Salivary Diagnosis: Detection of Several Intracellular Enzymes in Patients with Oral Lichen Planus

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Abstract: *Introduction*: Oral lichen planus is a chronic inflammatory disease, presenting malignant potential. An association between chronic inflammation and initiation and progression of cancer has long been established. Aspartate aminotransferase, lactate dehydrogenase, alkaline phosphatase and gammaglutamil transferase are intracellular enzymes associated with cell injury and cell death. The main aim of the present study is to evaluate changes of enzymatic activity of mentioned enzymes in saliva and serum of patients with oral lichen planus.

Materials and Methods: 20 patients with oral lichen planus and 20 healthy controls were included in the present study. Aspartate aminotransferase (AST), lactate dehydrogenase (LDH), alkaline phosphatase (ALP) and gammaglutamil transferase (GGT) were detected in both serum and saliva.

Results and Discussions: Salivary levels of ALP were decreased while LDH levels were increased in patients with oral lichen planus vs controls ($p<0.05$). At the same time GGT and AST levels were decreased (not significantly significant) in oral lichen planus patients and control groups. Serum levels of ALP were markedly increased while GGT was found decreased in patients vs. controls ($p<0.05$). AST and LDH were decreased but not significantly in oral lichen planus patient's as compared to controls.

Conclusions: Our results reflect increased levels for salivary LDH and serum ALP in patients with oral lichen panus. Saliva can be used as a new diagnostic fluid to detect certain biomarkers such as enzymes in patients with oral lichen planus.

Keywords: Oral lichen planus, saliva, inflammation, enzymes, cell injury.

INTRODUCTION

Oral lichen planus (OLP) is a chronic inflammatory disease, whose etiology is not fully elucidated which present malignant potential [1]. The World Health Organization (WHO) has categorized OLP as a precancerous condition which is a: generalized state associated with a significant increased risk of cancer (WHO) [2]. An association between chronic inflammation such as OLP and initiation and progression of cancer has long been established. The most important complication of OLP is the development of oral squamous cell carcinomas (OSCC) [3, 4]. During the past years, saliva has been proposed as a potential diagnostic fluid in many oral and systemic diseases [5-7]. Whole saliva is secreted by three major and many minor glands. Saliva contains both locally produced molecules as well as other molecules derived from systemic circulation. Whole saliva contains proteins, serum products, electrolytes immune and epithelial cells and gingival crevicular fluid (GCF) [7, 8].

Tissue response to inflammation, includes the release of several enzymes from stromal, bacterial, inflammatory or epithelial cells [9]. Elastases, collagenases, gelatinases, proteinases are enzymes of tissue degradation. Aspartate and alanine aminotransferases (AST and ALT), lactate dehydrogenase (LDH), alkaline phosphatase (ALP), gamaglutamil transferase (GGT), creatine kinase (CK) and acidic phosphatase (ACP), are intracellular enzymes that appear increased during cellular damage and cell death [9-11]. The main aims of the present study is to evaluate the activity of ALP, GGT, AST and LDH in patient's saliva with OLP, and to explore if this oral disease can effect general health by detecting the same enzymes in serum.

MATERIAL AND METHODS

Patients

The specimens were obtained by biopsy of lesional tissues from the oral cavity of patients treated at the

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Department of Oral Pathology, Faculty of Dentistry, University of Medicine and Pharmacy 'Carol Davila', Bucharest. The study was approved by the institutional human ethics committee of the university. Informed consent was obtained from each participant who agreed to participate voluntarily in this study.

Our study included 20 patients with OLP, 10 males and 10 females, with the median age 41.26±5.2. 20 healthy subjects formed by 15 males and 5 females, with the main age between 18-30 years old. Keratotic and erosive OLP forms were diagnosed following criteria defined by WHO. Patients with systemic diseases, systemic medications such as antibiotics, anti-inflammatory drugs, and smokers, were excluded from our study.

