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ORIGINAL ARTICLE

Evaluation of salivary biomarkers for early detection of oral cell carcinoma.

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ABSTRACT… Objective: To do a comparative analysis of salivary levels LDH, amylase, total protein, CYFRA 21-1 and CA19-9 in oral SCC, PML and healthy individuals. **Study Design:** Retrospective study. **Setting:** Department of Oncology, Women Medical College Abbottabad. **Period:** January 2021 to January 2022. **Material & Methods:** In a total of 200 patients. Study participants were divided into three groups. Group I (n=90) had cases diagnosed with oral SCC, Group II (n=60) cases with PML and Group III (n=50) had healthy controls. Saliva samples of patients with PML and SCC were collected before they were administered definitive therapy. ELISA was used for evaluation of CA19-9 and CYFRA 21-1, while standard kit method was used for evaluation of amylase, LDH and total protein level. **Results:** There was statistically significant association between oral lesions and alcohol (P=.0005), smoking (P=.0001) and tobacco chewing (P=.0005). In Group I, most common site of lesion was buccal mucosa (47%), while in Group II tongue (35%) was most commonly affected. 33.3% patients with SCC had lymphadenopathy. Level of total proteins, LDH and CYFRA 21-1 were higher, while amylase was lower in Group I and II compared to Group III. Level of CA19-9 was not significantly different in three study groups. ROC analysis showed that CYFRA21-1 had greater chance of identifying oral malignancies (P=.0001). **Conclusion:** The outcome shows that the concurrent evaluation of salivary biomarkers can help in early detection of malignancy.

Key words: Alcohol, Oral Squamous Cell Carcinoma, Pre Malignant Lesions, Salivary Biomarkers, Tobacco Smoking.

INTRODUCTION

Oral cancer is among the most prevalent carcinomas world wide.[1,2] In subcontinent, it is among the top three most prevalent types of cancers.[3] About 90% cases of oral malignancies are squamous cell carcinomas (SCC). In 70% cases of oral carcinoma there is a history of differentiated pre malignant lesion (PML). Early intervention can reduce progression of the lesion.[4]

Despite advancement in the treatment modalities, prognosis of oral cancer remains worse with 65% 5 year survival rate.[4] The poor survival is mainly associated with late diagnosis and failed treatment. Determination of tumor marker of SCC can help in early diagnosis and appropriate treatment. Recent studies reported that saliva has been increasingly used as diagnostic medium for oral cancers, and salivary biomarkers have re in detection of oral SCC.[4-6] Saliva is not only readily available, but can also be collected easily. CYFRA 21-1[7] is soluble fragment of cytokeratin 19 and is novel SCC marker. Lactate dehydrogenase (LDH), amylase, total protein and CA19-9 are also significantly deranged in SCC and can be used to differentiate it from healthy and premalignant patients.[8,9] The aim of the current study is do comparative analysis of salivary levels LDH, amylase, total protein, CYFRA 21-1 and CA19-9 in oral SCC, PML and healthy individuals.

MATERIAL & METHODS

The retrospective study was conducted in Oncology Department, Women Medical College Abbottabad from January 2021 to January 2022. The patients aged above 18 years who were diagnosed with oral SCC and PML were included in the study. Patients with chronic inflammatory diseases, acquired immune-deficiency, autoimmune disorders and those with history of

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head and neck cancer and radiation exposure were excluded. The study was conducted in total 200 patients. Informed consent of the participants was taken. Ethical board of the hospital approved the study (Ref.No.14/203).

Study participants were divided into three groups. Group I (n=90) had cases diagnosed with oral SCC, Group II (n=60) cases with PML and Group III (n=50) had healthy controls. Detailed history including socioeconomic status, occupation, smoking, alcohol and tobacco addiction, oral hygiene and use of dentures was taken. A thorough clinical examination of lymph nodes and oral cavity was done and status of oral hygiene and PML was recorded. Metastasis classification was used to ascertain stage of the disease.

Saliva samples of patients with PML and SCC were collected before they were administered definitive therapy. Whole saliva from the study and control group was collected between 10 am- 12 pm, 2 hours after the breakfast, so that diurnal variations would minimally affect salivary flow and composition. Samples were centrifuged for removing squamous cells. Supernatant was biochemically analyzed. Saliva samples were stored at −80°C before analysis. ELISA was used for evaluation of CA19-9 and CYFRA 21-1, while standard kit method was used for evaluation of amylase, LDH and total protein level SPSS version 23.0 was used for data analysis. Chi square test was used for the intergroup comparison of categorical data. Independent t-Test and analysis of variance (ANOVA) was used for inter group comparison of parametric data. Predictive value, sensitivity and specificity of the diagnostic test was evaluated though receiver operator characteristic (ROC) curve. $P < 0.05$ was considered statistically significant.

RESULTS

Of 90 patients with SCC, 83 (92.2%) were male and 7 (7.7%) were female. Mean age of the participants was 49.7 years. Patients with PML also had male predominance (88.3%). Mean age of the participants was 34.3 years. Control group had 45 (90%) male and 5 (10%) females. Mean age was 48.2%.

Majority cases with oral lesions were from lower socio economic status (P=.002). Tobacco smoking and chewing was a major predisposing factor. It had synergistic effect on concurrent alcohol consumption. There was statistically significant association between oral lesions and alcohol (P=.0005), smoking (P=.0001) and tobacco chewing (P=.0005). Poor oral hygiene had a contributing role in development of cancerous lesions. Poor oral hygiene had significant association with SCC and PML (P=.0005).

