

Salivary Enzymes Potential Markers for Periodontal Disease

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ABSTRACT

Objective: Periodontal disease results the production of different enzymes that are released by epithelial or inflammatory cells. There are important enzymes associated with cellular alteration and cell death like: Lactate dehydrogenase (LDH), Creatine Kinase (CK), Alkaline and Acidic phosphatase (ALP, ACP). Enzymatic activity changes results in changes health and metabolic changes in Gingival and Periodontal condition.

Design of study: We have examined the activity of CK, LDH, ALP and ACP in saliva from patients with periodontal disease before and after periodontal treatment (experimental group –40 samples) and in saliva from healthy patients (control group – 30 samples). Periodontal disease was determined based on clinical parameters (gingival index (GI), bleeding on probing (BOP), probing depth (PD)). Patients with periodontal disease were under periodontal treatment.

Results: Results showed statistically significant increases of activity of CK, LDH, ALP and ACP in saliva from patients with periodontal disease in relation to control group. There is positive correlation between the examined salivary enzymes and value of the gingival index. After periodontal therapy the

activity of all salivary enzymes was significantly decreased.

Conclusions: Based on these results, it can be stated that activity of these enzymes in saliva, as biochemical markers for periodontal tissue damage, may be useful in diagnosis, prognosis and evaluation of therapy effects in periodontal disease.

Key words: Salivary Enzymes, Periodontal Disease, Markers.

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INTRODUCTION

Saliva has been considered as an important biological marker to introduce new diagnostic criteria which may contribute to making a diagnosis and explaining the pathogenesis of many systemic diseases, such as: Sjogren's syndrome, AIDS, diabetes mellitus.¹ Salivary components are helpful to determine the severity of different pathological conditions. A response to any infectious microorganism to the periodontal infection includes production of several enzymes which are released from epithelial, inflammatory or bacterial cells. The study of these enzymes in salivary secretion, as well as in the gingival crevicular fluid, can contribute in understanding of the pathogenesis and treatment planning for the periodontal disease. Main functional enzymes of tissue degradation, such as: collagenase, gelatinase, proteinase and

intracellular enzymes are increasingly released from the damaged cells of periodontal tissues into the gingival crevicular fluid and saliva, as well as in the surrounding fluids this group of enzymes are the following: lactate dehydrogenase (LDH), creatine kinase (CK) alkaline phosphatase (ALP), acidic phosphatase (ACP). LDH can help to monitor the progression of the periodontal disease. These enzymes appear to be useful to test the progression of periodontal disease or to measure the effectiveness of periodontal therapy.²⁻⁴

Research objectives in this study were the following:

1. Analysis of the activities of CK, LDH, ALP and ACP enzymes in saliva of the healthy tested persons compared to the patients with periodontal disease.

- To evaluate the correlation between the activities of the salivary enzymes and the values of clinical parameters used for evaluation of clinical conditions of periodontium.
- Analysis of different activities of CK, LDH, ALP, ACP enzymes in saliva of the patients with periodontal disease before and after periodontal treatment.

MATERIALS AND METHODS

Examination included 40 persons, of both sexes, aged 25 – 50, with periodontal disease, and 30 healthy adult volunteers. Pregnant and lactating females were excluded; post-menopausal females or others on estrogen therapy were excluded. All subjects were good general health with no history of systemic diseases. In the initial, each subject were asked detailed medical questions and received a complete periodontal examination, which include: gingival index (GI), bleeding on probing (BOP), probing depth

(PD). Patients with periodontal disease were under periodontal treatment consisting of oral hygiene instructions, scaling and root planning and antibiotics.

Samples of unstimulated, mixed saliva were taken before and after treatment, 5 minutes after mouth rinses and before breakfast, directly from the mouth of the patient by an semi-automatic pipette (BenchSmart 96 is a semi-automated 96/384-well pipetting system) and were collected in sterile test tubes. After that, the saliva samples were centrifuged at 10000 rpm for 10 minutes. The function of enzymes in saliva was determined spectrometrically by the Vitros 250 autoanalyzer. The determination of enzymes activity was instant being aware that LDH activity decreases rapidly when frozen and we did not dispose other alternative method and device.⁵ The applied statistical analyses were the following: mean value, standard deviation, standard error, correlation co-efficient (Pearson), Student’s t-test.

Table 1: Differences between CK, LDH, ALP, ACP activity (U/L ± SD) in saliva of healthy and patients with periodontal disease, and before and after periodontal treatment

Enzyme	Healthy Patients	Patients With Periodontal Disease (Before Treatment)	Patients With Periodontal Disease (After Treatment)
CK	3, 62 ± 1,97 U/L	44,30 ±12,20 U/l *	23,10±5,11 U/l *
LDH	99,55 ± 12,07 U/l	1025 ±114,50 U/l *	215,50±28,39 U/l *
ALP	7,30 ± 2,05 U/l	38,40 +9,89 U/l *	25,12 +7,34 U/l *
ACP	20,55 ± 4,03 U/l	81,75 ± 15,40 U/l *	42,25 ±10,11 U/l *

CK-creatine kinase, LDH-lactate dehydrogenase, ALP-alkaline phosphatase, ACP-acidic phosphatase. statistically significant difference p < 0.001.

