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Salivary Biomarkers among Groups of Smokers in Relation to Oral Squamous Cell Carcinoma

A Thesis
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Abstract

Back ground: Saliva is not merely a digestive fluid, it may be the key element in the direction of new strategy for non-invasive diagnosis and follow of early oral squamous cell carcinoma. Saliva contains huge amounts of proteins like calcium binding protein (S100 proteins), sCD44, CD44v5, CD 59, profillin-1 and nuclear materials namely microRNA21that can serve this mission.

Clearly is the association between tobacco smoking and oral squamous cell carcinoma (OSCC). How is smoking linked to the above mentioned salivary biomarkers lacks sufficient understanding.

Aims of the study: the aims of this study were to evaluate the role of salivary microRNA21 and salivary proteins; calcium binding protein, profilin-1, sCD44, CD44v5 and CD59 in patients with oral squamous cell carcinoma and to study the association between smoking and the above mentioned salivary markers.

Subjects, Materials and Methods: The present study included 60 patients and control subjects with age ranging from 40-70 years, they were classified into three groups: the first one included 20 smokers patients with oral squamous cell carcinoma(OSCC), while the second group (20 persons) were smokers without OSCC and the last group (20 persons) were non-smokers without OSCC. From each patient and control subjects a sample of stimulated saliva (2 ml) was obtained between 8:00 a.m and 11:00 a.m. 1 ml of stimulated saliva treated with RNase inhibitor, and stored at -80ºC till the time of molecular analysis, while the other 1 ml of stimulated saliva stored at -80ºC without treating with RNase inhibitors. MicroRNA21 was quantified using steam loop real time PCR (Total RNA Extraction Kit AccuZol; Bioneer, Korea). While the salivary proteins were assessed by ELISA method. Data were analyzed using SPSS (Chicago version 20) software and Microsoft Office Excel 2010.

Results: In the present study the Males to Females ratio was (1.5:1). Majority of patients (55%) had lesions located at the lateral border of the tongue. In patients with

oral squamous cell carcinoma, there was no significant association between gender and duration of smoking(P >0.05). MicroRNA21 fold change was significantly highest in smoker patients with carcinoma compared to smoker persons without carcinoma and non-smoker subjects without carcinoma (P<0.001). On the other hand, smoker persons without carcinoma had a significantly higher miRNA21 fold change than non-smoker persons without carcinoma (P<0.001). No statistical significant difference was found in median microRNA 21 fold changes between Males and Females in all groups (P>0.05). there is a positive highly significant correlation between microRNA21 fold change and smoking (P<0.001).

Median Calcium binding protein value was significantly highest in smoker patients in comparison to other groups, P=0.001 and P<0.001, respectively. There is a positive highly significant correlation (P<0.001). No significant difference in median salivary profillin-1 among the three groups (P>0.05), and there is non-significant correlation was found between it and smoking (P>0.05). The median sCD44 level was significantly highest in smoker patients in comparison to other groups (P<0.05). There was no significant difference in mean sCD44 between smoker control subjects and non-smoker control subjects (P>0.05). there is a positive significant correlation was found between smoking and sCD44 (P<0.05).The median CD44v5 level was highest in group 1 (smokers with carcinoma) but there was no significant difference in CD44v5 between smoker patient and smoker control subjects (P>0.05). Median CD44v5 was significantly higher in patients group than that of non-smoker control subjects (P<0.05).There was no significant difference in CD44v5 level between control groups, smoker and non-smoker (P>0.05).A positive significant correlation (P<0.05) was found between smoking and CCD44v5. The median CD59 level was highest in group 1(smokers patients with OSCC). It was not significantly different in smoker patients with carcinoma in comparison with smoker control subjects (P>0.05). There was a significant difference in median CD59 between smoker patients and non-smoker control subjects (P<0.05). CD59 was significantly higher in smoker control subjects than non-smoker control subjects

(*P*<0.05). positive significant correlation was found between CD59 and smoking (*P*<0.05). Statistically no significant association (P>0.05) was found between mean miRNA21 fold change, salivary proteins and the following: age of patients, gender, duration of smoking, number of cigarette per day and site of the lesions.

Conclusion: Salivary microRNA21 and proteins can be used as a non-invasive additive way for diagnosis of suspicious oral lesions in smoker subjects. As a result of this smoker persons advice to quit smoking or reducing the amount of smoking to avoid development of future OSCC. It is also recommended that smokers should have regular clinical check up for early detection of suspicious oral lesions by using these salivary biomarkers.