Original Article

# Role of MMP1 and MMP10 in local invasion and distant metastasis in different levels of oral squamous cell carcinoma - A immunohistochemical comparative study

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## Abstract

**Background:** Matrix metalloproteinases (MMPs) are basically a part of a large family of proteolytic enzymes. They play an important role in degrading extracellular matrix and basement membrane, which is a basic mechanism in local invasion and tumour metastasis. The aim of this study was to evaluate immunohistochemically the expression of MMP1 and MMP10 in tumour invasion locally and at distant levels, including lymph nodes at different levels in oral squamous cell carcinoma (OSCC).

**Materials and Methods:** A total of 50 tissue samples with clinically confirmed OSCC and 15 normal oral mucosal tissues will be included in the study. Immunohistochemical staining will be performed for the demonstration of MMP1 and MMP10 in lesional tissue, perilesional tissue, and lymph nodes of different levels that were evaluated with respect to microscopic features.

**Results:** All OSCC cases had MMP1 and MMP10 expression levels. The expression increased as the nodal level increased from level I to level V. This difference was statistically significant at $P < 0.001$ Both MMPs were not expressed in normal epithelial cells. There was no significant correlation between MMP1 and MMP10 expression.

**Conclusion:** This study showed that MMP1 and MMP10 are expressed in the tissues of OSCC and may serve as prognostic indicators for the disease.

**Keywords:** Immunohistochemistry, lymph node, MMP1 & MMP10, oral squamous cell carcinoma oral

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## INTRODUCTION

Oral squamous cell carcinoma (OSCC) is the most frequently occurring malignancy in the oral and maxillofacial region, which has significant propensity for invasiveness and is consequently linked to a high mortality rate.[1] The main causes of oral SCC-related death are regional lymph node metastasis and distant organ metastasis.[2] The TNM staging system has been employed for many years to predict treatment responses and clinical outcomes. But even after receiving the appropriate treatment, many patients with stage I/II disease have died from oral SCC.[3] As a result, a more accurate assessment of invasion patterns and indicators is required.

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The MMPs are a group of highly conserved metal atom-dependent endopeptidases that may collectively degrade the majority, if not all, of the extracellular matrix and basement membrane components.[4] Particularly the MMPs have the only enzymes known to be capable of degrading fibrillar collagen. Fibrillar collagen refers to the polymeric structure adopted by collagens I, II, III, V and XI.[4,5] In humans, Type I collagen is the most abundant and comprises the principal collagen found in skin and bones.[4] MMPs are produced by a array of cell types, including epithelial cells, fibroblasts and inflammatory cells.[6-8]

Researchers have shown that MMP1 and MMP10, which are overexpressed in head and neck SCC and are all found on chromosome 11q22.3, may be useful as predictive markers. MMP10, which is induced in lymphoma cells and is known to speed up growth, degrades a variety of extracellular matrix components of lymphoid tumours in vivo.[9] It has been demonstrated that MMP10 can fully activate proMMP-1. Thus, this implies that MMP10 plays a role in triggering MMP1 in human cancer tissues.[10,11]

Studies have shown activated MMP1 has been found near the peripheral boundaries of tumour islands, where tumour cells have the ability to invade, during the invasion and metastasis phase of several malignancies.[12-14]

In spite of many studies, the current diagnostic procedures do not consider the correlation of oral squamous cell carcinoma with local invasion, which alters the tumour microenvironment and distant metastasis, including regional lymph node metastasis, which may show varied biochemical alterations. Hence, in our study, by taking MMP1 and MMP10, we are evaluating the tumour invasion locally and at a distant level, including lymph nodes of different levels. This should give us insights into the early diagnostic and better prognostic value of MMP1 and MMP10 markers.

## MATERIALS AND METHODS

### Patient selection

The proposed study involves the use of paraffin-embedded tissue blocks of previously diagnosed 50 cases of OSCC and metastatic lymph nodes of different levels in the test group, and 15 normal oral mucosal tissues in the control included in the study will be taken from the Department of Oral & Maxillofacial Pathology, Rajarajeswari Dental College and Hospital, Bangalore. This research was approved by the Ethical Committee of the college No.RRDCH ET/02 ORALP/2018-19.

### Inclusion criteria

1. Radical neck dissection cases of oral squamous cell carcinoma
2. Histopathologically confirmed cases of oral SCC of different grades with metastatic lymph node
3. Patients not treated previously for the same.

### Exclusion criteria

1. Oral squamous cell carcinoma without metastasis, incisional biopsy
2. Presence of other simultaneous primary tumours and patients who refused surgical treatment.

### Immunohistochemical staining

The following IHC procedure is used for both MMP1 and MMP10. The paraffin-embedded tissues were cut into 4 micron slices and used for IHC. The sections were subsequently deparaffinized and rehydrated in graded ethanol and xylene, respectively. The slides were washed with phosphate-buffered saline after being treated with 3% hydrogen peroxide/methanol for 30 minutes to inhibit the endogenous peroxidase activity.

