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KEY WORDS:

# A COMPARETIVE STUDY OF SALIVARY LACTATE DEHYDROGENASE ENZYME AS AN EARLY BIOMARKER IN ORAL SUBMUCOUS FIBROSIS AND ORAL SQUAMOUS CELL CARCINOMA

**Dr. Priyanka Kumari Mandia** B.D.S., MSc (Medical) Biochemistry

**Dr. Saroj Choudhary*** Senior Demonstrator, Department of Physiology, GMCH, Udaipur, Rajasthan, India.*Corresponding Author

**ABSTRACT**

Saliva an important and potential biomarker can be used as an adjuctive step for diagnosing oral cancer and precancers which improve the prognosis and outcome of the disease process. Aim of this study was 'To analyse salivary Lactate dehydrogenase level could be a reliable marker to diagnosis oral submucous fibrosis and oral cancer.' The present study include150 subjects of either sex among them:- Group a- 50 healthy control without any clinical symptoms or disease, Group b- 50 Oral submucous fibrosis patients and Group c- 50 Oral squamous cell carcinoma patients. Highly significant increase Salivary lactate dehydrogenase enzyme leve was found in Oral submucous fibrosis and oral squamous cell carcinoma as compared to healthy control. Salivary LDH level of OSMF patients shows significant difference with Salivary LDH level of OSCC Patients. Mean Salivary LDH level is higher in OSCC patients. Thus it can be concluded that Salivary lactate dehydrogenase is an early biomarker and supportive diagnostic tool in oral submucous fibrosis and oral squamous cell carcinoma.

## INTRODUCTION

Oral submucous fibrosis is a chronic ,complex, premalignant (1% transformation risk) lesion affecting any part of the oral cavity and sometime the pharynx. characterized by juxta-epithelial inflammatory reaction and progressive fibrosis of the submucosal tissues (the lamina propria and deeper connective tissue). As the disease progresses, the jaws become rigid to the point that the person is unable to open the mouth.[1]

Oral squamous cell carcinoma (OSCC) is the sixth most common human cancer affecting the oral cavity that encompasses at least 90% of all malignancy and is an important cause for cancer morbidity and mortality worldwide.[2]

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. 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.[3]

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 cell.[3] 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.[2]

Salivary LDH levels have not been studied rigorously in oral precancer and cancer. Hence this endeavor to measure and compare Salivary LDH levels in patients with oral submucous fibrosis and oral squamous cell carcinoma.

Many methods are available today for diagnosis of cancers but more emphasis is always given to a noninvasive and an accurate test for diagnosis, thus the saliva an important and potential biomarker can be used as an adjuctive step for diagnosing oral cancer and precancers which improve the prognosis and outcome of the disease process.

**AIM OF STUDY:** To analyse salivary Lactate dehydrogenase level could be a reliable marker to diagnosis oral submucous fibrosis and oral cancer .

## MATERIAL AND METHOD

This study was designed to evaluate a comparative study of salivary lactate dehydrogenase enzyme level in patients with Oral submucous fibrosis, Oral squamous cell carcinoma and healthy control.

**The present study include150 subjects of either sex among them:-**

Group a)- 50 healthy control without any clinical symptoms or disease
Group b)- 50 Oral submucous fibrosis patients and
Group c)- 50 Oral squamous cell carcinoma patients

**study design :-** Present study was conducted in the Department of Biochemistry, Jhalawar Medical college.The subjects in our study groups were selected from O.P.D. of Department of Dental of S.R.G.Hospital.

**Subject selection:-**

Based on the following inclusion and exclusion criteria selection of subjects for the study was made on the basis of detailed history and proper clinical examination.

**Inclusion criteria:-**

Patient's willingness to participate.
Subjects in the age group of 20-80 years irrespective of sex.
Histopathologically diagnosed patients of OSCC and OSMF.

