Salivary enzymes and periodontal disease

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ABSTRACT

Background: Host responses to periodontal disease include the production of different enzymes that are released by stromal, epithelial or inflammatory cells. There are important enzymes associated with cell injury and cell death like: aspartate and alanine aminotransferase (AST, ALT), lactate dehydrogenase (LDH), creatine cinase (CK), alkaline and acidic phosphatase (ALP, ACP), gama glutamil transferase (GGT). Changes in enzymatic activity reflect metabolic changes in the gingiva and periodontium in inflammation.

Design of Study: In this paper we have examined the activity of CK, LDH, AST, ALT, GGT, ALP and ACP in saliva from patients with periodontal disease before and after periodontal treatment (experimental group – 30 samples) and in saliva from healthy patients (control group – 20 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 conventional periodontal treatment.

Results: Obtained results were shown statistically significant increases of activity of CK, LDH, AST, ALT, GGT, ALP, ACP in saliva from patients with periodontal disease in relation to control group. There is positive correlation between the activity of examined salivary enzymes and value of the gingival index. After conventional periodontal therapy the activity of all salivary enzymes was significantly decreased.

Conclusions: Based on these results, it can be assume 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: *Saliva, enzymes, periodontal disease.*

RESUMEN

Objetivos: Las enfermedades que producen daño tisular producen la liberación de diferentes enzimas relacionadas con la muerte y destrucción celular, como son la aspartato y alanino aminotransferasa (AST, ALT), lactato dehidrogenasa (LDH), creatinin kinasa (CK), alcalina y ácida (ALP, ACP) y gamma glutamil transferasa (GGT). Al tratarse la enfermedad periodontal (EP) de un proceso inflamatorio con afectación de la encía y periodonto, parece lógico pensar que la actividad enzimática debe reflejar los cambios metabólicos secundarios a esta reacción inflamatoria.

Diseño del estudio: En este artículo examinamos la actividad de CK, LDH, AST, ALT, GGT, ALP y ACP en la saliva de pacientes con EP, antes y después del tratamiento periodontal (grupo experimental–30 muestras) así como en la saliva

de pacientes sin enfermedad periodontal (grupo control–20 muestras). La EP se diagnosticó en base a parámetros clínicos (índice gingival–GI, sangrado al sondaje-BOP y profundidad al sondaje-PD). Todos los pacientes con enfermedad periodontal recibieron tratamiento convencional de la misma. Se registró la actividad enzimática en todos los pacientes y se cuantificó por espectrofotometría
Resultados: Se observó un aumento estadísticamente significativo en la actividad de CK, LDH, AST, ALT; GGT, ALP y ACP en la saliva de los pacientes con enfermedad periodontal en relación a los resultados obtenidos en el grupo control. Se detectó una correlación positiva entre la actividad de las enzimas salivales examinadas y el valor del GI. Después del tratamiento periodontal convencional la actividad de estas enzimas salivales disminuyó significativamente.
Conclusiones: Basándonos en estos resultados. Podemos concluir que la actividad de estas enzimas puede ser útil en el diagnóstico y evaluación del tratamiento de la EP.

Palabras clave: *Saliva, enzimas, enfermedad periodontal.*

INTRODUCTION

Saliva has been discussed lately as an important biological material to the purpose to introduce new diagnostic tests which may contribute to making a diagnosis and explaining the pathogenesis of many systemic diseases, such as: leukemia, Sjogren's syndrome, AIDS, systemic lupus erythematosus, diabetes mellitus (1). Among important saliva components, which are in this context dealt with in the specialized literature, are also various enzymes. A response of an organism to the periodontal infection includes production of several enzyme families which are released from stromal, epithelial, inflammatory or bacterial cells. The analysis of these enzymes in salivary secretion, as well as in the gingival crevicular fluid, can contribute to clarification of the pathogenesis and to improvement of making a prompt diagnosis of the periodontal disease.

