RESEARCH ARTICLE

Lactate Dehydrogenase as a Tumor Marker in Oral Cancer and Oral Potentially Malignant Disorders: A Biochemical Study

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ABSTRACT

Introduction: Oral cancer is an alarming global concern accounting for an estimated 275,000 cases and 128,000 deaths annually. Oral cancer is often preceded by potentially malignant disorders with more emphasis being placed on early detection, since diagnosis at an early stage is comparatively easier and is the key to reduce mortality and morbidity. Tumor markers are biochemical substances elaborated by tumor cells due to either the cause or effect of malignant process. Several tumor markers in both serum and saliva have been identified. Lactate dehydrogenase (LDH) is one among them, which is a ubiquitous enzyme that plays a significant role in the diagnosis of pathologic processes. Lactate dehydrogenase activity in serum increases as a marker of cellular necrosis. The aim of the study is to estimate and compare salivary and serum LDH in normal healthy individuals, oral cancer, oral submucous fibrosis (OSMF), and oral leukoplakia.

Materials and methods: This study was conducted at the Department of Oral Medicine and Radiology, RajaRajeswari Dental College & Hospital, Bengaluru, India. The study comprised four groups as follow: Group I (OSMF), group II (oral leukoplakia), group III (oral cancer), and group IV (control group). Unstimulated whole saliva and 2 mL of blood were collected aseptically and were processed for LDH measurement using Agappe Diagnostic kit.

Results: Salivary and serum LDH levels were consistently higher in oral cancer followed by OSMF and oral leukoplakia. There was significant increase in salivary and serum LDH among study groups when compared with control group (p < 0.001, both serum and saliva).

Conclusion: Salivary diagnostics is a noninvasive, patient-friendly, effective tool which can substitute to serum LDH. It also serves as a valuable aid in early diagnosis, monitoring, treatment outcome, and prognosis.

Keywords: Lactate dehydrogenase, Oral cancer, Oral leukoplakia, Oral submucous fibrosis, Potentially malignant disorders, Tumor marker.

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INTRODUCTION

Oral cancer is an alarming global concern accounting for an estimated 275,000 cases and 128,000 deaths annually with an incidence rate of 300,000 new cases per year, accounting for 2 to 4% of all new cancers.[1] Oral cancer is often preceded by potentially malignant disorders (PMDs), with more emphasis being placed on early detection, since diagnosis at an early stage is comparatively easier and is the key to reduce mortality and morbidity.[2,3] Recently, the role of tumor markers in the management of head and neck cancer has increased for attention.[4] Tumor markers are biologically elaborated by tumor cells due to either the cause or effect of malignant process substances.[5] Several tumor markers in both serum and saliva have been identified. Lactate dehydrogenase (LDH) is one among them, which is a ubiquitous enzyme that plays a significant role in the diagnosis of pathologic processes.[1] This enzyme catalyzes the reaction of lactate production via pyruvate reduction during anaerobic glycolysis.[6] Lactate dehydrogenase is believed to vary according to the metabolic requirement of each tissue, and alteration in LDH levels has been observed during development, under changing biological conditions, and in response to pathological processes.[7] Lactate dehydrogenase activity in serum increases as a marker of cellular necrosis.[8] Increased LDH levels are due to increased mitotic index and more lactic acid production by tumor cells due to breakdown of glycoprotein.[9] It has been found that the serum LDH levels are increased in PMDs and malignancy.[6]

With the above background, the aim of the study is to estimate and compare serum and salivary LDH in oral cancer and oral PMDs.

MATERIALS AND METHODS

The study comprised 40 patients from the outpatient department of Oral Medicine and Radiology, RajaRajeswari

Dental College & Hospital, Bengaluru, India. The study was approved by the ethical committee of our institute. The study subjects included clinically diagnosed cases of oral cancer, oral submucous fibrosis (OSMF), and oral leukoplakia. Study subjects were divided into four groups as follow: Group I OSMF, group II (oral leukoplakia), group III (oral cancer), and group IV (control group). Informed consent was taken from the patients selected for the study. Duly signed informed consent was obtained from every individual participating in the study.

Inclusion Criteria

- Patients willing to participate
- Subjects in the age group of 20 to 60 years irrespective of sex
- Clinically diagnosed cases of oral cancer, oral leukoplakia, and OSMF

Exclusion Criteria

- Patients not willing for participation in the study
- Patients undergoing chemotherapy, radiotherapy, or any surgical procedure for oral cancer
- Patients with a history of heart failure (myocardial infarction) within past 2 weeks
- Patients taking procainamides and other drugs used to treat arrhythmia, pulmonary infarction, and stroke.
- Patients suffering from hepatitis, hypothyroidism, anemia (hemolytic or pernicious anemia), lung disease, liver disease, kidney disease, pancreatitis, muscle trauma, and muscular dystrophy.
- Patients with history of consumption of aspirin, narcotics or alcohol, and recent anesthesia.

