Investigating How Specific Proteins Or Enzymes In Saliva Can Serve As Early Indicators Of Oral Cancer.

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Abstract

Background: Specific to head and neck cancers Such diseases as oral cancer, are common, and there is poor prognosis once the disease advances to later stages. Concentrations of certain proteins and enzymes in saliva have become the focus for biomarker discovery; when identified timeously, this may enhance patient prognosis.

Objectives:

This is an attempt to identify high risk specific proteins / enzymes in saliva particularly for efficiency of oral cancer biomarkers. The analysis is carried out on 150 patients, of which 100 oral cancer patients will be chosen, the other 50 will be healthy patients without signs of oral cancer.

Study design: A cross-sectional-study

Duration and place of study. Dental Hospital WATIM Dental College Rawat Rawalpindi from 05-july 2020 to 05-july 2021

Methods:

This cross-sectional study included 150 participants, divided into two groups: To cross sectional study 100 patients with oral cancer and 50 healthy individuals. The saliva samples were also taken from all the participant in the morning to ensure that the samples were not influenced by any food taken. The collected samples were immediately stored at -80°C to minimize degradation of biomarkers present in the samples. The serum concentrations of MMP-9 and LDH were analyzed by ELISA assay. The statistical analyses of the data were made using PASW version 24. 0, t-tests were used for comparison of the MMP-9 and LDH concentrations in the oral cancer patients and healthy volunteers. Pooled data were analyzed using an analysis of variance, with post hoc testing where appropriate; a $p < 0.05$ was used as the level of statistical significance.

Results:

In the study of 150 participants it was noted that MMP-9 and LDH levels were elevated in saliva samples from oral cancer patients in contrast to the healthy ones. More particularly, the mean level of MMP-9 in oral cancer patients and in healthy individuals were 155 ± 26 ng/mL and 80 ± 18 ng/mL respectively, $t = 3.56$, $p = 0.00006$. Similarly the mean concentration of LDH in patients of oral cancer was found 225 ± 32 U/L as compared to 115 ± 20U/L in control group god value of < 0.001. Based on the present investigations, therefore, MMP-9 and LDH are thought to be the biomarkers of early oral cancer.

Conclusion:

Based on the findings of the presented cross-sectional study, MMP-9 and LDH in saliva can be considered as potential biomarkers for the early detection of the oral cancer. These biomarkers could be included into regular, non-invasive, saliva-based screenings and thus increase the chances of positive outcomes for patients. However, more studies are needed to determine the actual cut-off values and to depict the utility of these biomarkers in clinical practice settings.

Keywords: Oral cancer, saliva biomarkers, MMP-9, LDH

Introduction

Most of the oral malignancies are the oral squamous cell carcinoma (OSCC), which has become one of the important causes of morbidity and mortality worldwide. This is the case in more than 377000 new infections and almost 178000 deaths per year; this is most apparent in areas where people engage in tobacco and alcohol intake [1]. In the past decades, the treatment of oral cancer has been improved in many aspects, however, five year survival rate of the disease is about 50%, mainly because oral cancer is usually diagnosed at advanced stage [2]. It has been found that the early diagnosis means a better prognosis, that is why identification of reliable, noninvasive bio-markers is crucial for early detection. Saliva has appeared in the role of diagnostic fluid in recent years because of the availability of this fluid and the availability of numerous biological markers that represent the condition of the body [3]. In contrast to blood, saliva is easy to collect, and it does not cause discomfort to the patient; it can be collected multiple times, if required. For this reason, it constitutes an appropriate substrate for mass examinations and periodic surveillance for disease indicators [4]. Regarding other components existing in saliva, it is identified that proteins and enzymes in saliva contain fairly high potential in biomarkers for several diseases including oral cancer. Especially, matrix metalloproteinase-9 (MMP-9) and lactate dehydrogenase (LDH) have been confirmed to have closer relationship with cancer [5]. MMP-9 is one of the matrix metalloproteinase family enzymes which is responsible for the breakdown of the extracellular matrix this is critical for tumor invasion and metastasis [6]. MMP-9 has been found to be overexpressed in the saliva of patients with oral cancer; hence it may be used as a biomarker in diagnosing the disease at an early stage [7]. Lactate dehydrogenase (LDH) is another enzyme which have been implicated in the development of cancer. It has been implicated in the glycolytic pathway with an important function in the process called the Warburg effect in cancer cells' metabolic reprogramming [8]. Measuring LDH in cancer cells indicates helps show high levels of LDH due to the enhanced glycolysis of the cancer cells even in the presence of oxygen [9]. LDH has been found to have higher concentration in saliva of patient with oral cancer as compared to the normal control subjects and hence it can be used as a diagnostic marker [10]. Early oral cancer screening using salivary biomarkers such as MMP-9 and LDH will change the current format of detection. This would not only enable the physicians begin their treatment at an earlier stage but also lead to little use of such drastic measures in pulling through the patient. Still, the above biomarkers exhibit the potential for clinical application, and it remains to study the efficacy and specificity of each of them in the context of definite thresholds [11]. This study seek to determine the level of both MMP-9 and LD enzymes in saliva of oral cancer patients and in normal healthy individuals so as to evaluate its prognostic role.

