

# Immunohistochemical expression of TGF- $\beta$ in relation to invasion potential evaluated by MMP-2 in oral squamous cell carcinoma

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**ABSTRACT** Background: Squamous cell carcinoma is a malignant neoplasm of stratified squamous epithelium; it is by far the most common malignant neoplasm of the oral cavity representing approximately 94% of oral cancer. It is capable of local destruction and invasion with distant metastasis. This study evaluates the Immunohistochemical expression of TGF- $\beta$  and MMP-2 as markers of invasion and metastasis of OSCC and their correlation with the tumor grade and stage and with each other.

Materials and methods: Thirty blocks of OSCC were included in this study. An immunohistochemical staining was performed using anti matrix metalloproteinase-2 and anti-transforming growth factor- $\beta$  monoclonal antibodies.

Results: Positive immunohistochemical expression of MMP-2 and TGF-  $\beta$  was found in (100%) and (93.3%) of the cases respectively. No statistically significant correlation was found regarding either markers with respect to the tumor grade and stage .On the other hand a statistically significant correlation was found between the expressions of both markers (p = 0.036).

Conclusions: TGF- $\beta$  and MMP-2 immunoexpression showed no significant influence on tumor invasion and metastasis in relation to the tumor grade and stage. While the significant correlation seen regarding the expression of both markers with each other, suggests their cooperative role in oral squamous cell carcinoma.

Key words: Oral squamous cell carcinoma, TGF-  $\beta$ , MMP-2, invasion and metastasis. (J Bagh Coll Dentistry 2012; 24(4):62-66).

## INTRODUCTION

OSCC remains a lethal disease in over 50% of the cases diagnosed annually, due mostly to late detection of advanced stage cancer <sup>(1)</sup>. It is characterized by a high degree of local invasiveness and a high rate of metastasis to cervical lymph nodes, but a low rate of metastasis to distant organs. Death as a result of cancer is often the result of local recurrence or regional and/or systemic metastasis. Thus, metastases are a major problem in successful cancer treatment, and it is believed that they begin early in the growth of the primary tumor <sup>(2)</sup>. Local invasion and distant metastases are one of the most important determining factors in the prognosis of malignant tumors. Degradation of extracellular matrix (ECM) that surrounds tumor cells is one of the essential steps in tumor invasion and the development of metastasis <sup>(3)</sup>.

Matrixmetalloproteinase-2(MMP-2) (gelatin-ase A; also called type-IV collagenase) degrades type IV collagen of the basement membrane, and is believed to be involved in tumor invasion and metastasis through a degradation effect on ECM. Transforming growth factor- $\beta$  (TGF- $\beta$ ) is a multifunctional cytokine that has important roles in tumor formation, progression, and metastasis <sup>(4)</sup>.

Since invasion and metastasis are major problems in successful treatment of cancer and both the aforementioned markers play important roles in these biological aspects, therefore, this study was conducted to elucidate the role of these markers in OSCC.

## MATERIALS AND METHODS:

Thirty formalin-fixed paraffin-embedded tissue blocks of OSCC were collected from the Department of Oral Diagnosis / College of Dentistry / Baghdad University for the period from 1998 to 2011. Four-micrometer-thick sections were cut from each paraffin tissue block and stained with hematoxylin and eosin for diagnostic confirmation and histological grading. Another two 4- $\mu$ m section was cut from each tissue block and mounted on positively charged slides (Biocare,USA) to be stained with monoclonal antibodies to TGF-  $\beta$  and MMP-2 (USBiological-C2386-10). Negative and positive tissue controls were included into each immunohistochemical run (according to the manufacturer).

### Immunostaining

Five micrometer thick sections were cut and mounted on (Biocare, USA) positively charged slides, then deparaffinized and rehydrated. For immunohistochemical staining by MMP-2 and TGF- $\beta$  (US biological) monoclonal antibodies; then the sections were immersed in 0.3% hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>) to block the

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endogenous peroxidase activity, washed in phosphate-buffered saline (PBS), and then incubated in 10% normal serum to block any non-specific binding of antibodies. The tissue sections were incubated with monoclonal mouse anti-human MMP-2 (diluted 1:50) and TGF-β (diluted 1:30) antibodies over night at 37 °C. The bounded antibodies were detected by the streptavidin-biotin complex method, after an immunoreaction, the sections were counterstained with Hematoxylin.

**Scoring system**

The scoring of the markers was done by examining of at least 1000 cells per section in five different representative fields. The intensity of staining was not taken into consideration. For MMP-2: (0) point for negative staining of the considered cells, (1+) <10%, (2+) 10-50%, (3+) 51-100%,<sup>(5)</sup>. For TGF-β: (0) point was scored as negative, 1-25% (1+), 26-50% (2+), and 51-75% (3+) and 76%-100% (4+)<sup>(6)</sup>.

**Statistical analysis**

The data was compiled into statistical software, statistical package of social sciences (SPSS) version 18. All variables were compared using Chi- square test. While Pearson correlation coefficient was applied to plot a correlation matrix among the different immunohistochemical markers expression values altogether. P values of less than 0.05 were considered statistically significant.

