our present study we investigated salivary enzymes LDH, CPK, ALP and AST, as biochemical marker for the screening of Periodontitis. The enzymes selected for study have been routinely examined in serum at clinical Biochemistry laboratory. Thus our aim is to examine the feasibility and reliability of these parameters to adopt as a routine test in the diagnosis of Periodontitis. There by applying the available conventional clinical laboratory tests on saliva samples, which are technically less demanding, non-invasive and useful for public health.

MATERIALS AND METHODS

Subjects visited our Dental hospital for medical checkups and oral problems during the period of 2009 to 2011and clinically diagnosed as Periodontitis were enrolled as patients in the study group. The patient group population comprised of 205 males (74.1%) and 72 females (25.9%) with a mean age of 37.5 ± 6.9 years (range 32 to 57 years). Control group consists of 95 healthy age matched adult volunteers visiting Medical College Blood bank and having no history of any systemic disease.

Clinical examination:

Three trained Post Graduate dental students have conducted periodontal

examination. As per WHO direct, Subjects having probing depth of more than 3.5mm were diagnosed as Periodontitis. Before enrolment as study group, informed written consent was obtained from every subject for analysis of their saliva and blood sample. The experimental study was approved by college ethical committee. Subjects having any systemic disease involving increase of LDH, CPK, ALP or AST were excluded. Any known cases of Diabetes, renal disorders and subjects with the history of oral cancer, radio therapy or any treatment which decrease salivary flow and Periodontitis were debarred from the study population. Subjects having habit of smoking or alcoholic drinks were disqualified.

Collection of Saliva sample:

Unstimulated whole Saliva specimens were collected by draining method, between morning 9.00 am and 12.00 Noon, after 2 to 3 plain water mouth wash. Subjects were instructed to bend forward their head so the saliva moves towards the anterior region of the mouth and the pooled saliva is allowed to drool in to the wide bore sterile container. After collection, the saliva samples were centrifuged at 5000rpm for 10 minutes and supernatant was collected in to 2ml polypropylene sample storage vial and preserved at -20^0 centigrade until analyzed.

Estimation of enzymes:

Salivary level of Lactate dehydrogenase (LDH), Creatine Kinase (CPK), Alkaline Phosphatase (ALP) and Aspartate Transaminase (AST) were measured using commercially available kits developed for routine blood test. Enzyme Kits used in the study was IFCC method. All the measurements were performed on Hitachi -902 chemistry auto-analyzer present at our Clinical Biochemistry Laboratory.

RESULTS

We measured activity of LDH, CPK, ALP and AST enzymes in unstimulated whole saliva sample from control group and subjects having Periodontitis. As shown in the Table - 1statistically significant difference was observed in LDH, ALP and AST. Though the level of CPK was altered but remained non-significant.

TABLE NO 1

Comparison of LDH, ALP, CPK and AST enzymes activity in unstimulated whole saliva from Periodontitis and healthy control subjects.

	Control n= 95		Periodontitis n=205		P value
	Mean	± SD	Mean	± SD	
LDH U/L	352.31	157.68	1039.62	306.98	<0.001
ALP U/L	9.06	6.82	40.82	12.63	<0.001
CPK U/L	4.22	2.45	9.74	4.51	NS
AST U/L	22.20	7.86	69.75	24.60	<0.01

NS – non-significant

DISCUSSION

Levels of biomarkers in GCF have proved its usefulness in screening of Periodontitis, but sample obtained from single site failed to represent overall status of oral cavity. Sample from multiple site presented variation in analytes results requiring each sample to be treated as separate entity. Again requirement of skilled hand, expensive equipment and time factor are added limitation. Collection of saliva is less demanding and non invasive technique offers an alternative choice.

Saliva is derived from serum, gingival fluid and mucosal transudate. Whole saliva represents a pooled sample of periodontal sites and useful for overall assessment of Periodontitis or its risk status. [11]

Lactate dehydrogenase (LDH) is a ubiquitous enzyme detectable in cytoplasm of almost every cell of the human body. Significant increased level (<0.001) of salivary LDH enzyme activity (1039.62 ± 306.98 U/L) in Periodontitis as compared to control (352.31 ± 158.68) Observed in our present study correlates well with the finding of Smith et al and Atici K et al, [5, 12] Increased extracellular presence of LDH in saliva is the indication to cell necrosis or tissue breakdown and explains the leaking out of intracellular enzyme in GCF or saliva through membrane damage in sick gingival tissue due to inflammation.

Aspartate Transaminase (AST) is cytoplasmic enzyme having diagnostic value in cellular injury. In our study we observed significant increased (< 0.01) in salivary AST activity of Periodontitis (69.75 ± 24.60 U/L) as compared to control (22.20 ± 7.86 U/L). Our values are in agreement with earlier findings which also demonstrate similar increase of AST activity in Periodontitis. [13, 14, 15] The results further validates gingival cell injury or change of membrane permeability caused due to chronic bacterial infection.

Alkaline Phosphatase (ALP) is also an intracellular enzyme associated with bone metabolism. Significant Increase (<0.001) in activity level of ALP (40.82 ± 12.63U/L) in saliva from Periodontitis as compared to control (9.06 ± 6.82) may be correlated with the outcome of bone turnover phase responsible for mobility of tooth or tooth loss, a key feature of clinical examination, in Periodontics.[13] Totan A, et al and Yan F[16] have shown similar increase in the GCF which forms a part of whole saliva.

Intra cellular enzyme Creatine Kinase (CK) is among the other enzymes which is useful in interpreting the cellular necrosis. Though some workers have shown significant increase in ALP in GCF and whole saliva, in our present study we could not find any significant change in salivary ALP activity.

Our results signify increased activity of enzymes in unstimulated saliva, which is either derived from degenerating gingival tissue or systemic circulation due to infectious change in membrane permeability. Thus increased level of intracellular enzyme LDH, AST, ALP in unstimulated saliva can be used for the diagnostic tool in screening of periodontal diseases. Further studies are needed to assess the involvement of micro-organism in such increase by either isolating the organism or studying the isoenzymes pattern to pinpoint the origin of these enzymes.

CONCLUSION

Present study concludes that activity of enzymes like LDH, AST, and ALP in whole unstimulated saliva provides good insight of Periodontitis. Estimations are simple, convenient and do not need any expert examiner. With further affirmation on large population it can be adapted as test for screening Periodontitis in public health.

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