ORIGINAL RESEARCH

Immunoexpression of MMP-9 in Metastasis of Oral Squamous Cell Carcinoma

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Abstract

Introduction: Oral squamous cell carcinoma (OSCC) is the most common malignancy of the head and the neck. The unfavorable prognosis of OSCC is mainly due to extensive local invasion and frequent spread to the lymph node. Studies have supported that the metastatic potential of carcinoma might correlate with the degree of enzymatic degradation of basement membrane type IV collagen. MMP-9 a family member of zinc-dependent endopeptidases causes degradation of type IV collagen and plays a relevant role in tumor invasion and metastasis.

Aim and objective: To evaluate the role of MMP-9 in the invasion and metastasis of OSCC and to examine whether high levels of MMP-9 expression have any prognostic potential.

Materials and methods: MMP-9 expression was examined using the immunohistochemistry procedure in 30 OSCC cases with and without lymph node metastasis and in 10 normal controls. One set from each case and control was stained with the hematoxylin and eosin stain. The expression of MMP-9 was scored according to staining intensity and staining area. The relationship between MMP-9 expression and nodal metastasis was determined and statically analyzed.

Results: The staining for MMP-9 was observed in the cytoplasm of malignant keratinocytes within all the primary tumors as well as in the excised lymph nodes. MMP-9 positive expression was seldom found (less than 10% of cells) in the control group.

Conclusion: Higher MMP-9 expression in tumor proper and lymph node-positive cancers and overexpression in metastasized malignant cells indicate that MMP-9 plays an important role in invasion and metastasis of OSCC.

Keywords: Immunohistochemistry, MMP-9, Metastasis, Oral squamous cell carcinoma.

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Introduction

The major problem that the Asian continent is going through is head and neck cancers, with more than 2 lakh new cases diagnosed every year in India itself. India contributes up to 7.8% of the global cancer burden and 8.33% of global cancer deaths.[1] OSCC is the sixth most common malignancy of the head and the neck and is characterized by a high degree of local invasiveness and a high rate of metastasis to cervical lymph nodes.[2]

The unfavorable prognosis of OSCCs is mainly due to extensive local invasion and frequent spread to the lymph node.[3] The process of metastasis consists of tumor–host interactions that involve multiple extracellular matrix-degrading enzymes, including serine proteinases, cysteine proteinases, and matrix metalloproteinases (MMPs).[2] MMPs are a family of zinc-dependent endopeptidases, which cleave extracellular matrix. Cancer cells acquire this ability to become invasive. The type IV collagen is the main component of the basement membrane, and degradation of this structural protein is favored by MMP-2 and MMP-9 (known as type IV collagenases). Numbers of studies have supported the fact that metastatic potential of carcinoma might correlate with the degree of enzymatic degradation of the basement membrane type IV collagen.[4]

The study was carried out to evaluate the role of MMP-9 in the invasion and metastasis of OSCC and to examine whether high levels of MMP-9 expression have any prognostic potential. The expression of MMP-9 was investigated using immunohistochemistry in primary OSCC tissues and their cervical lymph node metastasis.

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Materials and Methods

Case Selection

After obtaining the institutional ethical approval for the study, a total number of 30 OSCC resection cases with concomitant neck dissections, comprising 15 cases positive for lymph node metastasis and 15 cases negative for lymph node metastasis, were retrieved from the archives of Department of Oral Pathology and Microbiology, MPDC, Vadodara, India.

Study samples were divided into two groups:

- Group I: 15 paraffin-embedded blocks of cases diagnosed as OSCC which showed histopathological evidence of regional lymph node metastasis.

- Group II: 15 paraffin-embedded blocks of cases diagnosed as OSCC without histopathological evidence of regional lymph node metastasis.

Ten tissue specimens of normal oral mucosa from noncancer patients with no history of tobacco or alcohol habits were included as the control. These tissues were taken during surgical extraction of impacted third molar teeth. All the tissues were routinely fixed in formalin and were subjected to tissue processing protocols and paraffin-embedded wax blocks were obtained. Two sections of 4 µ thickness were obtained from each case.