To retrieve antigen, the sections were immersed in a citrate solution (PH = 6) and heated in a microwave oven for 30 min. Sections were incubated for 1 hour at room temperature with primary mouse monoclonal anti-human antibodies MMP1 [code 6A5-MMP-1] and MMP10 [code 117239] Dilute 1:100 according to the manufacturer's instructions. After three PBS rinses at room temperature, the secondary antibody was then applied to the sections. Streptavidin peroxidase was used to incubate the immune complexes. They were visualized by immersing the slides in diaminobenzidine solution and counter-stained with Mayer's hematoxylin. The slides were cleaned in xylene and dehydrated before being mounted in DPX. Sections of paraffin-embedded human cervical cancer were used as a positive control.

### Evaluation of immunohistochemically stained sections

Two independent pathologists examined the slides under a light microscope. Hot spots, or the areas where cells are most densely populated, were examined under 40x magnification to measure the activity of MMP1 and MMP10. Expression of MMP1 was localized to the cytoplasm and outer membrane of tumour cells [Figure 1]. MMP10 is expressed by tumour cells in their cytoplasm [Figure 2]. Tumour cells were stained, and the staining was graded on a scale of 0–3.

Scoring of staining: score 0 (negative), score1 (low expression): <10%, score 2 (moderate expression): >10% <50% and score 3 (intensive expression): >50% positive staining (Mashhadiabbas *et al*., 2012)[15]

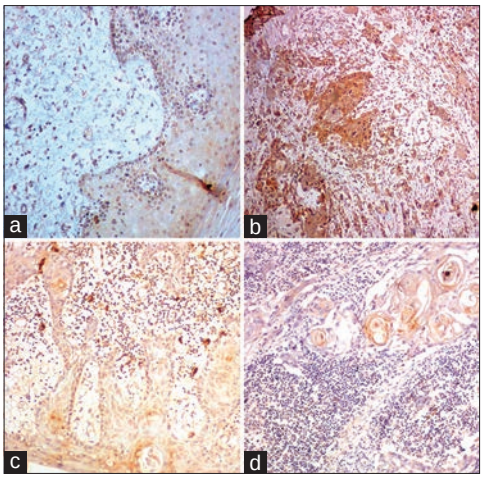


**Figure 1:** Photomicrograph shows expression of MMP1 in OSCC. (a) Negative (10x). (b) Intensive expression (10x). (c) Moderate expression (10x). (d) Low expression (10x)

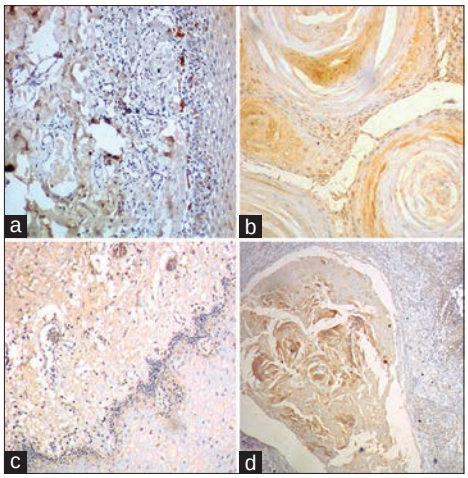


**Figure 2:** Photomicrograph shows expression of MMP10 in OSCC. (a) Negative (10x). (b) Intensive expression (10x). (c) Low expression (10x). (d) Moderate expression (10x)

### Statistical analysis

Data analysis was performed using SPSS for Windows Version 22.0, released in 2013, IBM Corp., Armonk, NY. The Chi-square test was used to compare the intensity of MMP1 and MMP10 expression in the lesion proper, peri-lesional tissue and lymph nodes at different nodal levels of OSCC. The Spearman's rank correlation was used to analyse the relationship between MMP1 and MMP10 expression. The level of significance was set at *P* 0.05.

## RESULTS

### Clinical characteristics

OSCC patients ranged in age from 30 to 75 years (mean age: 51.38%): female patients were more (58%), and male patients were (42%). According to histopathological grading, well differentiated were (48%), moderately differentiated were (30%) and poorly differentiated were (22%) taken for the study.

### Immunohistochemical findings

Immunohistochemical expression of MMP1 in lesion proper was compared with normal mucosa and different nodal levels of OSCC. MMP1 expression in lesion proper and at different nodal levels of OSCC showed low expression [40–60%], followed by moderate expression [30–40%] and intense expression [10–30%]. A significant difference was noted between the expression of MMP1 in lesion proper and normal tissue, which showed 100% negative expression. This difference was statistically significant at $P < 0.001$. However, there was no significant differences noted between lesion proper and different nodal levels of OSCC [P = 0.61] [Figure 3].

The expression of MMP1 levels in peri-lesion area between normal tissue and different OSCC nodal levels showed negative expression [100%] for normal tissue and predominant distribution in level I and level II with 60%, and with progressive levels from III to V, with low expression [50–70%]. However, no significant differences were noted in expression of MMP1 levels in peri-lesion area between normal tissue and different OSCC nodal levels [Figure 3].

A significant difference was noted in the expression of MMP1 levels in lymph nodes between normal tissues with 100% negative expression. The different OSCC nodal levels showed predominant low expression [60–70%] in levels I to III, followed by moderate expression [40–80%], signifying that MMP1 expression increased as the nodal level increased from level I to level V. This difference was statistically significant at $P < 0.001$. However, there was no significant difference noted in the lymph node level between different nodal levels of OSCC [P = 0.12] [Figure 3].