RESULTS

The obtained results have shown that the activity of examined enzymes in saliva of the patients with periodontal disease was significantly higher in relation to the control group. The established differences showed the statistical significance of a high level (p< 0.001) (table 1).

Correlation between the activities of the indicated salivary enzymes and the values of clinical indexes showed a high coefficient of correlation between the values of the gingival index (GI) and the activity of CK(r=0.815), LDH(r=0.844), ALP(r=0.801), AST(r=0.798).

Concerning the probing depth (PD), a good correlation was determined for ALP (r=0.631), LDH (r= 0,755).

After conventional periodontal treatment the activity of all salivary enzymes was significantly decreased (table 1).

DISCUSSION

Laboratory tests of serum are routinely used in estimation of many systemic disorders. In contrast, diagnosis of periodontal disease considered on clinical (GI, BOP and PD) and radiographic parameters (alveolar bone loss). These measures are helpful in detecting past diseases, or verifying periodontal health, but provide only limited information about patients and risk for future periodontal breakdown. Nowadays saliva has been proposed as diagnostic tool for periodontal as well as other oral and systemic disease such as intracellular enzymes (CK, LDH, ALP and ACP). Their functional activity has been proved in saliva, within some

normal limits, as these enzymes are determined even in blood of healthy persons. If there is periodontal pathology starts, or its cells get damaged due to external or internal environment, or destruction of a cellular membrane, these intracellular enzymes are being released in greater amount into the gingival crevicular fluid and saliva and their activity can be measured. These enzymes can be used as biochemical markers of the functional condition of periodontal tissues.²⁻⁷

CK, LDH are intracellular enzymes included in metabolic processes of cells and they are mostly present in cells of soft tissues.

The enzymes present in intra or extra cellular are predictive markers of a cellular damage.²⁻⁴ Other research shown the similar results, most of them related to functional activities of these enzymes in the gingival crevicular fluid but not in saliva.⁸⁻¹¹ Only a few papers have calculated on the activity of salivary enzymes relation to gingivitis and periodontal disease and shown similar results with our study.¹⁰⁻¹³

In this paper we have measured that the increased activity of certain tissue enzymes in periodontal disease can be proved in saliva as a marker of pathological changes in cells of periodontal tissues. The values of this study can reflect the depth of pathological changes and damage of periodontal tissues, this study shows a good correlation between the activities of CK, LDH, ALP and ACP in saliva and the value of gingival index, i.e. by increasing the value of gingival index, the activity of the above

mentioned enzymes was linearly increasing. This could be also stated on the basis of the typical enzyme profile in periodontal disease in relation to the healthy persons. The increased activity of CK, LDH, indicates the increasing probability of pathological changes in gingiva that leads to coincide with the initial stage of periodontal disease. However, the increased functionality of ACP, especially ALP, in saliva indicates that the pathological destructive process

ALP and ACP are intracellular enzymes present in most of the tissues and organs. Their increased levels in saliva is the consequence of vicious processes in the alveolar bone in advanced stages of development of periodontal disease that was proved by some other studies which shows positive correlation between the activity of ALP and the percentage of the alveolar bone loss.^{3,4} Some studies have ruled an amazingly increased activity of ALP in periodontal disease, and after the periodontal therapy, the levels of these enzymes comes to normal value as found with the healthy persons.⁹ The activity of these enzymes in saliva can be of helpful for curing periodontal disease.²⁻⁴

Most of the studies is been done on gingival crevicular fluid, which is in a closer contact with periodontal tissues and, due to this, it surely reflects better correlation with pathology. The problem with the gingival crevicular fluid is that the procedure to collect the fluid is technique sensitive and in routine practice it would be hardly practicable. Rather than this collecting saliva is easy. The procedure of its sampling is much easier and atraumatic for the patient and same enzymes as those in the gingival crevicular fluid can be detected. Because of this simple and non-invasive method of collecting saliva diagnosis of these pathological conditions will be easier in the future.^{2-4,10-12}

CONCLUSIONS

On the basis of results of this study it can be concluded that the functional levels of CK, LDH, ALP and ACP enzymes were significantly increased in the saliva of patients with periodontal disease in relation to those healthy. It can be a consequence of pathological processes in periodontal tissues where from these intracellular enzymes are increasingly released in the oral cavity. After periodontal treatment the activity of salivary enzymes was decreased, which is probably result of periodontal tissues repair.

On the basis of results of this study the salivary enzymes can be considered as the biochemical markers of pathological changes in periodontal tissues and other body organs that offer new opportunities in making diagnoses and following the efficiency of curing periodontal disease.

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