Staining Procedures

Out of the two sections, one was stained with hematoxylin and eosin stain, while for another section, immunohistochemical staining was performed for the expression of MMP-9 in metastatic and nonmetastatic tumor proper and lymph nodes. Immunohistochemical staining was performed where all the protocols for staining were followed. Formalin-fixed, paraffin-embedded-paraffin wax blocks of tumor proper, metastatic, and nonmetastatic lymph nodes were sectioned at 4 µ, mounted on poly-L lysine-coated slides, deparaffinized in xylene, and rehydrated through graded ethanol. To quench endogenous peroxidase, sections were incubated with fresh 3% H_2O_2 in methanol for 30 minutes at room temperature. After washing thrice in PBS and blocking endogenous biotin activity with normal goat serum for 30 minutes, sections were incubated overnight at 4°C with primary antibodies, then with secondary antibodies for 30 minutes at room temperature and followed again by washing thrice in PBS. The primary monoclonal antibodies, mouse anti-human MMP-9, were diluted 1:100 in 1% bovine serum albumin (BSA) in 1× PBS. Sections were counterstained with Mayer's hematoxylin. Positive and negative controls were taken to determine the false positive/negative expressions.

Evaluation of Staining

The expression of MMP-9 was scored according to staining intensity and staining area.[5] Four high power fields were randomly selected for evaluation. The staining intensity was scored as no staining (score 0), light yellow (score 1), yellow to brown (score 2), and dark brown (score 3). The staining area was scored as no staining (score 0), positive staining for less than one-third of tissue section (score 1), positive staining for one-third to two-thirds of tissue section (score 2), and positive staining for more than two-thirds of tissue section (score 3).

The sections were considered positive or negative according to the sum of the above two scoring systems and a score of ≥3 was considered as positive. Five high-power fields were randomly selected for observation. Percentages were calculated to determine the expression of these markers.

Statistical Analysis

The relationship between the expression of MMP-9 and nodal metastasis was determined by the Chi-square test using the SPSS 17 software. The *p* value below 0.10 was regarded as statistically significant.

Results

Staining for MMP-9 was observed in the cytoplasm of malignant keratinocytes within all the primary tumors as well as in the excised lymph nodes. MMP-9-positive expression was seldom found (less than 10% of cells) in the oral mucosa from the normal nonneoplastic group. Immunoexpression of MMP-9 in primary OSCC and regional lymph node metastasis.

Group I

Out of 15 cases, 13 (86.66%) cases were found to be positive for MMP-9 expression, whereas 2 (13.33%) cases were found to be negative for MMP-9 expression in tumor proper. Out of 15 cases, 8 (53.33%) cases were found to be positive for MMP-9 expression, whereas only 7 (46.66%) cases were found to be negative for MMP-9 expression in the lymph node (Figs 1 and 2).

Immunoexpression of MMP-9 in primary OSCC and nonmetastatic regional lymph node.

Group II

Out of 15 cases, 9 (60%) cases were found to be positive for MMP-9 expression, whereas 6 (40%) cases were found to be negative for MMP-9 expression in tumor proper. Out of 15 cases, 8 (53.33%) cases were found to be positive for MMP-9 expression, whereas 7 (46.66%) cases were found to be negative for MMP-9 expression in the lymph node (Figs 3 and 4).

The association between the two groups and immunoexpression of MMP-9 in tumor proper cases (positive and negative) was correlated using the Chi-square test and it was found to be statistically significant with a *p* value of 0.09 (statistically significant at $p < 0.10$) (Table 1). A similar association was found on comparing two groups and immunoexpression of MMP-9 in lymph node cases (positive and negative) using the Chi-square test and it was found to be statistically significant with a *p* value of 0.01 (statistically significant at $p < 0.10$) (Table 2). The altered expression of MMP-9 in tumor proper and metastatic and nonmetastatic lymph nodes suggest their distinctive role in invasion.

