

## RESEARCH ARTICLE

# Serum Levels of MMP9 and MMP2 in Patients with Oral Squamous Cell Carcinoma

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### Abstract

**Background:** Squamous cell carcinoma (SCC) is the most common cancer in the oral area. Matrix metalloproteinases (MMPs) and especially MMP-2 and MMP-9 are increased in malignancy and lymph node involvement in oral SCCs. We aimed to evaluate the serum levels of MMP-2 and MMP-9 in patients with oral SCC compared to normal subjects and their relation with clinicopathological findings. **Materials and Methods:** In this case control study, 20 patients with oral SCC and 20 healthy subjects were included and serum levels of MMP-2 and MMP-9 were compared between groups. Also, the correlation between these markers with clinicopathological findings including grade (T) and node (N) were evaluated. **Results:** Patients with oral SCC had significantly higher serum levels of MMP-2 ( $p=0.01$ ) and MMP-9 ( $p<0.001$ ) compared to healthy subjects. With increase in grade T, MMP-2 was significantly increased ( $p=0.001$ ), but in the MMP-9 case this was not significant ( $p=0.27$ ). The levels of MMP-2 ( $p=0.002$ ) and MMP-9 ( $p=0.01$ ) in cases with lymph node involvement and that of MMP-2 in subjects with smoking history ( $p=0.001$ ) were significantly high. There was significantly positive correlation between MMP-2 with grade T tumor ( $r=0.598$ ,  $p=0.005$ ), lymph node involvement ( $r=0.737$ ,  $p<0.001$ ) and smoking ( $r=0.674$ ,  $p=0.001$ ) and also between MMP-9 and lymph node involvement ( $r=0.474$ ,  $p=0.03$ ). **Conclusions:** Both markers are significantly increased in oral SCC compared to healthy subjects. However, MMP-2 was better for evaluating lymph node involvement and tumor grade.

**Keywords:** Squamous cell carcinoma - matrix metalloproteinase 2 - matrix metalloproteinase 9 - grade

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### Introduction

Oral carcinoma is amongst the leading malignancies worldwide, with squamous cell carcinoma (SCC) as the most common type accounting for over 90% of cases (Johnson et al., 2011). In spite of vast advance in the field of cancer research, availability of sophisticated diagnostic techniques and improved therapeutic options prognosis of patients presenting with this type of tumor still remains very poor (Saheb Jamee et al., 2008). The early diagnosis and treatment of oral SCC is important and it is possible that some biomarkers could be used for screening and demonstration of high risk patients.

Matrix metalloproteinases (MMPs) are a family of closely related enzymes that can degrade the extracellular matrix and play an important role in tumor invasiveness and metastasis (Knapinska and Fields, 2012; Pereira et al., 2012; Shuman et al., 2012; Abbasi et al., 2014; Yadav et al., 2014). MMP-2 and MMP-9 are the common MMPs involved in head and neck SCC. Both MMPs are found to be correlated with degree and stage of tumor and lymph node metastasis as well as poor prognosis. It is reported that MMP-2 activates MMP-9 (Forsyth et al.,

1999); however, MMP-9 compared to MMP-2 has shown to be more related to distant metastasis, lymph node involvement and higher stage of the disease (Katayama et al., 2004; de Vicente et al., 2005; Kondakova et al., 2008; Singh et al., 2010; Monteiro-Amado et al., 2013; Vilen et al., 2013; Bedal et al., 2014).

However, the studies in this regard have usually measured histologic expression of MMPs and not their serum levels (Katayama et al., 2004; de Vicente et al., 2005; Kondakova et al., 2008; Singh et al., 2010; 2012; Monteiro-Amado et al., 2013; Vilen et al., 2013; Bedal et al., 2014). There are few studies evaluated different MMPs role in oral SCC in Iran (Elahi et al., 2012; Tadbir et al., 2012; Mohtasham et al., 2013). In this study we aim to evaluate the serum levels of MMP-2 and MMP-9 in oral SCC patients compared to normal subjects and their correlation to SCC histopathologic findings.

### Materials and Methods

In this prospective study, 20 patients with confirmed oral SCC by pathology and 20 age- and sex-matched normal subjects were recruited. Patients and subjects with

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recent trauma, acute infection, burning, any laceration or previous history of surgery or chemoradiotherapy were excluded. Also, in control group, patients with no history of smoking or alcohol use were included. The study was approved by the institutional review board of Tabriz University of Medical Sciences and Informed consents were obtained from all patients.

