

Significance of Salivary *miRNA 21* Determined by Real Time PCR in Patients with Squamous Cell Carcinoma

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ABSTRACT

Background: Salivary biomarkers, a non-invasive alternative method to serum and tissue based biomarkers and it is consider as an effective modality for early diagnosis. Salivary microRNA 21, a nucleotide biomarker, was reported to increase in patients with oral squamous cell carcinoma. This study was conducted to measure the fold change of microRNA 21 in stimulated saliva and to study its association with smoking and occurrence of oral squamous cell carcinoma.

Materials and methods: A 20 patients with oral squamous cell carcinoma who used to be smokers was included in addition to 40 control subjects (20 smokers and 20 non- smokers health looking subjects). Stimulated saliva was collected under standardized condition. Salivary microRNA 21 was assessed by real time PCR.

Results: MicroRNA 21 fold change was significantly higher in both smoker patients with oral squamous cell carcinoma and in smoker control subjects compared to non-smoker control subjects.

Conclusions: Salivary microRNA21 can serve as a non-invasive tool aid in the diagnosis and follow up of squamous cell carcinoma patients.

Keywords: Oral squamous cell carcinoma, saliva, microRNA 21. (J Bagh Coll Dentistry 2016; 28(3):178-180).

INTRODUCTION

Biomolecules that are circulating in the blood are also found in human saliva. It consists of approximately about 2,000 proteins, 26% of these proteins are also found in blood, therefore emphasizes the saliva's importance as an added biological resource for disease diagnosis and monitoring, as well as an ultimate diagnostic medium to establish a person's response to treatment ⁽¹⁾.

The DNA, RNA and protein molecules derived from the living cancer cells can be conveniently obtained from saliva. Thus, salivary biomarkers, a non-invasive alternative to serum and tissue based biomarkers may be an effective modality for early diagnosis, prognostication and monitoring post therapy status. The miRNA-21 can also serve as a circulating tumor biomarker for the early diagnosis ⁽²⁾.

A key factor in the lack of improvement in prognosis of oral squamous cell carcinoma lesions over the years is the fact that a significant proportion are not diagnosed or treated until they reach an advanced stage. In Iraq oral cancer account for about 4.5 % of all cancer cases according to Iraqi cancer registry and squamous cell carcinoma represents about 91.5 % of all oral cancer and 37 % of head and neck cancer ⁽³⁾.

Among the list of well defined risk factors for oral squamous cell carcinoma, cigarette smoking stands at the top of the list and still blamed as a major risk factor. It is believed that oral squamous cell carcinoma is acquired following multistep genetic mutations which will ultimately give the epithelial cells the phenotypic properties of self sufficiency in growth, evasion of apoptosis, invasion and stimulation of angiogenesis, and tobacco is claimed to be one of the major environmental carcinogenic agents that are responsible for such mutations ⁽⁴⁾.

miRNA21 is thought to be an important target in the pathogenesis of oral squamous carcinoma. Over expression of miRNA21 is reported in many malignant tumors including oral squamous cell carcinoma miRNA21 can be detected in human saliva and used as a biomarker for early detection of malignant and dysplastic premalignant lesions in the oral cavity Saliva as such can serve as a non-invasive investigatory tool for early diagnosis, monitoring treatment and prognostication of oral squamous cell carcinoma. The aim of this study was to measure the fold change of microRNA 21 in stimulated saliva among patients with oral squamous cell carcinoma, and to study its association with smoking.

SUBJECTS, MATERIALS AND METHODS

The present study included 20 patients with oral squamous cell carcinoma who used to be smokers. For the purpose of comparison, 20

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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 μ L) was obtained between 8:00 am and 11:00 am. Treated with RNase inhibitor, and stored at -20C $^{\circ}$ till the time of molecular analysis. Micro RNA21 was quantified using steam loop real time PCR (Total RNA Extraction Kit AccuZol; Bioneer, Korea).