Collection of Sample

Collection of Saliva

Patients were asked to sit comfortably with head in upright position; they were asked to rinse the oral cavity using 30 mL of normal water and then were asked to accumulate the saliva in their oral cavities (unstimulated whole saliva) for 5 minutes. The patients were asked to spit the accumulated saliva in sterile, disposable container, until a minimum desired quantity of 2 mL was obtained.

Collection of Serum

Collection of serum sample was done by obtaining 2 mL of blood from median cubital vein under precautions by vein puncture and then transferred to a sterile test tube.

Both the samples were subjected to biochemical analysis. Estimation of LDH was with the help of Toshiba Semiautomatic Analyzer by using commercially available LDH assessment kit (Agappe kit; Agappe Pvt. Ltd, Kerala, India). It works on the principle that LDH catalyzes the reduction and conversion of the substrate pyruvate to lactate in the presence of NADH.

$$\text{Pyruvate} + \text{NADH} + \text{H}^+ \rightarrow \text{L-Lactate} + \text{NAD}$$
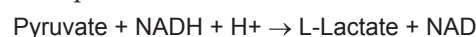
Statistical Analysis

The data were analyzed using Statistical Package for the Social Sciences statistical software version. Analysis of variance (ANOVA) followed by Tukey's *post hoc* analysis was used to compare among the groups and Pearson's correlation test was used to correlate salivary and serum LDH. The difference was considered to be statistically significant if p-values were 0.05 or less.

RESULTS

Salivary and serum LDH levels were consistently higher in oral cancer followed by OSMF and oral leukoplakia and control group.

Mean salivary LDH levels in OSMF, oral leukoplakia, oral cancer, and control group were 668.0, 563.6, 1126.0, and 376.1 respectively (Table 1 and Graph 1). We found statistically significant results while comparing among the study groups, with p-value <0.001. *Post hoc* test comparison between the intergroup was highly significant: Groups I to II (p = 0.22), groups I to III (p = 0.001), groups I to IV (p = 0.001), groups II to III (p = 0.006), groups II to IV (p = 0.001), and groups III to IV (p = 0.001) (Table 2).

Mean serum LDH levels in OSMF, oral leukoplakia, oral cancer, and control group were 512.7, 471.6, 883.3, and 251.5 respectively. There was statistically significant value while comparing among the study groups, with p-value <0.001 (Table 3 and Graph 2). *Post hoc* test comparison between intergroups was highly significant: Groups I to II (p = 0.7), groups I to III (p = 0.001), groups I to IV (p = 0.001), groups II to III (p = 0.001), groups II to IV (p = 0.001), and groups III to IV (p = 0.001) given in Table 4.
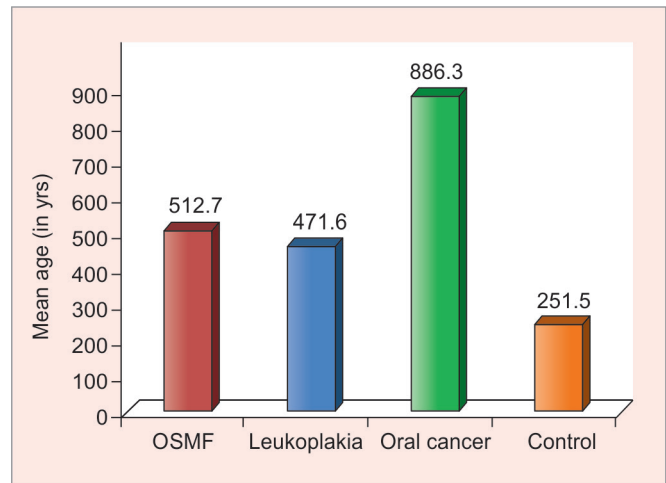
Table 1: Comparison of mean salivary LDH levels between study groups using one-way ANOVA test followed by Tukey's *post hoc* analysis

Groups	n	Mean	SD	Standard error	Minimum	Maximum	f-value	p-value
OSMF	10	668.0	75.1	23.7	580	785	72.863	<0.001*
Leukoplakia	10	563.6	80.6	25.5	425	652		
Oral cancer	10	1126.0	194.5	61.5	824	1456		
Control	10	376.1	76.5	24.2	221	500		