Saliva and Serum Sampling

Saliva collection followed well established protocols (8,9). The morning prior to sample collection, the patients were asked to refrain from eating, drinking or brushing their teeth. The procedure of collecting unstimulated whole saliva involved: mouth rinse with distilled water to remove exfoliated cells; sampling was done between 9 and 10 a.m into sterile tubes. After collection approximately 2 mL of saliva was centrifuged at 3000 rpm for 10 min to remove debris. The resultant supernatant was stored at -80ºC until further analysis. In the same session 5 mL of blood were collected and the resulted serum was used for further determinations.

Enzyme Assays

Aspartate Aminotransferase - Serum and Saliva Analysis

Aspartate aminotransferase was determined using AST Analyzing Kit (Biosystem, Spain, Barcelona). Aspartate aminotransferase catalyzes the transfer of amino groups from aspartate molecule to 2-oxoglutarate, forming oxaloacetate and glutamate. The catalytic concentration was determined from the rate of decrease of NADH, measured at 340nm, by means of the malate dehydrogenase (MDH) couplet reaction. Principle of the assay is based on the following reactions:

Aspartate + 2-oxoglutarate →L- glutamate + oxaloacetate

Oxaloacetate + NADH + H^+ → L-malate + NAD^+

Alkaline Phosphatase - Serum and Saliva Analysis

Alkaline phosphatase detection was done using Alkaline phosphatase detection kit (Biosystems, Spain, Barcelona). Under ALP action of p-nitrophenil-phosphate (colorless) is converted p- nitrophenol, the yellow colored compound. The color intensity is proportional to the activity of ALP in the sample (wavelength 405nm). The assay is based on the following reaction:

p-Nitro-phenilphosphate + H_20 → p-nitrophenol + phosphate

Lactate Dehydrogenase - Serum and Saliva Analysis

Lactate dehydrogenase was assayed using Lactate dehydrogenase kit from (Biosystems, Spain, Barcelona). Lactate dehydrogenase catalyzes the interconversion of pyruvate and lactate with concomitent interconversion of NADH and NAD^+ (wavelength 450 nm). The principle of the assay is based on the following reaction:

Pyruvate + NADH + H^+ ←→ L-lactate + NAD^+.

Gammaglutamil Transferase - Serum and Saliva Analysis

Gammaglutamil transferase (Biosystems, Spain, Barcelona) was used to detect the enzyme. Assay Kit GGT- catalyzes the transfer of γ-glutamate from L-γ-glutamyl-3-carboxy-4-nitroanilide in glycylglycine to form L-γ-glutamilglicilglicine and 5-amino-2-nitrobenzoate yellow. The absorbance is read at 405 nm. The assay follows the reactions: L-γ-glutamyl-3-carboxy-4-nitroanilide + glycylglycine → L-γ glutamilglicilglicine + 5-amino2-nitrobenzoate.

Statistical Analysis

Data distributions were expressed as means and standard deviations (SD) using ANOVA test. The data were analyzed statistically on the computer using StataIC 11 (StataCorp. 2009. Stata: Release 11. Statistical Software College Station, TX, USA). A p-value < 0.05 was considered statistically significant.

RESULTS

Salivary levels of ALP were decreased while LDH levels were increased in patients with OLP compared to control groups ($p<0.05$). At the same time, GGT and AST levels were decreased, but not statistically significance could be observed between OLP patients and control groups (Table **1**). Serum levels of ALP were significantly increased while GGT was found to be decreased in patients vs. controls. AST and LDH levels

Table 1: Salivary Enzymatic Levels of Patients with OLP and Controls

Parameters	Patients	Controls	p value
LDH U/mg	198.88±96	179.06±14	<0.05
ALP U/mg	47.3±25.81	66.82±26.27	<0.05
GGT U/mg	6.50±1.12	7.00±1.34	<0.3
AST U/mg	12.5±11.36	13.27±4.33	<0.4

Table 2: Serum Enzymatic Levels at Patients with OLP and Controls

Parameters	Patients	Controls	p value
LDH U/mg	211.83±49.70	224.6±45.36	<0.1
ALP U/mg	56.5±20.04	17.04±0.18	<0.05
GGT U/mg	7.71±2.21	14.04±3.37	<0.05
AST U/mg	11.11±0.70	13.58	<0.6

were decreased but no statistically significance could be found in OLP patients as compared to controls (Table **2**).

