International Journal of Oral & Maxillofacial Pathology. 2012;3(4):07-12
Available online at http://www.journalgateway.com or www.ijomp.org
ISSN 2231 – 2250

Original Research

Comparison Between Salivary and Serum Lactate Dehydrogenase Levels in Patients with Oral Leukoplakia and Oral Squamous Cell Carcinoma - A Pilot Study

Priya Shirish Joshi, Madhuri Chougule, Mahesh Dudanakar, Someshwar Golgire

Abstract

Background: The enzyme Lactate Dehydrogenase (LDH) is found in the cells of almost all body tissues. LDH is believed to vary according to the metabolic requirement of each tissue and alternation in LDH levels have been observed during development, under changing biological conditions, and in response to pathological processes. LDH activity in serum increases as a marker of cellular necrosis. Serum LDH levels have been used as a biochemical marker in diagnosis in various body cancers. The profile of salivary LDH is similar to that found in oral epithelium, indicating that the major source of salivary LDH is probably the oral epithelium-shedding cells. **Aims and Objectives:** To measure and compare the LDH levels in serum and saliva in patients of oral leukoplakia and oral squamous cell carcinoma. **Materials and Methods:** Clinically diagnosed seven cases each of Oral Leukoplakia (OL) and Oral Squamous Cell Carcinoma (OSCC) were selected and compared with control. After obtaining the consent of the patient, serum and unstimulated whole saliva was collected and processed for LDH measurement by using total LDH Span Kit. Incisional biopsy of selected cases was performed and clinical diagnosis of the cases was confirmed by histopathological examination. **Results:** LDH activity increased in serum as well as saliva in patients with oral leukoplakia and oral squamous cell carcinoma in comparison to normal control. **Conclusion:** Salivary LDH estimation can prove to be a valuable substitute to serum LDH as a biochemical marker, as it is a simple, non-invasive procedure and easily accepted by the patient.

Keywords: Lactate Dehydrogenase; Leukoplakia; Saliva; Serum; Squamous Cell Carcinoma.

Priya Shirish Joshi, Madhuri Chougule, Mahesh Dudanakar, Someshwar Golgire. Comparison Between Salivary and Serum Lactate Dehydrogenase Levels in Patients with Oral Leukoplakia and Oral Squamous Cell Carcinoma - A Pilot Stud. International Journal of Oral & Maxillofacial Pathology; 2012:3(4):07-12.

Received on: 13/02/2012 Accepted on: 21/10/2012

Introduction

Oral squamous cell carcinoma (OSCC) is the sixth most common human cancer that encompasses at least 90% of all oral malignancies.[1] OSCC is recognized to have 50%, five year survival rate.[1,2] The starting point of cancer is the mucosal epithelium which when subjected to exogenous and endogenous factors or carcinogens produce changes that are reactive and reversible. But with progressive loss of normal control mechanism these changes lead to precancerous state.[3] The most common precancerous lesions are oral leukoplakia (OL) and oral erythroplakia. If precancerous lesions are detected and treated early then the conversion to cancer is averted. Despite the advances made in the therapeutic modalities via multidisciplinary approaches, survival rate for OSCC has not significantly improved.[4] This motivates the search of factors which will help in the early diagnosis and management. Early diagnosis and prompt treatment will avert mutilating surgery, improve patient's quality of life and can decrease morbidity and mortality associated with cancer.

Recently, the role of tumor markers in management of head and neck cancer has received increasing attention. Tumor markers in serum, tissue and other body fluids during neoplastic process are of clinical value in the management of patients with various body cancers. Among all the body fluids, blood has been the media of choice for the study of the biochemical markers by the medical community but it does have some inherent disadvantages.[5] Collecting blood for investigation is an invasive procedure and has a potential risk of disease transmission through needle stick injuries. Despite the absence of charisma, however, a growing

ISSN 2231 - 2250

number of researchers are finding that saliva provides an easily available, non-invasive diagnostic medium for rapidly widening range of disease and clinical situations.[6]

Saliva is a complex and dynamic biologic fluid, which over the years has been recognized for the numerous functions it performs in the oral cavity. Modern technology, however, has unveiled a plethora of compounds never before detected in saliva like drugs, pollutants, hormones and also biomarkers of bacterial, viral, and systemic diseases. Saliva based diagnostics are more attractive as they are more accessible, accurate, less expensive and presents less risk of infection to the patient, health care worker and cross infection. With all these above mentioned added advantages saliva can serve as diagnostic tool as compared to serum.[7]