SD: Standard deviation
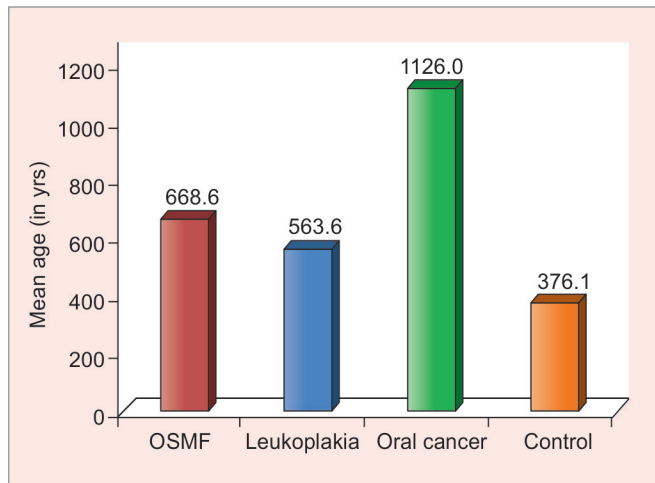
Graph 1: Mean salivary LDH

Graph 2: Mean serum LDH

Table 2: Multiple comparison using Tukey's honest significant difference *post hoc* analysis for salivary LDH

Groups	OS vs LP	OS vs OC	OS vs CT	LP vs OC	LP vs CT	OC vs CT
p-value	0.22	<0.001*	<0.001*	0.006*	<0.001*	<0.001*

OS = OSFM; LP = Lichen planus; OC = Oral cancer; CT = Control group

Table 3: Comparison of mean serum LDH levels between study groups using one-way ANOVA test followed by Tukey's *post hoc* analysis

Groups	n	Mean	SD	Standard error	Minimum	Maximum	f-value	p-value
OSMF	10	512.7	46.7	14.8	420	582	95.673	<0.001*
Leukoplakia	10	471.6	72.3	22.9	365	580		
Oral cancer	10	886.3	138.9	43.9	650	1120		
Control	10	251.5	48.3	15.3	175	326		

SD: Standard deviation

Table 4: Multiple comparison using Tukey's honest significant difference *post hoc* analysis for serum LDH

Groups	OS vs LP	OS vs OC	OS vs CT	LP vs OC	LP vs CT	OC vs CT
p-value	0.7	<0.001*	<0.001*	<0.001*	<0.001*	<0.001*

OS = OSFM; LP = Lichen planus; OC = Oral cancer; CT = Control group

We found statistically significant value in OSMF group (p = 0.001), oral cancer group (p = 0.004) while correlating salivary and serum LDH but we did not find statistically significant value for oral leukoplakia and control group (Table 5).

DISCUSSION

Oral cancer is the sixth most common malignancy and major cause of cancer mortality worldwide.[4] In India, oral cancer is highly prevalent due to habit of tobacco chewing.[6] Classic features may include a white lesion, red lesion, mixed red and white lesion, lump, and ulcer with exophytic raised margin.[9] The poor prognosis of oral cancer is owing to several factors including late diagnosis.[6] Oral cancer is often preceded by PMDs.[2] The World Health Organization (WHO) defined PMD as "the risk of malignancy being present in a lesion or condition either during the time of initial diagnosis or at future date."[1] Most common PMDs are leukoplakia, OSMF, and lichen planus.[9] Diagnosis at early stage is the key to reduce mortality, morbidity, prognosis, and response to therapy. Early detection followed by appropriate treatment can increase cure rate to about 80% and can greatly improve the quality of life by minimizing extensive and debilitating treatments.[9,10]

Therefore, there is essential need for developing new diagnostic aids that would improve early detection.[10] The identification of molecular markers in the body fluids that would predict the development of cancer in

Table 5: Correlation between serum and salivary LDH levels in different study groups – Pearson correlation test

Group	Variable	Values	Salivary LDH
OSMF	Serum LDH	r	0.87
		p-value	0.001*
Leukoplakia	Serum LDH	r	0.53
		p-value	0.11
Oral cancer	Serum LDH	r	0.82
		p-value	0.004*
Control	Serum LDH	r	0.57
		p-value	0.09

