

A Salivary Calcium Binding Protein in Patients with Oral Squamous Cell Carcinoma in Relation to Smoking

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ABSTRACT

Background: Calcium binding protein regulates many important cellular functions such as cell proliferation, cell motility and differentiation. Over-expression of calcium BP was detected in different human cancers, including oral squamous cell carcinoma (OSCC). Therefore the aim of the present study was to evaluate the role of calcium binding protein in OSCC, quantified in stimulated saliva, and its association with smoking.

Materials and methods: The present study included 20 patients with oral squamous cell carcinoma who used to be smokers, and 40 control subjects. Calcium binding protein was assessed by ELISA technique, in stimulated saliva collected from all groups.

Results: Salivary calcium binding protein was significantly higher in smoker patients with oral squamous cell carcinoma than smoker and non-smoker control healthy looking subjects.

Conclusions: Salivary calcium binding protein play a significant role, as a non-invasive approach, in the early diagnosis and follow up of oral squamous cell carcinoma patients.

Key words: OSCC, calcium binding protein, saliva, smoking. (J Bagh Coll Dentistry 2016; 28(2):149-151).

INTRODUCTION

The chronologic sequence of histologic stages of oral squamous cell carcinoma includes the transition from benign hyperplasia to dysplasia to carcinoma in-situ, and then ultimately followed by invasive squamous cell carcinoma. This phenotypic alteration is the results of multistep genetic mutations and altered gene expression ^(1,2). Tobacco is claimed to be one of the major environmental carcinogenic agents that are responsible for such mutations ^(3,4).

Analysis of saliva proteins, a non-invasive substitute to tissue biopsy, from patients with OSCC is a promising approach for diagnosis, monitoring and therapeutic targeting. The S100 proteins are a multi-gene calcium-binding family, comprising more than 20 different proteins which are encoded by a separate gene and are expressed in a controlled tissue specific or cell type-specific manner ⁽⁷⁾.

They are small, acidic proteins of 10-12 KDa and form the largest family of calcium binding proteins. They regulate many important cellular functions such as cytoskeleton organization, homeostasis, stress response, cell proliferation, cell motility and differentiation ⁽⁸⁾.

Over-expression of calcium protein was detected in different human cancers, presenting increased expression in neoplastic tumor cells as well as infiltrating immune cells ⁽⁹⁻¹²⁾. S100 A9 over-expression was also reported in oral squamous cell carcinoma (OSCC) ⁽¹³⁾.

Thus the aim of the present study was to evaluate the role of calcium binding protein in OSCC, quantified in stimulated saliva, and its association with smoking.

SUBJECTS, MATERIALS AND METHOD

The present study included 20 patients with oral squamous cell carcinoma who used to be smokers. For the purpose of comparison, 20 apparently healthy smokers and another 20 non-smokers were enrolled. Control subjects were chosen in such a way to be matched with age and gender of the patients. Patients age was from 40 to 70 years, included (8) females and (12) males.

The study was conducted in Ghazi Al-Hariri hospital, Al-Yarmook teaching Hospital and Al-Kadhimiya teaching hospital in Baghdad. The study was held from October 2014 through February 2015. From each patient and control subjects a sample of stimulated saliva (400^μL) was obtained between 8:00 am and 11:00 am. Saliva was stored at -80C° till the time of analysis. Calcium binding protein was assessed by ELISA (Elabscience; China)

Statistical analysis was performed using SPSS version 16. Being a non-normally distributed variable, comparison of mean calcium binding concentration among groups was done using the non-parametric Kruskal Wallis test and Mann Whitney U test. P-value was considered significant when it was less than or equal to 0.05 and highly significant when it was less than or equal to 0.001.

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RESULTS

Median values of calcium BP level was significantly higher in smoker patients in comparison to other two groups ($P < 0.001$), as shown in Table 1 and Figure 1. Also there was a significant difference in median values of calcium BP between smoker control subjects

and non-smoker control subjects ($P < 0.05$), as shown in Tables 1 and 2 and Figure 1.

Performing a regression analysis between calcium BP and smoking, represented by study groups, revealed a positive significant correlation, as shown in Figure 2.