Leading roles in this sense have the enzymes of tissue degradation, such as: elastase, collagenase, gelatinase, proteinase. The same 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. Those particularly relevant in this group of enzymes are the following: aspartate and alanine aminotransferases (AST and ALT), lactate dehydrogenase (LDH), gamma-glutamyl transferase (GGT), creatine kinase (CK) alkaline phosphatase (ALP), acidic phosphatase (ACP). LDH and AST can help monitor the progression of the periodontal disease. These enzymes appear to be useful to test the activity of periodontal disease or to measure the effectiveness of periodontal therapy (2-4).

Research objectives in this study were the following:

1) Analyse the activities of AST; ALT, CK, LDH, GGT, ALP, ACP enzymes in saliva of the healthy tested persons compared to the patients with periodontal disease.

2) Evaluate the correlation between the activities of the indicated salivary enzymes and the values of clinical parameters used for evaluation of clinical conditions of periodontal tissues.

3) Analyse the differences in activities of AST; ALT, CK, LDH, GGT, ALP, ACP enzymes in saliva of the patients with periodontal disease before and after periodontal treatment.

MATERIALS AND METHODS

Examination included 30 persons, of both sexes, aged 25 – 50, with periodontal disease, and 20 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 disease. As the initial examination, each subject completed a detailed medical questionnaire and received a complete periodontal examination, which included: gingival index (GI), bleeding on probing (BOP), probing depth (PD). Patients with periodontal disease were under conventional periodontal treatment consisting of oral hygiene instructions, scaling and root surface debridement and antibiotics.

Samples of a unstimulated, mixed saliva were taken before and after treatment, 3 minutes after mouth cleansing and before breakfast, directly from the mouth of the patient by an automatic pipette (Salivette, Sarsstadt, Germany) and were collected in sterile test tubes. After that, the saliva samples were centrifuged at 10000 rpm for 10 minutes. The activity of enzymes in saliva was determined spectrometrically by the IFCC method on the Hitachi 911 Automatic Analyser. 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 coefficient (Pearson), Student's t-test.

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($r=0.828$), GGT($r=0.835$), ACP($r=0.694$), ALT($r=0.854$), LDH($r=0.850$), ALP($r=0.815$), AST($r=0.804$).

ENZYME	HEALTHY PATIENTS	PATIENTS WITH PERIODONTAL DISEASE (BEFORE TREATMENT)	PATIENTS WITH PERIODONTAL DISEASE (AFTER TREATMENT)
CK	3, 60 ± 1,95 U/l	44,25 ±12,16 U/l *	23,12 ±5,13 U/l *
LDH	99,50 ± 12,02 U/l	1015 ±114,40 U/l *	215,25 ±28,14 U/l *
AST	21,20 ± 6,76 U/l	184,30 ±78,14 U/l *	50,25 ±14,18 U/l *
ALT	7,30 ± 1,76 U/l	98,15 ±20,72 U/l *	67,21 ±13,16 U/l *
GGT	4,60 ± 1,95 U/l	12,19 ±4,55 U/l *	8,03 ±1,21 U/l *
ALP	7,30 ± 2,05 U/l	38,40 ±9,89 U/l *	25,12 ±7,34 U/l *
ACP	20,53 ± 4,01 U/l	81,76 ± 15,40 U/l *	42,25 ±10,11 U/l *

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

Legend: CK-creatine kinase, LDH-lactate dehydrogenase, AST-aspartate aminotransferase, ALT-alanine aminotransferase, GGT- gamma glutamyl transferase, ALP-alkaline phosphatase, ACP-acidic phosphatase. *-statistically significant difference $p < 0.001$.

Concerning the probing depth (PD), a good correlation was determined for ALP (r=0.626), LDH (r= 0,750) and AST (r=0,728).

