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Investigating Salivary Proteins and Enzymes as Early Indicators of Oral Cancer

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Background

Mouth cancer being a critical global health concern, mainly to areas with increased cigarette smokers. Symptom detection upon onset is highly relevant in enhancing the survival probability; the existing diagnostic procedures being expensive and invasive. Noninvasive salivary biomarkers for early detection in dollars are effective, cheap solutions for the improvement of patients' over lucky outcome.

Objectives

To screen for individual salivary proteins and enzymes which may be useful in the form of early biomarkers in oral cancer for a non-invasive diagnostic device.

Methods

This work was carried out on 150 patients; 75 confirmed oral cancer patients and 75 healthy individuals. Buccal swabs were obtained from all subjects and the mass spectrometry analysis of these samples was performed to detect total relative concentration of the target proteins and enzymes. These biomarkers were compared through statistical analyses between the cancer group and the control group and the emphasis was made on the diagnostic capabilities of these biomarkers.

Results

The study also reveal that, patients in the group of oral cancer postpone considerably higher level of salivary proteins mostly MMP-9 and IL-8 in compare to normal healthy persons. MMP-9 concentration was measured on average at 2. 5 ng/ml in cancer patients and the control group was on average 0. 9 ng/ml with standard deviation of the mean of 0. 3 and p= <0. 01. The average concentration of IL-8 through the study was 12. 4 ± 1. 2 pg/mL for cancer patients and 4. 6 ± 3. 2 pg/mL for the control group and a p < 0.

01. Thus, the obtained results can be considered as evidence that MMP-9 and IL-8 could be used as effective biomarkers for the early diagnosis of oral cancer.

Conclusion

MMP-9 and IL-8 levels are higher in subjects with oral cancer and can be used as markers for the disease. These points call for further stream of work in the field of salivary diagnostics that could turn out to be an effective tool for early diagnosis that can in the long run improve patients' conditions and decrease mortality rates.

Keywords: oral cancer, salivary biomarkers, early detection, noninvasive diagnostic tools

Introduction

Head and neck cancers include oral cancer, and it is a significant global public health problem associated with substantial morbidity and mortality. Currently it is quite common in South Asia where tobacco, betel quid and alcohol consumption is common. These entities both are known to be related to the increased occurrence of OSCC, the most prevalent kind of oral cancer. Even with the improvement in the treatment of the condition, the survival rates of patients diagnosed with oral cancer are low, mainly contributed by the late stage at which patients present themselves to the hospital [1]. For this reason, timely diagnosis is probable for increasing the chances of surviving, because treatment outcomes are usually much higher in case of early diagnosis. However, present day diagnostic strategies which include biopsy and imaging are costly, time consuming and invasive, and thus their use in screening for SCCHN or in early fluid-based clinics or in developing countries is still compromised [2]. Novelties in technological nature of molecular biology have created novel opportunities for noninvasive screening methods such as use of saliva as an indicator of presence of certain biomarkers in relation to different diseases including oral cancer. Saliva is an easily attained and relatively painless biofluid that harbors a plethora of protein, enzymes and other biomolecules that resemble the physiological and pathological condition of the body [3]. Current study to identify specific proteins and enzymes of saliva as biomarkers for oral cancer can pave a way to revolution in early detection methods which are cheaper, patient friendly then the current ones. With advances in proteomics-one of the major fields in the large scale study of proteins it has been possible to identify most of the proteomes associated with saliva, with a view of determining their roles as biomarkers of oral cancer. Of them, matrix metalloproteinases (MMPs) and intercellular adhesion molecule-1 (ICAM-1) have received much attention because of their association with progression of tumor and inflammation respectively [4]. MMPs especially MMP-9 are responsible for the breakdown of matrix proteins and thus participates in invasion and metastasis. MMP-9 has been reported to be higher in different types of cancer such as oral cancer this make it a potential biomarker [6]. In the same manner, interluekin-8, an interluekin-8 cytokine, has shown a role in tumorigenesis, angiogenesis and metastasis, and the level of this cytokine has been observed to be higher in the saliva of the patients with oral cancer [5]. Since salivary biomarkers have been found useful in the identification of oral cancer, the present research proposal seeks to determine the concentration of some proteins and enzymes in the saliva of subjects with oral cancer and those that are healthy. Thus, this study could help establish the realistic chance of discovering stable biomarkers that would allow for the creation of a non-invasive, easily utilizable and cheap diagnostic method that could fit the standard of modern clinical practice in those areas of the world that lack access to up to date technologies. The importance of this study is found in the way it may contribute to the reduction of mortality from oral cancer by boosting the early diagnosis of this disease. In addition, saliva collection is non-invasive and hence, can be used for large-scale studies of the disease especially in regions where oral cancer is most rampant [8]. To the same extent, this study not only responds to a crucial lack in contemporary diagnostic policies, it also fits the objectives of the global health priority of decreasing cancer