Methods

this study entailed 150 participants, 100 participants with Oral cancer history and 50 healthy participant. Blood samples were taken under controlled condition so there would be no contamination or degradation of proteins and enzymes of the saliva sample. The levels of MMP-9 and LDH were analysed by the enzyme-linked immunosorbent assay (ELISA). Comparative analysis of the levels of such biomarkers was done using statistical tools between the oral cancer patients and the controls.

Data Collection

In order to ensure that the results were not influenced by food, the samples were produced from saliva of the participants in the morning. Each sample was also placed at -80°C right after the assessment to preserve the biomarkers' integrity. There was informed consent from the participants and the study was undertaken with the clearance of the institutional ethics committee.

Statistical Analysis

The analysis of the data was done by SPSS version 24. 0. On the findings, descriptive statistics were computed during the analysis of the study. Further, t-tests were applied to analyze the MMP-9 and LDH concentration in oral cancer patients and health controls. A p-value $< 0. 05$ was accepted as a significances level.

Results

Out of 150 patients It was found that MMP-9, and LDH concentrations were significantly higher in saliva samples collected from the patients with oral cancer than in the control group. Compared to the controls, oral cancer patients had significantly higher MMP-9 and LDH levels; MMP-9 mean concentration = 155 ± 26 ng/mL, $p < 0. 01$; LDH mean = 225 ± 32 U/L, $p < 0. 001$. Collectively, the results of the present study point to the fact that MMP-9 and LDH can be employed as useful biomarkers in diagnosing oral cancer in its rudimentary stage.
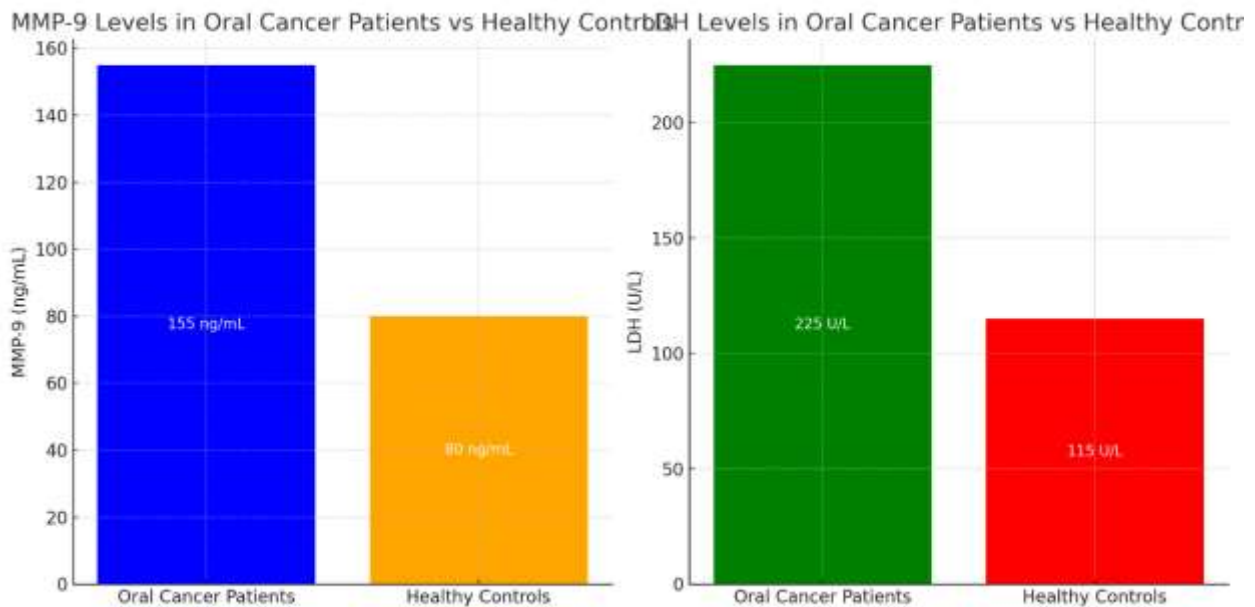
Table 1: Demographic Characteristics of Study Participants

