Original Article

Assessment of lactate dehydrogenase enzyme levels in saliva and serum of oral submucous fibrosis and leukoplakia patients

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Abstract

Background: Pathology involving the oral epithelium may alter the level of salivary concentration of LDH. Thus its estimation can be used as a non invasive screening tool for the early detection of OPMDs and also to predict its malignant transformation especially in high risk population.

Aims and Objectives: To evaluate the salivary and serum levels of lactate dehydrogenase (LDH) in patients having of oral submucous fibrosis (OSMF) and leukoplakia and compare it with healthy individuals.

Materials and Methods: A total of 120 subjects were selected and divided into three groups comprising clinically diagnosed cases of OSMF and leukoplakia and healthy subjects as controls. Unstimulated whole saliva and blood samples were collected under aseptic conditions for biochemical estimation of LDH by Semiautomatic Analyzer using LDH kit utilizing enzymatic UV-Kinetic method. The values obtained were statistically analyzed using the SPSS software version 20.0. P-value < 0.05 was considered significant.

Results: The mean salivary LDH level in Group I (OSMF) was 631.67 + 7.67, Group II (Leukoplakia) was 492.28 + 16.17 and Group III (Healthy Control) was 140.62 + 8.87. There was a statistically significant difference between the Serum and salivary LDH levels among the various groups of study population. A positive correlation between salivary LDH and serum LDH level was seen and the regression equation for OSMF and leukoplakia was computed.

Conclusion: A significant difference was found between mean salivary LDH Levels and serum LDH levels in patients with leukoplakia, OSMF and health controls. A positive correlation was also established between salivary and serum LDH levels in patients with OSMF and leukoplakia patients making saliva a potent non invasive tool for early prediction and detection of PMOD and its malignant transformation.

Keywords: Leukoplakia, oral submucous fibrosis, salivary biomarker, salivary lactate dehydrogenase, serum lactate dehydrogenase

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INTRODUCTION

There is an evident rise in the cancer of the upper aerodigestive tract among the Indians. It has been observed that cancer of mouth and tongue overshadowed the lung cancer. One-third of the global burden of cancers of lip and oral cavity is shouldered by India.[1] Oral squamous cell carcinoma (OSCC) is the 6th most common cancer among all kind. Entities such as tobacco, infectious organisms, unhealthy diet, inherited genetic mutations, hormones and immune conditions are blamed to be the causative factors.[2] Development of OSCC is believed to be a multistep process, preceded mostly by oral potentially malignant disorders (OPMDs).[3] "Potentially malignant disorders" are lesions which may have an increased potential for malignant transformations. Oral submucous fibrosis (OSMF) and leukoplakia are well-identified OPMDs.[4] OSMF is indigenous to Indian subcontinent having a malignant potential ranging from 4.5% to 7.6%.[5] Leukoplakia is the most commonly encountered entity in clinical practice among OPMDs. Global prevalence of leukoplakia has been estimated at 2.60%. The estimated overall (mean) malignant transformation rate of leukoplakia was estimated to be 3.5% as per a recent review.[6]

Lactate dehydrogenase (LDH) is an abundantly found cytoplasmic enzyme across all kinds of body tissues. Lactate gets converted to pyruvate by an oxidative process catalyzed by LDH. LDH is an intracytoplasmic component which is released to exterior upon cell death. Hence, mere extracellular presence of LDH can always be related to cell necrosis and tissue breakdown.[7] Numerous pathological entities such as myocardial infarction, toxic hepatitis, megaloblastic anemias, pyelonephritis, Hodgkin's lymphoma, cancer of the abdomen and lung, teratoma, liver metastases, leukemia, progressive muscular dystrophy and pulmonary embolism reflect an increased serum LDH level.[8]

LDH is also identified in saliva. The source of LDH in saliva is attributed to oral epithelium rather than the various salivary glands, as the profile of LDH isolated from saliva matches with that of oral epithelium.[9] Hence, any pathology involving the oral epithelium may lead to change in the level of salivary concentration of LDH. It is now an established fact that serum LDH levels are elevated in oral cancer (OC) and other potentially malignant disorders.[4] Very few studies are present in the literature about the serum and salivary expression of LDH in various OPMDs. Here, we tried to evaluate the salivary and serum levels of LDH in patients having OSMF and leukoplakia and compare it with healthy individuals.