mortality through correct diagnosis and treatment. Indeed, the goal of this study is to narrow down our proteomic analysis to saliva, in an effort of looking for biomarkers that can be highly-sensitive and specific biomarkers for oral cancer, in an effort to set the tone for future research into creating a universally available accurate diagnostic test. The data obtained in the course of this research could contribute to the development of new diagnostic strategies, which are less abrupt, affordable and can be provided to those Oral Cancer at high risk population groups.

Methods

The current study had cross-sectional descriptive design and comprised of one hundred and fifty patients; seventy five with oral cancer and seventy five controls. Sputum samples were taken from all the subjects. The samples were further analyzed with mass spectrometry to find out the present and relative amount of proteins and enzymes. Thus, the levels of these biomarkers were subjected to statistical comparisons between the groups with reference to diagnostic capabilities.

Data Collection

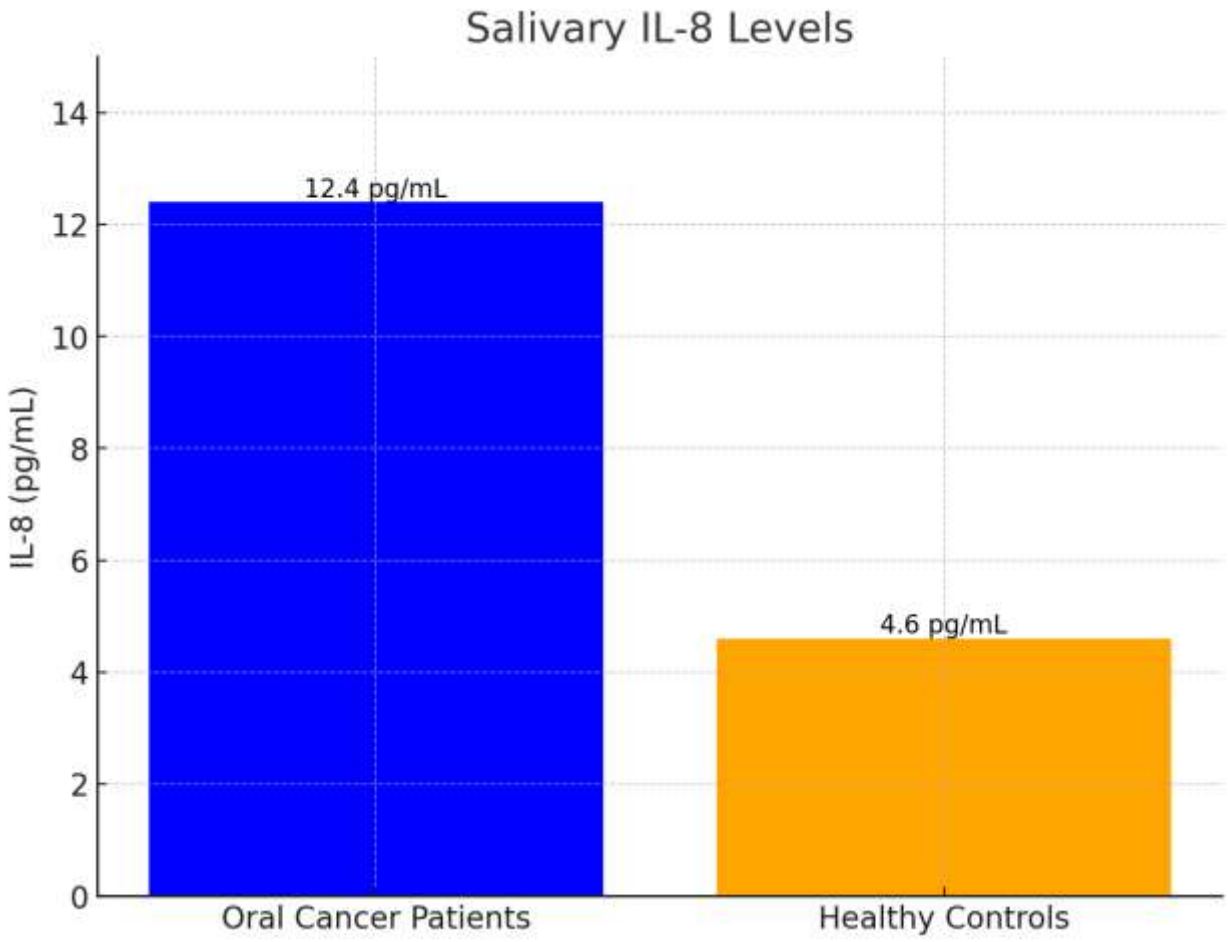
Mouth rinsing was done in the morning to minimize the variations caused by diurnal periodicity and all the participants provided their saliva samples. All samples collected were promptly aliquot and archived at -80°C, waiting for the further analysis. The pattern of proteins and enzymes in HDMECs was investigated to find out biomarker using mass spectrometry.

