INTRODUCTION

Oral cancer is an important global concern accounting for an estimated 2,75,000 cases and 1,28,000 deaths annually.[1] Oral squamous cell carcinoma (OSCC) is the sixth most common human cancer that encompasses at least 90% of all oral malignancies and one of the major cause of morbidity and mortality.[2,3] The 5 year survival rate of OSCC patients is 50%.[3,4] Despite the advances made in the therapeutic modalities via multidisciplinary approaches, survival rate for OSCC has not significantly improved.[1] This motivates the search for factors, which will help in the early diagnosis and better tailoring the individual management of OSCC patients.[3] Prevention and early diagnosis remains the best instrument in our armamentarium for reducing the death rate associated with oral cancer.[3] Saliva-based diagnostics are more attractive as they are more accessible, accurate, less expensive and present less risk of infection to the patient than current methodologies. The enzyme lactate dehydrogenase (LDH) is an ubiquitous enzyme that was discovered in the early periods of enzymology. This enzyme catalyzes the reaction of lactate production via pyruvate reduction during anaerobic glycolysis. LDH is found in the cells of almost all body tissues and is especially concentrated in the heart, liver, red blood cells, kidneys, muscles, brain and lungs.
The profile of salivary LDH isoenzymes is similar to that found in oral epithelium, indicating that the major source of salivary LDH is probably the oral epithelium, that is, derived from the surface exfoliated cells. Consequently, LDH concentration in saliva as an expression of cellular necrosis can be considered to be a specific indicator for lesions affecting the integrity of the oral mucosa.

Studies on analysis of salivary LDH either total or isoenzymes in oral leukoplakia (OL) and OSCC patients have not been carried out extensively. The present study was designed to evaluate salivary LDH isoenzyme pattern in OL and OSCC and to correlate between LDH isoenzyme levels and histopathologic grading in selected cases of OL and OSCC.

MATERIALS AND METHODS

Source of data

The study was approved by Institutional Ethics Committee. Patients were selected from those attending The Outpatient Department (OPD) of Oral Pathology and Microbiology Department of the institute and divided in three groups as follows:

- Group I: Thirty otherwise healthy and consenting OSCC patients
- Group II: Thirty otherwise healthy and consenting patients with leukoplakia
- Control group: Thirty healthy individuals of comparable age.

Exclusion criteria

Patients suffering from systemic conditions like cardiovascular disease, anemia, liver, kidney and pancreatic diseases, blood dyscrasias, stroke, muscular dystrophy, chronic generalized periodontitis, drugs like anesthetics, narcotics and aspirin.

Saliva collection

Informed written consent was obtained from the patients prior to saliva collection. Three milliliters of unstimulated whole saliva was aseptically collected in a wide mouthed container. Following the collection, saliva was centrifuged at 1000 rpm for 10 min and the resulting supernatant was used for biochemical estimation of LDH isoenzymes by using agarose gel electrophoresis method (SEBIA-HYDRAGEL ISO-LDH K-20 kit). Frozen samples were avoided.

Incision biopsy specimen obtained was processed and stained by hematoxylin and eosin. Sections of OL and OSCC cases were scrutinized histopathologically and appropriately graded for epithelial dysplasia and differentiation of carcinoma respectively.

RESULTS

Clinical data analysis

A wide variation in age was noted among the study groups ranging from 18 to 70 years. A mean age of 47.96 years was noted in OSCC group, whereas the mean age in leukoplakia group was 41.06 years. OSCC group comprised of 20 (66.6%) males and 10 (33.3%) females, while leukoplakia group comprised of 29 (96.66%) males and 1 (3.3%) female.

All patients in OL and OSCC groups had history of tobacco chewing in some or other form. Twenty male patients of OSCC group also had history of alcohol consumption.

Histopathological grading of OL and OSCC

Leukoplakia cases were classified based on clinical staging criteria according to Pindborg et al. On histopathological examination, 16 (53.33%) cases of leukoplakia showed only hyperkeratosis without any evidence of dysplasia (P1). Whereas 11 (36.66%), 2 (6.6%) and 1 (3.3%) cases were categorized under dysplasia grade I (P2 = mild dysplasia), II (P3 = moderate dysplasia) and III (P4 = severe dysplasia), respectively.

According to the Brynes grading system, 22 (73.33%) cases of OSCC were categorized under grade I (well differentiated), whereas 7 (23.33%) cases fell under the category of grade II (moderately differentiated) and only 1 (3.3%) case was categorized as grade III (poorly differentiated).

Total salivary LDH analysis

Total salivary LDH activity level was significantly increased in OL and OSCC group in comparison to control group [Table 1 and Figure 1].