A Spearman's correlation analysis was performed to assess the relationship between the expression of MMP1 in lesion proper, peri-lesion, and lymph node areas in different nodal levels of OSCC. In level I lesions, there was a significant positive correlation between the lesion proper and the lymph node [rho = 0.70] at $P = 0.02$. In level III lesions, there was a significant positive correlation between the lesion proper and the lymph node [rho = 0.83] at $P = 0.003$, and a significant moderate negative correlation between the lesion proper and the lymph node [rho =- 0.72] at $P = 0.02$. The peri-lesional tissue showed a negative correlation with the lymph node in level II [rho = -0.80] at $P = 0.005$ [Table 1].

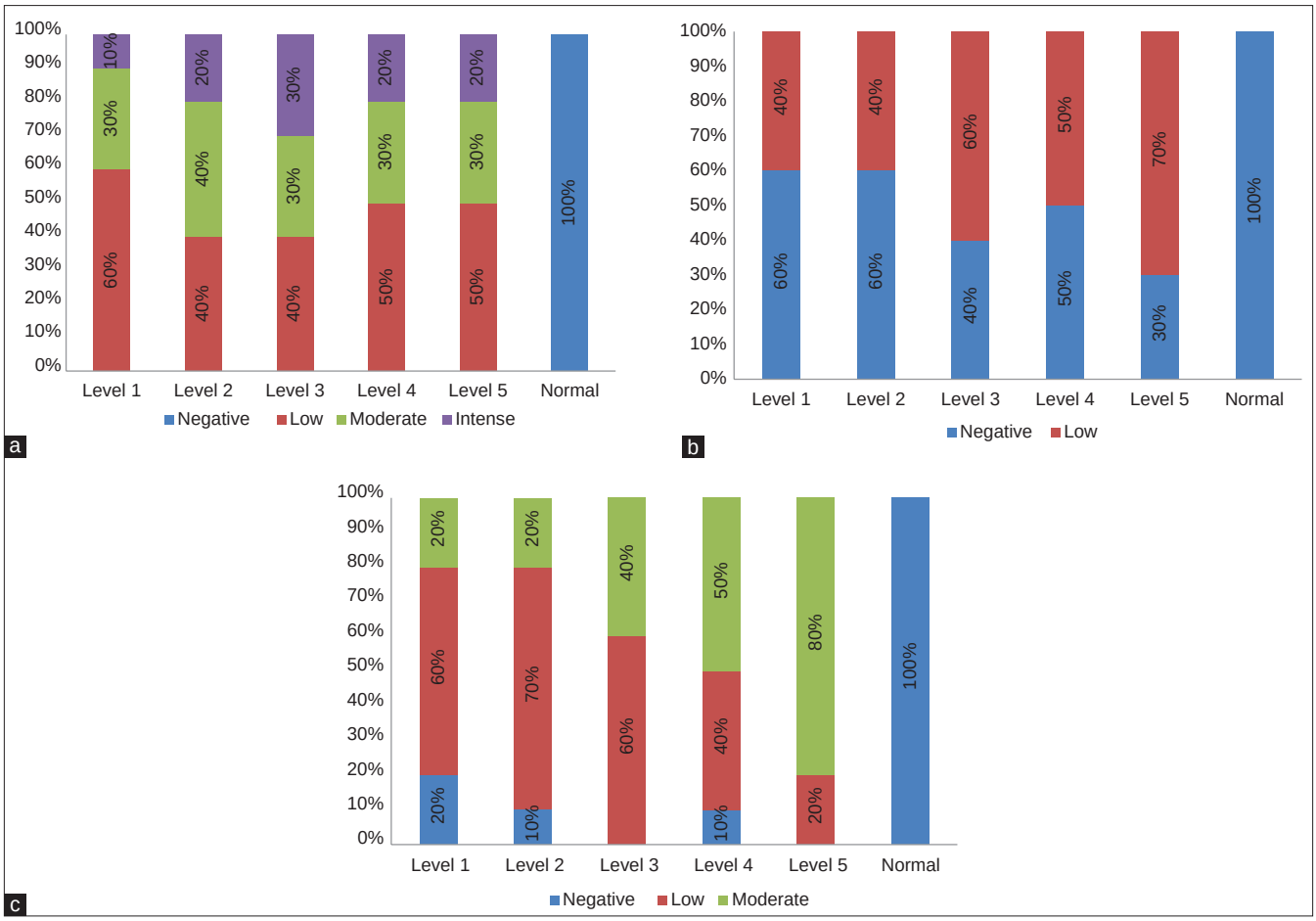


**Figure 3:** MMP1 expression in (a) lesion proper at different nodal levels. (b) Peri-lesional tissue at different nodal levels. (c) Lymph nodes at different nodal levels using Chi-square test

Immunohistochemical expression of MMP10 in lesion proper was compared with normal mucosa and different nodal levels of OSCC. MMP10 expression in lesion proper and at different nodal levels of OSCC showed low expression [10–40%], followed by moderate expression [40–70%] and intense expression [10–30%]. Significant difference was noted between the expression of MMP10 in lesion proper and normal tissue, which showed 100% negative expression. This difference was statistically significant at $P < 0.001$. However, there was no significant difference noted between lesion proper and different nodal levels of OSCC [P = 0.86] [Figure 4].