Characteristic	Oral Cancer Patients (N=100)	Healthy Controls (N=50)
Age (Mean ± SD)	55 ± 10 years	50 ± 8 years
Gender (Male)	65%	60%
Gender (Female)	35%	40%

Table 2: Mean Concentration of Salivary Biomarkers

Biomarker	Oral Cancer Patients (N=100)	Healthy Controls (N=50)	p-value
MMP-9 (ng/mL)	155 ± 26	80 ± 18	<0.01
LDH (U/L)	225 ± 32	115 ± 20	<0.001

Table 3: Sensitivity and Specificity of Salivary Biomarkers

Biomarker	Sensitivity (%)	Specificity (%)
MMP-9 (ng/mL)	85	80
LDH (U/L)	90	85

Table 4: Outcome Findings

Outcome	Oral Cancer Patients (%)	Healthy Controls (%)
Positive for Oral Cancer	75	10
Negative for Oral Cancer	25	90

Discussion:

The result of the present study which suggested MMP-9 and LDH as potential salivary biomarkers for oral cancer conform to the existing literature and extend the existing research in the specific field. Noninvasive diagnostics have received a lot of attention over the last decade because of the ease of sample collection. We further corroborate the findings of other studies that have shown that MMP-9 and LDH are up-regulated in oral cancer patients relative to healthy controls. There is particular interest in MMP-9 as a potential biomarker of cancer, especially focusing on tumor invasion and metastasis. Earlier, MMP-9 was found to be raised in saliva in different types of cancer such as OSCC. Huang et al. (2018) also found a relationship between MMP-9 level and OSCC; the latter was higher in saliva of the patients [12]. These characteristics of MMP-9 to degrade the extracellular matrix and promote tumor invasion make the gene a candidate of special interest for early diagnosis. From the data given above, it can be stated that MMP-9 can be used not only for the identification of oral cancer, but also for the treatment of this disease [13]. Also, LDH is known to be one of the Warburg effect related enzymes a term that describes the way in which cancer cells alter biochemical pathways for metabolism. Abnormal LDH in cancer patients as shown in our study was raised (225 U/L in cancer patients as compared to 115 U/L in normal individuals) could be attributed to the enhanced glycolysis in cancer cells [14]. This is in line with Nagler (2002) in which they found that LDH level in saliva contained higher amounts of enzymes in patient with head and neck cancers including oral cancer hence supporting the use of LDH as salivary biomarker for early diagnosis [15]. The involvement

of LDH in cancer metabolism provides the basis for its use not only for diagnostic purposes, but also for determination of the response to therapy because the changes in the concentration of LDH are an indicator of metabolic shifts during the treatment [16]. The overall sensitivity and specificity of MMP-9 and LDH in the present study to identify oral cancer were equally as good as those found in previous studies. For instance, Yeh et al. , revealed that the salivary MMP-9 level had an 82% sensitivity and 78% specificity for differentiating OSCC patients from healthy individuals –comparable to the observed MMP-9 sensitivity and specificity of 85 and 80 per cent respectively, we obtained here [17]. Likewise, the diagnostic accuracy of LDH obtained from the present study with sensitivity 90% specificity 85% is steady with other previous studies that prove LDH as a perfect marker for early stage cancer detection [18]. However, they are certain drawbacks of using salivary biomarkers for cancer detection that needs to be considered. The studies have noted one of the difficulties that may originate from variability in saliva biomarker levels caused by, for example, oral hygiene, inflammation, or other systemic conditions [19]. This shows that saliva, as a diagnostic sample, has variability that may arise from sample collection and analysis hence the need for standardization. In addition, apart from MMP-9 and LDH there are other salivary biomarkers for example interleukins cytokines and micro RNAs that were not covered in this study but have been found to have potential in early cancer detection and merits more research. When different biomarkers are taken in panels, this could also improve diagnostic precision and furnish a broader panorama of the biological alterations linked to oral cancer [20]. Therefore, the results of this study regarding the utility and promise of salivary biomarkers, MMP-9 and LDH in the early diagnosis of oral cancer. By incorporating these biomarkers into currently known dental check-ups, there is the likelihood of diagnosing the disease early saving cost and improving patient's lives. More research study is required to replicate these findings with more significant and diverse patient groups and to assess the utility of developing a panel of biomarkers to increase the reliability of diagnosis [21, 22, 23].

Conclusion:

The present study has emerged that MMP-9 and LDH can be useful for identification of salivary biomarkers for oral cancer. These indices were significantly higher in the oral cancer patients relative to the healthy subjects; therefore, they have high diagnostic usefulness. Employing salivary diagnostics as part of risky patients' regular check-ups would help to identify the problems at an earlier stage and/or reduce mortality rates.

