

Salivary Lactate Dehydrogenase Enzyme Activity in Oral Submucous Fibrosis: A Biochemical and Clinicopathological Study

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Abstract

Introduction: There has been an insidious yet steady rise in oral submucous fibrosis both in India and across the world; the reason is being attributed to the consumption of areca nut or gutkha. Numerous attempts are being made to develop non-invasive and reliable biochemical tests for the early detection and diagnosis. Of late, the role of specific salivary biomarkers has been studied extensively.

Methods: In this study, specific salivary biomarkers i.e. the level of lactate dehydrogenase enzyme among 160 subjects (80 oral submucous fibrosis patients and 80 healthy controls) were assessed. Concentrations of these biomarkers were compared in different grades of oral submucous fibrosis.

Result: The results revealed a positive correlation between the parameters in saliva samples of the two studied groups. There was significant increase in salivary lactate dehydrogenase levels in oral submucous fibrosis patients when compared to normal healthy individuals ($P < 0.001$). Positive correlation was observed between those who had submucous fibrosis and with the duration of areca nut chewing.

Keywords: Saliva, Salivary Biomarkers, Oral submucous fibrosis, Areca nut, Lactate dehydrogenase Enzyme

Abbreviations:

OSMF- Oral Submucous Fibrosis; LDH- Lactate Dehydrogenase Enzyme

Introduction

Most adults and young alike are subject to anxiety and pressure and unfortunately, they react to this stress by becoming enslaved to regretful habits like paan, betel chewing with or without tobacco, smoking and alcoholism. These habits, though believed to alleviate tension to some extent, inevitably produce detrimental and addictive effects on the body- especially the oral cavity [1,2]. The fact that oral precancers or cancers is still a major health problem in the Indian sub-continent is largely due to the chewing of the betel quid or pan masala or the frequent use of tobacco smoking and / or chewing with or without betel quid (product of chewing betel leaf with areca nut and lime) [1,2]. Thus the early diagnosis and treatment of precancers is vital. A thorough knowledge of these lesions is essential at this early stage as it offers the best prognosis and the most effective approach to reduce morbidity and mortality for oral cancers.

The development of oral cancer is a multistep process, arising from pre-existing Potentially Malignant Disorders. Globally, Squamous cell carcinoma of the head and neck region is the sixth most common cause of cancer-related deaths [3]. Squamous cell carcinoma of the head and neck region is the third most common cancer in India, which affects the oral cavity, oropharynx, hypopharynx, and larynx [4]. In India, head and neck cancers (HNCA) account for 30-40% cancers at all sites with about 700,000-900,000 new cases diagnosed every year [5].

In Madhya Pradesh alone, the prevalence of cancer cases was 15% in just 9.8% of the total population. [6] It has been found that Indian patients with oral submucous fibrosis have a higher risk in developing carcinomas than those without this disease. Clinically visible lesions are non- cancerous to begin and with a varying length of time may precede oral cancer [6]. The risk of an individual with pre-cancer for developing oral cancer is 69 times higher compared to those who do not have precancer [7].

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“Potentially Malignant Disorders”/“Epithelial Precursor Lesions” are described as lesions which may have an increased potential for malignant transformations. *Oral Submucous Fibrosis* is a potentially malignant disorder and a debilitating condition of the oral mucosa [8]. There is therefore, a mounting concern to recognize this dreadful disease in its early stages. Early detection is the key for effectual management of oral submucous fibrosis, better prognosis and helps to reduce mortality and morbidity of such cases.

Tumor markers are diagnostic aids that play a very important role in early diagnosis and interception of cancer. An increasing number of systemic diseases and conditions, amongst them oral cancer and precancer, have been shown to be reflected diagnostically in saliva which, unlike blood, is a non-invasive, highly sensitive and easily available media for investigation [9].

Lactate Dehydrogenase is an important salivary biomarker which could play a significant role in the diagnosis of pathologic process [10]. It could further aid as a disincentive in continuing the habit. There is however a paucity of available literature in the proposed fields of investigation concerning potentially malignant disorder (PMD) i.e. Oral Submucous Fibrosis and its relation to and lactate dehydrogenase enzyme as crucial biomarker in saliva. Also such studies have not been done in Bhopal.