**Exclusion criteria:-**

- Patient not willing for participation in the study.
- Patients undergoing chemotherapy, radiotherapy or any surgical procedure for OSCC.
- Patients with a history of heart failure (myocardial infarction) within past 2 weeks.
- Patient taking procainamides and other drugs used to treat arrhythmia, pulmonary infarction and stroke
- Patients with history of consumption of aspirin, narcotics or alcohol,and recent anaesthesia.

**Sample collection:**

**Saliva sample:-**

Unstimulated whole saliva was collected in a disposable and sterile plastic container by spitting method. Morning samples were preferred to avoid diurnal variations of salivary flow .Patients was advised to avoid intake of water or food one hour prior to sample collection to avoid interference of food and water with the enzyme level. Sample was centrifuged at 3000 rpm at 40 c for 30 minutes and supernatant was proceed for quantification of LDH by fully autoanalyser.(BECKMAN-COULTER Au680).

Lactate Dehydrogenase Test :- Lactate dehydrogenase, also called lactic dehydrogenase, or LDH, is an enzyme found in the cells of many body tissues, including the heart, liver, kidneys, skeletal muscle, brain, red blood cells, and lungs. It is responsible for converting muscle lactic acid into pyruvic acid, an essential step in producing cellular energy.

"Lactate dehydrogenase (LDH) is an enzyme present in a wide variety of organisms, including plants and animals". Its Enzyme Comission number is EC 1.1.1.27 where;

EC 1 = oxidoreductase
EC 1.1 = acting on the CH-OH group of the donor
EC 1.1.1 = With NAD or NADP as acceptor
EC 1.1.1.27 = L-lactate dehydrogenase

Thus, it is an oxidoreductase enzyme that catalyzes the interconversion of pyruvate and lactate accompanied by the interconversion of NADH and NAD+ (Butova, O.A. & Masalov, S.V. et al., 2009) The reaction is as following:

During anaerobic glycolysis (limited or no oxygen during intense muscular activity), the enzyme converts pyruvate to lactate.

## RESULT

**Table:1** Comparison of Salivary LDH among Healthy Control, OSMF Patient and OSCC Patient

| Groups | N | Mean | Std. Deviation | F value | P value |
|---|---|---|---|---|---|
| Healthy Control | 50 | 129.4600 | 41.61250 | 551.522 | <0.0001 * |
| OSMF Patient | 50 | 590.4400 | 181.51282 | | |
| OSCC Patient | 50 | 1248.1800 | 226.48482 | | |

**Table:2** Comparison of Salivary LDH between Healthy Control and OSMF Patient.

| Group | Mean | SD | T value | P value |
|---|---|---|---|---|
| Healthy | 129.4600 | 41.61250 | 17.5040 | <0.0001 |
| OSMF Patient | 590.4400 | 181.51282 | | |

**Table:3** Comparison of Salivary LDH between Healthy Control and OSCC Patient

| Group | Mean | SD | T value | P value |
|---|---|---|---|---|
| Healthy | 129.4600 | 41.61250 | 34.3525 | <0.0001 |
| OSCC Patient | 1248.1800 | 226.48482 | | |

The present study was conducted on total number of 150 subjects, out of which 50 were Oral submucous fibrosis,50 were Oral squamous cell carcinoma and 50 were the healthy control. Oral submucous fibrosis and Oral squamous cell carcinoma patients were studied with reference to age group and gender group. Level of salivary Lactate dehydrogenase enzyme were determined in Oral submucous fibrosis and Oral squamous cell carcinoma patients and healthy control. The values thus obtained were subjected to statistical analysis was done by help of SPSS 20.0 Software. The data in this study was expressed as mean ± SD,$p<0.05$ was considered as statistically significant. The present study was a comparative and case control study.

Table:1 show that Distribution of Salivary LDH according to Groups the mean value of healthy control was 129.46 and Standard deviation was 41.61. In OSMF patients mean value of Salivary LDH was 590.44 and SD was 181.51. OSCC patients show mean value of Salivary LDH was 1248.18 with SD of 226.48, difference of mean value of LDH was statistically significant.