The MMP10 expression in the peri-lesional tissue compared with normal tissue and various OSCC nodal levels revealed that normal tissue had negative expression levels of 100%. The MMP10 expression in levels I and II was predominately negative and low distributions of 40–60%, and levels III to V had low expression levels of [50–70%]. However, no significant differences were noted in expression of MMP10 levels in peri-lesion area between normal tissue and different OSCC nodal levels [p = 0.72] [Figure 4].

MMP10 expression levels in lymph nodes varied significantly between normal tissue, which had 100% negative expression, and various OSCC nodal levels, with low expression predominating in levels I to III [50–60%], followed by moderate expression [50–90%], signifying that MMP10 expression increased as the nodal level increased from level I to level V. This difference was statistically significant at $P < 0.001$. There was a significant difference noted in the lymph node level between different nodal levels of OSCC [P = 0.03*], signifying that as the nodal level increased from level I to level V, MMP10 expression increased [Figure 4].

There was no significant difference for MMP1 and MMP10 expression in different histopathological grades of OSCC cases.

Spearman's correlation analysis was performed to assess the relationship between the expression of MMP10 in

lesion proper, peri-lesion and lymph node areas in different nodal levels of OSCC. A significant positive correlation was found between peri-lesional tissue and lymph node [rho = 0.63] at $P = 0.04$ in level IV lesions. Similarly, a moderate positive correlation was found between lesion proper and lymph node [rho = 0.56] at $P = 0.04$ in level V lesions. However, there was no significant correlation found between the expression in lymph node levels and lesion proper and peri-lesion areas in other levels [Table 2].

**Table 1: Spearman's correlation test to assess the relationship between the intensity of expression in the MMP1 in lesion proper, peri-lesion and lymph node areas in different nodal levels of OSCC**

| Levels | Variables | Values | PL | LN1 | LN2 | LN3 | LN4 | LN5 |
|---|---|---|---|---|---|---|---|---|
| Level 1 | LP | rho | -0.12 | 0.70 | . | . | . | . |
| | | *P* | 0.74 | 0.02* | . | . | . | . |
| | PL | rho | 1 | -0.32 | . | . | . | . |
| | | *P* | . | 0.36 | . | . | . | . |
| Level 2 | LP | rho | -0.30 | 0.06 | 0.08 | . | . | . |
| | | *P* | 0.39 | 0.87 | 0.82 | . | . | . |
| | PL | rho | 1 | -0.32 | -0.80 | . | . | . |
| | | *P* | . | 0.36 | 0.005* | . | . | . |
| Level 3 | LP | rho | -0.38 | 0.10 | 0.05 | 0.83 | . | . |
| | | *P* | 0.28 | 0.99 | 0.90 | 0.003* | . | . |
| | PL | rho | 1 | -0.08 | -0.41 | -0.17 | . | . |
| | | *P* | . | 0.84 | 0.24 | 0.65 | . | . |
| Level 4 | LP | rho | -0.04 | 0.03 | 0.29 | 0.44 | 0.15 | . |
| | | *P* | 0.92 | 0.93 | 0.42 | 0.20 | 0.68 | . |
| | PL | rho | 1 | -0.23 | -0.22 | -0.27 | -0.20 | . |
| | | *P* | . | 0.52 | 0.55 | 0.45 | 0.58 | . |
| Level 5 | LP | rho | 0.08 | 0.15 | 0.09 | 0.45 | -0.72 | 0.35 |
| | | *P* | 0.82 | 0.69 | 0.80 | 0.19 | 0.02* | 0.32 |
| | PL | rho | 1 | -0.13 | -0.33 | -0.08 | -0.28 | 0.13 |
| | | *P* | . | 0.72 | 0.36 | 0.82 | 0.43 | 0.72 |

*Statistically significant; the correlation coefficients are denoted by 'rho'; Minus sign denotes negative correlation; correlation coefficient range; 0.0 - No Correlation; 0.01-0.20 - Very Weak Correlation; 0.21-0.40 - Weak Correlation; 0.41-0.60 - Moderate Correlation; 0.61-0.80 - Strong Correlation; 0.81-1.00 - Very Strong Correlation

**Table 2: Spearman's correlation test to assess the relationship between the expression in the MMP10 in lesion proper, peri-lesion and lymph node areas in different nodal levels of OSCC**