#### *Tumor characteristics including the TNM classification were recorded for all patients*

A total of 3 mL of blood was drawn and the serum separated and stored at  $-80^{\circ}\text{C}$ . Serum levels of MMP-2 and MMP-9 were measured using commercially available ELISA kits (Hangzhou Eastbiopharm Co., LTD., Hangzhou, China). Methods were as described in the manufacturer's protocol. All assays were performed in duplicate. Assay range for MMP-2 was 10-3000 ng/ml and for MMP-9 was 30-90000 ng/L. The sensitivity limit of the assay for MMP-2 was 5.64 ng/ml and for MMP-9 was 15.12 ng/L. All assays were done in duplicate. A subset of samples has been re-assayed for five times in every ELISA plate for quality control.

#### *Statistical analysis*

Statistical analyses were performed using the Statistical Package for Social Sciences, version 17.0 (SPSS, Chicago, Illinois). Quantitative data were presented as mean $\pm$ standard deviation (SD), while qualitative data were demonstrated as frequency and percent (%). The categorical parameters were compared by  $\chi^2$  tests (or Fisher's exact test, where appropriate), and the continuous variables were compared by independent samples T-test or One-way ANOVA. Pearson's correlation was used to evaluate the possible correlations between MMP-2 and MMP-9 levels with clinicopathological findings in patients with Oral SCC. A p value of  $<0.05$  was considered statistically significant.

## Results

In this study, 20 patients with oral SCC and 20 healthy subjects were included. SCC group comprised of 11 male and 9 female and control group was comprised of 10 male and 10 female subjects ( $p=0.75$ ). There was no significant difference regarding SCC and control group age (61.25 $\pm$ 11.82 vs 53.85 $\pm$ 23.56 years,  $p=0.21$ ). Among SCC patients, 6 were smoker and none were using alcohol.

SCC cases were lingual SCC in 15 cases including 9 in left lateral and 6 in right lateral and SCC of lips in 5 cases including 4 in upper lip and one in lower lip. Considering the TNM classification, 5 cases were in T1 grade, 11 cases in T2 grade and 4 cases in T3 grade. Lymph nodes involvement was observed in 6 cases including N1 in one case, N2 in 4 cases and N3 in one cases. There was no metastasis.

Oral SCC group had significantly higher serum levels of MMP-2 (661.73 $\pm$ 158.27 vs 247.38 $\pm$ 29.43 ng/mL;  $p=0.01$ ) and MMP-9 (1296.26 $\pm$ 334.18 vs 820.04 $\pm$ 244.23 ng/L;  $p<0.001$ ) compared to healthy subjects.

*MMP-2 and MMP-9 levels were compared between*

#### *different groups in oral SCC patients*

There was no significant difference between male and female patients regarding serum levels of MMP-2 (808.59 $\pm$ 242.32 vs 482.23 $\pm$ 187.55 ng/mL;  $p=0.17$ ) and MMP-9 (1357.81 $\pm$ 266.16 vs 1221.03 $\pm$ 406.33;  $p=0.37$ ).

Serum levels of MMP-2 and MMP-9 were 372.68 $\pm$ 255.75 ng/mL and 1119.80 $\pm$ 307.71 ng/L in grade T1, 420.97 $\pm$ 273.49 ng/mL and 1308.30 $\pm$ 372.92 ng/L in T2 and 1682.62 $\pm$ 1047.40 ng/mL and 1483.75 $\pm$ 134.85 ng/L in T3. With increase in T grade, the levels of MMP-2 was significantly increased ( $p=0.001$ ) but the increase in MMP-9 levels was not significant ( $p=0.27$ ).

SCC cases with lymph node involvement compared to non metastatic cases had significantly higher serum levels of MMP-2 (1347.51 $\pm$ 992.15 vs 367.82 $\pm$ 208.71 ng/mL;  $p=0.002$ ) and MMP-9 (1554.00 $\pm$ 262.93 vs 1185.80 $\pm$ 304.68 ng/L;  $p=0.01$ ).

SCC patients with smoking habit compared to non-smokers had significantly higher MMP-2 (1372.46 $\pm$ 944.17 vs 357.12 $\pm$ 237.31 ng/mL;  $p=0.001$ ), but the difference in MMP-9 was not significant (1506.16 $\pm$ 175.93 vs 1206.30 $\pm$ 349.66 ng/L;  $p=0.06$ ).