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

DISCUSSION

Diagnostic laboratory tests of serum are routinely used in evaluation of many systemic disorders. In contrast, diagnosis of periodontal disease relies primarily on clinical (GI, BOP,PD) and radiographic parameters (alveolar bone loss). These measures are useful in detecting evidence of past disease, or verifying periodontal health, but provide only limited information about patients and sites at risk for future periodontal breakdown. Numerous markers in saliva have been proposed as a diagnostic tests for periodontal disease such as intracellular enzymes (CK, LDH, AST, ALT, GGT, ALP, ACP). Their activity can be proved in saliva, within some normal limits, as these enzymes are determined even in blood of healthy persons. However, if a periodontal tissue becomes sick, or its cells become damaged, due to edema or destruction of a cellular membrane, i.e. of a cell as a whole, these intracellular enzymes are increasingly being released into the gingival crevicular fluid and saliva where their activity can be measured. Due to this, these enzymes can be bioche mical markers of the functional condition of periodontal tissues (2-4,6,7).

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

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 (2-4). Other studies reached similar results, although most of them related to testing the activities of these enzymes in the gingival crevicular fluid but not in saliva of oral cavity (8-11). The major number of studies were focused on AST activity (2-4,6-9,12). Only a few papers have focused on the activity of these enzymes in saliva in relation to gingivitis and periodontal disease and shown similar results with our study (10,13-15).

ALP and ACP are intracellular enzymes present in most of tissues and organs, particularly in bones. Their increased activity in saliva is probably the consequence of destructive processes in the alveolar bone in advanced stages of development of periodontal disease what was proved by some former research works as well where it was determined the positive correlation between the activity of ALP and the percentage of the alveolar bone loss (3,4). Some studies have shown a remarkably increased activity of ALP in the acute phase of periodontal disease, and after the periodontal therapy, the activity of these enzymes restored to the value as found with the healthy persons (16). Here again, these studies referred to the gingival crevicular fluid (17,18). Data on ACP activity

in gingival crevicular fluid and saliva in periodontal disease have not been found in the literature available.

This paper is a study which has shown that the increased activity of certain tissue enzymes in periodontal disease can be proved in saliva as a reflection of pathological changes in cells of periodontal tissues. The value of their activity can reflect the depth of pathological processes and damages of periodontal tissues, i.e. can show whether it is the matter of inflammation only or the destructive changes in soft tissues and bones have already commenced and can indicate the prognosis of the course of this disease. That is to say, this study shows a good correlation between the activities of CK, LDH, AST, ALT, GGT, 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, AST, ALT and GGT indicates the pathological changes located in soft tissues only, primarily in gingiva what could coincide with the initial stage of periodontal disease. However, the increased activity of ACP, especially ALP, indicates that the pathological destructive process has affected the alveolar bone what means that periodontal disease has significantly advanced and thus the prognosis is much worse. The activity of these enzymes in saliva can be of useful for the assessment of efficiency of changing the therapy in curing periodontal disease (2-4).

Previous studies mainly investigated the activities of these enzymes in gingival crevicular fluid, which is in a much closer contact with periodontal tissues and, due to this, it surely much better reflects the occurrences in them. However, the problem with the gingival crevicular fluid is in that the technique of collecting is rather complicated and that in a routine procedure, which possibly might be established, it would be hardly feasible in practice. Contrary to the gingival crevicular fluid, there is plenty of saliva, the procedure of its sampling is much easier and more bearable for the patient and, however, the same enzymes as those in the gingival crevicular fluid can be detected. Becouse of the simple and non-invasive method of collection, salivary diagnostic tests apper to hold promise for the future (2-4).

CONCLUSIONS

On the basis of results of this study it can be concluded that the activities of CK, LDH, AST, ALT, GGT, ALP and ACP enzymes were significantly increased in the saliva of patients with periodontal disease in relation to those healthy. This is probably a consequence of pathological processes in periodontal tissues where from these intracellular enzymes are increasingly released into the secretion which surrounds them – saliva. It was also established the correlation between the enzyme activity and the value of the gingival index. After periodontal treatment the activity of examined 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 the functional condition of periodontal tissues what provides new opportunities in making diagnoses and following the efficiency of curing periodontal disease.

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