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Salivary Lactate Dehydrogenase - A Biomarker of Potentially Malignant and Malignant Diseases of Oral Cavity?

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Authors' contributions

This work was carried out in collaboration among all authors. Author SSS gave the concept, did the design, drafting and critical revision of the article. Author KD did the clinical data, literature review and revision. Authors BS, RS and AV managed the Literature review and revision. All authors read and approved the final version of the manuscript.

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Original Research Article

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ABSTRACT

Introduction: Oral cancer is the sixth most common cancer occurring worldwide. In many cases, cancer develops from the site of potentially malignant lesion. Lactate dehydrogenase (LDH) is an ubiquitous enzyme the level of which is known to increase in serum and saliva as a result of shift of glycolytic pathway from aerobic to anaerobic in dysplastic cells.
Aim: This study aims to emphasize salivary LDH as a reliable bio-marker in diagnosing potentially malignant and malignant diseases.
Materials and Methods: Forty patients from the department of Oral Medicine and Radiology were enrolled in the study and divided into 2 groups: Group I consisting of patients with oral leukoplakia, oral submucous fibrosis and oral carcinoma; Group II consisting of healthy controls. Unstimulated

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saliva of about 2 ml was collected from all patients. LDH level in saliva was estimated using semi-automatic analyzer and LDH (P-L) kit. The obtained values were tabulated and statistical results were obtained.

Results: The mean salivary lactate dehydrogenase level was higher in case group than control group with value of 962.8 U/l in case group and 398.1 U/l in control group. There was statistically significant difference between the mean salivary LDH levels of the above groups.

Conclusion: This study concludes that Salivary LDH estimation can be used as an efficient, non-invasive, cost effective and friendly new tool for diagnosis of patients with oral potentially malignant and malignant diseases.

Keywords: Salivary lactate dehydrogenase; malignant; oral cavity.

1. INTRODUCTION

Oral cancer is the sixth most common cancer occurring worldwide which encompasses all malignancies that originate in the oral tissues with a five year mortality rate of approximately 50% and a high rate of morbidity [1,2]. India has one of the highest rates of occurrence of oral cancer due to tobacco chewing habit [3]. Oral cancer more commonly develops from the site of potentially malignant lesion and malignant transformation rate of these lesions ranges from 0.6% to 20% [4]. Inspite of the various advances made in therapeutic modalities via multidisciplinary approaches, there is no significant increase in the survival rate of patients with oral cancer [5]. Prevention and early diagnosis will aid in reducing the death rate associated with oral cancer [6].

Saliva based diagnostics are more attractive because of their advantages of easy accessibility, accuracy, cost-effectiveness with low risk of infection to the patient than current methodologies [6]. Lactate dehydrogenase (LDH) is an ubiquitous enzyme the main function of which is to catalyze the oxidation of lactate to pyruvate. LDH is always confined within the cell cytoplasm. Therefore, the extracellular presence of LDH indicates cell necrosis and tissue breakdown [1]. The purpose of this study is to determine the salivary LDH levels in patients with oral leukoplakia, oral submucous fibrosis, oral squamous cell carcinoma and compare the obtained values with normal healthy individuals. This study aims to emphasize salivary LDH as a reliable bio-marker in diagnosing potentially malignant and malignant diseases.

2. MATERIALS AND METHODS

This study was conducted in the Department of Oral Medicine and Radiology, Best Dental Science College, Madurai, Tamilnadu. The study samples were obtained from the patients attending the Outpatient Department of Oral Medicine and Radiology. Twenty patients fulfilling the inclusion criteria were enrolled for the case group of the study. Twenty normal healthy patients were enrolled for the control group of the study.

2.1 Grouping of Study Participants

Group I (Case group): 20 patients who were clinically and histopathologically diagnosed to have oral leukoplakia, Grade III oral submucous fibrosis and Stage 3 oral cancer.

Group II (Control group): 20 healthy patients who were age matched with those patients in Group I.

Inclusion criteria

- Patients under the age group of 40 – 65 years of age.
- Clinically and histopathologically diagnosed patients of oral leukoplakia, oral submucous fibrosis and oral cancer.
- Patients who were willing to participate in the study.

Exclusion criteria

- Patients with any other type of mucosal lesions.
- Patients who have received dental treatment about 48 hours before the sample collection such as extractions and scaling, which might affect the integrity of oral mucosa.
- Patients undergoing chemotherapy, radiotherapy or any surgical procedure for malignancy.
- Patients with history of previous malignancy.
- Patients with immunosuppressive diseases, HIV, hypercholesterolemia, other

infectious diseases, pulmonary diseases, endocrine disorders, cardiac diseases, hematological diseases, renal diseases, hepatic diseases, muscular diseases.