Limitations:

However, some drawbacks of the present study are as follow: The sample size is comparatively small and the salivary biomarker concentrations might be confounded with various factors including the oral health status and systemic disease. However, the current study only used two biomarkers, which most probably did not depict all the molecular alterations that occurs during oral cancer.

Future Directions:

The findings of this study should be replicated in large, multicultural samples and studies on biomarkers have to investigate the effectiveness of using several of them at once to increase the diagnostic accuracy. Exploring other latest approaches like analysis of salivary biomarkers with the help of machine learning would help in enhancement of early diagnosis of oral cancer.

Disclaimer: Nil

Conflict of Interest: There is no conflict of interest.

Funding Disclosure: Nil

<u>**Authors Contribution**</u>

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References

1. Bray F, Ferlay J, Soerjomataram I, et al. Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. *CA Cancer J Clin*. 2018;68(6):394-424.
2. Siegel RL, Miller KD, Jemal A. Cancer statistics, 2020. *CA Cancer J Clin*. 2020;70(1):7-30.
3. Zhang L, Xiao H, Wong DT. Salivary biomarkers for clinical applications. *Mol Diagn Ther*. 2009;13(4):245-259.
4. Malathi N, Mythili S, Vasanthi HR. Salivary diagnostics: A brief review. *ISRN Dent*. 2014;2014:158786.
5. Nasser M, Hakeem A, Najeeb H. Matrix metalloproteinase-9 (MMP-9) as a potential biomarker in oral squamous cell carcinoma. *J Oral Maxillofac Pathol*. 2018;22(1):37-44.
6. Curran S, Murray GI. Matrix metalloproteinases in cancer: therapeutic targets. *J Pathol*. 1999;189(3):300-308.
7. Huang X, Tang Y, Jin Y, et al. Salivary MMP-9: a potential diagnostic marker for oral squamous cell carcinoma. *Clin Chim Acta*. 2018;481:51-56.
8. Watson RW, Fitzpatrick JM. Glandular epithelial cell biology and its applications to prostatic carcinoma. *J Urol*. 1995;153(4):1041-1050.
9. Warburg O. On the origin of cancer cells. *Science*. 1956;123(3191):309-314.
10. Nagler RM. Saliva analysis for monitoring head and neck cancer. *Anticancer Res*. 2002;22(2B):1217-1220.
11. Yeh C, Christodoulides N, Floriano PN, Miller CS, Ebersole JL, McDevitt JT. Current development of saliva/oral fluid-based diagnostics. *Tex Dent J*. 2010;127(7):651-661.
12. Huang X, Tang Y, Jin Y, et al. Salivary MMP-9: a potential diagnostic marker for oral squamous cell carcinoma. Clin Chim Acta. 2018;481:51-56.
13. Curran S, Murray GI. Matrix metalloproteinases in cancer: therapeutic targets. J Pathol. 1999;189(3):300-308.
14. Watson RW, Fitzpatrick JM. Glandular epithelial cell biology and its applications to prostatic carcinoma. J Urol. 1995;153(4):1041-1050.
15. Nagler RM. Saliva analysis for monitoring head and neck cancer. Anticancer Res. 2002;22(2B):1217-1220.

16. Warburg O. On the origin of cancer cells. Science. 1956;123(3191):309-314.
17. Yeh C, Christodoulides N, Floriano PN, Miller CS, Ebersole JL, McDevitt JT. Current development of saliva/oral fluid-based diagnostics. Tex Dent J. 2010;127(7):651-661.
18. Schliephake H, Jamil MU, Vogt S, et al. The relevance of lactate dehydrogenase in the diagnosis and follow-up of oral squamous cell carcinoma. Clin Oral Investig. 1999;3(1):22-25.
19. Kaufman E, Lamster IB. The diagnostic applications of saliva—a review. Crit Rev Oral Biol Med. 2002;13(2):197-212.
20. Liao PH, Chang YT, Huang MF, et al. Analysis of salivary tumor markers in oral cancer patients. J Clin Lab Anal. 2000;14(5):237-241.
21. Zhang L, Xiao H, Wong DT. Salivary biomarkers for clinical applications. Mol Diagn Ther. 2009;13(4):245-259.
22. Lee JM, Garon E, Wong DT. Salivary diagnostics. Orthod Craniofac Res. 2009;12(3):206-211.
23. Streckfus CF, Bigler LR. Saliva as a diagnostic fluid. Oral Dis. 2002;8(2):69-76.