Dentist, in particular, the Oral Physician, is most often the first clinician consulted for general oral complaints and his consult is in an ideal situation to regularly screen patient’s oral mucosa for early signs of oral cancer or precancer. He or She has the unique opportunity in the routine mouth examination to detect malignant neoplasm while they are still asymptomatic, innocuous and unsuspected and therefore has the critical responsibility of differentiating benign from precancerous and malignant conditions.

Materials and Methods

Bhopal, Madhya Pradesh, the geographic centre of India, was selected as the area of investigation. All the study subjects were selected from the patient pool of oral health centres of Bhopal. The study got approval from the university’s institutional review board and the participants signed a free and informed consent form authorizing their voluntary involvement in the research. The survey period extended over a period of two years and six months, i.e. from June 2013 to December 2015. A total sample size of 160 patients was categorized equally into two groups i.e. Group I: 80 patients with clinically diagnosed oral submucous fibrosis and Group II: 80 apparently healthy patients who volunteered for the study. The cases and controls were selected from 4 oral health centers where 40 individuals (20 case and 20 controls) were taken from each center (Figure 1).

Both Group I and Group II patients were in age group of 15 to 65 years. Group I/Study Group had a habit history of areca nut chewing and clinically confirmed oral submucous fibrosis. They were clinically categorized into three stages based on the classification given by Khanna JN and Andrade NN, Bailoor D, and Nagesh KS and More CB, Gupta S, Joshi J and Varma SN [11-13]. Patients with any other oral mucosal change other than those related with oral submucous fibrosis. Subjects suffering from systemic conditions (like myocardial infarction, liver disease, renal disease and muscle dystrophy diabetes, hypertension,

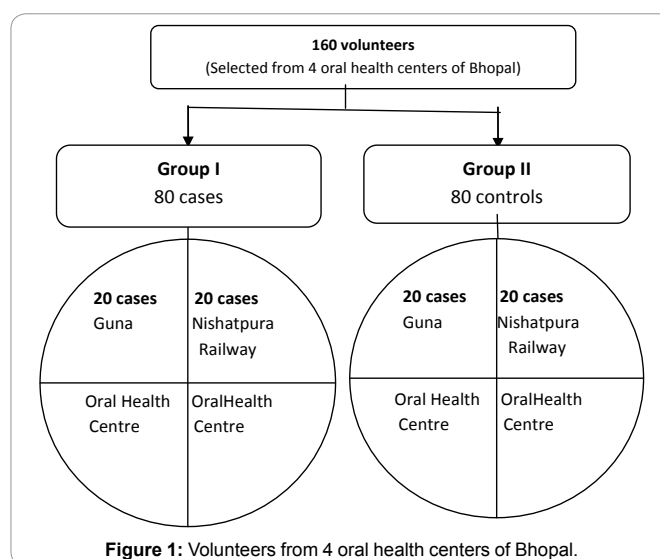


Figure 1: Volunteers from 4 oral health centers of Bhopal.

anemia) that alter the lactate dehydrogenase levels, subjects with a history of malignancy were excluded. Group I was further divided into 4 groups based on the duration of habit: < 6 months, 6-12 months, 12-24 months and >24 months.

Unstimulated whole saliva samples were collected between 9am to 12 pm from each of the patients by the spit method in calibrated sterile test tubes [14]. The participant was first asked to wash his mouth, allow saliva to accumulate in the mouth for 2 minutes and then to expectorate through a funnel into a sterile vial usually once every 60 seconds over a period of 10 minutes (Figure 2). The samples that contained visible blood were discarded. This was done to standardize the method of saliva collection and to minimize the changes in composition due to contamination and diurnal variation. These were stored in a polystyrene box containing dry ice and sent to the laboratory for analysis. Here they were centrifuged at 3,000rpm at 4°C for 30 minutes, diluted in 1:1 ratio with saline and assayed for lactate dehydrogenase enzyme. The LDH (P-L) kit, by modified IFCC method and a photometer analyzer at 340 nm, was used. The results obtained were multiplied by factor 2. It was based on the principle of reduction of pyruvate to lactate in the presence of NADH by the action of LDH. The pyruvate that remains unchanged reacts with