According to table:2, mean±SD Salivary LDH value of healthy control was 129.46 ±41.61 and mean±SD Salivary LDH value of OSMF patients was 590.44± 181.51.There was statistically significant difference between healthy control and OSMF LDH level ($p<0.0001$)

Table:2 show that mean± SD Salivary LDH value of healthy control was 129.46 ±41.6 and mean±SD Salivary LDH value of OSCC patients was 1248.18± 226.48. There was statistically significant difference between healthy control and OSMF LDH level ($p<0.0001$*)

## DISCUSSION

Oral submucous fibrosis and Oral squamous cell carcinoma patients exhibited increased levels of serum and salivary Lactate dehydrogenase enzyme. The present study was conducted with the aim to measure and compare LDH levels in saliva of patients with Oral submucous fibrosis and oral squamous cell carcinoma.

According to Rafael MN, et al.,(2005) have concluded that whole saliva LDH is nonglandular in origin and probably oral epithelium is the major source for this non glandular LDH. It is logical to assume that pathological alternations of oral epithelium like dysplasia or cancer may result in alternation of LDH levels in saliva.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.[4]

Tilakarathne, et al. (2008) stated that OSMF is a disease of connective tissue of the oral mucosa, there is alteration in the epithelium due to the abnormal changes occurring in the fibrous connective tissue. These altered epithelial cells might be the reason for the elevated salivary LDH levels in OSMF cases. Oral epithelial cells are the direct source of LDH in saliva rather than the salivary gland by itself.[5]

Shpitzer T et al.,(2007) who utilized comprehensive salivary analysis to evaluate biochemical and immunological parameters in the saliva of (OSCC) patients and found that in cancer patients, salivary concentration of LDH was significantly higher by 88% , When salivary LDH values were compared among the 30 cases of OSCC and 20 normal controls it was found that the LDH values in the 30 cases were significantly higher than in the controls ($p = 0.0001$). The p-value obtained was statistically significant which proved our first objective of using salivary LDH as a potential biomarker for OSCC detection and diagnosis.[6]

## SUMMARY AND CONCLUSION

This study was designed to evaluate salivary Lactate dehydrogenase enzyme as an early biomarker in Oral submucous fibrosis and Oral squamous cell carcinoma. This study include 150 patients of both sex ranging 20-80 year among them 50 normal healthy controls without any clinical symptoms or disease and 50 Oral submucous fibrosis, 50 Oral squamous cell carcinoma patients.

Highly significant increase Salivary lactate dehydrogenase enzyme leve was found in Oral submucous fibrosis and oral squamous cell carcinoma as compared to healthy control.

Salivary LDH level of OSMF patients shows significant difference with Salivary LDH level of OSCC Patients. Mean Salivary LDH level is higher in OSCC patients.

Thus it can be concluded that Salivary lactate dehydrogenase is an early biomarker and supportive diagnostic tool in oral submucous fibrosis and oral squamous cell carcinoma.

## References

1. Pindborg JJ, Sirsat SM. Oral submucous fibrosis Oral Surg Oral Med Oral Pathol.1966;22(6):764-79.
2. Lingen MW, Kalmar JR, Karrison T,Speight PM.Critical evaluation of diagnostic aids for the detection of oral cancer.Oral Oncology.2008;44:10-22.
3. 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.
4. Rafael MN,Denny YP, CM Ho. The oral fluid MEMS/NEMS Chips (OFMNC): diagnostic and translational application. Adv Dent Res 2005;18:3-5.
5. Tilakaratne WM, Iqbal Z, The MT, Pitiyage G. Upregulation of HIF 1alpha in malignant transformation of oral submucous fibrosis. J Oral Pathol Med. 2008; 37(6):372-377.
6. 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.