

## Evaluation of Serum Lactate Dehydrogenase Enzyme in Different Grades of Oral Sub Mucous Fibrosis among Patients Attending a Dental College Of Western India.

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**Abstract:** Oral submucous fibrosis (OSF) is a premalignant disorder associated with the chewing of areca nut (betel nut). OSF itself causes significant morbidity and may also cause mortality, if transformed into oral squamous cell carcinoma. Our study aims to evaluate lactate dehydrogenase enzyme activity in serum of different grades of oral sub mucous fibrosis and to compare them with healthy controls and to correlate the relationship between pathogenesis of OSF and the LDH enzyme among patients attending a dental college of Western India. A cross-sectional study was conducted among 30 patients of OSF and 30 healthy controls. Oral Punch Biopsy was taken from OSF patients and venous blood samples for LDH estimation was collected from both OSF patients and healthy control. Mean serum LDH level were significantly ( $p=0.004$ ) higher among cases than the control. One way ANOVA for mean serum LDH between Normal oral histology and different grades of submucous fibrosis also depicted that the serum LDH level were significantly higher among cases than controls. Increase in the mean serum LDH level was linear between different histological grades as advanced histological grades was associated with more increase in serum LDH level. Estimation of serum LDH level could be a reliable and cost effective marker to diagnose OSF (pre-malignant condition).

**Keywords-** Lactate dehydrogenase (LDH), Oral malignancy, Oral pathology, Oral submucosal fibrosis (OSF), Squamous cell Carcinoma (SCC).

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### I Introduction

Oral submucous fibrosis (OSF) is a premalignant condition first described by Schwartz in 1952 and he coined the term *Atrophia Idiopathica Mucosae Oris*.<sup>1</sup> OSF is defined as an "Insidious chronic disease affecting any part of the oral cavity and sometimes the pharynx, although occasionally preceded by and /or associated with vesicle formation, it is always associated with juxta epithelial inflammatory reaction followed by a fibro-elastic change of the lamina propria with epithelial atrophy leading to stiffness of mucosa and causing trismus and inability to eat".<sup>1</sup> In India this condition was first described as diffuse oral submucous fibrosis (Lal 1953) and as submucous fibrosis of the palate & pillars (Joshi 1953).<sup>2</sup>

Oral submucous fibrosis (OSF) is a premalignant disorder associated with the chewing of areca nut (betel nut). The habit is prevalent in South Asian populations but has been recognized nowadays also in Europe and North America. OSF causes significant morbidity and after transformation into oral squamous cell carcinoma (OSCC), it may also cause mortality. The combination of areca nut and tobacco has led to a sharp increase in the frequency of OSF.<sup>3</sup> The global incidence in 1996 of submucous fibrosis was estimated as 2.5 million individuals. In Indian populations the prevalence is 5% for women and 2% for men. Age groups below 20 years are more often contracted with submucous fibrosis.<sup>1</sup>

Increased LDH levels are due to increased mitotic index and more lactic acid production by tumor cells due to breakdown of glycoprotein. Increased serum lactate dehydrogenase (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 such as oral, laryngeal and breast cancer.<sup>4</sup> Studies have also suggested that increased levels of

LDH in serum are seen in patients with OSF, oral leukoplakia (OL) and oral squamous cell carcinoma (OSCC).<sup>5-6</sup> Value of LDH elevates in OSCC and potentially malignant disorders and this finding can be used for benefit of the patient in predicting prognosis.<sup>7-8</sup> This study aims to evaluate lactate dehydrogenase enzyme activity in serum of different grades of oral submucosal fibrosis and to compare them with healthy controls and to correlate the relationship between pathogenesis of OSF and the LDH enzyme among patients attending a dental college of Western India.

## II Material and Methods

A cross-sectional study was conducted among OSF Patients attending the Department of Oral Medicine & Radiology (OMR) of Vyas Dental College & Hospital, Jodhpur, Rajasthan (India), from June 2015- May 2016. Healthy individuals without OSF were chosen as the control group. Study was approved by the Institutional ethics committee of Vyas Dental College & Hospital.