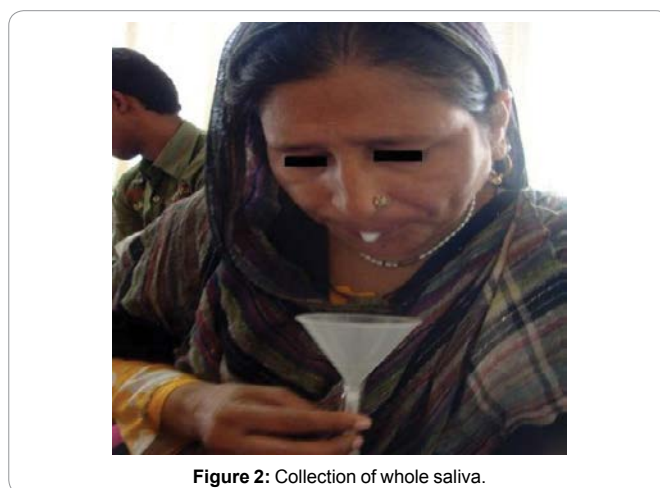


Figure 2: Collection of whole saliva.



Figure 3: Determination of Salivary LDH enzyme

2, 4-dinitro phenyl hydrazine to give the corresponding phenyl hydrazone, which is determined colorimetrically in an alkaline medium (Figure 3).

Results

The study design was a Prospective Case-Control study. Stratified Random Allocation Method was the sampling method used and the sample size was calculated using the following formula:

$$n = \frac{t^2 \times p(1-p)}{m^2}$$

where n = required sample size.

m^2 t = confidence level at 95% (standard value of 1.96)

p = estimated prevalence of depressive illness in the project area

m = margin of error at 5% (standard value of 0.05)

The data was analysed by using SPSS statistical software version 20.0. One-way ANOVA and Unpaired T-test was used to compare the data between the 2 groups and the difference was considered to be statistically significant if 'p' values were 0.05 or less. Tukey's Post HOC tests were used to compare intragroup variations.

Age and gender distribution

83.7% (67) participants in Group I were in the age range of 15-40 years, the youngest being 18 years and the oldest being 60 years (Table 1). 13.1% (21) Grade I OSMF and 20.6% (33) and 16.3% (26) had grade II and III OSMF respectively (Table 2). 63 (78.7%) participants of Group II were in the age group of 15-40 years (Table 3). The mean age of the participants was 30 years. 81.9% (131) of the total participants were males. Group I showed a male predominance with a male to female ratio of 17:3. 86.8% of males were in the age range of 15-40 years and maximum number of patients had grade II OSMF. In Group II, 63 out of 80 participants were males of which 55 (87.3%) were in the age range of 15-40 years (Table 2).

The mean salivary LDH level in males patients was 78.4644±7.76086, 511.3740±46.24769, 628.0897±99.18365 IU/L in Grades I, II and III OSMF respectively. The mean salivary

AGE (Yrs.)	MALE		FEMALE		TOTAL	
	No.	%	No.	%	No.	%
15-40	59	86.8%	8	66.7%	80	100
41-65	9	13.2%	4	33.3%		

Table 1: Gender distribution group I (study).

Group	Grade	Gender		Total	Chi Sq	P value
		Male	Female			
Group I OSMF	Grade I	19	2	21	4.744	0.191
		90.5%	9.5%	100.0%		
	Grade II	30	3	33		
		90.9%	9.1%	100.0%		
	Grade III	19	7	26		
		73.1%	26.9%	100.0%		
Group II Healthy Control	63	17	80			
	78.8%	21.2%	100.0%			
Total	131	29	160			
	81.9%	18.1%	100.0%			

Table 2: Distribution of gender according to groups.

AGE (Yrs.)	MALE		FEMALE		TOTAL	
	No.	%	No.	%	No.	%
15-40	55	87.3%	8	47.1%	80	100

Table 3: Gender distribution group II (control).

