Investigation of Salivary Enzyme Levels in Periodontitis Patient of Bangladeshi Population: A Priliminary Study

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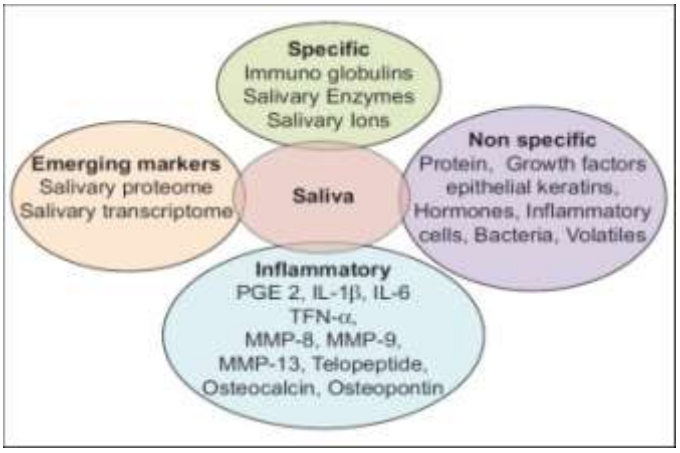
Abstract: **- The aim of the study was to find any association exists between salivary enzyme ALP, ALT, AST and CK level of periodontitis Bangladeshi individuals with their pathological oral condition. In this study levels of ALP, ALT, AST and CK were determined in saliva sample of periodontitis patient. The experimental group contains 50 (fifty) periodontitis patient and 50 (fifty) healthy individuals were served as control. Saliva sample was collected in sample tube and biochemical analysis was done in Biochemistry semi automatic analyzer. Periodontitis was diagnosed by a dental surgeon based on Gingival index (GI), Probing depth (PD) and Clinical attachment loss (CAL). Results were analyzed statistically by one way ANOVA test with SPSS software. Salivary enzyme level of periodontitis patient was compared with the salivary enzyme level of normal control group. ALP, ALT, AST and CK level of periodontitis patients were increased in the saliva than that of control group.**

Key words: **Alkaline Phosphatase (ALP), Alanine Transaminase (ALT), Aspertate Transaminase (AST), Creatine Kinase (CK), Periodontitis, Gingival Index (GI), Probing Depth (PD), Clinical Attachment Loss (CAL)**

I. INTRODUCTION

Periodontal disease is one of the commonest inflammatory diseases which are multi factorial in origin. Periodontitis is an inflammatory disease which affects the periodontium which is the collective form of free and attached Gingiva, Periodontal ligament, Cementum and alveolar bone. The most common form of periodontitis is chronic periodontitis which is irremediable and combined condition that damages tissue through the complex associations between periopathic bacteria and the host immune system (1). Among many diseases that affect teeth, periodontitis is a very common disease that causes the destruction of supporting structures of teeth, progressively results to tooth loss (2). Although periodontitis is an infectious disease of gingival tissue, destruction of alveolar bone is responsible for tooth loss. The commonest cause of alveolar bone damage during periodontitis is due to the progressive inflammation from the marginal gingiva to the underlying periodontal tissues (3). Biochemical markers like intracellular enzymes play a significant role in the detection of inflammatory responses. Saliva has been used as a diagnostic fluid in dentistry for long time. Salivary components used for periodontal diagnosis include enzymes, hormones, immunoglobulins and to investigate bacterial origin (4). Intracellular enzymes are released into the gingival crevicular fluid (GCF) and saliva from the damaged cells of periodontal tissues when these cells are stressed. There are several enzymes that are evaluated for the early diagnosis of periodontal disease include lactate dehydrogenase (LDH), aspartate transaminase (AST) and alanine transaminase (ALT), creatine kinase (CK), alkaline phosphatase (ALP), and gamma-glutamyltransferase (GGT). Diagnostic clinical biochemistry laboratory tests of serum are regularly used in assessment of numerous systemic disorders. In contrast, diagnosis of periodontal disease relies principally on clinical parameters such as Gingival Index, Clinical Attachment Loss, Pocket Depth and radiographic parameters to assess the loss of alveolar bone. These parameters are helpful in evaluation of previous disease, or elucidation of periodontal fitness, but it provides incomplete information about patient's present condition and sites at risk for future periodontal risk. When a periodontal tissue becomes damaged, or its cells become destroyed, destruction of a cellular membrane or edema formation occurs; these intracellular enzymes are released into the gingival crevicular fluid and saliva. These enzymes thus can be used as biochemical markers of the condition of periodontal tissues (5).



II. MATERIALS & METHOD

The study was conducted in Department of Biochemistry and Molecular Biology, Tejgaon College, Dhaka. Examination included 50 persons of both sexes, aged 25 – 50, with periodontal disease, and 50 healthy adult volunteers. Inclusion criteria for control group were the participants with at least 20 natural teeth and probing pocket depth of 2–3 mm with no attachment loss and bleeding on probing with <20 %. For the study group, the participants had to have five qualifying sites in two quadrants with a minimum of two affected teeth in each quadrant with each site having probing depth ≥5 mm, clinical attachment loss (CAL) ≥3 mm, and bleeding on probing. Patients with systemic diseases, smokers, pregnant women, patients who were not maintaining their oral hygiene post-menopausal females or others on steroid therapy were excluded from the study. All subjects were good general health with no history of systemic disease. Samples of non stimulated, mixed saliva were taken after three minutes of mouth cleansing and before breakfast, directly from the mouth of the patient by an automatic pipette. Saliva was collected in sterile test tubes. After that, the saliva samples were centrifuged at 1000 rpm for 15 minutes. The activity of enzymes in saliva was determined Quantimate Biochemistry Semi Automatic Analyzer. Determination of enzymes activity was done immediately after sample collection with corresponding detection kit by the International Federation of Clinical Chemistry (IFCC) method.