DISCUSSION

OLP is a chronic inflammatory disease affecting mostly oral mucosa. In patients with lichen planus, oral lesions are present in a proportion of 70-77%. Malignant transformation of OLP, particularly erosive forms, has been suggested to be between 0.5 to 2.5% [12]. Aside AST, LDH, and ALP, GGT is a also recently been included as a new biomarker of oxidative stress [13]. They have been detected especially at patients diagnosed with periodontal disease. Different research groups detected enzymatic alterations of AST, LDH, GGT and ALP in saliva of patients with periodontitis [9]. AST, ALP, LDH and GGT are intracellular enzymes present in cells of soft tissue associated with cell injury and cell death [9]. Saliva is considered today a new diagnostic fluid for both oral diseases but also for a number of systemic diseases [14-19]. ALP is intracellular enzymes concentrated mostly in bones, but present in liver, duodenum and kidneys [20]. Salivary levels of ALP was significantly decreased in patients with OLP group versus healthy subjects. It was observed that intestinal ALP promotes protection in a murine model of mucosal disease, such as colitis [21]. One possible mechanism for this salivary decreased level of ALP at OLP patients', this enzyme may able to detoxify lipopolysaccharide [22]. LDH enzyme activity was statistically increased in patients with OLP. The extracellular presence of LDH reflect cell damage or cell death [23]. Apoptosis is implicated in the pathogenesis of OLP. Brant JM and co-workers observed that erosive OLP present intense epithelial apoptosis compared with reticular OLP and controls [24]. This observation may add in explaining the salivary increased levels of LDH in our study. In saliva of patients' with OLP, we obtained decreased levels (no statistically significant) of AST and GGT compared with healthy patients group. Cells can release AST during tissue necrosis or trauma in peripheral circulation, such as gingival crevicular fluid and whole saliva [25, 26]. AST is a good marker for periodontal disease and seems not too sensitive for OLP. GGT is now regarded as a new marker of OS. Few studies observed an association between OLP and OS [13, 27]. The presence of oral OS at patients with OLP explain our salivary decreased level of GGT. The literature reports an association between this oral disease with potentially malignant and various systemic diseases such as liver disease [20, 28-30]. AST is an enzyme widespread, which is located in the liver, myocardium, muscle, being present in small amounts in the lungs, kidneys, pancreas and erythrocytes. Thus there is a marked increase in myocardial infarction, acute hepatitis, toxic liver damage. Moderate increases were observed in patients with chronic hepatitis, infectious mononucleosis [20]. In patients' serum with OLP we obtained an decreased serum level for AST (no statistically significant) compared with controls. Therefore, at patients with OLP studied, did not show any of the above systemic diseases. A number of

investigations have reported a correlation between OLP and liver diseases. The prevalence of this association varies in the literature. Erosive lichen planus appears to be associated with chronic liver disease, so it is recommended implementation liver tests in patients with lichen planus. In recent studies, hepatitis C virus (HCV) is common in patients with lichen planus. Elevated levels of transaminases occurred in patients with OLP. OLP relationship with HCV is controversial, some studies have reported positive results, others with negative results. An association between HCV infection and OLP is uncertain because the prevalence of HCV infection varies considerably from one geographic area to another [28, 29]. LDH is present in muscle, liver, myocardium, kidney and erythrocytes. Marked increase of LDH enzyme activity were found in myocardial infarction, toxic liver damage, megaloblastic, testicular cancer. There were moderate increases in LDH in muscle disease, hemolysis, malignant lymphoma [20, 31]. LDH enzyme activity was observed to be lower (no statistically significant) in serum from patients with OLP. Thus, we can't speak of the presence of cardiovascular, liver, muscle at OLP patients investigated. ALP present increased serum levels in skeletal damage associated with osteoblastic reaction, cholestasis [20, 32]. ALP enzymatic activity was statistically increased in patients with OLP group versus healthy patients, this may explain o possible association of this oral disease with one of the mentioned systemic diseases. More studies are need to be done to detect possible associations. Although there are discrepancies between for example ALP in saliva versus serum with an increase for serum no statistical difference could be found between the two body fluids. GGT is present in kidney, pancreas, liver. Significant increases in the GGT enzyme activity is associated with, alcoholism, hepatic tumors. Moderate increases were observed for chronic hepatitis, pancreatitis [20]. The enzymatic activity of GGT in patient's serum with OLP registered a statistically significant lower value. GGT is seen as a new biomarker of OS, so it can be explained statistically significant decrease observed in patients with OLP studied [13].