OSMF	Gender	N	Salivary LDH (IU/L)		T Value	P Value
			Mean	Std. Deviation		
Grade I	Male	19	78.4644	7.76086	1.125	0.275
	Female	2	72.0550	5.69221		
Grade II	Male	30	511.3740	46.24769	0.598	0.554
	Female	3	528.7667	68.92433		
Grade III	Male	19	628.0897	99.18365	0.223	0.825
	Female	7	618.3857	95.66914		

Table 4: Distribution of gender with salivary lactate dehydrogenase (IU/L) in different grades of OSMF.

LDH levels of female subjects in the corresponding groups were 72.0550±5.69221, 528.7667±68.92433 and 618.3857±95.66914 IU/L respectively (Table 4). The mean enzyme levels were statistically higher in females when compared to the male participants in Grade II OSMF. Though a positive correlation with age was observed in Grades II and III (r-value= 0.285 and 0.075 respectively), the Pearson's correlation coefficient was statistically not significant (P=0.643, 0.108 and 0.714) in all the 3 grades of OSMF (Table 5). Student T values of males when compared to females in all three grades of OSMF were 1.125, 0.598 and 0.223 respectively. The values were not statistically significant in the 3 grades of OSMF (P=0.275, 0.554 and 0.825). Thus age and gender were found to have no relation with enzyme lactate dehydrogenase in the saliva (Table 4).

Lactate dehydrogenase levels according to groups

The mean unstimulated salivary lactate dehydrogenase levels in Grade I OSMF was 77.85 +/- 7.717 IU/L, in Grade II OSMF was 512.955 +/- 47.550 IU/L and in Grade III OSMF was 625.477 +/- 96.430 IU/L. The mean salivary LDH level in Group II was 8.973 ±

Group I	Age		Ldh U/L		r value	p value
	Mean	Std. Deviation	Mean	Std. Deviation		
Grade I	29.0476	9.50514	77.8540	7.71653	-0.107	0.643
Grade II	29.6364	10.17294	512.9552	47.55022	0.285	0.108
Grade III	31.9615	12.68221	625.4771	96.43036	0.075	0.714

Table 5: Correlation of age with S Ldh U/L in group I.

0.83572 IU/L. Salivary lactate dehydrogenase enzyme showed a positive relation with severity of the disease. ANOVA test results between Group I and Group II participants was 1884.665, which was significant statistically ($p < 0.0001$) (Table 6).

Pair wise comparison of salivary lactate dehydrogenase levels between Healthy control and the 3 Grades of OSMF

The pair wise comparison of the mean lactate dehydrogenase level between healthy controls and OSMF (grade I, II and III) was 68.88121 ± 10.86059 IU/L, 503.98236 ± 9.16399 and 616.50433 ± 9.99928 IU/L respectively. Post Hoc tests comparison between the 2 groups were highly statistically significant ($p < 0.0001$). Intra group variations between all the 3 grades of OSMF were also highly significant ($p < 0.0001$) (Table 7).

Lactate dehydrogenase levels in OSMF with duration of areca nut habit

Salivary lactate dehydrogenase level in those with a habit history of less than 6 months was 77.46 ± 11.6 IU/L in Grade I

Group	N	Salivary Ldh (IU/L)		F Value	P Value
		Mean	Std. Deviation		
Healthy Control	80	8.9728	0.83572	1884.665	<0.0001*
OSMF Grade I	21	77.8540	7.71653		
OSMF Grade II	33	512.9552	47.55022		
OSMF Grade III	26	625.4771	96.43036		
Total	160	222.1418	267.75450		

Table 6: Distribution of salivary lactate dehydrogenase levels (U/L) according to groups.

(I) Group	(J) Group OSMF patients	Mean Difference (I-J)	Std. Error	Sig.
Group II	Grade I	68.88121*	10.86059	<0.0001*
	Grade II	503.98236*	9.16399	<0.0001*
Healthy Control	Grade III	616.50433*	9.99928	<0.0001*
	Grade II	435.10115*	12.36453	<0.0001*
OSMF Grade I	Grade III	547.62312*	12.99571	<0.0001*
	Grade III	112.52196*	11.61529	<0.0001*

Table 7: Pair wise comparison of salivary lactate dehydrogenase levels between the groups post hoc tests.