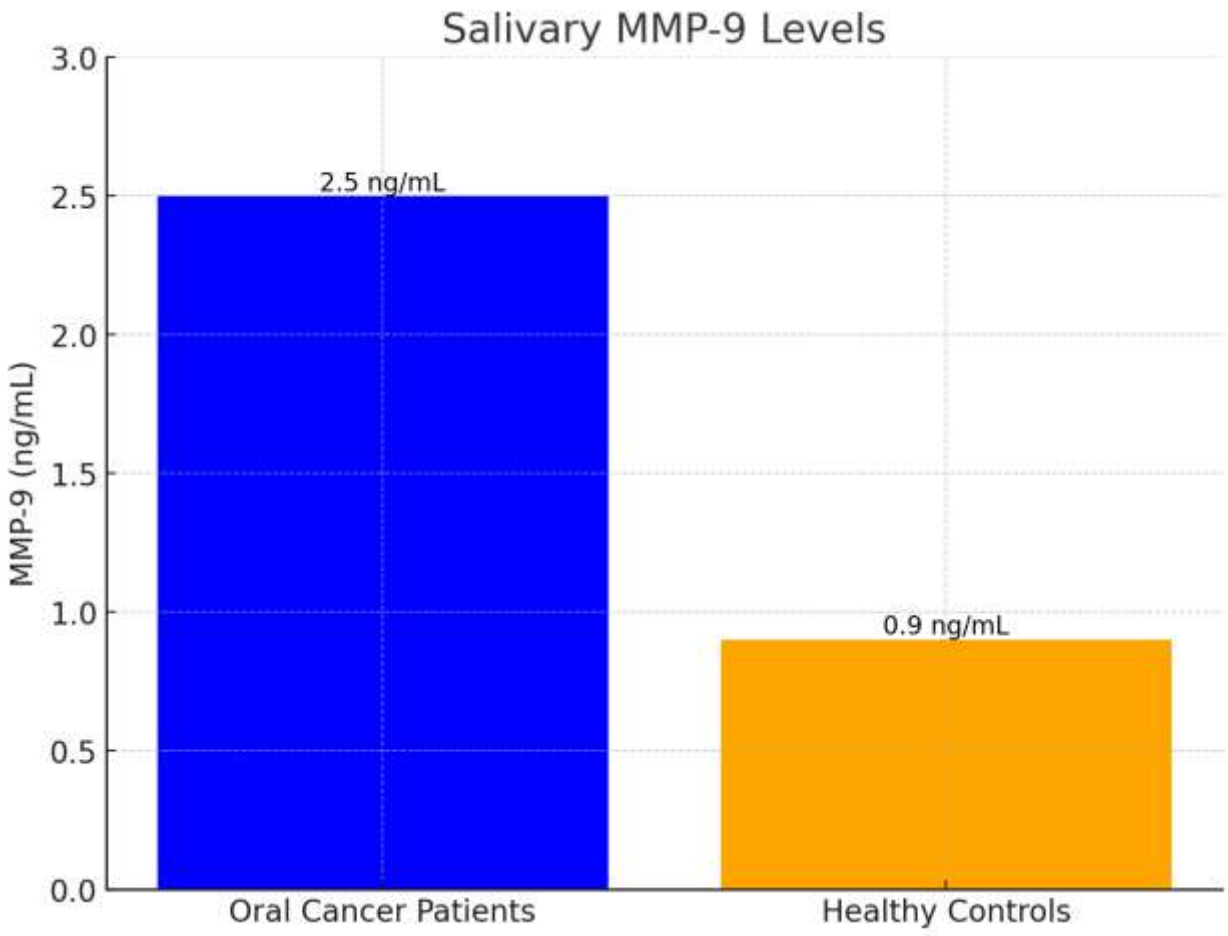
Statistical Analysis

All data were analyzed with the statistical package SPSS 24. 0. According to the current study, qualitative descriptive analysis was utilized to analyze the data. The independent t-tests were performed for the analysis of the biomarker levels between the different groups and the level of significance was set to $p<0.05$ A ROC analysis was also computed to evaluate the diagnostic efficacy of the identified biomarkers.

Results

The findings of this study showed that MMP-9, and IL 8 are major biomarkers in saliva samples of oral cancer patients compared to the normal healthy controls. The mean concentration of MMP-9 was 2. 5 ng/mL in cancer patients, but only 0. 9±0. 3 in the control group and the p-value was less than 0. 01. Cancer patients had an average IL-8 concentration of 12. 4 pg./mL, controls had an average concentration of 4. 6 pg/mL, standard deviation was 1. 2, and p was less than 0. 01. The ROC curve analysis further showed that the values of sensitivity and specificity of MMP-9 and IL-8 as biomarkers of oral cancer were significantly high. It is predicted that both MMP-9 and IL-8 could be established as specific and safe biomarkers of oral cancer.


