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Histopathological differentiation of oral squamous cell carcinoma and salivary lactate dehydrogenase: A biochemical study

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Abstract

Context: Early diagnosis of oral cancer is a priority health objective, in which oral health professionals may play a pivotal role. Detection should lead to less damage from cancer therapy and to a better prognosis. **Aims:** The aim was to estimate and compare the salivary lactate dehydrogenase (LDH) levels in various histological differentiation of oral squamous cell carcinoma (OSCC) patients and normal subjects. **Settings and Design:** Hospital-based setting, case-control study. **Subjects and Methods:** A case–control study was undertaken comprising 30 OSCC patients and 30 healthy controls. The OSCC patients were grouped into well-differentiated, moderately differentiated and poorly differentiated OSCC based on their histological tumor differentiation. Unstimulated whole saliva was collected, assayed for LDH levels using a standard kit and measured spectrophotometrically at 340 nm. **Statistical Analysis Used:** The results obtained were subjected to statistical analysis using Kruskal–Wallis and Mann–Whitney U-tests. Spearman's correlation was used to correlate the histological tumor differentiation with the salivary LDH levels. **Results:** The mean salivary LDH levels in the control group, well-differentiated OSCC group, moderately differentiated OSCC group, and poorly differentiated OSCC group were 117.33 ± 19.37 IU/L, 355.83 ± 16.73 IU/L, 484.18 ± 25.84 IU/L, and 620.35 ± 18.69 IU/L, respectively. The difference in the mean salivary LDH levels was statistically significant among the various groups (Kruskal–Wallis $\chi^2 = 50.820$, $P < 0.001$). Spearman's correlation showed significant difference between salivary LDH levels and histological differentiation of OSCC ($r = -0.689$, $P < 0.01$). **Conclusions:** The salivary LDH levels were higher in OSCC patients when compared to the healthy controls. The salivary LDH levels were found to be the highest among the poorly differentiated OSCC.

Key words: Histopathology, oral cancer, salivary lactate dehydrogenase

Introduction

Oral cancer refers to all malignancies arising from the lips, oral cavity, and pharynx and is a major public health problem as it causes significant morbidity and mortality. About 86% of world's oral cancer cases are reported in India.[1] In India, it accounts for over 30% of all cancers. It ranks first among all cancer cases in males and is the third most common among females in India. Oral squamous cell carcinoma (OSCC) is a multistage process from normal to dysplastic cells (precancerous lesions) and ultimately squamous cell carcinoma.[2] Early detection and treatment is the most effective means to reduce death from this disease.

Early diagnosis of oral cancer is a priority health objective, in which oral health professionals may play a pivotal role. Detection should lead to less damage from cancer therapy and to a better prognosis. There are two approaches in the early detection of oral dysplasia and cancer: (1) Oral cancer screening programs that identify asymptomatic patients with suspicious lesions and (2) employing specific diagnostic tools to identify dysplasia and early oral cancers in asymptomatic patients, with an oral abnormality. Saliva has been used as a diagnostic tool for oral diseases because it is noninvasive, requires minimal training and can be used for the mass screening of large population samples.[3]

The biomarkers for early cancer detection must meet the following criteria: (a) The altered can be objectively measured; (b) must be measurable in small specimens; (c) must be altered in high-risk tissues, but not in normal tissues; and (d) must be altered in the early stages of cancer development. Unlike the other deep cancers, OSCC occurring in the oral cavity is much easier to be monitored, specimens are easier to be collected for diagnosis, and the treatment is easier to be applied.