Duration (Months)	Grade I	Grade II	Grade III	T value	P value
<6	77.46 ± 11.65	525.54 ± 79.30	No case recorded	14.0441	<0.0001*
6-12	78.68 ± 5.51	533.52 ± 46.48	No case recorded	32.0244	<0.0001*
12-24	No case recorded	547.74 ± 40.22	630.47 ± 82.05	4.4770	0.0003*
>24	No case recorded	559.23 ± 34.65	658.92 ± 105.91	4.6983	0.0016*

Table 8: Salivary lactate dehydrogenase in relation to duration of OSMF.

and 525.54 ± 79.30 IU/L in Grade II OSMF. In those with habit duration of 6-12 months, it was 78.68 ± 5.51 IU/L in grade I and 513.52 ± 46.48 IU/L in grade II OSMF. Those with duration of 12-24 months, lactate dehydrogenase levels were increased to 507.74 ± 40.22 IU/L in grade II and 630.47 ± 82.05 IU/L in grade III OSMF and in those with a habit duration of 24 months or more it was 519.23 ± 34.65 IU/L and 618.92 ± 105.91 IU/L in grade II and grade III OSMF respectively. There were no cases of grade III OSMF recorded in patients with a habit history of less than a year. It was observed that there was a statistically significant increase in lactate dehydrogenase with increase in duration of the disease (Table 8).

Discussion

Oral submucous fibrosis is a chronic disease of oral cavity which is characterized by subepithelial inflammatory reaction followed by fibroelastic changes in the submucosa [15]. There has been an insidious yet steady rise in oral submucous fibrosis both in India and across the world; the reason being attributed to the consumption of areca nut or gutkha [16,17]. Numerous attempts are being made to develop non-invasive and reliable biochemical tests for the early detection and diagnosis. Of late, the role of lactate dehydrogenase enzyme, a specific salivary biomarker, has been studied extensively.

Lactate dehydrogenase enzyme is an oxidoreductase that catalyzes the interconversion of pyruvate to lactate. In recent years, total LDH and LDH isoenzyme activity has been used for screening oral potentially malignant disorders like OSMF [18], leukoplakia [16,19], lichen planus [20,21] and oral cancers [22-24]. The LDH enzyme activity in OSMF can be related to the following factors: hypoxia, alterations in glycolysis [19] and fibrosis [25]. Shpitzer T, et al. (2009) stated that lactate dehydrogenase enzyme is a potential salivary biomarker for cancer detection. Of the 8 salivary tumour markers analysed, they found that statistical significant increase in salivary LDH values (86%) and its sensitivity and specificity was 79% and 42% respectively. However he also commented that the existing literature about the levels of activity of LDH in saliva of the OSMF cases was very scarce [26]. Most patients in this study were in the second and third decade of life. 67 (83.7%) in Group I and 63 (78.7%) in Group II were in the age group 15-40 years. Akshata RO, Kaveri SH and Dhiraj JT (2015) in their study also selected an age range of 16-60 yrs for control and OSMF patients with a mean age of 24.3 and 29.2 respectively [27]. The affected age group of OSMF patients were akin to studies done by Sinor PN, et al. (1990) [17,20,28-30]. The mean age in the present study was 30 years. The results showed that most of the patients were in the third decade of life, which was analogous to studies by Maher R, et al. (1997) [31,32] that was 30, 30.6 and 30.7 years respectively. The predominance of this disease and the deleterious habit in the second and third decade maybe attributed to the changing lifestyles of the youngsters of our society.

Out of 80 OSMF patients 68 (85%) were males and 12 (15%) were females with male to female ratio of 17:3. A male predominance had also been reported by Akshata RO, Kaveri SH and Dhiraj JT (2015) [11,27,29,33], Shah N, et al. (1994), Khanna JN and Andrade NN (1995) and Lai DR, et al. (1995) However Pindborg JJ, et al. (1984) [34], Seedat, H. A. & van Wyk C. W. (1988) [35], Canniff JP, et al. (1986) Rajendran R, et al. (1986) [36] and Chaturvedi VN, et al. (1991) [37] had reported a female predominance. Male predominance in our study could have been due to easy accessibility by males acquire areca nut and its products as compared to females in our society. This gender variance could be due to the fact that the present study was conducted in a dental setting where the sample is constrained to a selective referral center only. Also the female predominance in some of the studies can be attributed to prevalence of various habits in different locations.