The enzyme lactate dehydrogenase (LDH) is found in the cells of almost all body tissues. It is especially concentrated in the heart, liver, red blood cells, kidneys, muscles, brain, and lungs. Increased serum LDH activity is considered as a marker of cellular necrosis and serum LDH levels have been used as a biochemical marker in diagnosis in various cancers like oral, laryngeal and breast cancer. LDH activity is mainly due to genomic changes during malignant transformation. Increased LDH levels are due to increased mitotic index and more lactic acid production by tumor cells due to breakdown of glycoprotein.[6] As the magnitude of dysplastic changes increase in leukoplakia it is logical to expect increase in values of LDH. Value of LDH elevates in OSCC cases as compared to OL cases and this finding can be used for benefit of the patient in predicting prognosis.

The profile of salivary LDH is similar to that found in oral epithelium, indicating that the major source of salivary LDH is probably the oral epithelium-shedding cells.[7] The similarity between the profile of LDH in whole saliva and the oral epithelium supports the hypothesis that salivary LDH is predominantly of extra glandular origin. Consequently, LDH concentration in saliva, as an expression of cellular necrosis, could be a specific indicator for oral lesions that affect the integrity of the oral mucosa. Therefore salivary LDH levels may be evaluated for possible oral mucosal pathologies.[8] Serum and Salivary LDH levels have not been studied rigorously in oral precancer and cancer. Hence this endeavor to measure and compare Serum and Salivary LDH levels in patients with oral leukoplakia and oral squamous cell carcinoma. We will also try to evaluate whether salivary analysis of LDH can substitute serum LDH analysis.

Materials and Methods

Clinically diagnosed seven cases each of OL and OSCC were selected for the study. Standard clinical criteria's were used for case selection.[9] Patients without leukoplakia and squamous cell carcinoma or any other known medical illness served as normal control. The cases were divided in three groups; Group I - Normal Control, Group II – Oral Leukoplakia and Group III – Oral Squamous Cell Carcinoma.

Exclusion criteria: Patients suffering from systemic conditions like cardiovascular disease, anemia, liver, kidney and pancreatic diseases, blood dyscrasis, stroke, muscular dystrophy, drugs like anesthetics, narcotics and aspirin. Individuals with other mucosal lesions were also excluded.

Institutional Ethical Clearance was obtained prior to begin the study. Written informed consent of the patient was obtained and case history was recorded. Unstimulated whole saliva was aseptically collected in wide mouth container by spitting method. The sample was centrifuged and supernatant was processed for LDH measurement by using Agarose Gel electrophoresis method with the help of SEBIA- HYDRAGEL ISO-LDH K-20 kit [Span Company]. Blood was collected by using standard aseptic precautions and processed for LDH. Since saliva supernatant was used which can be treated like serum, the same kit was used to process both the samples. Incisional biopsy of the selected cases was performed and clinical diagnosis of the cases was confirmed by histopathological examination.

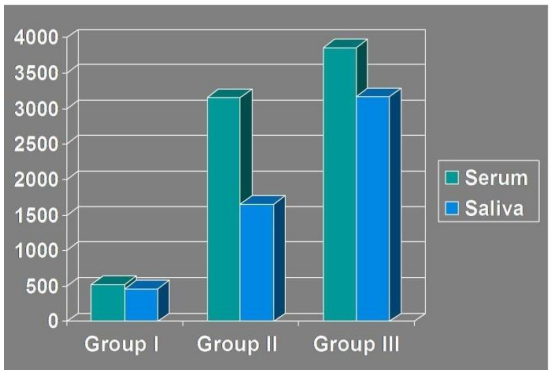
Results

On histopathological examination, all seven cases of leukoplakia showed only hyperkeratosis without any evidence of dysplasia. According to the Brynes grading system, all 7 cases of OSCC were categorized under grade I (Well differentiated OSCC).

Serum and Salivary LDH levels were found to be within normal limits in control (Group I) (225-550 IU/L). Serum and salivary LDH levels were found to be increased in leukoplakia (Group II) and in oral squamous cell carcinoma patients (Group III). (Table 1)

	Control Group		OL Group		OSCC Group	
Case	Serum Total LDH IU/L	Salivary Total LDH IU/L	Serum Total LDH IU/L	Salivary Total LDH IU/L	Serum Total LDH IU/L	Salivary Total LDH IU/L
Case 1	388.40	463.00	2238.4	1050.4	2873.0	1618.3
Case 2	204.30	384.70	1833.3	1893.0	3871.2	2328.2
Case 3	549.40	332.00	4086.0	1335.6	2234.1	4200.2
Case 4	282.50	430.58	3620.2	1830.2	4360.2	3338.4
Case 5	1295.00	640.00	1533.7	2104.0	4756.0	4437.6
Case 6	298.00	438.14	4734.5	1140.5	3850.6	3140.2
Case 7	681.40	530.00	4060.0	2238.0	5060.0	3128.0

Table 1: Serum And Salivary LDH Levels in Control, OL And OSCC Groups.