its earlier stage or in precancerous stage would constitute such tool.[11] Tumor markers are biochemical substances elaborated by tumor cells due to either the cause or effect of malignant process; it can be obtained from several body fluids, such as serum and saliva. Lactate dehydrogenase is one of the tumor markers that can be obtained from both serum and saliva.[5] Lactate dehydrogenase is a cytoplasmatic enzyme present essentially in all major organ systems.[1] The extracellular appearance of LDH is used to detect cell damage or cell death. Due to its extraordinarily widespread distribution in the body, serum LDH is abnormal in a host of disorders. It is released into the peripheral blood after cell death caused by, e.g., ischemia, excess heat or cold, starvation, dehydration, injury, exposure to bacterial toxins, after ingestion of certain drugs, and from chemical poisoning.[12] The basic mechanism for the increase in LDH level in malignancy is mainly due to necrosis and cellular degradation, induction process initiated by tumor and involving normal tissue, and lastly muscle degeneration caused by protein deficit.[1] Various studies have shown that LDH is released during tissue injuries. Serum LDHs have been studied extensively in various cancer and increased levels have been observed.[4] The LDH in the whole saliva within the oral cavity may originate from various sources, since 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, cellular and other debris.[12] Therefore, salivary LDH may be evaluated for possible oral mucosal pathologies. With the above background, our study was performed to estimate and to compare salivary as well as serum LDH among oral malignant disorders and PMDs.

Results of our study suggest that serum and salivary LDH levels are significantly higher in oral cancer groups. When serum LHD values were compared among oral cancer and control subjects, we found the LDH values were significantly higher in oral cancer group than healthy controls (p = 0.001). Our findings were in accordance with those of Rathore et al,[9] Joshi et al,[11] Pereira et al,[6] Hariharan et al,[13] Muralidhar et al,[14] and Görögh et al.[15]

We also found that salivary LDH levels in oral cancer cases were significantly higher than in control group. The comparison of salivary LDH levels between oral cancer and healthy subjects was statistically significant (p < 0.001). Our study finding was in agreement with the study done by Kallalli et al,[1] Lokesh et al,[8] Shetty et al,[16] Patel and Metgud,[4] and Joshi et al.[11]

Oral submucous fibrosis is a chronic disease of oral cavity which is characterized by subepithelial inflammatory reaction followed by fibroelastic changes in submucosa. The LDH enzyme activity in OSMF is mainly related to following factors: Hypoxia, alteration in glycolysis, and fibrosis.[3]

On comparing the serum LDH levels in OSMF groups and healthy controls in our study, we found that serum LDH levels in OSMF cases were greater than in healthy controls. This comparison was statistically significant (p < 0.001). Similarly, we found that salivary LDH levels in OSMF cases were significantly higher than in control group. The comparison of salivary LDH levels between OSMF and healthy subjects was statistically significant (p < 0.001). Our study findings were consistent with the study conducted by Sivaramakrishnan et al,[3] Kallalli et al,[1] Bhambal et al,[2] Shetty et al,[16] Rathore et al,[9] Pereira et al,[6] and Muralidhar et al.[14]

According to WHO 2005, oral leukoplakia is defined as a predominantly white patch or plaque that cannot be characterized clinically or pathologically as any other disorder; oral leukoplakia carries an increased risk of cancer development either in or close to the area of leukoplakia or elsewhere in the oral cavity or the head and neck region.[17] Malignant transformation rate of oral leukoplakia varies from 0.13 to 34%. The development of malignant transformation is often associated with a high glycolytic activity with a shift from aerobic to anaerobic glycolysis,[18] with increase in glycolytic activity which in turn increases LDH enzyme activity.[18] In our study, there was a significant difference in the salivary and serum LDH levels between oral leukoplakia and healthy controls (p = 0.001). Our results were in accordance with the study conducted by Patel and Metgud,[4] Pereira et al,[6] Rathore et al,[9] Joshi et al,[11] Shetty et al,[16] and Achalli et al.[19]

In our present study, while comparing among all the study groups, we found that serum and salivary LDH levels were significantly higher in oral cancer followed by OSMF and oral leukoplakia. Intergroup comparison for mean value of salivary LDH levels was significantly higher in oral cancer group than control, oral leukoplakia, and OSMF groups (p < 0.0001). Similarly mean value of serum LDH levels showed significantly very high levels in oral cancer group as compared with OSMF and oral leukoplakia (p < 0.0001). On comparing serum and salivary LDHs, we found statistically significant value in OSMF (p = 0.001) and oral cancer (p = 0.004). However, we found no statistical significance while comparing serum and salivary LDH in oral leukoplakia (p = 0.11) and control group (p = 0.09).

To our knowledge, ours is the first study which estimates both salivary and serum LDH levels in oral cancer along with PMDs, such as OSMF and oral leukoplakia. Also, our study is the first preliminary study reporting that salivary LDH levels are comparative higher than serum LDH in all the study groups, which strongly suggests that salivary LDH can replace serum LDH.

CONCLUSION

Lactate dehydrogenase has extensive potential benefits as a screening aid. Clinical diagnosis accompanied by estimation of salivary and serum LDH can gain diagnostic importance in the future. Salivary LDH can prove to be a valuable substitute to serum LDH as a biomarker, since it is simple, has noninvasive procedure, and is easily accepted by patient.

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