In a study done by M. Sivaramakrishnan, B. Sivapathasundharam, M. Jananni (2014) where the levels of lactate dehydrogenase were evaluated only in males, the age group was 18-60 years and the mean age of the study group was 31.27. [38] Shetty, et al. (2012) reported that the mean salivary LDH levels significantly higher in males (90.67 ± 4.67 IU/L) as compared to female (68.34 ± 3.90 IU/L) healthy controls [10]. Ranganathan, et al. (2004) in a study done in Chennai reported the prevalence of OSMF was greater in the third decade of life. The study also showed a male predominance [39]. Langvad E, Zachariah J and Pindborg JJ (1970) carried out a study on the LDH enzyme patterns in south Indians suffering from submucous fibrosis. They reported an increase in the LDH enzyme activity [40].

In the present study an increase in the lactate dehydrogenase level was observed in 21 (26.25%) cases of grade I, 33 (41.25%) cases of grade II and 26 (32.5%) cases of grade III severity of OSMF. There was progressive increase in the enzymes levels with increase in the severity of disease in saliva, which was found to be statistically significant ($P < 0.0001$). ANOVA test results between Group I and Group II participants were significant statistically ($p < 0.0001$). Audrey M. D'Cruz and Varsha Pathiyil (2015) stated that the mean salivary LDH levels in a control group of 30 participants was 117.33 ± 19.37 IU/L which was much lower when compared to those suffering from oral squamous cell carcinoma [41]. M. Sivaramakrishnan, B. Sivapathasundharam, M. Jananni (2014) the average salivary LDH value OSMF patient was 606.83 ± 60.09 U/l and for healthy subjects was 80.73 ± 20.06 U/l. They also stated that theirs was the first study in which the total LDH levels in serum and saliva of the OSMF cases were estimated [38].

SR Shetty, et al. (2012) in their study stated that among seventy-five patients there was very high significant difference between the mean salivary LDH levels in healthy controls (90.67 ± 4.06 IU/L) and in patients with OPMDs (145.69 ± 3.72 IU/L). They concluded that salivary LDH could be a reliable marker for oral cancer and thus alteration in salivary LDH levels could be a crucial factor in pathogenesis of oral cancer [10]. Sonika Achalli, et al. (2012) reported a definite significant increase in the LDH levels in malignant and potentially malignant disorders when compared to healthy controls (i.e 606.83 ± 60.09 U/l and for Group B was 80.73 ± 20.06 U/l respectively). They also stated that theirs was the first study that was done to estimate the total LDH levels in serum and saliva of the OSMF cases [41].

The elevated salivary lactate dehydrogenase levels maybe also related to the hypoxic state seen in OSMF. Hypoxia triggers glycolytic pathways where the end product is lactate. This reaction is mediated by lactate dehydrogenase enzyme. Thus in these conditions by reflex LDH levels are increased [42]. Tilakarathne, et al. (2008) stated that increased hypoxia plays a role in malignant transformation and progression of OSMF. Although 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 [43].

Oral submucous fibrosis and its potential malignant transformation can be mainly attributed to fibrosis, hypoxia, muscle fatigue and a shift to anaerobic glycolysis (i.e. the conversion of pyruvate to lactate). These conditions are usually associated with increase in lactate dehydrogenase enzyme [44]. Pair wise comparison of salivary lactate dehydrogenase levels between Healthy control and the 3 Grades of OSMF was highly significant statistically. Audrey M. D'Cruz and Varsha Pathiyil (2015) in a study on 60 volunteers reported a statistically significant correlation among various groups. Post hoc test using Mann-Whitney U-test for pair-wise comparison of groups also showed statistical significance.