Table 1: Total LDH in various study groups

| Groups          | Control | OL       | OSCC      |
|-----------------|---------|----------|-----------|
| Total LDH IU/L  | 267.2   | 519.3667 | 788.7333  |

LDH: Lactate dehydrogenase, OL: Oral leukoplakia, OSCC: Oral squamous cell carcinoma

Figure 1: Total LDH variation in study groups
Salivary LDH Isoenzymes analysis [Table 2, Figure 2]

Control group
Levels of salivary LDH 5 isoenzyme was high, LDH 1 isoenzyme levels were low and levels of isoenzymes LDH 2, LDH 3 and LDH 4 were intermediate.

OL group
There was statistically significant increase in LDH 2 \( (P < 0.001) \), LDH 3 \( (P < 0.001) \), LDH 4 \( (P < 0.001) \) and LDH 5 \( (P < 0.001) \) in comparison with control group.

OSCC group
There was statistically significant increase in levels of LDH 3 \( (P < 0.001) \), LDH 4 \( (P < 0.001) \) and LDH 5 \( (P < 0.001) \) in comparison to control group. There was no difference in levels of LDH 2 \( (P < 0.955) \) isoenzyme between control and OSCC group, whereas levels of LDH 1 \( (P < 0.054) \) isoenzyme was lower in OSCC group in comparison to control group.

Correlation of salivary isoenzymes patterns with histopathologic grades of OL and OSCC

OL group
There was statistically significant increase in levels of salivary isoenzymes LDH 5, LDH 4, LDH 3 and LDH 2 with increasing grades of dysplasia [Tables 3 and 5, Figure 3].

No change was noted in levels LDH 1 isoenzyme.

OSCC group
There was significant increase in levels of isoenzyme LDH 5 in well, moderate and poorly differentiated OSCC, whereas levels of LDH 4, LDH 3, LDH 2 and LDH 1 did not show any significant variation among well, moderately and poorly differentiated OSCC [Tables 4 and 5, Figure 4].

Table 2: LDH isoenzymes variation in study groups

| Values Groups | LDH 1 IU/L±SEM | LDH 2 IU/L±SEM | LDH 3 IU/L±SEM | LDH 4 IU/L±SEM | LDH 5 IU/L±SEM |
|---------------|----------------|----------------|----------------|----------------|----------------|
| Control       | 29.417±5.007   | 43.960±3.145   | 50.640±3.785   | 65.316±4.589   | 77.865±5.758   |
| OL            | 46.105±6.823   | 64.933±4.29    | 75.872±4.183   | 139.097±6.822  | 194.714±10.668 |
| OSCC          | 11.852±3.468   | 43.610±5.346   | 87.566±5.396   | 144.717±9.113  | 502.108±21.323 |

LDH: Lactate dehydrogenase, OL: Oral leukoplakia, OSCC: Oral squamous cell carcinoma, SEM: Standard error of mean

Table 3: LDH Isoenzyme pattern in histopathological grades of Leukoplakia

| Values Dysplasia grades | LDH 1 IU/L±SEM | LDH 2 IU/L±SEM | LDH 3 IU/L±SEM | LDH 4 IU/L±SEM | LDH 5 IU/L±SEM |
|-------------------------|----------------|----------------|----------------|----------------|----------------|
| P1 (no dysplasia)       | 40.024±11.389  | 65.415±4.797   | 79.356±6.541   | 141.963±10.362 | 206.673±13.634 |
| P2 (mid dysplasia)      | 47.752±8.896   | 57.915±8.525   | 70.446±6.555   | 128.342±11.201 | 172.743±20.182 |
| P3 (moderate dysplasia) | 63.285±30.861  | 84.474±24.258  | 75.141±5.082   | 172.242±12.459 | 225.858±5.742  |
| P4 (severe dysplasia)   | 46.35          | 69.75          | 58.95          | 133.65         | 141.3          |

LDH: Lactate dehydrogenase, SEM: Standard error of mean
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Table 4: LDH isoenzyme pattern in histopathological grades of oral squamous cell Carcinoma

| Grades SCC            | LDH 1 IU/L±SEM | LDH 2 IU/L±SEM | LDH 3 IU/L±SEM | LDH 4 IU/L±SEM | LDH 5 IU/L±SEM |
|-----------------------|----------------|----------------|----------------|----------------|----------------|
| Well differentiated   | 14.032±4.625   | 41.174±6.471   | 91.462±6.528   | 144.227±11.201 | 519.861±26.561 |
| Moderately differentiated | 6.017±2.103    | 53.885±10.139  | 77.221±10.216  | 148.874±18.145 | 438.143±27.067 |
| Poorly differentiated  | 4.74           | 25.28          | 74.26          | 126.4          | 559.32         |