| Levels | Variables | Values | PL | LN1 | LN2 | LN3 | LN4 | LN5 |
|---|---|---|---|---|---|---|---|---|
| Level 1 | LP | rho | 0.24 | -0.28 | . | . | . | . |
| | | *P* | 0.50 | 0.43 | . | . | . | . |
| | PL | rho | 1.00 | -0.32 | . | . | . | . |
| | | *P* | . | 0.36 | . | . | . | . |
| Level 2 | LP | rho | -0.22 | -0.01 | 0.31 | . | . | . |
| | | *P* | 0.54 | 0.98 | 0.39 | . | . | . |
| | PL | rho | 1.00 | -0.19 | 0.02 | . | . | . |
| | | *P* | . | 0.59 | 1.00 | . | . | . |
| Level 3 | LP | rho | 0.15 | 0.28 | 0.09 | -0.14 | . | . |
| | | *P* | 0.68 | 0.44 | 0.80 | 0.70 | . | . |
| | PL | rho | 1.00 | 0.15 | -0.50 | 0.15 | . | . |
| | | *P* | . | 0.68 | 0.14 | 0.68 | . | . |
| Level 4 | LP | rho | 0.08 | -0.04 | 0.34 | 0.20 | 0.06 | . |
| | | *P* | 0.84 | 0.92 | 0.34 | 0.57 | 0.87 | . |
| | PL | rho | 1.00 | 0.08 | -0.26 | -0.34 | 0.63 | . |
| | | *P* | . | 0.83 | 0.47 | 0.34 | 0.04* | . |
| Level 5 | LP | rho | -0.08 | 0.04 | -0.09 | 0.48 | 0.56 | 0.15 |
| | PL | *P* | 0.82 | 0.91 | 0.80 | 0.17 | 0.04* | 0.68 |
| | | rho | 1.00 | -0.13 | -0.33 | 0.08 | -0.28 | 0.25 |
| | | *P* | . | 0.72 | 0.36 | 0.82 | 0.43 | 0.48 |

*Statistically significant; the correlation coefficients are denoted by 'rho'; Minus sign denotes negative correlation; correlation coefficient range; 0.0 - No Correlation; 0.01-0.20 - Very Weak Correlation; 0.21-0.40 - Weak Correlation; 0.41-0.60 - Moderate Correlation; 0.61-0.80 - Strong Correlation; 0.81-1.00 - Very Strong Correlation

## DISCUSSION

A number of studies have attempted to explain which, if any, of the MMPs are required for head and neck squamous cell carcinoma (HNSCC) to grow and spread.[16] Until now, the predictive value of the MMPs in invasion and metastasis of HNSCC has been controversial, partly because of the different methods used to detect the expression of MMPs. Because the components of the extracellular matrix are complex, the combined action of various MMPs is essential for the efficient degradation of the structure. Therefore, a thorough examination of the expression of various MMPs is necessary to understand the intricate mechanisms by which cancers develop the ability to invade and spread.[17,18] MMP1 and 10 might be related to early consequences that occurred in this field as indicators or markers for the initiation of OSCC.[19] So we have taken two MMPs, MMP1 and MMP10.

In this study, we examined the expression of MMP1 in lesional tissue. The results showed that MMP1 appeared in tumoural samples, whereas its expression in normal epithelium was negative. Our results are consistent with the hypothesis that MMP1, which is highly active against interstitial collagen and is produced by cancer cells or mesenchymal cells, may contribute to the breakdown of the extracellular matrix and, in turn, may promote the invasion of cancer cells into stromal tissues.[20-22] Also there is existence of the MMP1/PAR-1 signalling axis in oral SCC and its close relationship to tumour angiogenesis. This relationship with angiogenesis not only provides necessary nutrients to the tumour cells, but also facilitates tumour invasion and migration.[23,24] Furthermore, these findings are in accordance with those of previous studies.[25,26]

MMP1 expression in peri-lesional tissue was compared to different levels of lymph nodes, with level I and level II lymph nodes having predominant expression. In our study, we examined tumour margins on the hypothesis that cellular processes at the tumour-stromal interface could be more closely related to the metastatic potential of the tumour than the lesion proper (tumour tissue).[18] Similar to our study, Pornchai *et al.* study showed expression of MMP1, MMP2, MMP3, MMP7, MMP9 and MMP10 was significantly higher in malignant tissues (primary tumours

