

Matrix metalloproteinase 9 (MMP-9) expressions in oral cancer and adjacent normal tissue

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Abstract

Background Despite advances the oral cancer in India is continued to be diagnosed in late stages and have poor prognosis. The disease has preponderance for local recurrence and development of second primaries. To explain this theory of field cancerization has been proposed. In this study we looked at the expression of MMP-9 in tumor tissue and in the adjacent microscopically normal tissue, to explain higher failures in oral cancer after successful treatment.

Patients and Methods MMP-9 expression was looked at by immunohistochemistry in 100 patients with oral cancer in the tumor and adjoining normal tissue. Data on stage of the disease, treatment and other demographic and pathological features was collected in presetproforma.

Results Frequent expression of MMP-9 was observed in Oral Squamous Cell Carcinoma (OSCC), with 85% and 80% of tumor and adjacent tissue samples expressing the protein. The expression in patients with neck node metastasis was significantly higher compared to those with no nodal metastasis.

Conclusions The results of the present study show that MMP-9 overexpression is a good indicator of aggressive nature of the disease and is associated with increased nodal metastasis.

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Introduction

In oral squamous cell carcinoma, poor prognosis is related with loco-regional recurrence and metastatic disease. Positive histological margins, when reported, are associated with the local or recurrent disease. However, failures have been observed in

Table 1: Sites for Sample Collection

S.No.	Site	Number of Samples
1.	Buccal mucosa (Cheek)	44
2.	Tongue	12
3.	Alveolus	19
4.	Lip	14
5.	GingivoBuccal Sulcus (G. B. Sulcus)	11

surgical negative margin due to lack of sensitivity in evaluation of margin, despite high specificity [1, 2]. This has led to identification of cells, at the surgical negative margin, that have transformed into the cancer cell genetically but, not expressed phenotypically. Such genetically transformed cells could not be identified in surgical margin on histological assessment, and later, progress to produce recurrent disease. The role of genetic alteration at the molecular level has been investigated and several molecular factors, such as, TP53 mutations, VEGF and MMP-9 expression, telomerase activity and microsatellite instability, which alter the prognosis in the margin negative cases have been identified [3, 4].

One important hallmark of cancer progression is the degradation of the extracellular matrix (ECM), which allows cancer cells to invade the surrounding tissue. Matrix metalloproteinases (MMPs) are comprise a group of structurally related zinc-dependent endopeptidases that play major roles in tissue regeneration [3-6] and are of importance in virtually any process that involves remodeling, ranging from angiogenesis and cell proliferation to wound healing and degradation of dysfunctional tissue, and also in pathological processes such as inflammation, tumor invasion and metastasis[7].

Matrix metalloproteinase-9 (MMP-9), a 92 kDa gelatinase, that plays important roles in tumor invasion and angiogenesis [8, 9], and is

associated with the aggressive nature of many cancers, including OSCC. It is thought to cause type IV collagen degradation, a main component of basement membranes [10]. Increased MMP-9 protein levels, due to transcriptional upregulation of MMP-9, leads to increased MMP-9 activity. MMP expression increases a chance for local relapse, and lymph node and distant metastasis. High MMP-9 expressions are associated with the progression of lymph-node spread which is correlated with poor disease free survival. However, no correlation between MMP9 expression with clinical variables, such as, tumor stage and recurrence rate has been observed [5, 6]. It has been suggested that MMP-9 expression at histologically negative surgical margins could predict OSCC recurrence [11].

In this study, the ability of MMP-9 expression in identifying tumor-related alterations in histologically negative surgical margins has been evaluated. In this way, it is hypothesized that the expression profile of this gene in histologically negative margins could act as a more sensitive and useful marker for the detection of molecular alterations associated with local disease control in OSCC patients.

Material and Methods

Patient and Sample Selection

A cohort of 100 patients was selected for the purpose of study. These patients were diagnosed with primary HNSCC at the Department of Surgical Oncology, IMS, BHU, during 2006-2012, and were treated with surgery as primary curative modality of treatment. In all the cases, the surgical margins were reported to be histological negative by histopathologist at Department of Pathology, IMS, and BHU. Tumor tissue specimens from various sites in the oral cavity were obtained, along with the histological negative surgical margins from clinically normal mucosa 1 cm from the tumor (Table 1).