CONCLUSIONS

In conclusion, the present study reflect enzymatic changes of LDH in patient's saliva with OLP. Serum level for ALP were also increased at patients with OLP compared with controls. The pathological process involved in the progression of OLP are very complex. Saliva can be used to detect certain enzymes which appear to be involved in the pathogenesis of this oral disease.

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REFERENCES

[1] Mattsson U, Jontell M, Holmstrup P. Oral lichen planus and malignant transformation: Is a recall of patients justified? Crit Rev Oral Biol Med 2002; 13: 390-92. http://dx.doi.org/10.1177/154411130201300503

[2] World Health Organization: Tobacco or health: a global overview. In Tobacco or health: a global status report. WHO. 1997; Geneve 5-65.

[3] Chaiyarit P, Ma N, Hiraku Y, Pinlaor S, Yongvanit P, Jintakanon D, *et al*. Nitative and oxidative DNA damage in oral lichen planus in relation to human oral carcinogenesis. Cancer Sci 2005; 96: 553-59. http://dx.doi.org/10.1111/j.1349-7006.2005.00096.x

[4] Ohshima H, Tatemichi M, Sawa T. Chemical basis of inflammation-induced carcinogenesis. Arch Biochem Biophys 2003; 417: 3-11. http://dx.doi.org/10.1016/S0003-9861(03)00283-2

[5] Wong DT. Salivary diagnostics powered by nanotechnologies, proteomics and genomics. JADA 2006; 137: 313-21.

[6] Lee JM, Wong DT. Salivary diagnostics. Orthod Craniofac Res 2009; 12: 206-11. http://dx.doi.org/10.1111/j.1601-6343.2009.01454.x

[7] Spielmann N, Wong DT. Saliva: diagnostics and therapeutic perspectives. Oral Dis 2011; 17: 345-54. http://dx.doi.org/10.1111/j.1601-0825.2010.01773.x

[8] Miller CS, King CP, Chris-Langub Jr M, Kryscio J, Thomas MV. Salivary biomarkers of existing periodontal disease: A cross-sectional study. J Am Dent Assoc 2006; 137: 322-29.

[9] Todorov T, Dozic I, Barrero MV, Ljuskovic B, Pejovic J, Marjanovic M, *et al*. Salivary enzymes and periodontal disease. Med Oral Patol Oral Circ Bucal 2006; 11: E115-9.

[10] Miller CS, Foley JD, Bailey AL, Campell CL, Humphries RL, Floriano NCP, *et al*. Curent developments in salivary diagnostics. Biomarkers Med 2010; 4: 1-19. http://dx.doi.org/10.2217/bmm.09.68

[11] Dabra S, China K, Kaushik A. Salivary enzymes as diagnostic markers for detection of gingival/periodontal disease and their correlation with the severity of the disease. J Indian Soc Periodontol 2012; 16: 358-64. http://dx.doi.org/10.4103/0972-124X.100911

[12] Aly DG, Shahin RS. Oxidative stress in lichen planus. Acta Dermatoven APA 2010; 19: 3-11.

[13] Battino M, Greabu M, Totan A, Bullon P, Tovaru S, Mohora M, *et al*. Oxidative stress markers in oral lichen planus. Biofactors 2008; 33: 301-10. http://dx.doi.org/10.1002/biof.5520330406

[14] Zhang l, Xiao H, Wong DT. Salivary biomarkers for clinical applications. Mol Diagn Ther 2009; 23: 245-59.

[15] Greabu M, Battino M, Mohora M, Totan A, Spinu T, Totan C, *et al*. Could constitute saliva the first line of defence against oxidative stress? Rom J Int Med 2007; 45: 209-13.