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Lactate dehydrogenase (LDH) is an intracellular enzyme that catalyzes the reaction of lactate production via pyruvate reduction during anerobic glycolysis. Its extracellular presence is always related to cell necrosis and tissue breakdown. Serum LDH nonspecifically increases in many pathological conditions such as myocardial infarction, megaloblastic anemias, liver and renal diseases. LDH concentration in saliva could be a specific indicator for oral lesions that affect the integrity of the oral mucosa.[4]

Previous study has reported that the LDH isoenzyme profile of the oral epithelium was found to be similar to that of the whole saliva and the study concluded that the major source for whole saliva LDH is nonglandular.[5] It has been shown that saliva as a diagnostic fluid meets the demand for an inexpensive, noninvasive, and accessible diagnostic methodology for oral cancer detection.[3]

A study has reported higher levels of salivary LDH in oral cancer and oral leukoplakia than normal subjects.[2] Comparison of tissue LDH isoenzyme ratios in biopsy specimen of oral cancer, oral submucous fibrosis, oral leukoplakia with normal controls, showed altered ratios in oral cancer and oral submucous fibrosis.[6] Salivary LDH levels were high in OSCC patients when compared to normal subjects in various studies.[3]

Salivary LDH levels in histological differentiation of OSCC have not been reported in the literature. A possible correlation between tumor differentiation and the levels of salivary LDH may exist. The objectives of the present study were to estimate and compare the salivary LDH levels in - various histological differentiation of OSCC patients and normal subjects.

Subjects and Methods

Thirty patients visiting the outpatient department of the A. B. Shetty Memorial Institute of Dental Sciences and Mangalore Institute of Oncology who were clinically diagnosed and histopathologically proven with OSCC formed the study group. Thirty healthy age and gender matched subjects formed the control group. They were grouped into further categorized based on Broder's histopathological grading into three groups well-differentiated ($n = 10$), moderately differentiated ($n = 10$), and poorly differentiated OSCC ($n = 10$). Patients with the

previous history of malignancy and treated for cancers (surgery, chemotherapy, radiotherapy) and patients with systemic diseases known to increase serum LDH levels such as myocardial infarction, liver disease, renal disease, muscular dystrophy were excluded from the study. The subjects were informed about the procedure of the study, and informed consent was obtained. Ethical clearance was obtained from the Institutional Ethical committee.

About 2 ml of unstimulated whole saliva was collected by the spit method. The patients were instructed not to consume food, smoke or chew tobacco or gum 1 h before the saliva collection procedure. The saliva was collected between 9 am and 12 noon to prevent any variations in the composition of saliva due to circadian rhythm according to the method of Navazesh.[7] The subjects were asked to rinse the mouth with distilled water prior to saliva collection to remove any food debris. They were then directed to spit into a sterile plastic container for 10 min. The subjects were instructed not to spit forcibly as it can cause blood contamination, if any, from inflamed gingival tissue. The saliva samples were then sent for biochemical analysis. The samples were diluted in 1:1 ratio with saline and assayed using a standard kit and measure spectrophotometrically at 340 nm. The LDH levels were expressed as IU/L.

The results obtained were tabulated and subjected to statistical analysis using SPSS statistical package 17.0 (SPSS Inc., Chicago, IL, USA). As the tests of normality showed significant results, the data were deemed nonnormal distribution and hence, nonparametric tests were applied. Kruskal–Wallis test was used to compare the data among the various groups followed by Mann–Whitney U-test for pairwise comparison among the groups. Spearman's correlation was used to correlate the histological tumor differentiation with the salivary LDH levels. The level of significance was set at $P < 0.05$.

Results

The present study was conducted to estimate and compare the salivary LDH levels in OSCC patients and normal subjects and to correlate the histological differentiation of OSCC with salivary LDH levels. The results of the present study showed that the mean salivary LDH levels in the control group, well-differentiated OSCC group, moderately differentiated OSCC group, and poorly differentiated OSCC group were 117.33 ± 19.37 IU/L, 355.83 ± 16.73 IU/L, 484.18 ± 25.84 IU/L, and 620.35 ± 18.69 IU/L, respectively. The salivary LDH levels among various groups were statistically significant among the various groups [Table 1]. *Post-hoc* test using Mann–Whitney U-test for pair-wise comparison of groups showed statistical significance. Spearman's correlation coefficient showed a significant difference between salivary LDH levels and histological differentiation of OSCC ($r = -0.689$, $P < 0.01$).

