International Journal of Research in Health and Allied Sciences

Journal home page: www.ijrhas.com

Official Publication of "*Society for Scientific Research and Studies*" [Regd.]

ISSN 2455-7803 Index Copernicus value 2016 = 68.10

Original Article

Evaluation and Comparison of Salivary Alkaline Phosphatase and Lactate Dehydrogenase in Patients of Potentially Malignant Disorders and Oral Squamous Cell Carcinoma

Geeta Sharma[1], Randhir Kumar[2]

[1]Professor & HOD, Department of Oral and Maxillofacial Pathology, Sarjug Dental College, Darbhanga, Bihar, India,
[2]Professor & HOD, Dept of Periodontology, Patna Dental College, Patna, Bihar, India

***ABSTRACT*:**
Aim: To estimate the value and the significance of Alkaline phosphatase and Lactate dehydrogenase in salivary samples of an individual with oral potentially malignant disorders, oral squamous cell carcinoma cases and control groups and to establish the salivary alkaline phosphatase and salivary lactate dehydrogenase as biomarkers for assessing the malignant potential in order to improve the quality of life of the patients. **Methods**: 5 ml of unstimulated saliva was collected from the patients by spit method in a calibrated test tube. It was then immediately centrifuged at 2500 rpm for 15 minutes. The resulting supernatant was then separated into 1 ml aliquots and subjected for further biochemical assay analysis using standard kit method. The samples will then be diluted in 1:1 ratio with saline and assayed using standard kit and measured using semi auto analyzer. The salivary LDH & salivary ALP concentrations were expressed in terms of IU/L. Results: A highly significant association was observed in Alkaline phosphatase group with different conditions (p=0.00) with 122.06 F value. A highly significant association was observed in lactate dehydrogenase group with different conditions (p=0.00) with 17.25 F value. **Conclusion**: Both Alkaline phosphatase and Lactate dehydrogenase are sensitive markers for the detection of pre cancer and cancer hence helpful in early detection of oral carcinoma. Statistical analysis also proves that ALP levels are consistently higher in oral pre cancer and cancer hence it could be more reliable marker in detection of oral carcinoma in comparison with S- LDH. The roles of S-LDH have to be further evaluated with larger sample size.

Key words: Leukoplakia, Oral Squamous Cell Carcinoma, Saliva, Lactate dehydrogenase, Alkaline phosphatase.

Received: 5 January 2019 Revised: 25 January 2019 Accepted: 28 January 2019

Corresponding author: Dr. Randhir Kumar, Professor & HOD, Dept of Periodontology, Patna Dental College, Patna, Bihar, India

This article may be cited as: Sharma G, Kumar R. Evaluation and Comparison of Salivary Alkaline Phosphatase and Lactate Dehydrogenase in Patients of Potentially Malignant Disorders and Oral Squamous Cell Carcinoma. Int J Res Health Allied Sci 2019; 5(1):41 -44.

INTRODUCTION:

Oral squamous cell carcinoma (OSCC) represents about 3% of malignancies in the western population; however, it accounts for over 30% of all malignancies in the Indian population[1]. India has one of the highest rates of oral cancer in the world due to the habit of tobacco chewing; the habit, which is associated with the rural population, illiterate individuals, and those of lower socioeconomic status, is responsible for half of the cancers in men, and quarter of the cancers in women. The frequency of chewing tobacco varies greatly between states (8%–60% in men, <1%–61% in women).

The poor prognosis of OSCC is mostly due to the fact that a significant proportion of patients are not diagnosed or treated until they reach an advanced stage. However, the prognosis for patients with OSCC that is treated early has 5-year survival rates as high as 80%, with improved quality of life and fewer invasive surgeries. Thus, prevention of oral carcinoma is divided into two major categories: The reduction of exposure to tobacco and early screening with detailed oral examination and biochemical analysis. Detection and appropriate treatment at the level of pre malignancy can greatly reduce the changes of further development into oral cancer, especially if the tobacco chewing habit is discontinued. Early detection can also be a key to reducing the mortality, morbidity and reducing the cost of treatment.[2]

Though biopsy is mandatory before making any treatment plans[3], biopsy is an invasive procedure and all patients may not be willing for biopsy as the lesion is usually asymptomatic. Saliva, the most available and non-invasive biofluid of the human body, permanently "bathes" the oral cavity and is trying to cope with an ever changing milieu. The oral cavity, a very complex and

unique milieu due to its dual function, is the only place in the body where the mineralized tissue is exposed to the external environment in which there are complex interactions between various surfaces: host soft and hard tissues, food, air, and microorganisms.