MATERIALS AND METHODS

Study group subjects were recruited from the patients attending the Outpatient Department of Kalinga Institute of Dental Sciences, Bhubaneswar. A total of 120 subjects with age range of 20–70 years were selected and divided into three groups: Group I comprised 40 cases clinically diagnosed as OSMF, Group II comprised 40 cases clinically diagnosed as leukoplakia and Group III consisted of 40 apparently healthy subjects as controls.

Patients with systemic diseases known to alter serum LDH levels such as myocardial infarction, liver diseases, pulmonary disorders, renal disease and muscle dystrophy; immunocompromised patients; patients under corticosteroid therapy and patients with other mucosal lesions other than OSMF and leukoplakia were excluded from the study. Ethical clearance was obtained from the Institutional Ethical Committee, KIIT Deemed to be University (Reference no: KIMS/KIIT/IEC/75/2015) before the study. Written informed consent was obtained from all participating subjects.

Two milliliters of unstimulated whole saliva was collected in a sterile and disposable container during 9 AM–11 AM by spitting method. Patients were asked not to eat, drink water and smoke at least 1 h before sample collection. Morning samples were preferred to avoid diurnal variations of salivary flow. The collected saliva was immediately centrifuged at 2500 rpm for 15 min. Five milliliters of blood samples was collected under aseptic conditions by venipuncture and allowed to clot for 30 min at room temperature. The samples were then centrifuged for 15 min to get a clear serum. Biochemical estimation of LDH in both the salivary and serum samples was done with the help of Semiautomatic Analyzer (Accurex-ACCULAB AT300D). The LDH kit that was used utilized enzymatic

Table 1: Distribution of age groups among the study population

Age groups (years)	OSMF	Leukoplakia	Healthy control	Total	*P* (Chi-square test)
20-29	9	3	4	16	0.164
30-39	11	8	15	34	
40-49	12	15	15	42	
50-59	6	13	5	24	
60-69	2	1	1	4	
Total	40	40	40	120	

OSMF: Oral submucous fibrosis

Table 2: Distribution of gender among the study population

Gender	OSMF	Leukoplakia	Healthy control	Total	*P* (Chi-square test)
Male	38	35	35	108	0.435
Female	2	5	5	12	
Total	40	40	40	120	

OSMF: Oral submucous fibrosis

UV-Kinetic method in which LDH catalyzes the reduction of pyruvate by NADH to form lactate and NAD^+. The enzymatic activity was observed at a wavelength of 340 nm.

The values obtained were statistically analyzed using the Statistical Package for the Social Sciences (SPSS) software for windows version 20.0 (IBM, SPSS Inc., IL, Chicago). $P < 0.05$ was taken as significant. Chi-square test was used to find out if there exists any bias among the study groups in terms of age and gender. ANOVA test was used for comparison of LDH levels between various groups. Pearson's correlation and *Post hoc* comparison was also applied for statistical analysis.

RESULTS

Age and gender distribution of the study population is outlined in Tables 1 and 2. Statistical evaluation revealed no bias among the study groups.

The mean serum LDH level in Group I (OSMF) was 534.58 ± 12.6, Group II (leukoplakia) was 288.71 ± 13.54 and Group III (healthy control [HC]) was 217.09 ± 38.07. The mean salivary LDH level in Group I (OSMF) was 631.67 ± 7.67, Group II (leukoplakia) was 492.28 ± 16.17 and Group III (HC) was 140.62 ± 8.87. There is a statistically significant difference between the serum LDH levels among the groups of study population. It is quite evident that serum LDH level in OSMF is significantly high in comparison to the other two groups. Moreover, serum level of LDH in leukoplakia was significantly higher than their healthy counterpart. A similar type of significant difference was also noticed between the salivary LDH levels among the study groups as evident in Table 3.

A *post hoc* comparison of LDH levels between each group revealed that there is a significant difference between OSMF and healthy, leukoplakia and healthy as well as OSMF and leukoplakia groups in terms of salivary and serum LDH expressions [Table 4].

Among both study and control groups, correlation was evaluated between salivary LDH Levels and serum LDH levels which revealed a highly significant $P = 0.00$, suggesting a positive correlation between salivary LDH and serum LDH level [Table 5].