Discussion

MMPs play important roles in tumor biology, from early carcinogenesis, through tumor development to the breakdown of ECM and invasion.[4–6] MMP-9 is a Zn^{2+}-dependent endopeptidase that mediates the degradation of extracellular matrix protein and is associated with tumor invasion and metastasis. It is synthesized and secreted in a monomeric form as the zymogen and belongs to the gelatinase group.[7] Invasive and metastatic cancers such as colorectal cancer, gastric carcinoma, pancreatic carcinoma, breast cancer, and oral cancer showed increased expression of MMP-9.[7,8]

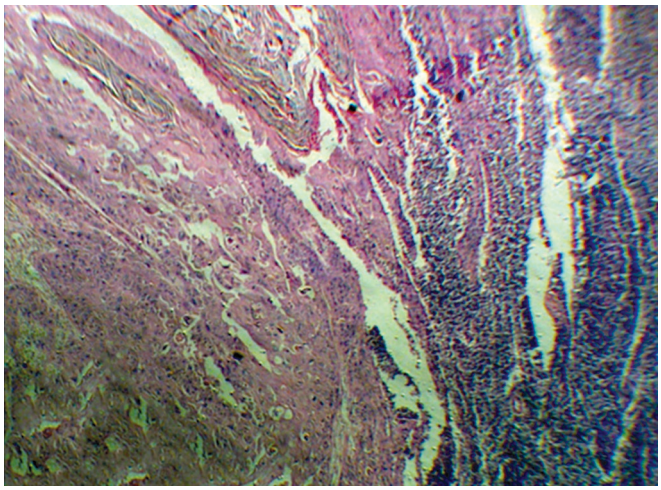

Fig. 1: Metastatic lymph node (H and E staining, 40×)

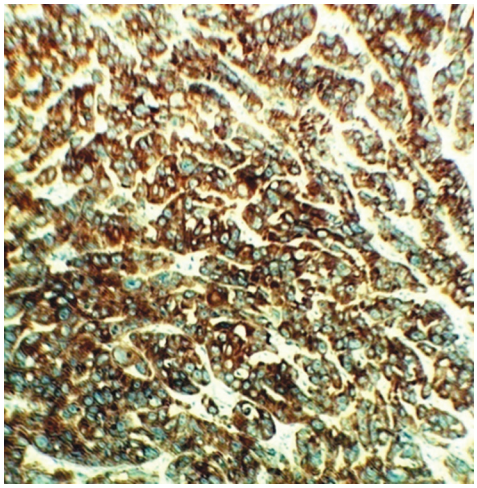

Fig. 2: Immunoexpression of MMP-9 in the metastatic lymph node (IHC, 40×)

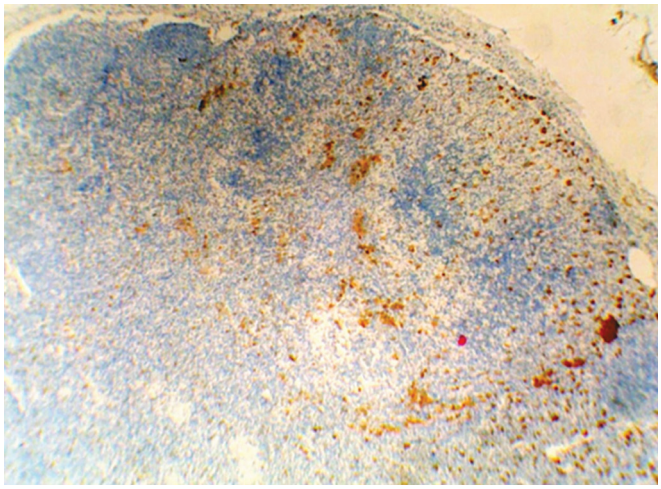

Fig. 4: Immunoexpression of MMP-9 in the metastatic lymph node (IHC, 10×)

Table 1: Correlation between groups I and II and positive and negative cases of tumor proper

Tumor proper	*Group I*	*Group II*	*Marginal row total*
Positive	13	9	22
Negative	2	6	8
Marginal column total	15	15	30

The Chi-square statistics is 2.7273. The *p* value is 0.098648
The result is statically significant at $p < 0.10$

Table 2: Correlation between groups I and II and positive and negative cases of lymph nodes

Lymph nodes	*Group I*	*Group II3*	*Marginal row total*
Positive	14	8	22
Negative	1	7	8
Marginal column total	15	15	30

The Chi-square statistics is 6.1364. The *p* value is 0.013243
The result is statically significant at $p < 0.10$