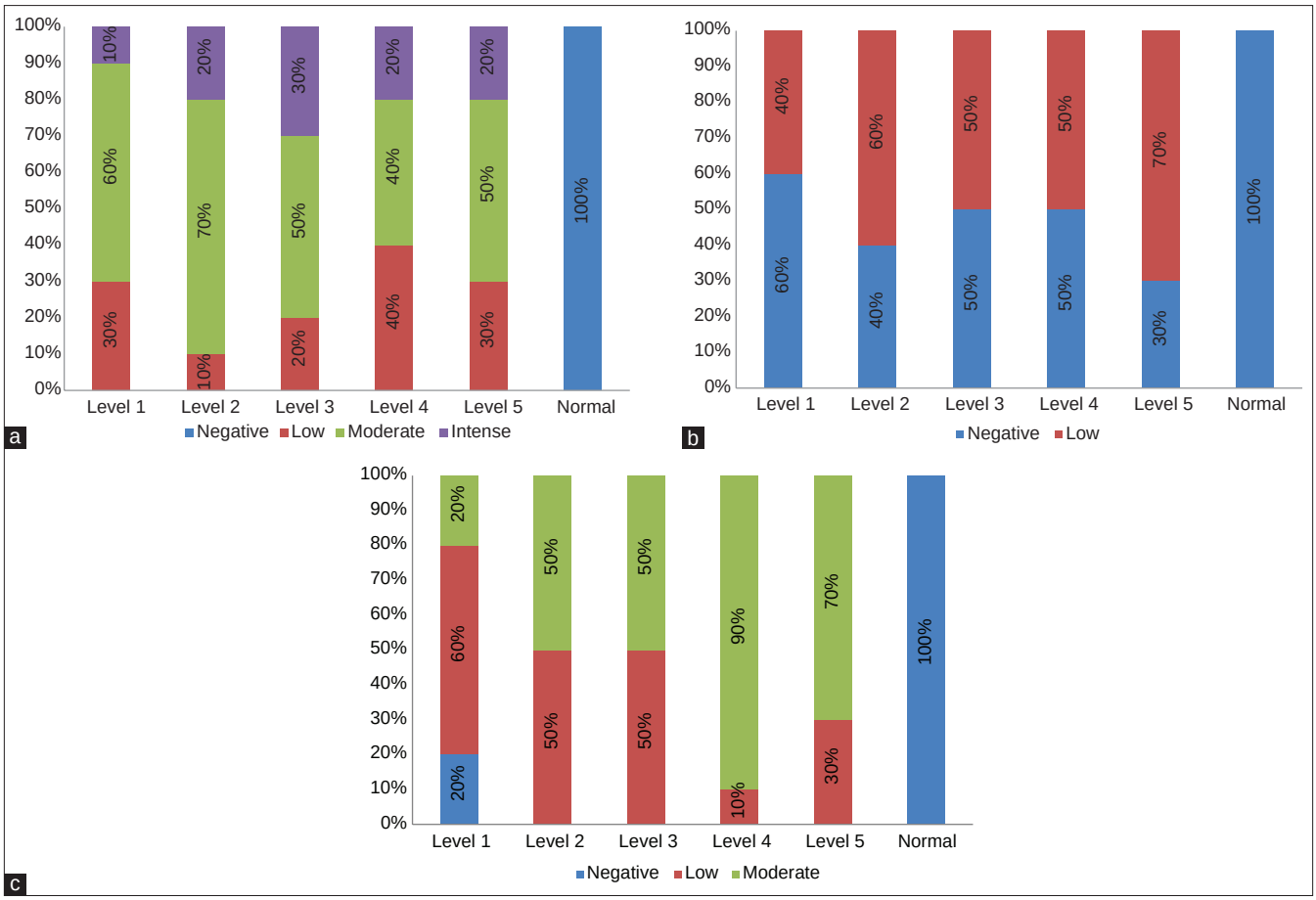


**Figure 4:** MMP10 expression in (a) lesion proper at different nodal levels. (b) Peri-lesional tissue at different nodal levels. (c) Lymph nodes at different nodal levels using Chi-square test

and lymph nodes) compared to levels in histologically normal adjacent mucosa (P =0.02 to. 001).[18]

Our results indicating that MMP1 expression increased as the nodal level increased from level I to level V. Consistent with these results, the study by Ha Ixia Fan *et al*.[23] showed correlation between MMP1 protein expression and lymphatic metastasis has been amply demonstrated, and high MMP1 expression indicates poor prognosis. To our knowledge, there are no earlier studies which have considered lymph nodes of different levels (level I–level V). Our findings are consistent with those of Pornchai *et al*.[18] who found that MMP1 protein levels in primary tumours were 4.1-fold higher and lymph node metastasis was 3.8-fold higher (p <.001) when compared to control tissues. The up-regulation of several MMPs in lymph node-positive patients raises the possibility that the evaluation of MMPs in HNSCC tissues at the time of presentation may allow identification of a subset of patients with HNSCC who are more susceptible to metastatic spread via lymphatic pathways and enable appropriate therapy to be provided.[27]

Sutinen *et al*.[28] investigated the expression of MMP1, MMP2 and TIMPs in oral premalignant lesions, OSCC and lymph node metastases. The results demonstrated that the MMPs and TIMPs under investigation were up-regulated during invasion in oral SCC.

A significant MMP1 correlation was found between the lesion proper and lymph node levels I and III, whereas this correlation was not found in other levels may be due to smaller sample size.

Our results showed MMP10 expression in the lesion proper of all the studied tumoural samples, whereas its expression in normal epithelium was negative. This result is significant, which signifies MMP10 protein may be one of the important possible factors in transforming a normal epithelium into OSCC. The work by Tsang *et al*.[29] supported our findings by demonstrating that tongue cancer tissues had more MMP10 expression than normal epithelium. Additionally, OSCC treatment (tongue area) utilizing curcumin, a substance derived from the root of Curcuma longa, can reduce and inhibit cell migration

and invasion in OSCC by lowering MMP10 protein production.

Mashhadiabbas *et al*.[15] investigated MMP2, MMP10, TIMP-1 and TIMP-2 markers in OSCC. MMP10 was expressed in all OSCC samples, and the protein expression had a moderate staining intensity, similar to the current study. Additionally, no correlation was found between MMP10 expression in OSCC and histopathologic gradings; this finding is consistent with our findings.

In the study of Impola *et al*.,[30] the tumoural epithelium of OSCC expressed more than 90% of the MMP10 protein. Finally, it was proposed that the invasive behaviour of oral cancer may be correlated with the expression pattern of MMPs.