In Group I, most common site of lesion was buccal mucosa (47%), while in Group II tongue (35%) was most commonly affected. 33.3% patients with SCC had lymphadenopathy. Majority of the patients were at stage I (n=43, 47.7%) followed by stage IV A (n=21, 23.3%), stage II (n=11, 12.2%), stage III (n=12, 13.3%) and stage 0 (n=3, 3.3%).

Histopathologically, 31.1 % SCC lesions were well differentiated, 65.5% moderately differentiated and 3.4% were poorly differentiated. Of 60 patients with PML, 33 (55%) had leukoplakia, 13 (21.6%) had oral submucous fibrosis, 7 (11.6%) had lichen planus and 7 (11.6%) squamous papilloma.

Details of salivary biomarkers in different study groups are summarized in Table-I. Level of total proteins, LDH and CYFRA 21-1 were higher, while amylase was lower in Group I and II compared to Group III. Level of CA19-9 was not significantly different in three study groups.

ROC analysis for CYFRA21-1 revealed sensitivity and specificity to be 90% and 97% respectively for cancerous lesions. Positive predictive value and negative predictive values were 96.5% and 91.6% respectively. ROC analysis showed that CYFRA21-1 had greater chance of identifying oral malignancies (P=.0001).

Bivariate analysis showed statistically significant association among amylase, total proteins, LDH and CYFRA21-1. There was positive correlation between total proteins, LDH and CYFRA21-1,

while amylase was negatively correlated with them. Association between clinical parameters and study variables is shown in Table-II.

Salivary Biomarker	Group I	Group II	Group III	P-Value
Total proteins	193.6 ±58.4	135.8 ±19	95.8 ±18.5	< .0005
Amylase	627.6 ±447.5	1117.9 ±276.5	1285.4 ±298	<.0005
LDH	456.6 ±156.8	276.4± 61	107.7 ±68.5	<.0005
CA 19-9	20.3 ±8.1	18.6 ±3.6	20.6 ±5.7	<.929
CYFRA 21-1	16.7 ±15.6	5.8 ±2.5	3.8 ±2.1	<.0005

Table-I. Mean values of various salivary biomarkers in all study groups

Salivary Biomarker	Tumor Size	Clinical Stage	Status of Lymph Nodes
	(χ2) P	(χ2) P	(χ2) P
Total proteins	10.908 .001	17.376 .0005	9.7 .002
Amylase	10.908 .001	17.376 .0005	9.7 .002
LDH	6.132 .012	11.765 .001	5.5 .020
CA 19-9	8.432 .004	8.28 .004	3.2 .067
CYFRA 21-1	10.908 .001	17.376 .0005	9.7 .002

Table-II. Association between salivary biomarkers and clinical findings (χ2)

DISCUSSION

Oral cancer is the common cause of morbidity and mortality worldwide. Oral cancer occur on the surface and can be recognized at the early stage. The usual etiologic factors include smoking, alcohol consumption and older age (> 50 years).[10] The result of current study also showed that oral lesions were predominant among middle to older aged men addicted to alcohol and tobacco. Mean age of patients with oral cancer was 49.7 years, while for PML was 34.3 years. This major age difference is crucial and early detection of potential premalignant lesion can lead to more effective management. In this study there was significant association between development of oral lesions and consumption of alcohol and tobacco, this was in line with the findings of previous studies.[11,12] Association with alcohol was seen in 96.5% patients, with tobacco smoking in 86.6% and with tobacco and betel nut chewing in 96.6% patients. This percentage was higher than reported by a previous study.[12] Like previous studies, this study showed that tobacco had synergistic effect on concurrent alcohol consumption.[13,14]

Though saliva is not commonly used for diagnosis of oral lesions, many studies have reported that saliva is a preferable diagnostic medium as its readily available, cost effective, non invasive and non complicated procedure.[15,16] In current study, saliva had an excellent diagnostic accuracy for oral lesions. In patients with oral SCC and PML, salivary level of CYFRA 21-1 were 4.5 times higher compared to normal controls. Various studies have reported significance of CYFRA 21-1 in detection of oral lesions, and have reported its increased levels in malignant and pre malignant conditions.[6,17] However, a study reported no association between both at the initial diagnosis; however, it was useful during follow up.[18] Current study found no correlation between CA 19-9 and oral lesions, same are reported by a previous study.[6]

In current study, there was 3.8 folds increase in LDH and 2.1 fold increase in total proteins in SCC, while 0.5 fold decrease in amylase compared to controls. In PML, there was 2.6 folds increase in LDH and 1.5 fold increase in total proteins while 0.7 fold decrease in amylase, these findings are in line with the previous study.[19] There was significant association between salivary biomarkers and clinical stage, lymph node status and tumor size. Higher level of total protein, CYFRA 21-1 and LDH was associated with increased tumor size, lymph nodes and advanced stage of cancer. However, a previous study did no report correlation between CYFRA 21-1 and these parameters.[7]

The limitation of this study is small sample size, a multicenter study is recommended for detailed analysis.

CONCLUSION

Patients with oral squamous cell carcinoma and pre malignant lesions have increased level of salivary total protein, CYFRA 21-1 and LDH and lower level of amylase compared to normal individuals. Fluctuation in salivary biomarkers is more apparent in oral squamous cell carcinoma compared to premalignant lesions. The outcome shows that the concurrent evaluation of salivary biomarkers can help in early detection of malignancy.

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4	Sadia Hassan	Data design and AUthorized.	
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