Clinical utility of Salivary micro RNA (miRNA-125a, miRNA-200a, miRNA-93) for Detection of Oral Squamous Cell Carcinoma

A Thesis
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Abstract

Background

Oral Squamous Cell Carcinoma is a common human malignant tumor with an increasing incidence. Around the world, the 5-year mortality rate of oral cancer is about 50%which has not changed significantly in recent 50 years despite of the advances in surgery, radiotherapy and chemotherapy. This may be due to the fact that most of the oral squamous cell carcinoma cases are diagnosed at a late stage and no reliable early diagnostic marker is available. In addition, oral squamous cell carcinoma has a very high recurrence rate, the early identification of recurrence or second primary tumors remain a challenge.

microRNAs, a family of an average 22 nucleotide long are non coding miRNA which play an important role in gene regulation. They are important in many biological and cellular processes including development, differentiation, cell cycle control and oncogenesis.

Aims

To measure the level of hsa-miR-200a, hsa-miR-125a and hsa-miR-93 in saliva supernatant of oral squamous cell carcinoma patients and healthy control groups to asses the validity of saliva miRNAs as a tool in diagnosis of oral squamous cell carcinoma. Also to correlate salivary miR-200a,miR-125a and miR-93 with the clinical data which include age, sex, tumor size, tumor grade and shape of tumor.

Subjects, materials and methods

Twenty seven patients with oral squamous cell carcinoma were recruited at the Maxillofacial surgery clinic of Ghazi Al- Hariri Hospital, Al-Kadhimia, Al-Ramadi and Al-Yarmouk Teaching Hospital .

The general information were taken from each patient including the name, age and sex. The clinical data about the tumor which included grading, tumor size and site were recorded. Patients consents for participation in the study were also taken. A group of apparently healthy individuals with age and sex matching to patients served as a control group.

All patients and controls were asked to give a sample of saliva which was immediately centrifuged, then saliva supernatant mixed with SUPERase (RNase inhibitor). The level of hsa-miR-200a, hsa-miR-125a and hsa-miR-93 in saliva supernatant were measured by using quantitative Real- time Polymerase Chain Reaction.

Results

After normalization of CT (Cycle Threshold) values for the studied three miRNAs the result of this study showed the mean of normalized CT values for miR-200a was obviously higher in oral squamous cell carcinoma group (1.06) compared with healthy controls group (1.01) with statistically significant difference ($p=<0.001$).

miR-200a was the strongest parameter (most affected by disease status) in the context of differentiation between OSSC and healthy controls (having the highest ROC area of 0.781 which is significantly higher than the area associated with equivocal test).Coming next in order of importance in the context of case-control differentiation was normalized CT values for hsa-miR-93, which has a reasonably high ROC (0.650), but failed to show statically significance differences, $P>0.05$.

Salivary hsa-miR-200a was down regulate while salivary hsa-miR-93 was up regulated in females of oral squamous cell carcinoma compared with females of healthy control group with a significant difference between the two groups (p=<0.001, p=0.016) respectively.

For tumor grade the normalized CT values of salivary hsa-miR-200a showed that each tumor grade group had a mean normalized CT value which was higher than that of controls with statistically significant differences, P<0.05.On the other hand the normalized CT values for hsa-miR-93 showed a significant difference between normalized CT values of healthy controls and poorly differentiated tumor grade group (0.041).

From the linear correlation coefficient for healthy control group there was a significant difference and strong positive linear correlations between normalized CT values of miR-200a and miR-93and in between normalized CT values of miR-125a and miR-93. While for the oral squamous cell carcinoma group there was a significant difference (p<0.001) and a strong positive linear correlation (r=77) between the normalized CT values of miR-200a and miR-125a. According to the normalized CT values of miR-200a and miR-93 there was a significant difference and strong negative linear correlation (p=<0.001,r=0.843) respectively. The normalized CT values of miR-125a and miR-93 showed a significant difference and a strong positive linear correlation (p=<0.001,r=0.799)respectively.

Conclusions

The detection of miRNAs in saliva can be used as noninvasive and rapid diagnostic tool for the diagnosis of oral cancer,miR-200a was the strongest parameter (most affected by disease status) in the context of differentiation between OSSC and healthy controls.

Also both miR-200a and miR-93 could be used as biomarkers for poorly differentiated and aggressive cancer.