

# Local Expression of MMP-7 in Oral Cancer

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## Abstract

**Background:** Squamous cell carcinoma of the oral cavity (OSCC) is a highly invasive neoplasm. Many MMPs play role in human cancer invasion and metastases.

**Aim:** Estimating The MMP-7 expression level in HPV-16 positive and HPV-16 negative OSCC paraffin embedded sections.

**Method:** Biopsies from thirty three patients with oral squamous cell carcinoma (OSCC) were obtained and investigated for the presence of HPV-16 RNA with the application of ISH and the MMP-7 expression level using IHC .

**Results:** Expression level of MMP-7 found to be high in OSCC sections 29 (87.8%) cases with no significant difference in its expression level between HPV-16 positive and HPV-16 negative OSCC cases  $p=1.00$ .

**Conclusion:** MMP-7 found to be expressed in high level in OSCC with no significant relevance to HPV-16.

**Keywords:** oral squamous cell carcinoma, HPV-16, MMP-7

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## Introduction:

Matrix metalloproteinase-7 (MMP-7) is a member of the Matrix metalloproteinases (MMPs) family and has a wide spectrum of varied substrates. It is reported to play an important role in carcinoma invasion and metastasis through extracellular matrix degradation<sup>(1)</sup>. Squamous cell carcinoma (SCC) of the oral cavity is a highly invasive tumor of stratified squamous epithelium that spreads through degradation of the basement membrane (BM) and extracellular matrix (ECM). This process involves multiple proteolytic enzymes including matrilysin (MMP-7)<sup>(2,3,4)</sup>. HPV is an important risk factor for development of cervical cancer as well as head/neck and anal cancer<sup>(5,6,7,8)</sup>. The precise mechanisms of the growth and transformation of oral epithelia into oral cancers and the relationships of HPV infection to this transformation have not yet been elucidated<sup>(8)</sup> therefore the aim of this study was to evaluate the level of expression of MMP-7 in HPV-16 infected and HPV-16 non infected OSCC sections.

## Methods:

The study group consisted of pathological specimens from thirty three patients with oral squamous cell carcinoma attending The Maxillofacial Center in Surgical Specialty Hospital in Baghdad, from January/2006 to January/2008. Ten normal oral tissues, as negative controls, were obtained from buccal mucosa of individuals underwent plastic surgery at the same Hospital. All samples were fixed in paraffin embedded blocks and then were investigated for the expression of HPV-16 and MMP-7 using insitu hybridization (ISH) and immunohistochemistry (IHC) respectively. All slides were examined by histopathologist and scored under high power field.

**Immunohistochemistry:** Fixed, paraffin embedded tumor tissues and control tissues were placed inside a hot air oven at 65°C overnight then dipped in xylene and ethanol (100%, 95% & 70%). After deparaffinization and rehydration, slides were incubated in 3% H<sub>2</sub>O<sub>2</sub> for 30 minutes. The slides were placed in 10 mM citric acid buffer at pH 6.0 and underwent antigen retrieval for 10 min at 680 W in a microwave oven. Mouse monoclonal antibody against MMP-7 in 8Ug/ml (Chemicon International,USA) was added. Slides were incubated at 37 °C for one hour followed by overnight incubation at room temperature. After application of peroxidase labeled secondary antibody and the DAB, the sections were finally counterstained and mounted. Immunostaining was scored semi quantitatively, ten representative areas containing 100 SCC cells were analyzed. The intensity of staining was quantified between zero (white) and 225 (black). These values were categorized into four scores (zero, 1, 2 and 3)<sup>(9)</sup>. paraffin embedded sections of ovarian cancer were immunostained as a positive control (as indicated in manufacturer protocol)

**InSitu Hybridization:** InSitu Hybridization procedure was conducted according to the instruction of the manufacturer using DNA probe Hybridization/detection System InSitu Kit (Maxim Biotech, USA). The biotinylated cDNA probe for HPV-16 ((Maxim biotech, USA) was diluted to 7%. Positive control was made with housekeeping gene probe while the negative control by applying hybridization solution without probe. Determination of positive reaction was made with nuclear and/or cytoplasmic blue staining of cells<sup>(10)</sup>.

Statistical analysis of observed data was performed utilizing spss with the application of Chi-Square and Fisher Exact test.