Graph 1: Comparison of Serum and Salivary LDH Levels In Control, OL and OSCC Groups

Graph 1 shows increased levels of salivary and Serum LDH in OL and OSCC as compared to healthy controls. The increase in LDH levels was less in saliva as compared to serum in group II (OL) patients. The increase in LDH levels was consistent in saliva and serum of OSCC patients.

Discussion

The present study was conducted with the aim to measure and compare LDH levels in serum and saliva of patients with leukoplakia and oral squamous cell carcinoma and to evaluate whether salivary analysis of LDH can substitute serum LDH analysis. Quantitative estimation of total LDH was carried out by Agarose Gel Electrophoresis, which is the best method available. Immunohistochemical studies for qualitatively analysis of LDH activity in gastric cancers have been done.[10,11]

Lactate dehydrogenase is a hydrogen transfer enzyme and is involved in the final step in the metabolic chain of anaerobic glycolysis. LDH catalyses the oxidation of L-lactate to pyruvate with nicotinamide-adenine dinucleotide (NAD) + as hydrogen acceptor.[12] The enzyme is composed of four peptide chains of two types: M (Muscle) and H (Heart), each under separate genetic control. Lactate dehydrogenase (LDH), a cytoplasmic enzyme is present essentially in all major organ systems. The extracellular appearance of LDH is used to detect cell damage or cell death. Due to its extraordinarily widespread distribution in the body, serum LDH is abnormal in a host of disorders. It is released into the peripheral blood after cell death caused by, e.g. ischemia, excess heat or cold, starvation, dehydration, injury, exposure to bacterial toxins, after ingestion of certain drugs, and from chemical poisonings.[12]

The LDH in the whole saliva within the oral cavity may originate from various sources, because whole saliva is a combination of secretions from both major and minor salivary glands, fluids diffused through the oral epithelium and gingiva, material originating from gastrointestinal reflux, and cellular and other debris. Rafael MN, et al have concluded that whole saliva LDH is nonglandular in origin and probably oral epithelium is the major source for this non glandular LDH.[5] It is logical to assume that pathological alternations of oral epithelium like dysplasia or cancer may result in alternation of LDH levels in saliva. Therefore, salivary LDH may be evaluated for possible oral mucosal pathologies in a manner similar to that used for evaluating tissue pathologies in heart, muscle, or liver by LDH detection in plasma.[7]

ISSN 2231 - 2250

Carcinogenic changes have tremendous influence in increasing LDH activity. These carcinogenic changes may lead to decreased lactate to pyruvate conversion resulting in anomaly in the regeneration of NAD^+ which may interfere with glycolysis part of carbohydrate metabolism. Malignant tumor tissue or contiguous tissue damaged by tumor liberates enzymes into circulation which contributes towards abnormal increase in enzyme levels. Increased LDH levels are due to increased mitotic index and more lactic acid production by tumor cells due to breakdown of glycoprotein.[6]

LDH and its isoenzymes have been of considerable interest to the biochemical oncologist and serum LDH isoenzyme levels have been studied extensively in lung, breast & cervical and gastric cancers. We found increased serum and salivary LDH levels in all cases of OL and OSCC groups in comparison to control group but the increase in LDH levels was less in saliva as compared to serum in group II (OL) patients. The increase in LDH levels was consistent in saliva and serum of OSCC patients. Our findings are consistent with the results presented in studies done so far. Rotenberg et al in 1988 in their study of LDH in serum of patients with non-small cell lung cancer have concluded that this inexpensive laboratory method should be carried out in every patient with such type of cancer as it may indicate clinical stage and tumor burden.[13] Study on estimation of serum LDH values in patients with nasopharyngeal carcinoma by Liaw CC et al in 1997 have shown higher serum LDH values in patients with metastatic disease. They concluded that serum LDH levels correlate with the clinical responsiveness to systemic chemotherapy. Cases with normal serum LDH before treatment had a better chance of survival than those with elevated levels.[14] Visnja Bog danovic, et al., in 2008 found elevated serum LDH levels in patients with breast cancer and they have opined that elevated level of LDH might be a prognostic sign of disease progression.[15] The study conducted by Subramanian N et al in 2009 on cervical carcinoma has concluded that LDH isoenzyme is an important biochemical marker in assessing the grade of malignancy.[16]