Statistical analysis was performed using SPSS version 16. Being a non-normally distributed variable, comparison of medians of microRNA 21 fold change among groups was done using the non-parametric Kruskal Wallis 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 0.01.

RESULTS

Salivary microRNA21 expression fold change was higher in patients with squamous cell carcinoma than smoker control subjects. Also it was higher in smoker patients than in non-smoker control subjects.

On the other hand, smoker control subjects showed a higher microRNA21 fold change than non-smoker control subjects Figure 1 and Table 1. The difference was highly significant ($P < 0.001$) as shown in Table 2.

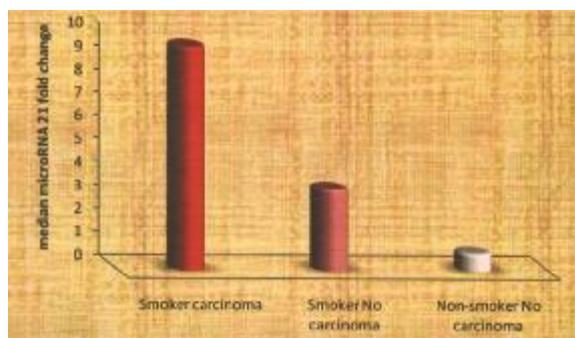


Fig. 1: Median microRNA 21 fold change in the study groups

Table 1: Descriptive statistics of miRNA21 fold change among the three study groups

| Groups | N | Mean | S.D. | Min. | Max. |
|-------------------------|----|-------|-------|-------|--------|
| Smoker carcinoma | 20 | 9.971 | 3.488 | 4.230 | 17.480 |
| Smoker No carcinoma | 20 | 3.813 | 1.409 | 2.000 | 7.940 |
| Non-smoker No carcinoma | 20 | 0.851 | 0.389 | 0.380 | 1.700 |

Kruskal Wallis H test = 50.930, d.f. = 2, p-value = <0.001

Table 2: Statistical differences among the study groups

| Groups | Mann-Whitney U test | d.f. | p-value |
|---------------------|---------------------|------|---------|
| Group 1 vs. Group 2 | 12 | 1 | <0.001 |
| Group 1 vs. Group 3 | 0 | 1 | <0.001 |
| Group 2 vs. Group 3 | 0 | 1 | <0.001 |

DISCUSSION

The present study showed a highly significant over-expression of microRNA 21 fold changes in smoker subjects and further over-expression in smoker patients with OSCC in comparison with non-

smoker subjects. In accordance with results of the present study, Soga et al. ⁽⁷⁾ stated that microRNA21 was up regulated more than four folds in oral cancer compared to normal subjects. Sio et al. ⁽⁸⁾ also stated that microRNA 21 was over-expressed (11.4 fold change) in comparison to normal subjects, in accordance with the result of the present study ⁽⁸⁾.

The explanation for the role of microRNA 21 in OSCC may be due to the fact that miRNA-21 causes over-expression of Ras oncogene and subsequently increased cell proliferation, as shown in the study done by Ren et al. ⁽⁹⁾. On the other hand, microRNA 21 may cause suppression of Programmed Cell Death 4 (*PDCD4*) mRNA, and hence it's corresponding protein. PDCD4 is a known tumor suppressor protein, the role of which is to prevent uncontrolled cell proliferation, and its suppression may play an important role in the pathogenesis of OSCC, as stated by Miranda ⁽¹⁰⁾ and Yong et al. ⁽¹¹⁾.

An interesting finding of the present study is the highly significant over-expression of microRNA 21 fold changes in healthy smoker subjects in comparison with healthy non-smoker subjects, opening the scope for future studies about the relation between smoking and microRNA 21 and augmenting the insight to word

smoking cessation as an important early measure to prevent OSCC development.

In conclusion, salivary miRNA21 can serve as a non-invasive tool to aid in the diagnosis and follow up of squamous cell carcinoma patients.

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