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# Estimation of Salivary Lactate Dehydrogenase Levels in Patients of Leukoplakia and Oral Squamous Cell Carcinoma

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## ABSTRACT

**Aim**: To establish salivary Lactate dehydrogenase as biomarkers for assessing the malignant potential in leukoplakia and oral squamous cell carcinoma patient in order to improve the prognosis and outcome of the disease.

**Materials and Methods**: Total 90 subjects and their saliva samples were taken to estimate lactate dehydrogenase level in oral squamous cell carcinoma, leukoplakia and healthy subject. 2 ml of unstimulated saliva was collected from the patients by spit method in a wide mouthed sterile container. It was then immediately centrifuged at 2500 rpm for 15 minutes to remove squamous cell and cell debris. 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 concentrations were expressed in terms of IU/L.

**Results:** A highly significant association of lactate dehydrogenase was observed in leukoplakia and squamous cell carcinoma patient. The present study also showed correlation between tumor differentiation and the level of salivary LDH in oral squamous cell carcinoma patient and different grade of dysplasia.

**Conclusion:** 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 lactate dehydrogenase levels are consistently higher in oral pre cancer and cancer hence it could be more reliable marker in detection of oral cancer.

**Keywords:** Leukoplakia, Oral Squamous Cell Carcinoma, Saliva, Lactate dehydrogenase.

## INTRODUCTION

**O**ral 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

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examination and biochemical analysis. According to Warburg's hypothesis and findings, malignant tissue has high glycolic activity. This pointed to major difference between normal and malignant tissue. LDH is a major enzyme in glycolysis which reversibly catalyses pyruvate to lactate with increased glycolic activity, which may be reflected in tissue fluid and serum. 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]

## MATERIALS & METHODS

The study involved 90 subjects with age range of 25-65 years reporting to the Department of Oral Medicine and Radiology. Thirty individuals with leukoplakia formed the first group. Thirty clinically and histopathologically diagnosed cases of Oral Squamous Cell Carcinoma formed the second group. Thirty healthy individuals comprised the third group. Patients with Systemic diseases like acute infections, autoimmune diseases, inflammatory conditions, etc. were excluded from the study. 2 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 concentrations were expressed in terms of IU/L.

## RESULTS

The present study was carried out on 90 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 Lactate Dehydrogenase was 964 , 1579 and 615 in group 1 , group 2 and group 3 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 lactate dehydrogenase was 395±152, 1010 ± 105 and 1975±257 in healthy subject, leukoplakia and oral squamous cell carcinoma respectively.

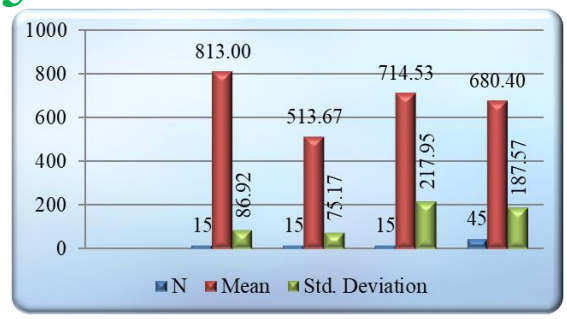
Table 1: Distribution of sample.

| Groups | Sample size | Categories |
|---|---|---|
| Group 1 | 30 | Leokoplakia |
| Group 2 | 30 | Oral squamous cell carcinoma |
| Group 3 | 30 | Control/Healthy subjects |

Table 2: Comparison of Enzymes with Different Conditions

| Enzymes | Condition | N | Mean | Std. Deviation |
|---|---|---|---|---|
| Lactate Dehydro-Genase | Leukoplakia | 30 | 1010.74 | 105.30 |
| | Oral squamous cell carcinoma | 30 | 1975.57 | 257.98 |
| | control group | 30 | 395.73 | 152.10 |

Intergroup comparison was done among the different conditions using ANOVA test. 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 LDH group, a highly significant association was observed in Potentially malignant disorders with Oral squamous cell carcinoma and control.




Graph 1: Comparison between mean LDH level of well differentiated, moderately differentiated, and poorly differentiated squamous cell carcinoma.

Likewise, a highly significant association was found in Oral squamous cell carcinoma 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 ANOVA comparison among different groups (Tukey's HSD Test) was done in Lactate dehydro-genase group, a highly significant association was observed in different grades of Oral squamous cell carcinoma (p=0.00) i.e.well differentiated, moderately differentiated and poorly differentiated. Mean LDH level is highest among poorly differentiated and lowest mean LDH level in well differentiated squamous cell carcinoma. A highly significant association was found in Oral squamous cell carcinoma with leukoplakia and control group (p=0.00). Association between control group and leukoplakia… was found non-significant (0=.15) but found highly significant association with Oral squamous cell carcinoma (p=0.00).

## 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. 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]

The present study revealed that the mean value of salivary lactate dehydrogenase (S- LDH) is 1010.74+105.30 and 395.73+152 in leukoplakia and control group respectively. There was a rise in the level of salivary LDH from healthy control group to different grade of dysplasia 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 increased in case of OSCC i.e. 1975+257.98 which was significantly high in our study. The causes of such levels can be attributed due to increased mitotic index and more lactic acid production by tumor cells due to breakdown of glycoprotein. Rise in LDH concentration in saliva, as an expression of cellular necrosis reflects inflammation and destruction of healthy tissue

Thus, Salivary 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

Saliva has been viewed as an important diagnostic fluid for a very long time. In recent times because of the improved efficiency of genomic and proteomic technologies 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.

## CONFLICTS OF INTEREST

The authors declare they have no potential conflict of interests regarding this article.

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