

## RESEARCH ARTICLE

# Serum Level of Matrix Metalloproteinase-2 and -9 in Patients with Laryngeal Squamous Cell Carcinoma and Clinical Significance

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### Abstract

**Background:** Laryngeal cancer is an important malignancy in head and neck area and squamous cell carcinoma (SCC) is the most common type accounting for 95% of cases. Increase in matrix metalloproteinases (MMPs) in different tumors and their correlation with tumor invasiveness has been documented. However, most studies have evaluated MMP-2 and MMP-9 expression and few have evaluated serum levels. The aim of current study was to evaluate serum levels in patients with laryngeal SCC compared to normal subjects and assess any relation with tumor clinicopathological findings. **Materials and Methods:** In this case control study, 20 patients with oral SCC and 20 healthy subjects were included. Serum levels of MMP-2 and MMP-9 were compared between groups and correlations with findings including grade (T) and node involvement (N) were evaluated. **Results:** Patients with laryngeal SCC had significantly higher serum levels of MMP-2 ( $p=0.01$ ) and MMP-9 ( $p=0.03$ ) compared to healthy subjects. Patients with higher T stage (T3,4) had significantly higher MMP-2 ( $p=0.04$ ) and MMP-9 ( $p=0.01$ ). There was significant positive correlation between serum levels of MMP-2 with T stage ( $r=0.45$ ,  $p=0.04$ ) and lymph node involvement ( $r=0.563$ ,  $p=0.01$ ) and between levels of MMP-9 with T stage ( $r=0.527$ ,  $p=0.01$ ). **Conclusions:** Our results showed that compared to healthy subjects, both MMP-2 and MMP-9 are significantly increased in serum of laryngeal SCC cases. MMP-2 was correlated with lymph node involvement while MMP-9 has stronger correlation with T stage compared to MMP-2.

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

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### Introduction

Laryngeal cancer is one of the main and common malignancies in the head and neck area. Laryngeal squamous cell carcinoma (SCC), which originates from the laryngeal epithelium, is the predominant histological type accounting for over 95% of laryngeal cancers (Ramroth et al., 2011). Patients with invasion and metastasis of laryngeal carcinoma have much worse prognosis, with a 5-year survival rate of approximately 60% (Marioni et al., 2006). Although therapeutic strategies targeting LSCC have improved, including surgery, radiotherapy and chemotherapy, the mortality rate of LSCC has not changed (Ferlay et al., 2010; Ramroth et al., 2011).

Thus, early diagnosis is considered to be critical for improving the survival of patients. Therefore, the identification of novel biomarkers for laryngeal SCC tumor staging and new treatment strategies is necessary and may provide valuable information for clinical treatment.

Matrix metalloproteinases (MMPs) are a family of closely related enzymes that has the ability to 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; Lotfi et al., 2015). The most common MMPs found in head and neck SCC are MMP-2 and MMP-9. The correlation between these MMPs and degree and stage of tumor and lymph node metastasis and poor prognosis are well shown. MMP-9 has shown to be better predictor of poor prognosis compared to MMP-2 (Sun et al., 2007; Kondakova et al., 2008; Gou et al., 2010; Cao et al., 2011; Mallis et al., 2012; Colovic et al., 2013).

However, evidence regarding the role of MMP-2 and MMP-9 has shown conflicting results. There is no previous study evaluating the levels of MMP-2 and MMP-9 in laryngeal SCC in Iran. In this study, we aimed to evaluate the serum levels of MMP-2 and MMP-9 in laryngeal SCC and their correlation to the tumor clinicopathological findings.

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## Materials and Methods

In this prospective study, 20 patients with confirmed laryngeal SCC by pathology and 20 age- and sex-matched normal subjects were recruited. Patients and subjects with 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. Pearson's correlation was used to evaluate the possible correlations between MMP-2 and MMP-9 levels with clinicopathological findings in patients with laryngeal SCC. A p value of  $<0.05$  was considered statistically significant.

## Results

In this study we evaluated 20 male with laryngeal SCC and 20 normal male subjects. Patients mean age was  $65.35 \pm 10.41$  years and control group mean age was  $61.30 \pm 16.36$  years ( $p=0.35$ ). Among laryngeal SCC group, one patient reported history of alcohol use and 18 cases were smoker.