**RESULTS**

The results of 30 oral squamous cell carcinoma cases were designed as follows: most of the cases (69%) aged > 50 years; the majority of the cases were males (63.3 %). The most common site was tongue 11 cases (36.7%). Most of the cases presented as mass 17 cases (56.7%). Histopathological examination showed that 15 cases (50%) were moderately differentiated, followed by 12 cases (40%) well differentiated and 3 cases (10%) were poorly differentiated.

**Table 1: Age and sex distribution of the study sample**

Age	No.	%
50>	20	69
50≤	9	31
	29*	100
sex		
Male	19	63.3
female	11	36.7
	30	100

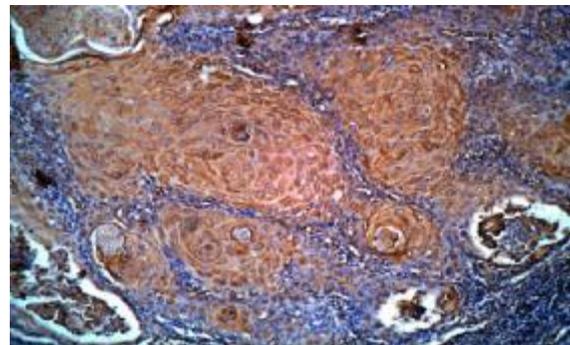
\*1 case the age was not recorded.

**Table 2: MMP-2 IHC expression in OSCC cases**

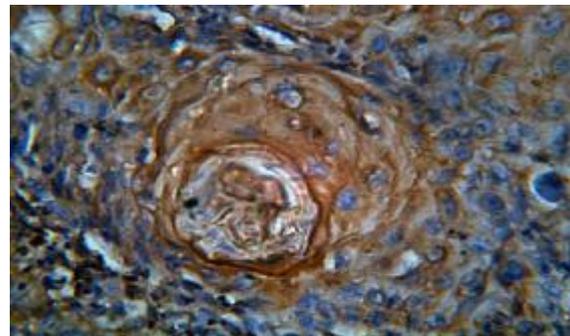
MMP-2 Score*	Frequency	Valid Percent
1	1	3.3
2	16	53.3
3	13	43.3
<b>Total</b>	<b>30</b>	<b>100.0</b>

\*1 (weak expression), 2 (moderate expression), 3 (strong expression)

Positive immunohistochemical expression of Matrix metalloproteinase-2 was found in all cases (100%) as follows: 16 cases (53.3%) were moderate positive; 13 cases (43.3%) were strong positive and weak positive only in one case (3.3%).



**Figure 1: Positive brown cytoplasmic immunostaining of MMP-2 in well differentiated OSCC-Lower lip (100X).**

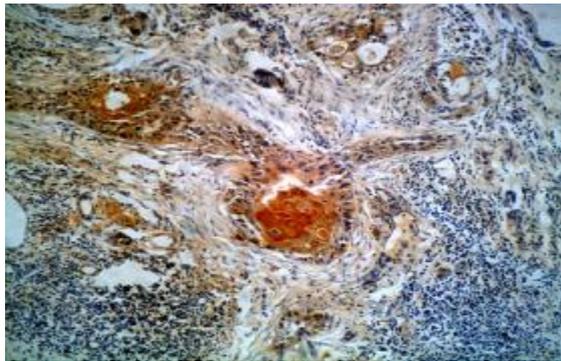


**Figure 2: Positive brown cytoplasmic immunostaining of MMP-2 in well differentiated OSCC-Lower lip (400X).**

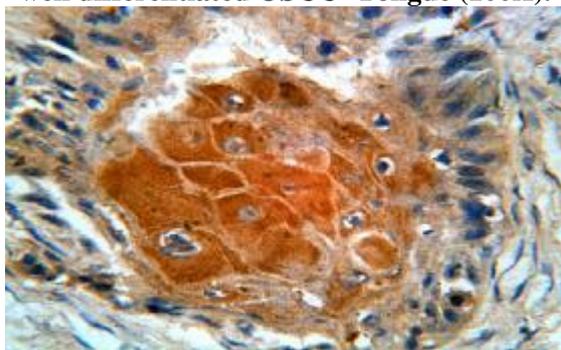
**Table 3: TGF-β IHC expression in OSCC cases**

TGF-β score * TGF-β	Frequency	Percent
1	10	33.3
2	12	40.0
3	6	20.0
<b>Total</b>	<b>28</b>	<b>93.3</b>
Missing (- ve)	2	6.7
<b>Total</b>	<b>30</b>	<b>100.0</b>

\*1 (weak expression), 2 (moderate expression) ,3 (strong expression).



**Figure 3: Positive brown membranous /cytoplasmic immunostaining of TGF-β in well differentiated OSCC -Tongue (100X).**



**Figure 4: Positive brown membranous /cytoplasmic immunostaining of TGF-β in well differentiated OSCC -Tongue (400X).**

The positive immunohistochemical expression of transforming growth factor -β of the studied cases was (93.3%) which was scored as follows: 12 cases (40%) were moderate positive; 6 cases (20%) strong positive and weak positive in 10 cases (33.3%).