- Patients under corticosteroid medication.

2.2 Clinical Examination

Patients were asked to sit comfortably on the dental chair and detailed case history was recorded. Habit history was obtained comprising the type of habit like usage of tobacco in smoking or smokeless form, alcohol usage, duration of the habit and the amount of consumption per day. History regarding symptoms like pain, burning sensation to normal or spicy food, difficulty in speech and swallowing, increased or decreased salivation with altered taste sensation were recorded. Clinical examination was carried out by trained oral medicine specialist under artificial illumination wearing hand gloves and mouth mask. Biopsy specimen was collected and histopathological examination was done. Fig. 1 shows histological picture of a patient with oral squamous cell carcinoma whose report reveals a section showing acanthotic stratified squamous epithelium with moderate dysplastic changes and underlying stroma showing infiltrating islands of malignant squamous epithelium with surrounding lymphocytic infiltrate.

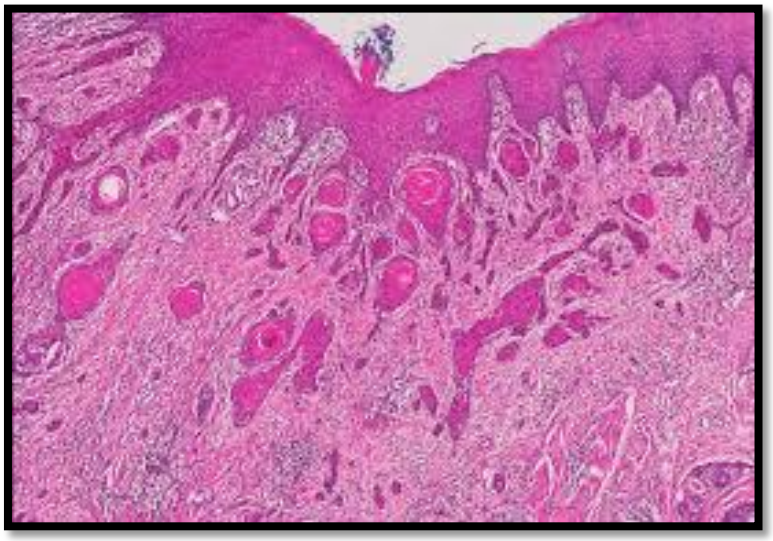

Fig. 1. Histological image of the lesion of one of the study participants and that corresponds to Oral squamous cell carcinoma

Fig. 2. Saliva collection tube

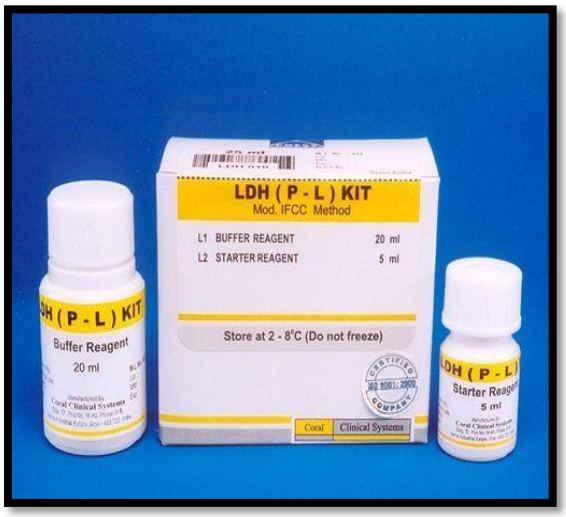

Fig. 3. LDH P-L Kit

2.3 Sample Collection

Aseptically unstimulated whole saliva was collected from all the study participants. Patients were asked to refrain from drinking, eating or smoking for one hour before the collection of saliva. Patients were asked to sit comfortably with head in upright position and were asked to rinse the oral cavity with normal water and then asked to accumulate saliva in their oral cavities for 5 minutes. The patients were asked to spit the accumulated saliva in a sterile, disposable eppendorf vial until a minimum desired quantity of 2 ml was obtained. The vials containing saliva were then stored in an ice box, refrigerated, transported to the laboratory and processed within 12 hours.

2.4 Estimation of Lactate Dehydrogenase Enzyme in Saliva

Lactate dehydrogenase enzyme level in saliva was calculated using semi-automatic analyser with the help of LDH (P-L) kit. The normal value of LDH enzyme in saliva is 230 to 460 U/l. The data was then entered into Microsoft Excel spread sheet and analyzed using SPSS Software version 26.0. Descriptive statistics was used. For all the tests, $p<0.05$ was considered statistically significant. Unpaired t-test was used for comparison of statistically significant difference between the groups.