**Study design:** Cross-sectional

**Study location:** Department of Oral Medicine & Radiology (OMR), Vyas Dental College & Hospital, Jodhpur, Rajasthan (India).

**Study Duration:** June 2015-May 2016.

**Sample size:** 30 cases of OSF and 30 healthy control.

**Sampling technique:** Non probability, Consecutive.

**Study Population:** Cases: Patients with OSF, Control: healthy individual.

**Inclusion criteria:** For cases Diagnosed patients of OSF and for control healthy individuals.

**Exclusion criteria:** Moribund patients, Individuals using steroids, individuals having severe hepatic disease, Acute heart attack, anemia, muscle trauma, bone fractures, and other cancers.

**Data Collection:** After taking informed written consent, study participants were interviewed using semi structured, pre tested schedule. Data regarding socio-demographic characteristics and addiction details with special emphasis on the frequency and duration of the oral habits of chewing areca nut, paan (betel quid), gutkha and smoking beedi or cigarette were taken. After interview OSF patients were clinically examined and OSF was clinically classified as per the Nagesh and Bailoor criteria.<sup>9</sup> Punch Biopsy of the OSF lesion were taken with the help of 5mm disposable punch, from the most representative area of the buccal mucosa under local anesthesia and with full aseptic measures. The tissue was then fixed in 10% formalin solution and stained by routine technique for Hematoxylin and Eosin stain. After staining, the sections were observed under a binocular microscope. The cases were histopathologically graded according to the Pindborg and Sirsat criteria.<sup>10</sup> 5 ml of venous blood were drawn from both OSF patients and healthy individuals under full aseptic condition and sent to laboratory for serum LDH estimation. Evaluation of LDH was done on patient's serum stored at 20-25°C by using Flex reagent cartridge (LDI method), supplied by Dimension clinical chemistry system. The LDI method used L-lactate as a substrate buffered at a pH of 9.4. Lactate dehydrogenase oxidizes the substrate in the presence of NAD<sup>+</sup> to yield pyruvate & NADH which absorbs at 340nm. Lactate dehydrogenase activity concentration is measured as a rate reaction at 340/700nm, proportional to the amount of lactate dehydrogenase in the sample.

**Statistical analysis:** Data was analyzed using SPSS software, version 20.0. Independent t test and one way ANOVA used to show the statistical association between mean differences among different grades of OSF.

## III Results

There was 30 study subjects in both control as well as cases groups. Among Controls 56.7 % and 43.3% were male and female respectively, while in case groups 56.7 % were females and 43.3 % were male. 63.3% of cases were ≥ 41 years of age followed by 26.7% were in 21-40 years age group and 10.0% were < 10 years of age. In control group 60.0% were ≥ 41 years of age, 33.3 % were in 21-40 years of age group and 6.7% were less than 20 years. 80.0% of cases & 90.0% of control belonged to Hindu community, while 13.3% of cases and 6.7% of control belonged to Muslim Community.

Mean serum LDH level were significantly (p-0.004) higher among cases than the control (table-1).

One way ANOVA for mean serum LDH between Normal oral histology and different grades of submucous fibrosis also depicted that the serum LDH level were significantly higher among cases than controls (table-2), increase in the mean serum LDH level was linear between different histological grades as advanced histological grades was associated with more increase in serum LDH level (Figure-1).

**Table-1: Independent sample ‘t-test’ for Comparison of Serum LDH levels in Group 1(cases) and Group 2 (Healthy Subjects), n=30 in each group**