Yu Lin Song et al[10] have noted increased LDH in gastric cancer cells by immunohistochemistry. They are of the opinion that the increase is mainly due to increased LDH isoenzme 5and that the invasion and spread of gastric cancer cells could be indirectly promoted through the elevated activities of acid hydrolase due to the decrease of pH caused by the elevation of lactate. They feel that intestinal metaplasia and dysplasia might be a borderline lesion between normal gastric mucosa and gastric cancer.

Literature review shows very few studies on comparison between total serum and salivary LDH analysis in oral precancer and cancer. Masahiro et al correlated clinical and experimental activity of serum LDH and its isoenzymes in oral cancer and noted increased levels.[17] Hariharan S, et al[18] studied serum LDH and its isoenzymes in buccal mucosa cancer. They made apparent that isoenzymes LDH 4 and LDH 5 are higher in cancer patients as compared to normal controls. Muralidhar M, et al[19] in 1988 also reported a definite rise of serum LDH levels from normal in premalignant and malignant cases. T Gorogh et al [20] studied serum LDH isoenzymes in squamous cell carcinoma of oral cavity. They concluded that percentage distribution of serum LDH isoenzymes may represent useful parameter of disease activity in patients with OSCC. Milind Naphade and Ujwala Naphade studied major immunoglobulin status and LDH isoenzymes profile in oral premalignancy and malignancy and found increased LDH levels in premalignancy and malignancy.[21] Thomas Shpitzer et al in 2007 have comprehensively analyzed saliva in oral cancer and they found total salivary LDH (88%, p=0.002) to be elevated in comparison to normal healthy individuals.[22] Salivary isoenzymes have been studied in lichen planus by Balwant Rai et al in 2007.[23] They have noted that salivary LDH3, LDH4, LDH5 and M subunit were significantly increased in patients with oral lichen planus as compared to controls and no significant differences in levels of LDH isoenzymes in gender. According to them increased levels of isoenzymes LDH in oral lichen planus may be due to carcinogen enhanced activity of LDH. They have concluded that measuring Salivary LDH may be a feasible, simple and convenient approach for screening of oral precancer.[23]

In various research settings it has been observed that, saliva diagnostics have potential capabilities in the diagnosis and screening of oral precancer and cancer. [24]This diagnostic capacity is based on the ever continuous and intimate contact between saliva and the mucosa where the cancer evolves. Usages of saliva for diagnostics has many benefits in comparison to serum, as it is simple, easy, noninvasive collection procedure and carries less chances of cross-contamination than blood. Literature review has revealed very few studies analyzing salivary and serum LDH levels in OL and OSCC. Further studies are required on larger sample size to confirm the reliability of this parameter in screening of oral precancer and cancer.

Conclusion

Salivary and serum LDH levels increase in oral mucosal pathologies like OL and OSCC. Salivary LDH estimation can prove to be a valuable substitute to serum LDH as a biochemical marker, as it is a simple, non-invasive procedure and easily accepted by the patient.

Acknowledge

We gratefully acknowledge Dr. Ulhas Mali for his advice, supervision and crucial contribution during the lab procedures.

Author Affiliations

1. Dr.(Mrs). Priya Shirish Joshi, Professor & Head, 2. Dr. Madhuri Chougule, Reader, 3. Dr. Mahesh Dudanakar, Senior Lecturer, 4. Dr. Someshwar Golgire, Department Of Oral Pathology & Microbiology, Vasantdada Patil Dental College & Hospital, Kavalapur, Sangli, Maharashtra, India.