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*p : 14*

## Results:

Immunoreactivity of OSCC cells to MMP-7 was demonstrated in 29 (87.8%) cases with no such reactivity in the control group (figure1 A & B). The MMP-7 expression scores were illustrated in table (1). An important observation in this study was the clear vascular endothelial expression of MMP-7 in all sections of OSCC (figure 1C). Nuclear hybridization signals for HPV-16 were demonstrated in 27 (81.8% )

of OSCC cases (figure1D) with no observed signals in the negative control ones. Regarding MMP-7 immunoreactivity, no significant difference was observed between MMP-7 expression level in HPV-16 infected and non infected OSCC sections (table 2). In addition no significant difference was observed between HPV-16 infected and non infected OSCC sections considering lymph node metastases (table 3).

**Table (1 ): MMP-7 immunoreactivity in HPV-16 positive versus HPV-16 negative OSCC.**

MMP-7			
	Positive (%)	Negative(%)	Total
HPV Positive (%)	24(82.7)	3(7.5)	27(81.8)
Negative(%)	5(17.2)	1(2.5)	6(18.1)
Total	29(87.8)	4(12.1)	33

**P=1.00**

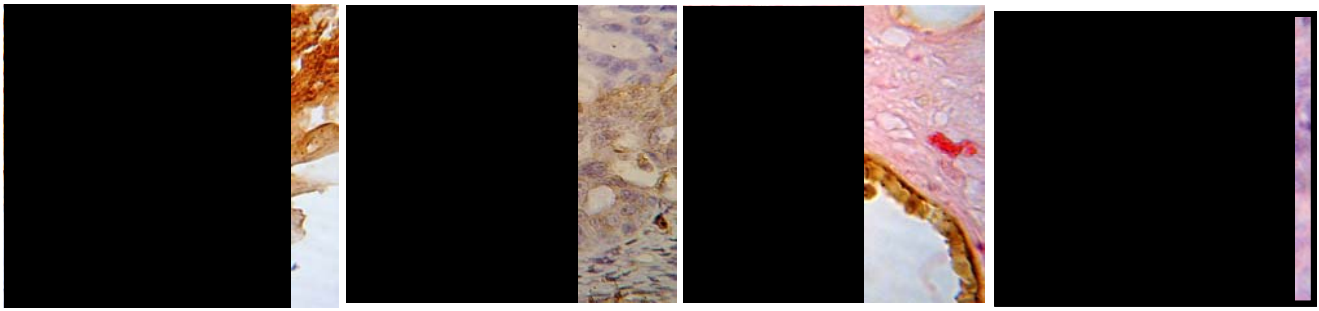
**Table (2):MMP-7 Immunoreactivity scores in OSCC.**

MMP-7			
Score zero(%)	Score 1(%)	Score2(%)	Score3(%)
4(12.1)	15(45.5)	12(36.4)	2(6.1)

**Table (3): Association of MMP-7 Immunoreactivity score and lymph node metastases in OSCC.**

Lymph Node Involvement(%)		MMP-7 Score(%)		
		Low	Intermediate	High
	Positive	14(93.3)	9(75)	2(100)
	Negative	1(6.6)	3(25)	zero
	Total	15(51.7)	12(41.3)	2(6.8)

**P =0.47**



**A** **B** **C** **D**  
**Figure 1: A- MMP-7 expression in OSCC (400X).**  
**B-MMP-7 expression in ovarian cancer as a positive control (400X).**  
**C- vascular endothelial expression of MMP-7 in OSCC(400X).**  
**D- Nuclear hybridization signals for HPV-16 in OSCC(400X).**

### **Discussion:**

We investigated biopsies from thirty three OSCC cases for immunoreactivity to MMP-7, which was found to be highly expressed in 29 (87.8%) cases, a finding that is supported by others <sup>(2,4)</sup>. Many researchers demonstrated the overexpression of MMP-7 in other types of cancer as well <sup>(7,11,12)</sup>. In this study MMP-7 was expressed in tumor cells in addition to its very clear expression in angiogenic endothelial cells where its expression was not dependent on the proximity of malignant MMP-7 expressing epithelial cells, suggesting an endogenous endothelial origin <sup>(3)</sup> reflecting an important role for MMP-7 in angiogenesis. Therefore MMP-7 may be potentially valuable as a prognostic indicator of cancer progression and that theoretically specific inhibition of its expression could contribute to inhibition of angiogenesis and anticancer therapy in general.

Another line in this study was to investigate the presence of any significant difference in local expression level of MMP-7 between OSCC sections infected and those not infected with HPV-16. Although a recent study has demonstrated such difference <sup>(13)</sup> we couldn't do so (P=1.00). However larger number of cases may be needed for confirming such association. In conclusion, MMP-7 was found to be highly expressed in OSCC sections with its clear expression in angiogenic endothelial cells reflecting its role in pathogenesis and angiogenesis of oral cancer.

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