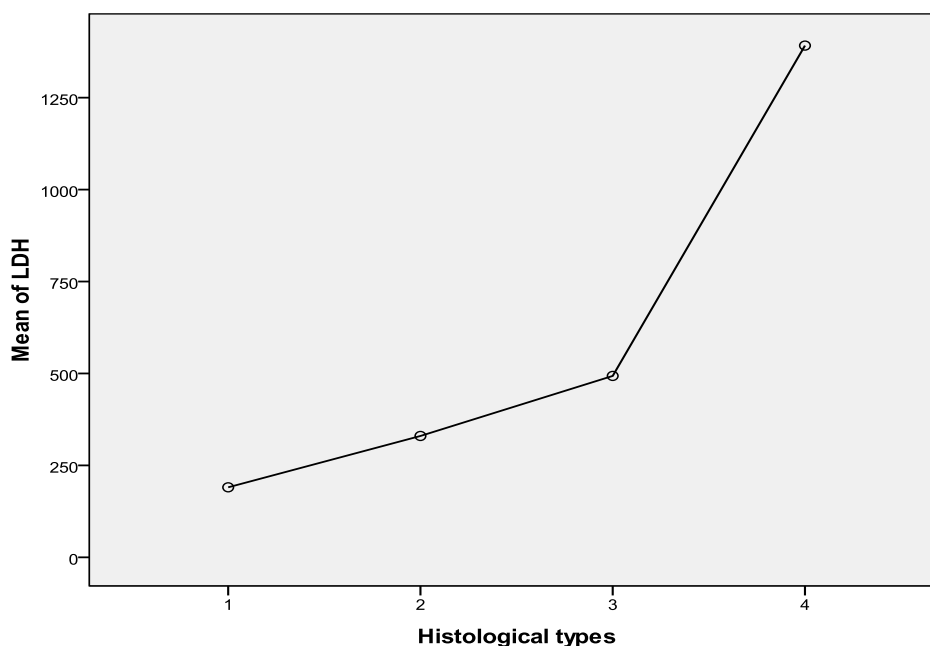
Groups	n	Mean	SD	Mean difference	SE difference	t	p- value	95% C.I of the difference	
								lower	upper
Serum LDH Group 1 Group 2	30	828.13	1175.695	637.733	214.865	2.968	0.004	207.634	1067.833
	30	190.40	52.48	637.733	214.865				

**Table-2: Comparison of normally distributed Serum LDH between the normal (1) and 3 Histological groups (2-early, 3-moderately advanced, 3-advanced) of Submucous Fibrosis using– One way ANOVA**

One Way-ANOVA  
LDH

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	1.349E7	3	4498147.343	7.686	.000
Within Groups	3.277E7	56	585205.780		
Total	4.627E7	59			

**Figure-1: Showing Means Plots of Serum LDH among control and different histological grades of cases.**



- 1: Control
- 2: Early Submucous Fibrosis
- 3: Moderately Advanced Submucous Fibrosis
- 4: Advanced Submucous Fibrosis

#### IV Discussion

Oral submucous fibrosis is a chronic debilitating disease of the oral cavity characterized by inflammation and progressive fibrosis of the submucosal tissues, well recognized for its malignant potential and is particularly associated with areca nut and tobacco chewing which is a habit practiced predominately in Southeast Asia and India. Increased serum lactate dehydrogenase (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 such as oral, laryngeal and breast cancer.<sup>4</sup>

In our study 30 OSF cases was compared with 30 matched healthy controls. The mean levels of LDH were significantly higher in OSF group subjects compared to control subjects. Similar observations were made in studies conducted by various other investigators.<sup>11-12</sup> Rajendran et al.<sup>7</sup> confirmed positive relation between OSF and increased serum LDH isoenzyme pattern. Hariharan et al. found that isoenzymes LDH-4 and LDH-5 are higher in cancer patients as compared to normal controls.<sup>13</sup> Kamath et al. in 2013 carried out a review of

various biochemical markers including LDH, and concluded that increased levels of LDH are seen in patients with oral sub mucous fibrosis.<sup>12</sup>

The results of the present study showed that there was a progressive increase in the serum LDH levels from early to moderately advance and from moderately advance to advanced histologic grade of OSF. This abnormality may be due to an altered amount of the enzyme forming tissue, an altered rate of synthesis of these enzymes within the tissue of origin, or an alteration in the permeability of the cell membrane brought about by the pathological condition. Muralidhar et al. reported a definite rise of serum LDH levels from normal in premalignant and malignant cases.<sup>14</sup> Görögh et al. studied LDH isoenzymes in human epithelial cells from squamous cell carcinomas and healthy tissues of the oral cavity and concluded that gradual changes in the percentage distribution of LDH isoenzymes may represent a useful parameter of disease activity in patients with squamous cell carcinoma.<sup>15</sup> Pereira T et al. also reported that Serum LDH levels increase in oral premalignant lesions/conditions and OSCC.<sup>16</sup>

## V Conclusion

Serum LDH rises in OSF and OSCE. Serum LDH level increases with increasing histologic grades of OSF. Serum LDH can be used as a marker of disease progression in cases of OSF.