In our investigation, peri-lesional tissue displayed 100% expression of MMP10, but normal tissue did not. This finding is concordant with other studies Mashhadiabbas *et al*.,[15] and Tsang *et al*.[29] showed the immunohistochemistry staining for MMP10 in paired tissue arrays from tongue cancer and adjacent normal tissue aims to confirm that tongue cancer cells actually overexpress MMP10. Overall, 85% tongue SCC tissues demonstrated high-to-medium expression of MMP10. In comparison with the adjacent normal tongue epithelial tissue, low-to-medium expression of MMP10 was demonstrated.

Our results showed MMP10 expression in different levels of lymph nodes increased as the nodal level increased from level I to level V. According to our research, Deraz *et al*.[31] study revealed a substantial relationship between MMP10 expression and lymph node metastases ($P = 0.001$). Twenty-nine of the 89 HNSCC cases with high MMP10 expression showed lymph node metastases, while only 5 of the 27 HNSCC cases with low MMP10 expression did. Mashhadiabbas *et al*.[15] also found a positive relationship between MMP10 expression and lymphatic vessel density (LVD) in TIF; therefore, MMP10 expression may be associated with the lymphatic metastasis in OSCC tumours.[32]

MMP10 level has also been demonstrated to be correlated with the size of the local tumour, the invasiveness of the tumour and distant metastasis in oesophageal cancer, indicating that it played a key part in the development of the disease.[33]

We also found significant MMP10 positive correlation between peri-lesional tissue and lymph node in level IV lesions. Similarly, a moderate positive correlation was found between lesion proper and lymph node in level V lesions. In consistent with our study, Deraz *et al*.[31] found 76.7% of OSCC had significant MMP10 protein expression. MMP10 expression, invasion pattern, disease stage and lymph node metastases, however, were all significantly correlated with one another.

Overall, MMP1 and MMP10 expressions did not significantly differ with respect to lesion proper, peri-lesion area and lymph node in different nodal regions from level I to level V. We did not find this correlation, which may be due to small sample size in the present study.

In conclusion, according to our study, MMP1 and MMP10 proteins play a role in the transmission of non-neoplastic oral epithelium to OSCC. These proteins have potential role in progression and metastasis of OSCC. Therefore, we established that MMP1 and MMP10 are possible prognostic or predictive markers for oral cancer.

**Declaration of patient consent**

The authors certify that they have obtained all appropriate patient consent forms. In the form, the patient(s) has/have given his/her/their consent for his/her/their images and other clinical information to be reported in the journal. The patients understand that their names and initials will not be published and due efforts will be made to conceal their identity, but anonymity cannot be guaranteed.

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**Conflicts of interest**

There are no conflicts of interest.

**REFERENCES**

1. Byers RM, El-Naggar AK, Lee YY, Rao B, Fornage B, Terry NH, *et al*. Can we detect or predict the presence of occult nodal metastases in patients with squamous carcinoma of the oral tongue? Head Neck 1998;20:138-44.
2. Mamelle G, Pampurik J, Luboinski B, Lancar R, Lusinchi A, Bosq J. Lymph node prognostic factors in head and neck squamous cell carcinomas. Am J Surg 1994;168:494-8.
3. Chang YC, Nieh S, Chen SF, Jao SW, Lin YL, Fu E. Invasive pattern grading score designed as an independent prognostic indicator in oral squamous cell carcinoma. Histopathology 2010;57:295-303.
4. Curran S, Murray GI. Matrix metalloproteinases in tumour invasion and metastasis. J Pathol 1999;189:300-8.
5. Itoh Y. Membrane-type matrix metalloproteinases: Their functions and regulations. Matrix Biol 2015;44-6:207-23.
6. Rosenthal EL, McCrory A, Talbert M, Carroll W, Magnuson JS,