Figures 1 and 2, respectively, showcase the regression equation for OSMF and leukoplakia. For OSMF patients and leukoplakia patients, serum LDH level can be predicted for a given value of salivary LDH using the regression equation as given below.

Table 3: Comparison of salivary and serum lactate dehydrogenase levels between the study groups and healthy controls

	Mean±SD	*F*	*P* (ANOVA)
Salivary LDH levels			
OSMF	631.67±7.67	19250.53	0.00*
Leukoplakia	492.28±16.17		
Healthy control	140.62±8.87		
Total	421.52±207.80		
Serum LHD levels			
OSMF	534.58±12.61	1857.140	0.00*
Leukoplakia	288.71±13.54		
Healthy control	217.09±38.07		
Total	346.79±138.67		

OSMF: Oral submucous fibrosis, LDH: Lactate dehydrogenase, SD: Standard deviation. *P value ≤ 0.00 is Highly Significant

Table 4: *Post hoc* comparison of salivary and serum lactate dehydrogenase levels among various groups

Post hoc test	Significance
Salivary LDH levels	
OSMF and Leukoplakia	0.00*
Healthy control and OSMF	0.00*
Healthy control and Leukoplakia	0.00*
Serum LHD levels	
OSMF and Leukoplakia	0.00*
Healthy control and OSMF	0.00*
Healthy control and Leukoplakia	0.00*

*P value ≤ 0.00 is Highly Significant. OSMF: Oral submucous fibrosis, LDH: Lactate dehydrogenase

Table 5: Correlation between salivary lactate dehydrogenase levels and serum lactate dehydrogenase levels among various groups

	Pearson correlation	*P*
All samples	0.834	0.00*
OSMF	0.58	0.00*
Leukoplakia	0.852	0.00*
Control	0.71	0.00*

OSMF: Oral submucous fibrosis. *P value ≤ 0.00 is Highly Significant

Serum LDH level in OSMF = 0.9615 × salivary LDH in OSMF = 72.777

Serum LDH level in leukoplakia = 0.7128 × salivary LDH in leukoplakia = 62.195

The coefficient of determination R^2 for OSMF and leukoplakia was 0.3426 and 0.7253, respectively.

DISCUSSION

The most common oral malignancy occurring worldwide today is OSCC. These carcinomas are usually diagnosed at a later stage causing difficulties in its treatment as well as its prognosis. However, most of them are preceded in the form of OPMDs, which aids the practitioners as well as physicians to diagnose and educate the patients at an early stage.[3]

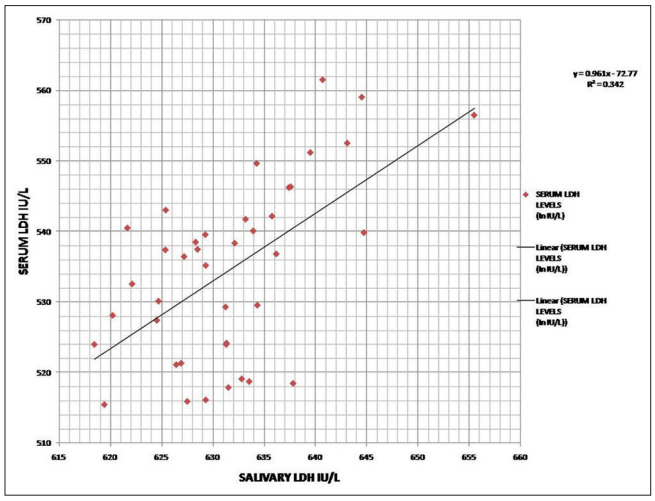

Figure 1: Correlation between salivary lactate dehydrogenase level and serum lactate dehydrogenase level in oral submucous fibrosis patients

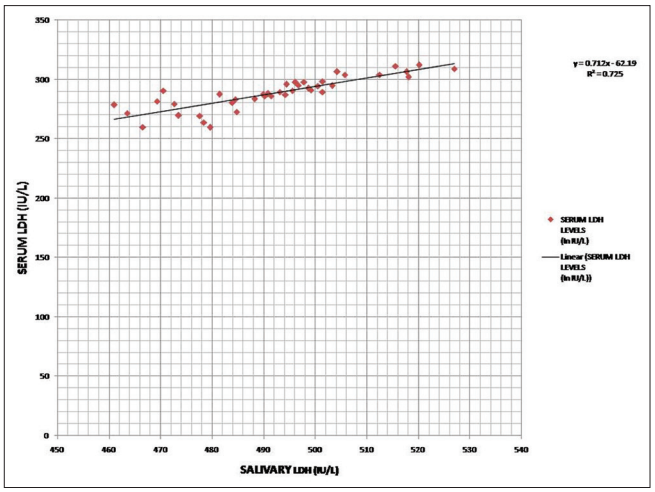

Figure 2: Correlation between salivary lactate dehydrogenase level and serum lactate dehydrogenase level in leukoplakia patients