**Conflict of Interest:** Nil

**Source of Financial Support:** Nil

## References

- [1]. Basoya S, Etiopathogenesis and management of oral sub mucous fibrosis, *Quality in Primary Care (2015) 23 (6): 327-332*.
- [2]. Gupta M, Pachauri A, Singh SK, Ahuja R, Singh P, Mishra SSS, Recent Advancements in Oral Sub mucous Fibrosis Management: an Overview, *Bangladesh Journal of Dental Research & Education*, Vol. 04, No. 02, July 2014
- [3]. Wollina U, Verma SB, Ali FM, Patil K, Oral sub mucous fibrosis: an update, *Clinical, Cosmetic and Investigational Dermatology 2015;8 193–204*.
- [4]. Lingen MW, Kalmar JR, Karrison T, Speight PM. Critical evaluation of diagnostic aids for the detection of oral cancer. *Oral Oncol. 2008;44:10-22*.
- [5]. Rajendran R, Rani V, Shaikh S. Pentoxifylline therapy : A new adjunct in the treatment of oral sub mucous fibrosis, *Indian J Dent Res.2006 | Volume : 17 | Issue : 4 | Page : 190198*
- [6]. Deshmukh SN, Dive AM. Oral sub mucous fibrosis review, *I. J. Of Applied Research*, March 2015,5(3).
- [7]. Liaw CC, Wang CH, Huang JS, Kiu MC, Chen JS, Chang HK. Serum lactate dehydrogenase level in patients with nasopharyngeal carcinoma. *ActaOncol. 1997; 36:159-64*.
- [8]. Rajendran R, Paul S, Mathews PP, Raghul J, Mohanty M. Characterization and quantification of mucosal vasculature in oral submucous fibrosis. *Indian J Dent Res 2005; 16:83-91*.
- [9]. Maher R, Sankaranarayanan R, Johnson NW. Evaluation of inter-incisor distance as an objective criteon of the severity of oral submucous fibrosis in Karachi, Pakistan. *Oral Oncology Eur Journal of Cancer 1996; 32:362-4*.
- [10]. Katharia SK, Singh SP, KulshreshthaVK. The effects of placenta extract in management of oral submucous fibrosis. *Ind J Pharma 1992; 24; 181-83*.
- [11]. Anuradha CD, Devi CS. Studies on enzymes of clinical signifcance in oral sub mucous fibrosis. *J ClinBiochem Nut. 1998;24:45-52*.
- [12]. KamathVV, Satelur K, Komali Y. Biochemical markers in oral sub mucous fibrosis: A review and update. *Dent Res J (Isfahan) 2013;10:576-84*.
- [13]. Hariharan S, Dhawan IK, Sharma U, Srivastava LM. Serum LDH and its isoenzymes in buccal mucosa cancer. *Indian J Med Res 1977;65:566-77*.
- [14]. Muralidhar M, Raghavan MR, Bailoor DN, Kamath VV. Evaluation of serum Lactate Dehydrogenase (LDH) in oral premalignant and malignant lesions. *Ann Dent 1988;47:11-5*.
- [15]. Kamath VV, Satelur K, Komali Y. Biochemical markers in oral submucous fibrosis: A review and update. *Dent Res J (Isfahan) 2013;10:576-84*.
- [16]. Pereira T, Shetty S, Pereira S. Estimation of serum lactate dehydrogenase level in patients with oral premalignant lesions/ conditions and oral squamous cell carcinoma: A clinicopathological study. *J Can Res Ther 2015;11:78-82*.

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