[16] Beck JD, Slade G, Offenbacher S. Oral disease, cardiovascular disease and systemic inflammation**.** Periodontol 2000; 23: 110-20. http://dx.doi.org/10.1034/j.1600-0757.2000.2230111.x

[17] Bullon P, Morillo JM, Ramirez-Tortosa MC, Quiles JL, Newman HN, Battino M. Metabolic Syndrome and periodontitis: is oxidative stress a common link? J Dent Res 2009; 88: 503-18. http://dx.doi.org/10.1177/0022034509337479

[18] Kaufman E, Lamster I. The diagnostic applications of saliva-a review. Int American Assoc Dent Res 2002; 13(2): 197-21. http://dx.doi.org/10.1034/j.1600-051x.2000.027007453.x

[19] Kaufman E, Lamster I. Analysis of saliva for periodontal diagnosis. J Clin Periodontol 2000; 27: 453-65.

[20] Lal H. A textbook of Biochemistry for dental students 4th Edition, CBS Publishers & Dristibutors Pvt. Ltd., 2010; Chapter 6, pp. 54-68.

[21] Campbell EL, MacManus CF, Kominsky DJ, Keely S, Glover LE, Bowers BE, *et al*. Resolvin E1-induced intestinal alkaline phosphatase promotes resolution of inflammation through LPS detoxification. Proc Natl Acad Sci USA 2010; 107: 14298-303. http://dx.doi.org/10.1073/pnas.0914730107

[22] Tuin A, Koelstra K, Jager-Krikken A, Lok L, Raaben W, Velders MP, *et al*. Role of alkaline phosphatase in colitis in man and rats. Gut 2009; 58: 379-87. http://dx.doi.org/10.1136/gut.2007.128868

[23] Drent M, Cobben NAM, Henderson RF, Wouters EFM, Dieijen-Visser van M. Usefulness of lactate dehydrogenase and its isoenzymes as indicators of lung damage or inflammation. Eur Respir J 1996; 9: 1736-42. http://dx.doi.org/10.1183/09031936.96.09081736

[24] Brant JMC, Aguiar MCF, Grandinetti HAM, Rodrigues LV, Vasconcelos AC. A comparative study of apoptosis in reticular and erosive oral lichen planus. Braz Dent J 2012; 23: 564-69. http://dx.doi.org/10.1590/S0103-64402012000500016

[25] Totan A, Greabu M, Totan C, Spinu T. Salivary aspartate aminotransferase, alanine aminotransferase and alkaline phoshatase: possible markers in periodontal diseases? Clin Chem Lab Med 2006; 44: 612-15. http://dx.doi.org/10.1515/CCLM.2006.096

[26] Sheth TS, Verma SJ. Analysis of aspartate aminotransferase in gingival crevicular fluid: A study with initial therapy. J Indian Soc Periodontol 2011; 15: 235-39. http://dx.doi.org/10.4103/0972-124X.85666

[27] Ergun S, Trosala C, Warnakulasuriya S, Özel S, Önal AE, Ofluoğlu D, *et al*. Evaluation of oxidative stress and antioxidant profile in patients with oral lichen planus. J Oral Pathol Med 2011; 40: 286-93. http://dx.doi.org/10.1111/j.1600-0714.2010.00955.x

[28] Ali AA, Suresh CS. Oral lichen planus in relation to transaminase levels and hepatitis C virus. J Oral Pathol Med 2007; 36: 604-608. http://dx.doi.org/10.1111/j.1600-0714.2007.00581.x

[29] Konidena A, Pavani BV. Hepatitis C virus infection in patients with oral lichen planus. Niger J Clin Pract 2011; 14: 228-31. http://dx.doi.org/10.4103/1119-3077.84025

[30] Annoni G, Chirillo R, Swanie D. Prognostic value of mitochondrial aspartate amniotrasferase in acute myocardial infarction. Clin Biochem 1986; 19: 235-38. http://dx.doi.org/10.1016/S0009-9120(86)80033-9

[31] Kornberg A, Polliak A. Serum lactate dehydrogenase levels in acute leukemia. Marked elevations in lymphoblastic leukemia. Blood 1980; 56: 351-55.

[32] Chapman JF, Woodard LL, Silverman LM. Alkaline phosphatase izoenzymes In: Kaplan and Pesce (Editors). Clinical Chemistry CV Comp Toronto Princeton 1984; pp. 1098-1101.

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