Immunohistochemistry

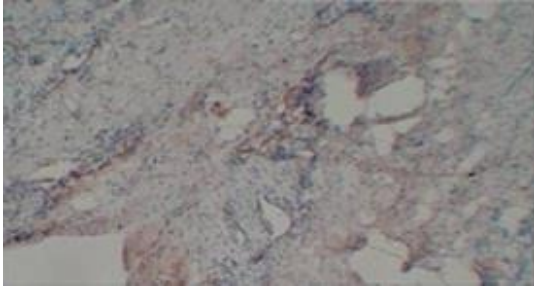


Figure 1: Photomicrograph showing negative staining for MMP9

Immunohistochemical staining technique was carried out to determine the MMP-9 protein expression levels in tumor specimens and respective surgical margins. Deparaffinized slides of 4-6 micron thick tissue sections were subjected to antigen retrieval in sodium citrate buffer (pH 6.0) with microwave irradiation for 10 minutes. Endogenous peroxidase activity was then quenched incubating specimens in peroxidase block for 5 minutes. Prior to application of primary antibodies, the sections were incubated with serum block for 20 minutes. Diluted primary antibody (1:200 μ l) (HPA001238 Sigma Aldrich) was then added to specimen and sections incubated for 20 minutes at room temperature. Washed specimens were then incubated with secondary antibody for about 30 minutes.

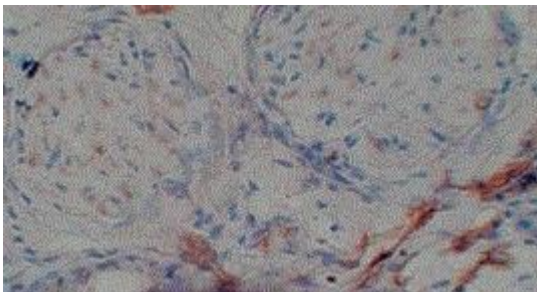


Figure 2: Photomicrograph showing cytoplasmic 1+ expression of MMP 9 in grade I squamous carcinoma (x100)

Immunohistochemical reactions were developed by addition of HRP-Streptavidin complex followed by HRP substrate, resulting in brown staining of specimens. After counterstaining with haematoxylin, the sections were observed under light microscope and the staining was graded and scored. Also, the type of staining was recorded.

Statistical Analysis

Statistical analysis of the obtained results was carried out using Fisher's Exact Test using GraphPad Quickcalcs software, available online, in order to determine any correlation between the expression of MMP-9 protein and various findings in the OSCC.

Results

Frequent expression of MMP-9 was observed in Oral Squamous Cell Carcinoma (OSCC), with 85% and 80% of tumor and adjacent tissue samples expressing the protein (Figure 1-4). With respect to the tumor grade, the expression in adjacent tissue was decreased in well differentiated tumors, while observed to be increased in case of poorly differentiated tumors (Figure 2-4). Increased immunohistochemical expression of MMP-9 was observed in tongue OSCC at the invasion front.

MMP-9 expression was significantly higher ($p < 0.0001$) in patients with regional lymph node metastasis compared to those with non-metastatic tumors. All cases with lymph node negative disease were negative for MMP-9 expression. [Table 2]

Discussion

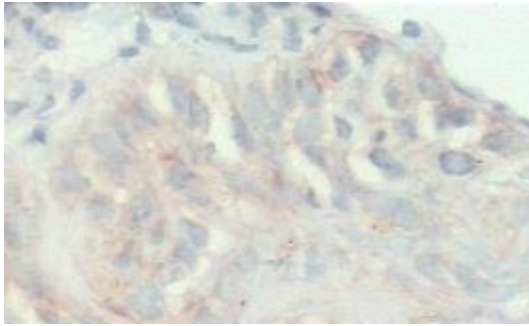


Figure 3: Photomicrograph showing moderate cytoplasmic (2+) expression of MMP 9 in moderately differentiated squamous cell carcinoma (x100)