LDH: Lactate dehydrogenase, SEM: Standard error of mean, SCC: Squamous cell carcinoma

Table 5: Statistical correlation between various study groups

| Group compare | First | Second | P value | MD* | P value | MD* | P value | MD* | P value | MD* | P value |
|---------------|-------|--------|---------|-----|---------|-----|---------|-----|---------|-----|---------|
| Control OL    | -16.69| 0.054  | <0.001  | -20.97| <0.001  | -25.23| <0.001  | -73.78| <0.001  | -116.8| <0.001  |
| OSCC          | 17.57 | 0.006  | 0.35    | 0.955| 0.007   | -36.93| <0.001  | -79.4 | <0.001  | -424.2| <0.001  |
| OL OSCC       | 34.25 | <0.001 | 21.32   | 0.003|         | -11.69| 0.093   | -5.6 | 0.624   | -307.4| <0.001  |

MD* denote mean difference. Comparison between various study groups and LDH isoenzyme values. Two sample t test for testing the significance of difference between two group means. LDH: Lactate dehydrogenase, MD: Mean deviation

DISCUSSION

OSCC is one of the most common head and neck malignancy with a worldwide incidence of over 300,000 new cases emerging per year and accounting for 2-4% of all new cancers.[9] There is an imperative need for developing new diagnostic tools that would improve early detection. The identification of molecular markers in body fluids that would predict the development of cancer in its earliest stage or in precancerous stage would constitute such a tool.[9] The major competent among all the body fluids is blood but compelling reasons of being in-expensive, noninvasive and easiness to handle wins saliva over blood as a diagnostic fluid to monitor health and disease.[10] For patients, the noninvasive collection technique dramatically reduces anxiety, discomfort and simplifies procurement of repeated samples for longitudinal monitoring over time. Therefore the present study was conducted to measure salivary LDH isoenzyme levels in OL and OSCC and to correlate between LDH isoenzyme levels with histopathologic grading in cases of OL and OSCC.

LDH is a hydrogen transfer enzyme that catalyses the oxidation of L-lactate to pyruvate with nicotinamide adenine dinucleotide (NAD) as hydrogen acceptor, the final step in the metabolic chain of anaerobic glycolysis. All isoenzymes catalyze the same biochemical reaction but differ in their molecular structure and are more or less organ specific. Isoenzyme patterns can be used to localize the source of LDH release and are separable electrophoretically into LDH-1 (H4); LDH-2 (H3M); LDH-3 (H2M2); LDH-4 (HM3); and LDH-5 (M4).[11] There are various methods for separation of LDH isoenzymes like gel electrophoresis, isoelectric focusing and immunochemical staining out of which gel electrophoresis method was found to be more effective than others because LDH banding pattern obtained by gel electrophoresis demonstrates simple tetrameric as compared with other methods. Hence we used this method effectively to our advantage.

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. Nagler et al. concluded that major source for whole saliva LDH is nonglandular and that the oral epithelium is the major source.[12] LDH-1 and LDH-2 isoenzymes, which originate from the heart, predominate in plasma, while isoenzymes LDH-4 and LDH-5 predominate in saliva. Though the profile of salivary LDH isoenzymes is entirely different from that found in plasma it is similar to that found in oral epithelium. Since the major source of salivary LDH is probably the surface exfoliating epithelial cells, it is logical to assume that pathological alternations of oral epithelium like dysplasia or cancer may result in alternation of LDH isoenzyme profile. Therefore, salivary LDH isoenzymes may be evaluated for possible oral mucosal pathologies in a manner similar to that used for evaluating pathologies in heart, muscle or liver for LDH detection in plasma.[12]

Tobacco usage either in smoking or nonsmoking form and alcohol consumption are considered to be most important causes of oral precancer or cancer. Nicotine affects a variety of cellular processes ranging from induction of gene expression to secretion of hormones and modulation of enzymatic activity. Cigarette smoking, which is one of the potent oxidant and radiation exposures that may exacerbate its effect, may induce functional and chemical change in living systems.[11] Hedayat and Azab [13] demonstrated high activity of serum LDH following nicotine administration in their experiments on Gamma-irradiated rats. They also concluded that vitamin E due to its free scavenging potential inhibits lipid peroxidation and may reverse many enzymes and changes induced by radiation exposure and/or nicotine administration. Increased LDH levels are usually found due to cell death and/or leakage from the cells. In a study on biochemical changes in alcoholics
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by Usharani increased serum LDH concentration was noted in alcoholic patients.