There was no statistically significant correlation regarding either markers with respect to the tumor grade and stage .On the other hand a statistically significant correlation was found between the expressions of both markers.

**Table 4: The correlation of MMP-2 & TGF-β IHC expressions**

		MMP-2 Per	TGF-β Per
MMP-2 Per	Pearson Correlation	1	0.398
	Sig. (2-tailed)		<i>*0.036</i>
	N	30	28
TGF-β Per	Pearson Correlation	0.398	1
	Sig. (2-tailed)	<i>*0.036</i>	
	N	28	28

\*p value less than 0.05 is considered significant

## DISCUSSION

Concerning the epidemiological parameters, including age, sex, site, clinical presentation, studies showed variable results; These inconsistent findings could be credit with the fact that the current study and some of the others are not an epidemiological type of studies, therefore the limited number and the random selection of the cases according to what is available preclude for definitive clinical findings.

### Assessment of MMP-2 immunohistochemistry

The results of this study showed MMP-2 positive immunostaining in all OSCC cases (100%). High MMP-2 expression could be explained by that OSCC is a highly invasive cancer with poor prognosis <sup>(7, 8)</sup>. MMPs have been reported to play an important role in tumor invasion, metastasis, and aggressive behavior <sup>(9)</sup>.

Regarding tumor grade, although the expression percentage of MMP-2 increased as the tumor grade increases, yet they didn't reach the level of statistical significance.

Concerning the tumor stage; no statistically significant correlation was seen between MMP-2 expression and tumor stage. The variations in results with other studies <sup>(10, 11)</sup> regarding MMP-2 expression correlation with tumor stage and grade may be due to the limitation in sample size.

### Assessment of TGF-β immunohistochemistry

This study revealed high TGF-β immuno-reactivity (93.3%). High TGF-β expression in cancerous tissues can be explained by its oncogenic functions in cancer progression including proliferative signaling, evasion of growth suppressors, resistance to cell death, replicative immortality, angiogenesis, invasion, and metastasis <sup>(12)</sup>.

Regarding the clinicopathological parameters; the present study showed no correlation between TGF-β expression with respect to histopathological grade and stage of the tumor.

These variations mentioned above with other studies <sup>(13, 11)</sup> regarding the expression correlation of TGF-β with tumor grade and stage may be due to the small sample number, since it is not a longitudinal or epidemiological study, besides the difference in behavior among different cancers.

### Assessment of the correlation between TGF-β and MMP-2 IHC expression:

TGF-β super family members and MMPs have long been regarded as important factors in cancer cell invasion and metastasis. TGF-β1 and MMP-2 have been widely accepted as important mediators, or initiators in many malignancies, including OSCC <sup>(14, 15)</sup>.

It has been reported that on one hand TGF- $\beta$  can enhance the activities of MMPs, which then hydrolyze basement membranes through a process involving EMT<sup>(16)</sup>. On the other hand, MMP-2 is able to cleave the latency associated peptide of TGF- $\beta$  releasing active TGF- $\beta$ <sup>(17, 18)</sup>.

Furthermore, Over-expression of MMP-2 was reported to occur in caveolin null mice and this giving rise to an invasive breast tumor phenotype associated with increased TGF- $\beta$  activation<sup>(19)</sup>. In addition, TGF- $\beta$  signaling was found to induce the expression, secretion and activation of MMP-2 in immunoblotting analysis of Tongue SCC<sup>(20)</sup>.

The present study showed statistically significant correlation between MMP-2 and TGF- $\beta$ . Malaponte et al (2010) also found a correlation between plasma MMP-2 and TGF- $\beta$  levels in melanoma patients<sup>(21)</sup>. Moreover they reported that MMP-2 and TGF- $\beta$  can be considered as biomarkers and indices of disease progression in several human cancers.

Many researchers have shown that TGF- $\beta$  induce the expression of MMPs (like MMP-2, MMP-9, MMP-10) in several human cancer cell lines, such as prostate<sup>(22)</sup>, bladder<sup>(23)</sup>, breast<sup>(24)</sup>, endometrial<sup>(25)</sup>, ovarian cancers<sup>(26)</sup>, and melanoma<sup>(21)</sup>.

Lee et al (2010) stated that TGF- $\beta$  enhances MMP-2 production and activity. They recorded an increase in the expression of TGF- $\beta$  and MMP-2 in Tuberous Sclerosis Complex (TSC) tumors compared with those in normal skin, as well as they found a significant co-localization of TGF- $\beta$  and MMP-2 in the TSC tumors<sup>(27)</sup>.

Finally, the statistically significant correlation between MMP-2 and TGF- $\beta$  expression revealed in this study suggests their close and synergistic, cooperation and co-activation in cancer. Therefore, they could be considered important biomarkers acting together in the invasion and metastasis of OSCC.

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