3. RESULTS

The mean salivary LDH level in case group is 962.8 U/l and the mean salivary LDH level in control group is 398.104 U/l which has been depicted in Table 2. The mean salivary LDH level in patients with malignant and potentially malignant diseases was significantly higher than the mean salivary LDH level in normal healthy patients of control group.

4. DISCUSSION

Oral cancer presents a major clinical diagnostic challenge to many dental practitioners especially in its early stage. They often tend to be preceded by a potentially malignant stage for a long time before transformation into cancer [7]. WHO defined potentially malignant disorders as “the risk of malignancy being present in a lesion or condition either during the time of initial diagnosis or at future date” [8]. Early detection followed by appropriate treatment can increase the cure rates and greatly improve the quality of life by minimizing extensive, debilitating treat-ments [5]. There has been an ever-growing effort focusng on the identification of biological indicators for the diagnosis of malignancy [1].

Altered gene expressions resulting from genetic aberrations of cancer cells can be identified long before the resulting cancer phenotypes are manifested. Changes that arise specifically or exclusively in cancer compared to normal tissue of the same origin can be used as molecular biomarkers [5]. Tumor markers in serum, tissue and other body fluids help in early diagnosis during a neoplastic process. Though blood has been the main choice of medium for detection of biochemical markers, it has some inherent disadvantages. On the other hand, saliva produces a non-invasive, easily available diagnostic medium for diagnosing the rapidly widening range of diseases [1].

Lactate dehydrogenase (LDH) is an ubiquitous enzyme that plays a significant role in the diagnosis of pathologic process [2,4]. LDH is an enzyme which is detectable in the cytoplasm of all cells in the human body the extracellular presence of which indicates cell death [9]. LDH is a hydrogen transfer enzyme that catalyses the oxidation of L-lactate to pyruvate with nicotinamide-adenine dinucleotide (NAD)+ as hydrogen acceptor [6]. Transformation of normal tissue to dysplastic tissue and further to cancerous tissue manifests as a shift from aerobic to anaerobic glycolysis [9]. As whole saliva is a combination of secretions from both minor and major salivary glands, fluids diffused through the oral epithelium and gingiva, material originating from gastrointestinal reflex and cellular and other debris, LDH in whole saliva may originate from various sources within the oral cavity. Nagler et al stated that oral epithelium is the major source for whole salivary LDH [6].

Table 1. Mean Salivary LDH Levels among the subgroups of group I

Subgroups	Mean LDH
Leukoplakia	1073.63
Oral Submucous Fibrois	747.1
Oral Cancer	1172.5

The possible reasons for increased LDH activity in patients with cancer are: (i) tissue damaged by tumor releases enzymes into body fluids (ii) increased mitotic index and increased lactic acid production by tumor cells (iii) breakdown of glycoprotein into lactic acid [1]. Pereira et al. and Sivaramakrishnan et al. in 2015 concluded from

Table 2. Mean Salivary LDH levels in the study groups

Groups	N	Mean LDH	Std Deviation	p - value
Cases	20	962.8	945.58	
Controls	20	398.104	523.08	0.03

their study that there was significant increase in serum LDH level in patients with oral cancer and potentially malignant disease in comparison with controls [8].

In our study the mean salivary LDH level in patients with Oral Cancer, Oral Leukoplakia and Oral Submucous Fibrosis is higher than the normal healthy patients in the control group, which is statistically significant (p value -0.03). The results of our study is in accordance with the results obtained by Achalli et al. in 2012 and Joshi et al. in 2014 whose studies also revealed statistically significant increase in salivary LDH level in cases than in controls [8].

Ninomiya et al. in 1985 stated that morphologic and enzymatic changes indicating incomplete cellular destruction were exhibited by neoplastic cells along with the presence of dilatation of capillary vessels [3,8]. Dreyfuss et al. in 1992 found that there was correlation between the presence of distant metastasis and elevated LDH and carcino embryonic antigen levels [7]. LDH levels showed increase from well differentiated to moderately differentiated to further increase in poorly differentiated oral squamous cell carcinoma patients in Patel and Metgud's study in 2018 [5]. Further interventional studies have to be carried out to find out the correlation existing between increase in salivary LDH level and histopathological gradings of oral cancer.

5. CONCLUSION

Our study shows significant increase in salivary LDH level in patients with oral cancer and potentially malignant diseases compared to normal healthy individuals. This concludes that salivary LDH estimation can be used as a reliable, non invasive, cost effective and friendly new tool for diagnosis of oral potentially malignant and malignant diseases in early stage itself.

CONSENT AND ETHICAL APPROVAL

Institutional Ethical Committee approval was obtained for the study. Written informed consent was obtained from the willing patients after explaining in detail the purpose of the study in their own language.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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