Table 1: Comparison of mean salivary LDH levels (IU/L) among various groups

Group	n	Mean salivary LDH IU/L (SD)	Spearman's correlation
Well-differentiated[a]	10	355.83 (16.73)	r=−0.689, P<0.001*
Moderately differentiated[b]	10	484.18 (25.84)	
Poorly differentiated[c]	10	620.35 (18.69)	
Control[d]	30	117.33 (19.37)	
Kruskal-Wallis ANOVA[#]	χ^2 (df)=50.820 (3), P<0.001*		

*P<0.001=Significant, [#]*Post-hoc* test using Mann-Whitney U-test for pair-wise comparison of groups-ad, bd, cd, ab, ac, bc showed statistical significance (P<0.001). LDH=Lactate dehydrogenase SD=Standard deviation, ANOVA=Analysis of variance

Discussion

Oral squamous cell carcinoma is the sixth most common human malignancy, with a 5-year mortality rate of approximately 50%, which has not changed significantly in more than 50 years and a high rate of morbidity.[1] Salivary testing, a noninvasive alternative to serum testing, can be an effective modality for diagnosis and prognosis predicting of oral cancer as well as for monitoring the patient's post-therapy status.[1,3] Saliva may provide a cost-effective and practical approach for the screening of large populations.[3] Salivary "tools" are those focused on measuring changes of specific salivary macromolecules such as proteins or nucleic acids (as fatty acids are rather scarce in saliva), that is, examining genomic or proteomic targets like enzymes, cytokines, growth factors, metalloproteinases, endothelin, telomerase, cytokeratines, mRNA's, DNA aberrations, etc.[1,3]

Various studies on urinary LDH, serum LDH, salivary LDH, and intracellular tissue LDH in a variety of pathological conditions have been reported in literature. Immunohistochemical evaluation of LDH levels in various types of malignant disorders has shown that irrespective of the malignancy, LDH levels were higher in malignant cells than the normal cells.[1,3]

The results of the present study showed that there were increased levels of salivary LDH levels in OSCC group when compared to the healthy age and gender matched controls. Similar results have been obtained in previous studies reported in the literature. A study evaluated the salivary LDH levels in 25 OSCC, 25 oral leukoplakia, and 25 control groups. It was reported that salivary LDH levels were higher in oral cancer and precancer group when compared to healthy age and gender matched controls.[2] A study conducted on South Indian population also showed increased levels of salivary LDH levels in oral cancer patients compared to oral leukoplakia and oral submucous fibrosis.[6] The authors have used molecular markers for analysis of salivary samples for early detection of OSCC. Another study considered five different salivary parameters; it was found that salivary LDH was significantly increased in patients with carcinoma of the tongue than healthy controls.[8] Joshi *et al*. reported that the increase in LDH levels was consistent in saliva and serum of OSCC patients.[9] It has been reported that salivary LDH_3, LDH_4, LDH_5, and M subunit were significantly increased in patients with oral lichen planus as compared to controls. The authors have stated that the increased levels of isoenzymes LDH in oral lichen planus may be due to carcinogen enhanced activity of LDH. They have concluded that measuring salivary LDH may be a feasible, simple, and convenient approach for screening of oral precancer.[10]

The present study also aimed to assess the correlation between tumor differentiation and the levels of salivary LDH in OSCC. The results of the present study showed that there was a progressive increase in the salivary LDH levels from well-differentiated to the poorly differentiated OSCC. 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 member brought about by the pathological condition.[1,3,5] The mitotic differentiation is higher in moderately differentiated squamous cell carcinoma than the well-differentiated carcinoma, and it is the highest in the poorly differentiated OSCC. This may be the reason for increased levels of salivary LDH in the poorly differentiated than the moderately and the well-differentiated groups.

Conclusion

The present study revealed that the salivary LDH levels were higher in OSCC patients when compared to the healthy controls. The salivary LDH levels were found to be the highest among the poorly differentiated OSCC.

Salivary lactate dehydrogenase levels could be a valuable biomarker for detection of oral cancer. Its alteration in histological tumor differentiation can be an indicator for the treatment and prognosis of oral cancer.

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