References

1. Massano J, Regateiro FS, Januario G, et al. Oral squamous cell carcinoma: Review of prognostic and predictive factors. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 2006;102:67-76.
2. Robert E. Marx, Diane Stern. Oral and maxillofacial pathology - A rationale for diagnosis and treatment. Quintessence publishing co. Inc: New-Delhi; 2003. p326-29.
3. Burkhardt A, Maerker R. Leukoplakia and precancerous lesions. A colour atlas of Oral cancers. Wolfe medical publication Ltd; London: 1981: 9p.
4. Liu L, Satish K, Sedghizadeh PP, et al. Oral squamous cell carcinoma incidence by sub site among diverse racial and ethnic populations in California. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 2008;105:470-80.
5. Denny YP, CM Ho. The oral fluid MEMS/NEMS Chips (OFMNC): diagnostic and translational application. Adv Dent Res 2005;18:3-5.
6. Bigler LR, Streckfus CF, Dubinsky WP, et al. Salivary biomarkers for the detection of malignant tumors that are remote from the oral cavity. Clin Lab Med 2009;29:71-85.
7. Nagler RM, Lischinsky S, Diamond E, Klein I, Reznick AZ. New insight salivary lactate dehydrogenase of human subjects. J Lab Clin Med 2001;137;5:363-9.
8. Lingen MW John R Kalmar,TheodoreKarrison. Critical evaluation of diagnostic aids for the detection of oral cancer. Oral Oncol 2008;44:10–22.
9. Lester Burket, Martin s.Greenberg ,Jonathan Ship et al:Burket's Oral Medicine .11th Edition, PMPH Publications-USA, 2008.
10. Yu Lin Song, Guang Lin Yang, Yu Ming Dong. Immunohistochemical study of lactate dehydrogenase isoenzymes in gastric cancer, China Natl J New Gastroenterol 1995;1(1):13-7.
11. Song Y, Yang G, Dong Y. Immunohistochemical study of lactate dehydrogenase isoenzymes in gastric cancer: light and electron microscopic observations Chinese J Oncol 1996;18(4):266-9.
12. Drent M, Cobben NA, Henderson RF, Wouters EF, Van Dieijen-Visser M. Usefulness of lactate dehydrogenase and its isoenzymes as indicators of lung damage or inflammation. Eur Respir J 1996;9:1736-42.
13. Rotenberg Z, Weinberger I. Total Lactate Dehydrogenase and its isoenzymes in serum of patients with Non-small-cell carcinoma. Clin Chem 1988;34(4):668-70.
14. Liaw CC, Wang CH, Huang JS, Kiu MC, Chen JS, Chang HK. Serum lactate dehydrogenase level in patients with nasopharyngeal carcinoma. Acta Oncol 1997;36(2):159-64.
15. Visnja BD, Turshijan S. Activity of lactate dehydrogenase and superoxide dismutase in the circulation of patients with breast

carcinoma. Arch Oncol 2008;16(3-4):39-41.
16. Subramanian N, Krishnan MH, Venkatachalam P, Kamatchi PA. A Study of Lactate Dehydrogenase (LDH) Isoenzyme is a Biochemical Tumour Marker in Cervical Carcinoma Patients. Int J Hum Genet 2009;9(1):5-12.
17. Masahiro Dohijyo. Clinical and experimental studies of serum LDH activity and LDH isoenzymes of patients with oral cancer. Kyushu Dental College, Kitakyushu Japan: 1971. PDF source : internet
18. Hariharan S, Dhawan IK, Sharma U, et al. Serum LDH and its isoenzyme in buccal mucosa cancer.Indian J Med Res 1977;65:566-77.
19. Muralidhar M, Raghavan MR, Bailoor DN, et al. Evaluation of Serum Lactate dehydrogenase(LDH) In oral premalignant and malignant lesions. Ann Dent 1998;42(2):11-15.
20. Gorogh T, Eickbohm JE, Ewers R, et al. Lactate Dehydrogenase Isoenzymes in Squamous cell carcinomas of the oral cavity. J Oral Pathol Med 1990:19:56-9.
21. Naphade M, Naphade U. Major Immunoglobulin status and Lactate Dehydrogenase isoenzyme profile in Oral Premalignancy and Malignancy. Dental Dialogue 2011:37;1:16-20.
22. Shpitzer T, Bahar G, Feinmesser R, et al. A comprehensive salivary analysis for oral cancer diagnosis. J Cancer Res Clin Oncol 2007;133:613-7.
23. Rai B, Jain R, Anand SC, Kharb S. Salivary lactate dehydrogenase isoenzymes in oral lichen planus. Adv Med Dent Sci 2008;2(1):1-3.
24. Boyle JO, Mao L, Brennan JA, et al. Gene mutations in saliva as biomarkers for head and neck squamous cell carcinomas. Am J Surg 1994;168:429-32.

Corresponding Author
Dr.(Mrs). Priya Shirish Joshi,
B/3, Avani Appartment,
Datta Nagar, 80 Feet Road,
Vishrambaug, Sangli.
Email: opathvpdc@gmail.com
Ph: +91 9890712016

Source of Support: Nil, Conflict of Interest: None Declared.