Saliva includes a large number of inorganic and organic compounds, which act as a "mirror of the body's health."[4] Use of saliva in evaluating the biomarkers for early diagnosis of cancer risk potential may be more appropriate in oral cancer, as saliva reflects most of the oral diseases & effects of oral mucosa in cancer can be better reflected in saliva as it bathes the entire oral cavity.[5] Cellular Alkaline phosphatase (ALP) is increasingly recognized as an important marker of induction of tumor cell differentiation5. The development of cancer is associated with a high glycolytic activity with a shift from aerobic to anerobic glycolysis. With the increase in the glycolytic activity the concomitant increase in lactate dehydrogenase (LDH) enzyme may be reflected in certain tissues.[6]

MATERIALS & METHODS:

The study involved 45 subjects with age range of 20-70 years reporting to the Department of Oral Medicine and Radiology. Fifteen individuals with potentially malignant disorder (PMD) formed the first group. Fifteen clinically and histopathologically diagnosed cases of Oral Squamous Cell Carcinoma formed the second group. Fifteen healthy individuals comprised the third group. Patients with Systemic diseases like acute infections, autoimmune diseases, inflammatory conditions, etc. were excluded from the study. Ethical clearance was obtained from the institution. 5 ml of unstimulated saliva was collected from each of the patients by asking the patient to expectorate gradually over a period of 5 minutes after getting a prior informed consent from them. Care was taken to see that the volunteers did not consume food or chew gum at least one hour before the collection procedure. Following collection, saliva was immediately centrifuged at 2500 rpm for 15 minutes to remove squamous cells and cell debris. The resulting supernatant was separated into 1 ml aliquots and subjected for further biochemical assay analysis using standard kit method. The samples will be diluted in 1:1 ratio with saline & assayed using the standard kit & measured using semi auto analyzer & the LDH & ALP concentrations were expressed in terms of IU/L.

Groups	Sample size	Categories
Group 1	15	Premalignant disorders
Group 2	15	OSCC
Group 3	15	Control/Healthy subjects

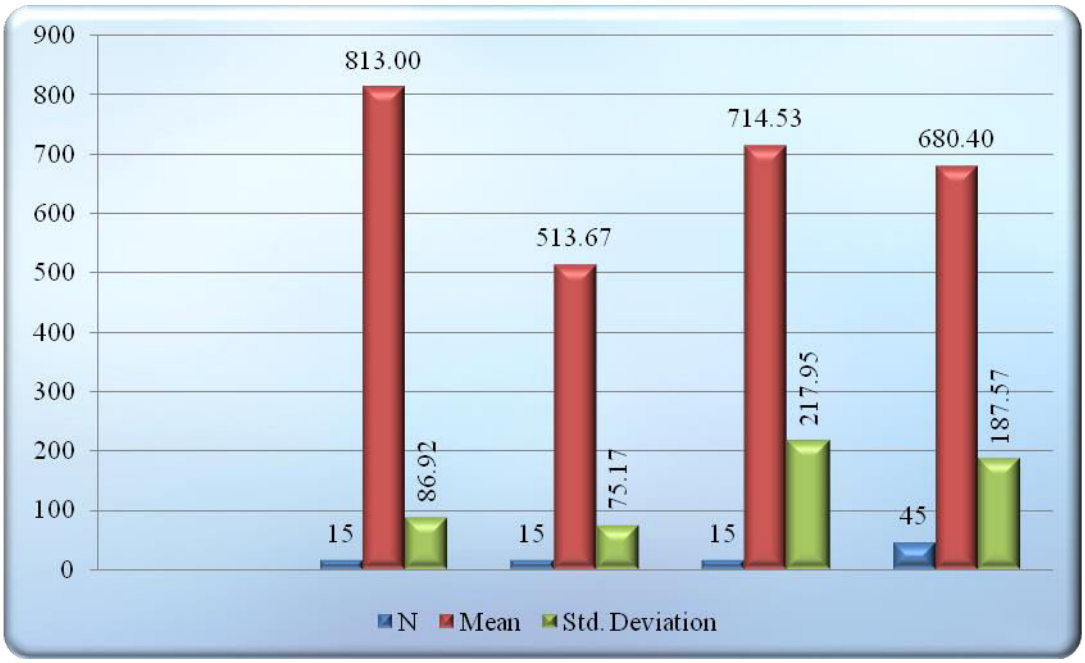
RESULTS:

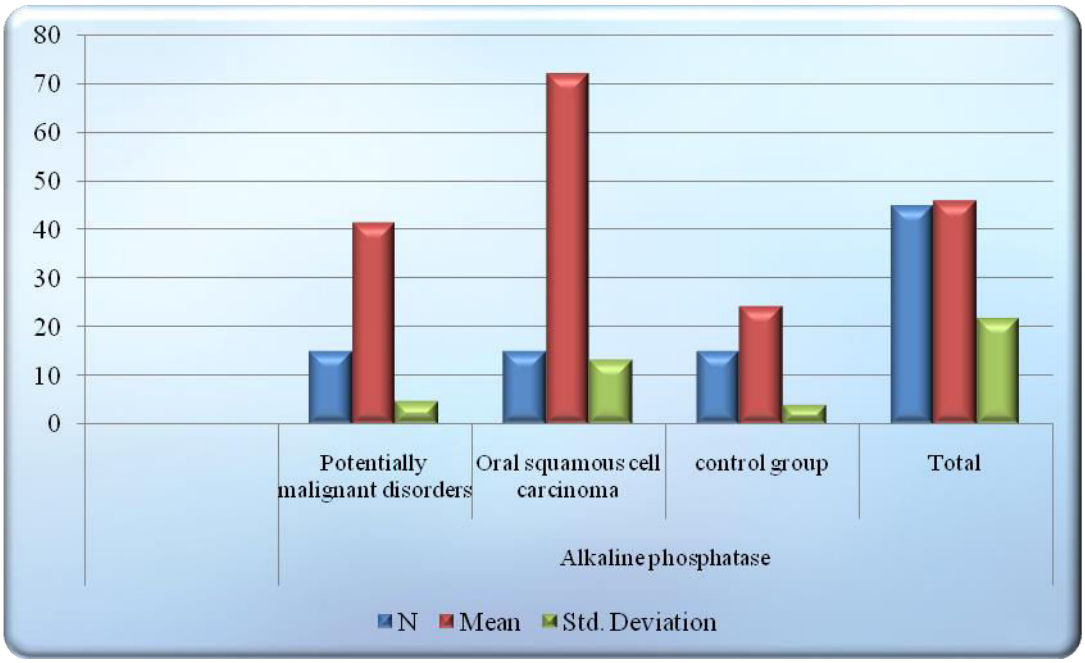
The present study was carried out on 45 subjects with mean age of the study participants was 37.96+14.73. The saliva of all the individuals was collected and measured. The mean value of Alkaline Phosphatase and Lactate Dehydrogenase was 45.96+21.63 and 680.40+187.57 respectively. Gender wise distribution was assessed among different groups, there were 10 (66.7%) male and 5 (33.3%) female, 9 (60.0%) male and 6 (40%) female, and 13 (86.7%) male and 2 (13.3%) female in potentially malignant disorders, oral squamous cell carcinoma group and control group respectively. The mean value of alkaline phosphatase was 41.33+4.79, 72.13+13.28 and 24.40+4.07 in potentially malignant disorders, oral squamous cell carcinoma and control group respectively. The mean value of lactate dehydrogenase was 813+86.92, 513.67+75.17 and 714.53+217.95 in potentially malignant disorders, oral squamous cell carcinoma and control group respectively.

Intergroup comparison was done among the different conditions using ANOVA test. A highly significant association was observed in Alkaline phosphatase group with different conditions (p=0.00) with 122.06 F value. A highly significant association was observed in lactate dehydrogenase group with different conditions (p=0.00) with 17.25 F value. A post hoc comparison among different groups (Tukey's HSD Test) was done in Alkaline Phosphatase group, a highly significant association was observed in Potentially malignant disorders with Oral squamous cell carcinoma and control group (p=0.00). Likewise, a highly significant association was found in Oral squamous cell carcinoma with Potentially malignant disorders and control group (p=0.00). Control group was also found highly significant with Potentially malignant disorders and Oral squamous cell carcinoma (p=0.00). Similarly, when post hoc comparison among different groups (Tukey's HSD Test) was done in Lactate dehydro-genase group, a highly significant association was observed in Potentially malignant disorders with Oral squamous cell carcinoma (p=0.00) but a non-significant association was observed with control group (p=.15). A highly significant association was found in Oral squamous cell carcinoma with Potentially malignant disorders and control group (p=0.00). Association between control group and Potentially malignant disorders was found non-significant (0=.15) but found highly significant association with Oral squamous cell carcinoma (p=0.00).