Peters GE. Expression of proteolytic enzymes in head and neck cancer-associated fibroblasts. Arch Otolaryngol Head Neck Surg 2004;130:943-7.
7. Stetler-Stevenson WG. Matrix metalloproteinases in angiogenesis: A moving target for therapeutic intervention. J Clin Invest 1999;103:1237-41.
8. Rydlova M, Holubec L Jr, Ludvikova M Jr, Kalfert D, Franekova J, Povysil C, *et al*. Biological activity and clinical implications of the matrix metalloproteinases. Anticancer Res 2008;28:1389-97.
9. Van Themsche C, Alain T, Kossakowska AE, Urbanski S, PotworowskiEF, St-Pierre Y. Stromelysin-2 (matrix metalloproteinase 10) is inducible in lymphoma cells and accelerates the growth of lymphoid tumors in vivo. J Immunol 2004;173:3605-11.
10. Nicholson R, Murphy G, Breathnach R. Human and rat malignant-tumor-associated mRNAs encode stromelysin-like metalloproteinases. Biochemistry 1989;28:5195-203.
11. Yen CY, Chen CH, Chang CH, Tseng HF, Liu SY, Chuang LY, *et al*. Matrix metalloproteinases (MMP) 1 and MMP10 but not MMP12 are potential oral cancer markers. Biomarkers 2009;14:244-9.
12. Boire A, Covic L, Agarwal A, Jacques S, Sherifi S, Kuliopulos A. PAR1 is a matrix metalloprotease1 receptor that promotes invasion and tumorigenesis of breast cancer cells. Cell 2005;120:303-13.
13. George A, Ranganathan K and Rao UK. Expression of MMP1 in histopathological different grades of oral squamous cell carcinoma and in normal buccal mucosaan immunohistochemical study. Cancer Biomark 2010;7:275-83.
14. Zhou J, Brinckerhoff C, Lubert S, Yang K, Saini J, Hooke J, *et al*. Analysis of matrix metalloproteinase1 gene polymorphisms and expression in benign and malignant breast tumors. Cancer Invest 2011;29:599-607.
15. Mashhadiabbas F, Mahjour F, Mahjour SB, Fereidooni F, Hosseini FS. The immunohistochemical characterization of MMP-2, MMP-10, TIMP-1, TIMP-2, and podoplanin in oral squamous cell carcinoma. Oral Surg Oral Med Oral Pathol Oral Radiol 2012;114:240-50.
16. Thomas GT, Lewis MP, Speight PM. Matrix metalloproteinases and oral cancer. Oral Oncol 1999;35:227-33.
17. Curran S, Murray GI. Matrix metalloproteinases: Molecular aspects of their roles in tumour invasion and metastasis. Eur J Cancer 2000;36:1621-30.
18. O-Charoenrat P, Rhys-Evans PH, Eccles SA. Expression of matrix metalloproteinases and their inhibitors correlates with invasion and metastasis in squamous cell carcinoma of the head and neck. Arch Otolaryngol Head Neck Surg 2001;127:813-20.
19. Sareh F, Mehdi M. Significance of MMPs expression on OSCC clinical outcome. SL Dent Oral Disord Ther 2017;1:111.
20. Gray ST, Wilkins RJ, Yun K. Interstitial collagenase gene expression in oral squa-mous cell carcinoma. Am J Pathol 1992;141:301-6.
21. Muller D, Wolf C, Abecassis J, Millon R, Engelmann A, Bronner G, *et al*. Increased stromelysin 3 gene expression is associated with increased local invasiveness in head and neck squamous cell carcinomas. Cancer Res 1993;53:165-9.
22. Bryne M, Koppang HS, Lilleng R, Stene T, Bang G, Dabelsteen E. New malignancy grading is a better prognostic indicator than Broders' grading in oral squamous cell carcinomas. J Oral Pathol Med 1989;18:432-7.
23. Fan HX, Chen Y, Ni BX, Wang S, Sun M, Chen D, Zheng JH. Expression of MMP-1/PAR-1 and patterns of invasion in oral squamous cell carcinoma as potential prognostic markers. Onco Targets Ther 2015;8:1619-26.
24. Cai M, Zheng Z, Bai Z, Ouyang K, Wu Q, Xu S, *et al*. Over expression of angiogenic factors and matrix metalloproteinases in the saliva of oral squamous cell carcinoma patients: Potential non-invasive diagnostic and therapeutic biomarkers. BMC Cancer 2022;22:530.
25. Eck SM, Blackburn JS, Schmucker AC, Burrage PS, Brinckerhoff CE. Matrix metalloproteinase and G protein coupled receptors: Co-conspirators in the pathogenesis of autoimmune disease and cancer. J Autoimmun 2009;33:214-21.
26. Blackburn JS, Rhodes CH, Coon CI, Brinckerhoff CE. RNA interference inhibition of matrix metalloproteinase-1 prevents melanoma metastasis by reducing tumor collagenase activity and angiogenesis. Cancer Res 2007;67:10849-58.
27. Zhang Z, Pan J, Li L, Wang Z, Xiao W, Li N. Survey of risk factors contributed to lymphatic metastasis in patients with oral tongue cancer by immunohistochemistry. J Oral Pathol Med 2011;40:127-34.
28. Sutinen M, Kainulainen T, Hurskainen T, Vesterlund E, Alexander JP, Overall CM, *et al*. Expression of matrix metalloproteinases (MMP-1 and -2) and their inhibitors (TIMP-1, -2 and -3) in oral lichen planus, dysplasia, squamous cell carcinoma and lymph node metastasis. Br J Cancer 1998;77:2239-45.
29. Tsang RK-Y, Tang WW-Y, Gao W, Ho WK, Chan JY, Wei WI, *et al*. Curcumin inhibits tongue carcinoma cells migration and invasion through downregulation of matrix metallopeptidase 10. Cancer Invest 2012;30:503-12.
30. Impola U, Uitto VJ, Hietanen J, Hakkinen L, Zhang L, Larjava H, *et al*. Differential expression of matrilysin-1 (MMP-7), 92 kD gelatinase (MMP-9), and metalloelastase (MMP-12) in oral verrucous and squamous cell cancer. J Pathol 2004;202:14-22.
31. Deraz EM, Kudo Y, Yoshida M, Obayashi M, Tsunematsu T, Tani H, *et al*. MMP-10/stromelysin-2 promotes invasion of head and neck cancer. PLoS One 2011;6:e25438.
32. Ren ZH, Wu K, Yang R, Liu ZQ, Cao W. Differential expression of matrix metalloproteinases and miRNAs in the metastasis of oral squamous cell carcinoma. BMC Oral Health 2020;20:24.
33. Mathew R, Khanna R, Kumar R, Mathur M, Shukla NK, Ralhan R. Stromelysin-2 overexpression in human esophageal squamous cell carcinoma: Potential clinical implications. Cancer Detect Prev 2002;26:222-8.