M. Sivaramakrishnan, B. Sivapathasundharam, M. Jananni (2014) conducted as study at a dental college in Chennai and found that the salivary LDH levels in OSMF cases were significantly higher than healthy subjects. The comparison of salivary LDH levels between OSMF cases and healthy subjects was statistically significant ($P < 0.001$). On comparing the salivary LDH in OSMF patients with the clinical staging of OSMF, the results were not statistically significant ($P = 0.999$). Although salivary LDH level was slightly increased in grade III as compared to grade II OSMF, the results were not statistically significant. They suggested that further larger trials were needed to establish a definitive relationship between the staging of OSMF and the LDH levels [38]. Nagler, et al. (2001) in their study stated that salivary LDH level showed a slight increase in Stage III than Stage II. However, the findings were not statistically significant. (90.1 ± 28.8 U/l) [46] Unfortunately so far no sound literature is available for comparison of the staging of OSMF and the LDH levels. Kiran K, et al (2007) stated that the severity of the clinical staging may not have direct correlation with the severity of the disease [8]. Based on the relation between salivary lactate dehydrogenase enzyme levels in OSMF patients and the duration of areca nut habit, it was observed that, in the present study grade I and grade II OSMF cases with a habit duration of less than 6 months showed an increase in salivary lactate dehydrogenase (i.e. 77.46 ± 11.6 IU/L and 525.54 ± 79.30 IU/L respectively). In those with habit duration of 6-12 months, salivary lactate dehydrogenase levels were 78.68 ± 5.51 IU/L in grade I and 513.52 ± 46.48 IU/L in grade II OSMF. While in those with duration of 12-24 months, lactate dehydrogenase levels were increased to 507.74 ± 40.22 IU/L in grade II and 630.47 ± 82.05 IU/L in grade III OSMF and in those with a habit duration of 24 months or more it was 519.23 ± 34.65 IU/L and 618.92 ± 105.91 IU/L in grade II and grade III OSMF respectively. It was observed that no cases of grade III OSMF were reported with a habit history less than a

year. Similarly, no cases with grade I OSMF was found in areca nut chewers with a history of more than 1 year. The present study showed a significant increase in lactate dehydrogenase levels that was proportionate to the increase in duration areca nut chewing habit ($p < 0.05$). On comparing the salivary LDH in OSMF subjects with duration of habit, M. Sivaramkrishnan, B. Sivapathasundharam and M. Jananni (2014) found that the mean LDH in saliva of cases with habit ≤ 5 years was higher than those with habit > 5 years although the relationship was not statistically significant ($P = 0.539$). They suggested that further larger trials were needed to establish a definitive relationship [39]. However Sonika Achalli, et al. (2012) found no statistically significant relationship on comparing the salivary LDH in Group A (OSMF subjects) with duration of habit. ($P = 0.539$) [41]. Lactate dehydrogenase (LDH) isoenzymes are of considerable interest to the biochemical oncologists. N. Subramanian, et al. (2009) had stated that Lactate dehydrogenase (LDH) isoenzyme is one of the important biochemical tumor markers in cervical carcinoma patients for diagnosis and to assess the grade of malignancy [45].

Conclusion

Salivary harvesting is a noninvasive, patient friendly, effective tool, which can be used as an alternative to serum testing. Salivary enzyme analysis can be used as an early diagnostic aid for PMDs and for planning comprehensive treatment protocol. Oral submucous fibrosis has always been a challenging disease with high prevalence in India. Lactate dehydrogenase is an enzyme, which helps in the conversion of pyruvate to lactate in anaerobic glycolytic pathway. An increase in the level of salivary lactate dehydrogenase may increase the risk of malignant transformation and thus causing direct damage to the tissue. This study shows significant difference in salivary LDH level between healthy controls and OSMF subjects and also with the duration of habit in OSMF subjects. So estimation of salivary LDH level could be a reliable marker to diagnose OSMF, potentially malignant disorder. It is an efficient, non-invasive and cost effective investigation which will be helpful to diagnose the disease in early stages itself. Clinical diagnosis of oral submucous fibrosis supplemented with LDH levels can gain diagnostic importance in future. It can be concluded that the potential benefits of salivary lactate dehydrogenase enzyme as a diagnostic marker in OSMF patients is colossal and can also be used as a valuable aid in monitoring treatment outcomes in the OSMF patients. Also it would be highly desirable and beneficial if salivary tumor marker analysis could be performed on a routine basis. This is especially important for people who live far from treatment centers and for those at high risk for developing oral cancer.

Conflict of interest

This was an unsponsored and self financed study. I declare that there exists no financial interest or any conflict of interest.

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