Table 1: Descriptive statistics of calcium BP in the three study groups

Groups	N	Median	Mean	S.D.	Min.	Max.
Smoker carcinoma	20	11.943	14.88	5.19	0.58	73.93
Smoker No carcinoma	20	4.897	5.884	3.09	1.36	11.63
Non-smoker No carcinoma	20	2.482	3.384	2.77	0.21	7.79

Kruskal Wallis H test = 20.331, d.f. = 2, p-value = < 0.001

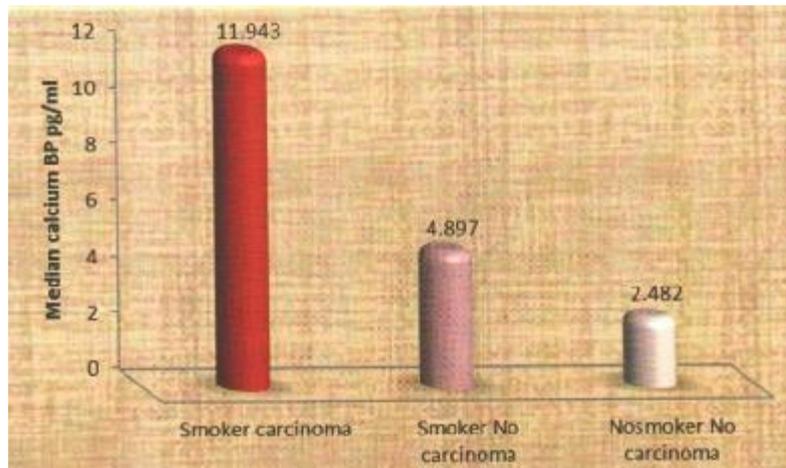
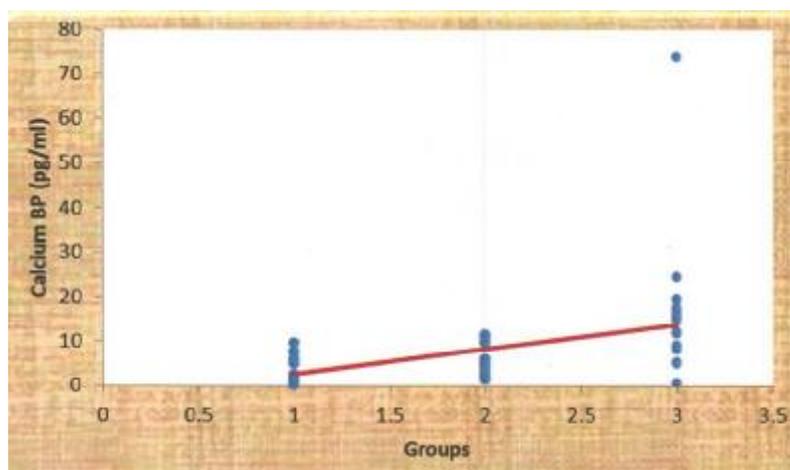


Fig.1: Comparison of Median values of Calcium BP among the three studied groups

Table 2: Statistical differences among the study groups

Groups	Mann-Whitney U test	d.f.	p-value
Group 1 vs. Group 2	77	1	< 0.001
Group 1 vs. Group 3	49	1	< 0.001
Group 2 vs. Group 3	123.5	1	< 0.039



$R=0.464, R^2=0.215, P<0.001$

Fig. 2: Regression analysis between smoking and salivary CB protein level in groups enrolled in the present study (1= non-smoker no carcinoma, 2= smoker no carcinoma, 3=smoker with carcinoma)

DISCUSSION

The results of the present showed an over-expression of salivary calcium binding protein in patients with OSCC in comparison with smoker and non-smoker control subjects with statistically highly significant differences. Over-expression of calcium binding protein in patients with OSCC was shown by several other studies, in agreement with the result of the present study⁽¹⁴⁻¹⁷⁾.

Also several studies showed over-expression of calcium binding protein in smoker patients without carcinoma^(18,19). Tobacco smoking may induce the formation of reactive oxygen species that alter the function of rough endoplasmic reticulum, subsequently increasing the expression of several transcriptional factors that ultimately induces up-regulation of salivary proteomics including calcium binding proteins.

The role of the calcium binding protein in OSCC is not fully understood, nevertheless Andrea Mueller *et al.*⁽²⁰⁾ suggested that the interaction among Calcium, p53 (a tumor suppressor protein) and calcium binding protein will ultimately lead to suppression of p53 and enhancement of cell growth and evasion of apoptosis. Thus salivary calcium binding proteins may have a key role as a non-invasive marker in the diagnosis and follow up of patients with OSCC.

In conclusion, salivary calcium binding protein can be used as a non-invasive alternative method for the diagnosis and monitoring of OSCC.

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