The laryngeal SCC comprised of supraglottic SCC in 9 cases and glottic SCC in 11 cases. In TNM classification, 2 cases were T1, 4 cases were T2, 11 cases were T3 and 3 cases were in T4. Lymph node involvement was seen in 3 cases (15%) which was N1 in 1 case and N2 in 2 cases. There was no metastasis.

Laryngeal SCC group had significantly higher MMP-2 ( $708.06 \pm 488.44$  ng/mL vs.  $399.71 \pm 239.11$  ng/mL;  $p=0.01$ ) and MMP-9 levels ( $1761.30 \pm 887.54$  ng/L

vs.  $1287.79 \pm 343.56$  ng/L;  $p=0.03$ ) than healthy subjects.

There was no significant difference between supraglottic and glottis SCC regarding MMP-2 ( $757.00 \pm 619.26$  vs.  $668.01 \pm 377.61$  ng/mL;  $p=0.69$ ) and MMP-9 levels ( $1840.85 \pm 1046.64$  vs.  $1696.20 \pm 781.00$ ;  $p=0.72$ ).

SCC in grade T3 & T4 compared to SCC in grade T1 & T2 had significantly higher serum levels of MMP-2 ( $853.33 \pm 519.23$  vs.  $385.70 \pm 97.35$  ng/mL;  $p=0.04$ ) and MMP-9 ( $2059.95 \pm 905.63$  vs.  $1064.45 \pm 168.75$  ng/L;  $p=0.01$ ).

Using Pearson's correlation we observed significant correlation between serum levels of MMP-2 with T grade ( $r=0.45$ ,  $p=0.04$ ) and with lymph node involvement ( $r=0.563$ ,  $p=0.01$ ) and between MMP-9 levels with grade T ( $r=0.527$ ,  $p=0.01$ ).

## Discussion

It is shown that MMPs play a relevant role in several steps of tumor progression, including invasion, angiogenesis, and metastasis (Liotta, 1986; Rosenthal and Matrisian, 2006). In some tissues including larynx, it is demonstrated that the MMPs have additional enzymatic activity that may affect the cancer invasion (Christopoulos et al., 2006). Previous studies have tried to show the role of MMPs in the cancer progression and metastasis in different cancers (Christopoulos et al., 2004). In our study, we observed that the serum levels of MMP-2 and MMP-9 are significantly increased in laryngeal SCC compared to normal healthy subjects.

Similar to our findings, Sun et al. (2007), Gou et al. (2010), and Uloza et al. (2011) reported higher expression of MMP-2 and MMP-9 in laryngeal SCC compared to normal subjects. However, unlike these studies which have evaluated the expression of these markers in the tissue, we evaluated the serum levels of these markers which is more available and may be more useful in early diagnosis and prediction of laryngeal SCC.

We also evaluated the correlation between MMP-2 and MMP-9 with different variables in patients with laryngeal SCC. It is possible that MMP-2 and MMP-9 activity increase and be affected by age (Yu et al., 2013). However, we found no correlation between levels of MMP-2 and MMP-9 with age. Wang et al. (2006) also reported that age had no effect on MMP-2 expression.

The type of SCC would have effect on the levels of MMP-2 and MMP-9, as Cao et al. (2011) observed increased expression of MMP-9 in supraglottic cancer cells compared to glottis SCC. However, we failed to show such correlation. It is possible that the difference in both studies could be due to different techniques of MMP evaluation.

We also observed significant correlation between serum levels of MMP-2 and MMP-9 with tumor T grade, but found only significant correlation between MMP-2 and lymph node involvement. Findings in this regard are controversy. Some studies have reported significant correlation between MMP-2 or MMP-9 expression with laryngeal SCC clinicopathological findings including clinical stage, grade T, grade N and metastasis (Gou et al., 2010; Cao et al., 2011; Lin et al., 2012; Colovic et al.,

2013; Tang et al., 2013), while some other studies have failed to show these correlations (Krecicki et al., 2001; Peschos et al., 2006; Wittekindt et al., 2011; Mallis et al., 2012; Akdeniz et al., 2013). These 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. However, it is obvious that MMPs have considerable role in evaluating the advanced or early stage of the tumors and may be used as early predictor of their prognosis.

Both markers are significantly increased in laryngeal SCC compared to healthy subjects. However, MMP-2 was correlated with lymph node involvement and MMP-9 has stronger correlation with stage T compared to MMP-2.

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