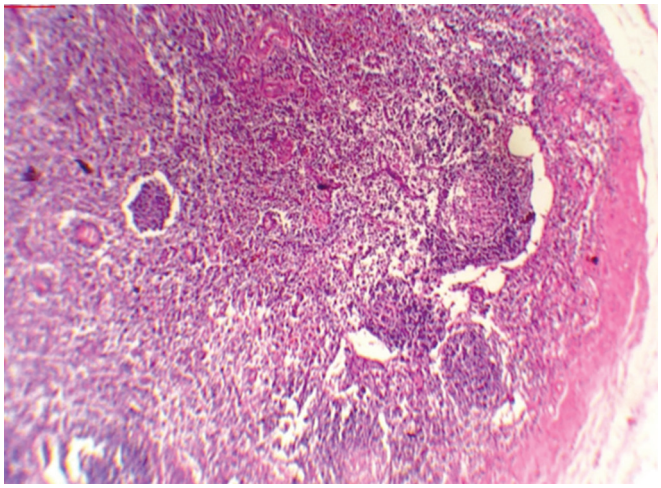

Fig. 3: Nonmetastatic lymph node (H&E staining, 10×)

In the present study, the expression of MMP-9 was investigated in tissues of the primary tumor in OSCC and in metastatic cervical lymph nodes. Expression of MMP-9 was seldom found in the normal oral epithelium, while out of 15 cases of primary oral carcinomas and lymph node metastasis, 13 (86.66% cases) were found to be positive for MMP-9 expression and 14 (93.33% cases) of the lymph node were found to be positive for MMP-9 expression, respectively. In the present study, MMP-9 protein was overexpressed with lymph node metastasis which is consistent with the study done by Zhou et al., who found that MMP-2 and MMP-9 played an important role in invasion and metastasis of tongue cancer and overexpression of MMP is strongly associated with nodal metastasis of tongue cancer and represents a strong prognostic value for a locoregional spread. Patel et al. in their study found that MMP-2 and MMP-9 levels were significantly elevated in malignant tissues as compared to their normal counterpart, the results of which are consistent with the present study.[3,4] de Vicente et al. observed stronger immunoexpression of MMP-2/MMP-9 in patients with lymph node metastasis and concluded that MMP-9 is related to poor prognosis in the subset of patients with neck node metastasis.[2] The present study suggests that MMP-9 could be important in the progression of OSCC which was also suggested by Ruokolainen et al. who found positive immunostaining for MMP-9 in 82% of the head and neck carcinomas and concluded that MMP-9 has a role in tumor progression of head and neck carcinomas, as well as in the estimation of the prognosis of these diseases and also stated that MMP-9 expression was independent of the stage or grade of the tumor.[9] In a study conducted by Kale et al. to confirm the establishment of field change by the expression of cytokeratins 8/18, 19, and MMP-9 in apparently normal mucosa adjacent to SCC, they found overexpression of MMP-9 in all cases of SCC, and also in 90% tissues of adjacent normal mucosa predicting invasive tumor progression, similar overexpression of MMP-9 was found in the present study.[5] Similarly increased expression of MMP-9 was found in nasopharyngeal carcinoma tissues as compared to normal nasopharyngeal tissues in the study conducted by Liu et al. Relationship between MMP-2/MMP-9 and lymph node metastasis with a statically significant correlation was obtained by studies conducted by Kusugawa et al., Hong et al., Tokumaru et al., Miyajima et al., and Kawamata et al.[7,10–14] Liu et al. concluded that high level of MMP-9 expression is a potential prognostic factor for patients with nasopharyngeal carcinoma, which was also reflected in the present study as stronger immunoreactivity was obtained for MMP-9.[7]

Tumor invasion and metastasis are multistep phenomena involving degradation of the basement membranes, rearrangement of the extracellular matrix, and angiogenesis.[4] The present study indicates that MMP-9 is the key member of MMP family involved in the processes of tumor invasion and metastasis. In the present study, it is shown that MMP-9 is strongly associated with nodal metastasis and represents a strong prognostic factor for a locoregional spread. It is understood from the present study that upregulated MMP expression contributes to the progress of cancer and inhibition of these proteases may be effective targets for cancer therapy.

Conclusion

The present study has shown that expression levels of MMP-9 are much higher in tumor proper and lymph node-positive cancers than in node-negative cancers and that overexpression is commonly found in the malignant cells which have metastasized to cervical lymph nodes. Thus, it indicates that MMP-9 plays an important role in cell invasion and metastasis in OSCC.

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