Table 1: Comparison of Enzymes with Different Conditions

Enzymes	Condition	N	Mean	Std. Deviation
Alkaline Phosphatase	Potentially malignant disorders	15	41.33	4.79
	Oral squamous cell carcinoma	15	72.13	13.28
	control group	15	24.40	4.07
	Total	45	45.96	21.63
Lactate Dehydro-Genase	Potentially malignant disorders	15	813.00	86.92
	Oral squamous cell carcinoma	15	513.67	75.17
	control group	15	714.53	217.95
	Total	45	680.40	187.57





DISCUSSION:

Biochemical changes have been occurring in biological fluids and tissues of different types of malignancies. Most molecules found in blood and urine are found in saliva, but their concentrations were estimated to be one tenth to one thousandth of that in the blood.[12] There are many invasive & non-invasive diagnostic techniques to assess the malignant risk potential in cancer risk patients. One such non-invasive diagnostic technique to evaluate cancer risk potential in potentially malignant disorders such as leukoplakia is sialodiagnosis. Salivary biomarkers are used in diagnosis of oral & systemic diseases.[13] A simple biochemical parameter that helps to diagnose the disease in early stage is lactate dehydrogenase enzyme and alkaline phosphatase. LDH is present in almost all the cells of our body. According to Warburg hypothesis due to hyperplasia the LDH level increases due to increased level of anaerobic glycolysis.[14] Applications and limitations of alkaline phosphatase as a tumor marker are discussed in literature.

The present study revealed that the mean value of salivary alkaline phosphatase (S-ALP) is 41.33+4.79, 72.13+13.28 and 24.40+4.07 in potentially malignant disorders, oral squamous cell carcinoma and control group respectively. This is in agreement with the previous reports.[6,12,15]

Recently, Prakash AR et al, in 2016 established that Alkaline phosphatase (ALP) is a hydrolase intracellular enzyme participating in the metabolic processes of cells[16]. Rise in salivary ALP (S-ALP) levels reflects inflammation and destruction of healthy tissues

suggesting it as a clinical biomarker. In addition to this, the present study also reported the mean value of salivary lactate dehydrogenase (S- LDH) as 813+86.92 and 714.53+217.95 in potentially malignant disorders and control group respectively. There was a rise in the level of salivary LDH from healthy control group to PMD which is according to numerous analysis in the literature.[6,16,17,27,32,34,36,37,38]

Hirschhaeuser F et al in 2011,[18] advocated increased glucose uptake and accumulation of lactate, even under normoxic conditions (i.e., aerobic glycolysis or the Warburg Effect), is a common feature of cancer cells.

Moreover, in 2016, Gracia SR et al supported the thought that malignant transformation of cells leads to enhanced glucose uptake and the conversion of a larger fraction of pyruvate into lactate, even under normoxic conditions; this phenomenon of aerobic glycolysis is largely known as the Warburg effect. The role of lactate as an immuno suppressor molecule that contributes to tumor evasion was discussed and the possibility of targeting lactate metabolism for cancer treatment, as well as of using lactate as a prognostic biomarker was explored.[19]

But the value of S-LDH levels declined in case of OSCC i.e. 513.67+75.17 which was significantly low in our study. The causes of such levels can be attributed due to the patients of OSCC undergoing radiotherapy or chemotherapy resulting decrease in the salivary quantity and flow of saliva in the patients, or there would have been a technical error. The possibility of the cause has to be further investigated.

Thus, Salivary ALP and LDH estimations can prove to be a valuable biochemical markers. The method is a simple, non-invasive and can be advocated as tool for diagnosis and monitoring of oral precancer and cancer. Therefore, this study proves that we should not rely only upon clinical diagnosis and a co-relation between clinical diagnosis and investigations has to be made to direct the patients toward proper treatment.

CONCLUSION:

Metabolomics approach complements the clinical detection of premalignant disorders and OSCC, leading to an improved disease diagnosis and prognosis. Salivary ALP and LDH estimation can prove to be a valuable substitute to serum ALP and LDH as a biochemical marker, as it is a simple, non-invasive procedure and easily accepted by the patient. Salivary ALP levels are consistently higher in oral pre-cancer and cancer hence it could be future marker for potential significance in clinical diagnosis and prognosis of cancer. But, the roles of S-LDH have to be further evaluated with a larger sample size. Salivary diagnosis should be performed in all the dental institutions to assess the malignant risk potential of potentially malignant disorders and thus quality of life of patients can be improved.