Using Pearson's correlation we observed significant correlation between serum levels of MMP-2 and T grade ( $r=0.598$ ,  $p=0.005$ ), Lymph node involvement ( $r=0.737$ ,  $p<0.001$ ) and smoking ( $r=0.674$ ,  $p=0.001$ ) as well as between MMP-9 levels and Lymph node involvement ( $r=0.474$ ,  $p=0.03$ ).

## Discussion

The ability of cancer cells to invade other tissues and spread to distinct organs is an often-fatal characteristic of malignant tumors. Numerous clinical and experimental studies have demonstrated an increase in particular MMPs, especially MMP-2 and MMP-9 with cancer progression (17,18). MMP-2 and MMP-9 are type IV collagenases, and thus can degrade the major structural protein of extracellular membrane and basement membrane. Several studies have documented enhanced expression of these MMPs with tumor progression and metastasis (Folgueras et al., 2004; Rosenthal and Matrisian, 2006; Di Domenico et al., 2011; Wang et al., 2013).

In this study, we evaluated the serum levels of MMP-2 and MMP-9 in patients with oral cavity SCC and compared them with normal subjects and observed that patients with oral SCC had significantly higher serum levels of MMP-2 and MMP-9. The significant increase in serum levels of MMPs especially MMP-2 and MMP-9 are also shown in other cancers compared to control subjects (Ylisirnio et al., 2000; Nikkola et al., 2005; Wu et al., 2008;). Although previous studies on oral SCC have not measured serum levels of MMPs, but they have also shown significant differences between expression of MMP-2 and MMP-9 in oral SCC compared to normal tissues and normal subjects (Hong et al., 2006; Singh et al., 2010).

We evaluated the possible correlation between demographic and clinicopathological findings in oral SCC patients with serum levels of MMP-2 and MMP-9. We found no correlation between age and gender of oral SCC patients with MMP-2 and MMP-9 levels. Similarly,

Zhang et al. (2014) and Mardani et al. (2014) evaluating breast ductal carcinoma and salivary gland tumor, did not find any correlation between MMPs and age and gender. However, de Vicente et al. (2005) reported higher levels of MMP-2 in patients under 60 years old. Although there is a possibility that the activity of the MMPs increase by age, the findings have controversy in the literature.

We observed that MMP-2 levels were significantly higher in patients with smoking history, but the difference in MMP-9 levels was not significant. Unlike our findings, Garvin et al. (2008) showed positive correlation with MMP-9 and smoking on healthy subjects.

Studies evaluating oral SCC have reported different results regarding the value of MMP-2 and MMP-9 on the cancer diagnosis, progression and metastasis. Erdem and colleagues (2007) reported that MMP-9 have more important role than MMP-2 in metastasis. Liu and colleagues (2001) observed that both MMP-2 and MMP-9 are increased in patients with metastasis and those with lymph node involvement compared to patients without metastasis or lymph node involvement. However, Makinen et al. (2012) found no correlation between MMP-2 and MMP-9 and tumor clinicopathologic findings including invasion and size of the tumor.

We observed that higher serum levels of MMP-2 are significantly correlated with increase in T grade and lymph node involvement. However, we only found that MMP-9 was only correlated with lymph node involvement and not with Tumor T grade. Similar to our findings, some previous studies have shown significant correlation between MMP-2 and MMP-9 with lymph node involvement (Hong et al., 2006; Singh et al., 2010; Monteiro-Amado et al., 2013; Li et al., 2014). Unlike our findings, Katayama et al. (2004) found no correlation between MMP-2 and lymph node involvement. Also, although we found no correlation between tumor T grade and MMP-9, most other studies have reported significant correlation between MMP-9 and metastasis, differentiation and stage of the tumor (de Vicente et al., 2005; Singh et al., 2010; Elahi et al., 2012).

The differences between studies could be due to different study sample, variable stage of the disease and absence or presence of metastasis as well as the method of evaluating these markers. Although there is no integration between findings of different studies, all studies are indicative of role of MMPs in the evaluation of the advanced or early stage of oral SCC and may be used as early predictor of their prognosis.

In conclusion, both markers are significantly increased in oral SCC compared to healthy subjects. However, MMP-2 compared to MMP-9 was better marker in evaluating the lymph node involvement and tumor grade (T).

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