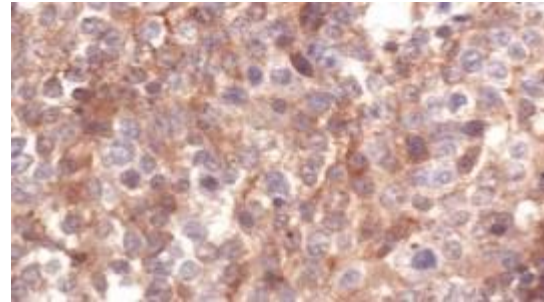


Figure 4: Photomicrograph showing 3+ cytoplasmic expression of MMP9 in squamous cell carcinoma.

It has been supposed that tumor cells undergoing genetic alteration in surgical margins reported negative by the histologist are responsible for tumor recurrence and prognosis of the patient in head and neck squamous cell carcinoma.

In this study, we have demonstrated the overexpression of MMP-9 in the tumor cell and

adjacent negative margin in the surgically treated case of oral squamous cell carcinoma and its correlation with lymph node metastasis. The MMP-9 over expression (in the tumor cell) has been well correlated with the lymph node metastasis [5, 6].

The patients having positive cervical lymph nodes with adjacent tissue reported normal were also investigated for the higher expression of MMP-9. These are the cells which are probably responsible for the local or

Table 2: Showing expression profile by various pathological parameters

Variable	MMP-9 Positive Tumor Samples	MMP-9 Negative Tumor Samples	MMP-9 Positive Adjacent Tissue Samples	MMP-9 Negative Adjacent Tissue Samples	P value
Overall					
	85	15	80	20	NS
Tumor Grade					
Well differentiated	43	15	34	20	
Moderately differentiated	32		28		
Poorly differentiated	10		18		
Lymph Node Metastasis					
Lymph node positive	75	10	70	15	<0.0001
Lymph node negative	-	15	-	15	

lymph node recurrence. Many other studies have reported high MMP-9 expression associated with the progression of lymph node spread, which are correlated with poor disease free survival (DFS). It has been shown that increased expression of this gene leading to enhanced synthesis of this protein (MMP-9), independently on other factors, increases a chance for local relapse, and distant metastasis. Consequently, patients with oral cancer have poor disease-free survival, as well as poor overall survival. No statistical correlation was found between the level of MMP-9 expression and histological and nuclear grade, tumor size, disease relapse or patients overall survival.

Our results of expression of MMP-9 in tumor tissue are comparable to the literature and investigation of positive expression of MMP-9 in adjacent normal tissue throws new light in explaining the cause of the recurrence of oral cancer even when reported negative by the pathologist. Thus, MMP-9 expression in adjacent normal tissue may play an important role in progression of HNSCC. Many other researchers have also demonstrated that overexpression of tumor-related genes in histologically negative surgical margins is a frequent event by the use of quantitative RT-PCR and may be an useful tool in detecting actually negative HNSCC surgical margins and the overexpression of specific genes in these margins could be helpful in the identification of patients with a higher risk of developing second primary tumor and local recurrences [3, 11].

Conclusions

Based on these results, we may speculate that the expression of these genes in HNSCC surgical margins, diagnosed as tumor-free by conventional histopathology, could be a helpful biomarker to identify subjects at risk of new disease. It can also be used as an alternative tool to guide the surgeon in the delineation of the HNSCC resection extent and also during follow-up in the planning of adjuvant therapy.

Authors' Contribution

DKR: Literature search and preparation of manuscript.

MK: Editing the manuscript and interpretation of pathological results

GS: Concept and design, editing of manuscript.

SBR: Concept and design, editing of manuscript.

TC: Literature search and preparation of manuscript.

AKS: Concept and design, editing of manuscript.

MP: Concept and design, editing of manuscript.

Conflict of Interests

The authors declares that there are no conflicts of interests.

Ethical Considerations

The study was approved by the Institute Ethics Committee.

Funding

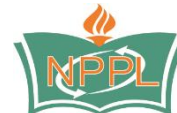
None

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