Saliva has always been an effective and a noninvasive tool for investigations of various oral diseases. Salivary analyses have proven to be emerging trends in diagnosing several diseases including oral premalignant lesions and conditions.[10,11] OSMF and leukoplakia are the most common OPMDs and have high potential to convert into malignancy. LDH is a metabolic enzyme seen to be related to cell necrosis and tissue breakdown and this marker can be obtained from serum as well as saliva.[12-14]

In 2012, Shetty *et al.*[15] analyzed salivary LDH levels in patients with oral leukoplakia (OL) OC and HCs and found a significant difference between mean salivary LDH levels of OL and HC and thus concluded that LDH could be a future marker for malignant transformation. Similar findings were also seen in our study which showed a significant difference between mean salivary LDH levels in HCs (140.62 ± 8.87) and leukoplakia (492.28 ± 16.17) patients.

Joshi and Golgire[16] collected saliva from patients with OL and controls for the estimation of LDH and found that there was significantly higher LDH levels associated with OL as compared to the controls. Our results also showed similar findings in patients affected with OL and in HCs [Table 3].

Comparison of values for LDH levels in OL between our study (492.28 ± 16.17) and a study done by Patel and Metgud[17] (497.00 ± 100.404) showed similar results and the values in both the cases were significantly higher than the HCs.

A study by Sivaramakrishnan *et al.* in 2015[18] on evaluation of LDH enzyme activity in saliva and serum of OSMF patients showed that both salivary and serum LDH levels were greater in OSMF patients (salivary LDH = 606.83 ± 60.09 U/L; serum LDH = 521.0 ± 27.30 U/L) than HCs (salivary LDH = 80.73 ± 12.06 U/L; serum LDH = 289.43 ± 26.86 U/L), and this was statistically significant. The findings were in consonance with the results of our study.

Kallalli *et al.*[12] evaluated LDH as a biomarker in OC, OSMF and HCs and found the mean salivary LDH levels of 606.28 ± 30.22 and 182.21 ± 34.85 in OSMF patients and healthy controls, respectively. The LDH levels in controls were much less than compared to OSMF denoting higher rate of cell death. The result of our study also revealed similar findings in patients with OSMF (631.67 ± 7.67) and in controls (140.62 ± 8.87).

The hypoxic states in OSMF are seen to be related to elevated salivary LDH levels. This increased hypoxia also plays some role in the progression of OSMF toward its malignant transformation. The altered epithelial cells in OSMF might be a reason for the elevated salivary LDH levels as these oral epithelial cells are the direct source of LDH in saliva.[19] The other factors which could be responsible for the LDH activity in OSMF are alteration in glycolysis and fibrosis.[18,20]

Our study showed a good correlation between salivary and serum LDH levels in OSMF patients [Figure 1], whereas in patients with leukoplakia, a high positive correlation was observed [Figure 2]. Thus, estimation of salivary LDH level can be the best noninvasive alternative to serum LDH analysis. To our knowledge, our study is the first study to estimate, compare and correlate the serum and salivary LDH levels in OSMF and leukoplakia patients.

CONCLUSION

Today, the prevalence of OPMDs has increased worldwide and mostly seen in developing countries. They have a higher chance to transform into malignancy if not detected and treated at an early stage. The current study was framed to establish a noninvasive screening tool for the early detection of OPMDs and also to predict its malignant transformation, especially in high-risk population. We found a significant difference between mean salivary and serum LDH level in subjects with leukoplakia, OSMF and HCs. A positive correlation between mean salivary and serum LDH levels in both OSMF and leukoplakia made saliva a promising tool for early prediction and detection of OPMDs and its malignant transformation.

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Conflicts of interest
There are no conflicts of interest.

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