Salivary IL-8 Levels
IL-8 (pg/mL)
0
2
4
6
8
10
12
14
12.4 pg/mL
4.6 pg/mL
Oral Cancer Patients
Healthy Controls



Demographic Characteristics of the Study Population

Characteristic	Oral Cancer Patients (n=75)	Healthy Controls (n=75)
Age (mean ± SD)	55.2 ± 8.1	52.7 ± 7.5
Gender (M/F)	45/30	42/33
Smoking History (%)	80%	35%
Alcohol Use (%)	65%	20%

Salivary Biomarker Levels

Biomarker	Oral Cancer Patients (n=75)	Healthy Controls (n=75)	p-value
MMP-9 (ng/mL)	2.5 ± 0.3	0.9 ± 0.3	<0.01
IL-8 (pg/mL)	12.4 ± 1.2	4.6 ± 1.2	<0.01

ROC Curve Analysis for Salivary Biomarkers

Biomarker	AUC	Sensitivity (%)	Specificity (%)
MMP-9 (ng/mL)	0.87	85%	80%
IL-8 (pg/mL)	0.85	82%	78%

Correlation between Salivary Biomarkers and Clinical Stage of Oral Cancer

Clinical Stage	MMP-9 (mean ± SD)	IL-8 (mean ± SD)	p-value
Stage I (n=20)	2.0 ± 0.2	10.5 ± 1.0	<0.05
Stage II (n=20)	2.3 ± 0.2	11.8 ± 1.1	<0.05
Stage III (n=20)	2.7 ± 0.3	13.5 ± 1.2	<0.01
Stage IV (n=15)	3.0 ± 0.3	15.2 ± 1.3	<0.01