REFERENCES:

1. Kumar SA, Shakunthala G.K., Burde KN. Developments in diagnostic applications of saliva in oral and systemic diseases- A comprehensive review. Journal of Scientific and Innovative Research 2014; 3(3): 372-387.
2. Chari A, Rajesh P, Prabhu S. Estimation of serum lactate dehydrogenase in smokeless tobacco consumers. Indian J Dent Res 2016; 27:602-8.
3. Rao P.K. Potentially malignant lesion – oral leukoplakia. Global Advanced Research Journal of Medicine and Medical Sciences 2012; 1(11):286-291.
4. Greabu M, Battino M, Mohora M et al. Saliva- a diagnostic window to the body, both in health and in disease. J Med Life 2009;2(2):124-132.
5. Xiao B, Guo J, Liu D, Zhang S. Aloe Emodin induces in vitro G2/M arrest & alkaline phosphatase activation in human oral cancer KB cells, Ora Oncol 2007;43(9).905-10.
6. Dhivyalakshami M and Uma Maheshwari TN. Expression of Salivary Biomarkers – Alkaline Phosphatase & Lactate dehydrogenase in Oral Leukoplakia. International Journal of Chem Tech Research 2014; 6(5): 3014-3018.
7. Narayanan S. Alkaline Phosphatase as Tumor Marker. Institute for Clinical Science, Inc.1983;13(2):300-318.
8. Naglar RM, Liechinsky S, Diamond E, Klein I et al. New insights into salivary lactate dehydrogenase of human subjects. J Lab Clin Med 2001;137(5):363-369.
9. Saif WM, Alexander D, Charles M et al. Serum Alkaline Phosphate Level as a prognostic Tool in Colorectal Cancer: A study of 105 patient. J Appl Res. 2005; 5(1): 88–95.
10. De La Pena VA, Diz Dios P, Tojo Sierra R. Relationship between lactate dehydrogenase activity in saliva and oral health status. Arch Oral Biol.2007;52(10):911-5.
11. Desai S, Shinde H, Mudda J, Patil V. Levels of Alkaline Phosphatase (ALP) In Saliva of Patients with Chronic Periodontitis; a Clinical and Biochemical Study The Internet Journal of Dental Science.2008;8(1).
12. Merza KS, Alaaraji SB, Abdullah BH. Comparative study on lactate dehydrogenase, Alkaline phosphatase and immunoglobulins in Serum and saliva of acute leukemia and oral Squamous cell carcinoma patients. Iraqi Journal of Science 2010;51(2):262-270.
13. Umamaheswari TN. Salivary biomarkers in oral leukoplakia-A review. Int J Pharm Bio Sci 2014;6(5):3014 – 3018.
14. Kadiyala SV. A Study of Salivary Lactate Dehydrogenase (LDH) Levels in Oral Cancer and Oral Submucosal Fibrosis Patients among the Normal Individuls. J. Pharm. Sci. & Res 2014;7(7):455-457.
15. Deepika V, Priya V, Bedre A, Harsha L Salivary AST, ALP and CK Levels in Patients with Periodontitis. Pharm. Sci.& Res 2015;7(6):341-343.
16. Prakash AR, Indupuru K, Sreenath G, Kanth MR et al. Salivary alkaline phosphatase levels speak about association of smoking, diabetes and potentially malignant Diseases. Journal of Oral and Maxillofacial Pathology 2016;20(1).
17. Joshi PS, Chougule M, DudanakarM, Golgire S. Comparison Between Salivary and Serum Lactate Dehydrogenase Levels in Patients with Oral Leukoplakia and Oral Squamous Cell Carcinoma - A Pilot Study. International Journal of Oral & Maxillofacial Pathology 2012;3(4):07-12.
18. Hirschhaeuser F, Sattler U G.A, Klieser WM. Lactate: A Metabolic Key Player in Cancer.2011;129: 2207-2217
19. Gracia SR, Morewno Altamirano MMB, Gracia HP, Gracia FJS. Lactate Contribution to the Tumor Microenvironment: Mechanisms, effects on immune Cells and Therapeutic Relevance. Frontiers in Immunology 2016;7: Article 52.