Statistical analysis

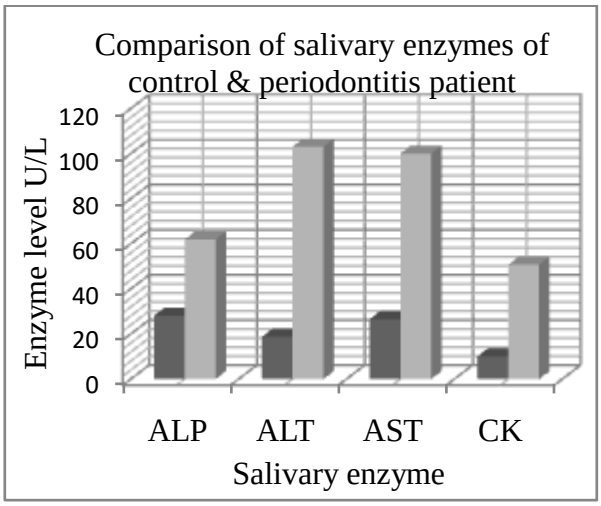
To compare the enzymatic activities among subjects without periodontal disease, subjects with gingivitis and subjects with periodontal disease, one-way analysis of variance (ANOVA) was used. To determine the significance of differences among groups, the P values were adjusted by the Bonferroni method for multiple comparisons.

III. RESULT

The obtained results showed that the levels of enzymes in saliva of the patients with periodontal disease were significantly higher (P<0.001) than that of control group [Tables: 1, Figure: 1].

Table 1: Differences between ALP, ALT, AST and CK activity (Mean ± SD) in saliva of healthy control and periodontitis patient

Enzyme of saliva	Control (U/L)	Periodontitis patient (U/L)	P value
ALP	28.82±4.32	61.95±8.60	<0.001*
ALT	18.33±4.88	103.03±8.35	<0.001*
AST	26.57±2.59	100.07±7.07	<0.001*
CK	10.17±1.37	50.79±4.89	<0.001*



IV. CONCLUSION

The obtained results showed that the levels of ALP, ALT, AST and CK enzymes in saliva of the patients with periodontal disease were significantly higher than that of control group by one way ANOVA. It is strongly suggested that salivary enzymes can be considered as biochemical markers for the indication and can serve as diagnostic parameter for periodontal tissues damage. Salivary enzymes will serve as rapid method in monitoring and well-organized management of periodontal disease.

V. DISCUSSION

Serum enzymes have been used in diagnosis of various diseases and evaluation of serum enzymes constitutes as the major parameters of clinical biochemistry tests. Various body fluids are used for biochemical diagnosis, such as, serum, plasma, cerebrospinal fluid, broncho alveolar lavage, synovial fluid etc. ALP, ALT, AST and CK are intracellular enzymes present in various metabolic pathways of cells and play a critical role in cellular functions. These enzymes presence in body fluid higher than normal level are indicators of a higher level of cellular damage and their increased activity is an effect of their increased discharge from the damaged cells of soft and attached gingiva, cementum, periodontal ligament etc. Increased level of these enzymes directly indicates the abrupt metabolic, inflammatory and physiological changes in the inflamed soft and attached gingiva (6). Previous studies mainly investigated the levels of these enzymes in the Gingival Crevicular Fluid (GCF), which is directly in contact with periodontal tissues. Althouth the collection of GCF is very difficult and hardly feasible. During the screening and diagnosis of periodontitis in large number of patients is very meticulous and huge effort is required, since in every patient one index teeth is checked for pocket depth and clinical attachment loss in every sextant. The levels of these enzymes can also be found in saliva as well as in the blood of healthy people. When periodontal tissue becomes damaged due to destruction of cellular membrane and as a result these intracellular enzymes are released into the GCF and saliva

where their activity or level can be calculated (7). Saliva is more abundant than that of GCF in oral cavity; and sampling techniques of saliva is much easier and more suitable for the patient. Since the sample collection process is simple and non-invasive salivary diagnostic tests hold promise for the rapid diagnosis. Alkaline Phosphatase (ALP) is an intracellular enzymes present in most of tissues but particularly found in bones. Their increased activity in saliva is most likely due to the destructive processes in the alveolar bone during advanced stages of periodontitis (8). There is a positive correlation between the increased activity of ALP and the percentage loss of the alveolar bone (9, 10). Other studies have shown an extremely increased activity of ALP in the acute phase of periodontitis, and after the treatment of periodontal disease the activity of these enzymes are returned to normal level (11).This research has shown that the increased level of ALP, ALT, AST and CK enzymes in periodontal disease can be found in saliva as a result of inflammatory changes in cells of periodontal tissues. Not only that but their value of activity can indicate the severity of pathological processes and destruction of periodontal tissues and can lead toward the prognosis of the disease. The activity of these enzymes in saliva can be very helpful for the evaluation of efficiency of changing the treatment of periodontal disease (12). As far we know this is the first reported research work on the salivary enzyme level periodontitis Bangladeshi patients. But this is only a preliminary study which needs further research in this field to check the reliability of these parameters for the diagnosis of periodontitis. It is also noteworthy that the serum enzyme level of periodontitis patient was not evaluated in this study, so there might be a chance that patients with higher enzyme level is serum are included as subject in this research which may also effect the evaluated findings. There is also some chance that the saliva was in contact with blood since periodontitis may damage the tissue. So, further study with large sample size should be done to support the verification of the present investigation.

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