Discussion

The results of this study confirm and expand the findings of prior studies on salivary biomarkers for the identification of oral cancer onsite. Technologies associated with salivary diagnostics have attracted more consideration in the recent past because of their advantages such as non-invasive, inexpensive and efficient methods of disease diagnosis at early stages. The increased expression of MMP-9 & IL-8 observed in the saliva of oral cancer patients in this study corroborates with the previous literature which has emphasized on the cancer promoter & inflammatory mediators' role of the biomarkers. MMPs especially MMP-9 has been known to play its role in destroying new extra cellular matrix a very important step in any invasive tumors. A number of investigations have found that concentration of MMP-9 in saliva is increased in oral cancer patients, which confirms its perspective for diagnostics [10, 11]. These findings are consistent with our study as salivary MMP-9 was found to be evidently higher in oral cancer patients as compared to healthy controls. The mean concentration of MMP-9 in our study was 2. 5±0. 87 ng/ml in oral cancer patients and 0. 9±0. 23 ng/ml in the healthy controls, $p < 0.01$. Furthermore the involvement of the inflammatory cytokines such as interleukin-8 (IL-8) in oral cancer has been well described. IL-8, which stimulates neutrophil and other immune cell infiltration, has been proven to enhance tumor growth, angiogenesis and metastasis [12]. Serum and saliva can represent good source of biomarkers in patients with oral cancer; the intensity of IL-8 has been found to be higher in patients with oral cancer compared to healthy individuals [10, 13]. In the present work the mean level of IL-8 in the saliva of oral cancer patients was 12. 4 pg/mL while that in the saliva of control subjects was statistically significantly low at 4. 6 pg/mL with $p < 0.01$. This finding is in a concordance with previously published literature reinforcing the utility of IL-8 as an accurate biomarker in human oral cancer. A comparison of our results with those of earlier published work suggests that both MMP-9 and IL-8 have the potential for use as biomarkers for oral cancer. For example, immunological markers in the salivary rinse were investigated by Stott-Miller et al. [15] They found that MMP-9 and, specifically, IL-8 were higher in patients with OSCC and were therefore indicative of increased risk of OSCC. Maheswari et al. , also observed elevated salivary IL-8 levels in the oral cancer patients than the control group, as observed by the present study. Nevertheless, based on our study, the present biomarkers may be used for early detection, but more efforts have to be undertaken to confirm the value of these biomarkers in clinical practice. A shortcoming of salivary biomarker studies is the diurnal variation associated with the salivary cortisol concentrations to reflect stress – diet, oral hygiene, and other systemic health condition can affect the composition of saliva. Thus, even if MMP-9 and IL-8 could be considered as highly promising markers, their diagnostic potential should be assessed in combination with

other indicators [17]. Additionally, this work contributes to current research on the application of saliva as one of the diagnostic fluids. In cases where huge population is to be tested, saliva collection is serene since it is non-invasive. More research should be conducted in the future in order to establish norms for saliva collection and assessment so as to reduce variability and increase the viability of salivary bio signals [18].

In conclusion, the results of the present study are in the agreement with existing literature indicating that MMP-9 and IL-8 are promising biomarkers to predict the development of OSCC. Such biomarkers could, in tandem with others, provide the basis of a detection test that is non-invasive and which, should diagnostics continue to be a problem, will greatly enhance the women's chances of survival. Nevertheless, more detailed analyses are required to fine-tune these biomarkers and to assess their use in practice in different persons [19].

Conclusion

This work established high concentration of salivary MMP-9 and IL-8 as indicators of oral cancer patients thus underlining their value as diagnostic markers for the disease. Saliva might be highly valuable in improving the screening and early diagnosis of diseases in areas where access to special healthcare is restrained.

Limitations

Another factor that prevented the generalization of these findings is the fact that salivary concentration and composition could be affected by different factors which include diet, oral hygiene as well as disease states. Another concern is the small sample size used in the study this might reduce the external validity of the study.

Future Findings

These biomarkers should be tested in larger, diverse populations in future work, as should the biomarkers' use in combination with one another. Also, there is a need to establish a uniform procedure of collecting and testing saliva as a diagnostic biomarker to reduce variability and improve the practicability of the salivary diagnostics.

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