The LDH isoenzyme pattern analysis has been found to be more specific than total activity alone and has been of considerable interest to the biochemical oncologist. Serum LDH isoenzyme levels have been studied extensively in various body cancers and increased levels have been observed. Malignant tumor tissue or contiguous tissue damaged by tumor liberates enzymes into circulation, which contributes toward abnormal increase in enzyme levels. Irrespective of tissue of origin, studies on isoenzyme distribution in different types of malignant tumors have shown cathodic isoenzymes LDH 3, LDH 4 and LDH 5 to be abnormally increased. LDH isoenzyme determination in carcinomas is found to be useful not only in diagnosis but also is an important prognostic parameter. Masahiro et al. correlated clinical and experimental activity of serum LDH and its isoenzymes in oral cancer. They found LDH activity to be elevated in oral cancer and marked increase was noted in levels of LDH 4 and LDH 5 and decrease in levels of LDH 2 activity. Naphade and Naphade studied major immunoglobulin status and LDH isoenzyme profile in oral premalignancy and malignancy and concluded that increase in the density of the LDH 2, LDH 3 and LDH 4 from premalignancy to malignancy is definitely suggestive of increased isoenzyme activity in malignancy.

Studies on analysis of salivary LDH either total or its isoenzyme levels in OL and OSCC patients have not been carried out extensively. We found significant increase in total salivary LDH in OL and OSCC groups. This finding is in agreement with the studies done by Shpitzer et al. and Shpitzer et al. who found total salivary LDH (88%, P = 0.002) level to be high in oral cancer patients. Our results are comparable with the study done by Shetty et al. who have reported consistently higher salivary LDH levels in oral precancer and cancer and mean salivary LDH levels to be higher in males in comparison to females in all three study groups of leukoplakia, cancer and healthy controls. In our study, there was only one female patient in OL group (LDH = 230 IU/L) and the value was less elevated as compared with total LDH in male patients (mean LDH = 529.34IU/L). In OSCC group, there were 10 females (mean LDH = 822.66IU/L) and 20 males (mean LDH = 774.19IU/L) and values in females were marginally higher than in males.

Salivary LDH 3, LDH 4 and LDH 5 isoenzyme levels were increased in OL group in comparison to control group. This result is consistent with the finding presented in the only study of its kind done by Rai et al. who have reported increased levels of LDH 3, LDH 4 and LDH 5 isoenzymes in lichen planus. In OL group of our study, there was generalized increase in levels of salivary isoenzymes LDH 5, LDH 4, LDH 3 and LDH 2 with increasing grades of dysplasia. There was a significant correlation between levels of salivary LDH isoenzymes and histologic grades of dysplasia but this finding is in contrast to the results of the study by Langvad et al. who did not find any correlation in between LDH isoenzyme pattern and epithelial thickness.

Literature review reveals very few studies on analysis of salivary LDH isoenzymes in oral cancer. In our study, LDH 3, LDH 4 and LDH 5 isoenzyme levels were increased in OSCC group in comparison to control group. In OSCC group, there was a significant increase in levels of isoenzyme LDH 5 in well, moderate and poorly differentiated OSCC, whereas levels of LDH 4, LDH 3, LDH 2 and LDH 1 did not show any significant variation among well, moderately and poorly differentiated OSCC.

There was a significant correlation between histopathologic grades of OSCC and levels of LDH 5 isoenzyme. The biochemical configuration of tissues in premalignant lesions differ from that of the normal and frank malignant group in that they may represent a sort of transient phase in the eventual progression to cancer. With increased dysplastic changes, the tendency toward utilization of anaerobic phase of the glycolytic pathway is increased. This would then cause a rise in LDH levels as LDH is a basic enzyme utilized in this process. Thus findings of different LDH values in groups of near normal to potentially malignant to frankly malignant can be correlated to varying degree of dysplasia.

Since literature review shows few studies on salivary LDH and its isoenzyme profiles in oral precancer and cancer, we suggest that the findings of our study need to be reinforced by including larger sample size with long term follow up.

CONCLUSION

The present study was carried out to determine whether salivary LDH isoenzymes can be used as a biochemical marker in OL and OSCC and to study the efficacy of saliva as a diagnostic tool in early detection of oral precancer and cancer. Total salivary LDH level was increased in both OL and OSCC group. There was a significant correlation between levels of salivary LDH isoenzymes and histopathologic grades of dysplasia in OL and OSCC. We propose that salivary analysis could be used as an efficient, noninvasive and friendly new tool for diagnosis and monitoring of oral precancer and cancer.

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