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Original Article

Clinical Utility of MMP-9 Expression in Oral Malignant and Premalignant Lesions

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ABSTRACT

Introduction: The main histological form of oral cancer is oral squamous cell carcinoma (OSCC) which predominantly arises from oral potentially malignant lesions. Matrix metalloproteinases-9 (MMP-9) can degrade different elements of the ECM and basement membrane. In the present study, MMP-9 expression was assessed in tissue samples among cases of oral squamous cell carcinoma, oral potentially malignant disorders (OPMDs) and healthy individuals to assess its important role in OSCC and OPMDs.

Objective: This study aims to assess the immunohistochemical expression of MMP-9 in tissue samples of diagnosed cases of oral squamous cell carcinoma, OPMDs, and normal oral mucosa.

Material and Methods: A total of 60 histologically diagnosed cases of OSCC (n=25), OPMDs (n=25), and Healthy individuals (n=10) as control were included. Immunohistochemical staining was performed for all cases. Results were analyzed using SPSS version 20.

Results: Increased expression of MMP-9 was observed in OSCC cases (n=23/25; 92%) as compared to OPMDs and healthy individuals. Statistically, a significant relation was observed while comparing the grade of staining intensity and MMP-9 expression among OSCC cases (p=0.001) and OPMD cases (p= ≤ 0.01).

Conclusion: Increased expression of MMP-9 was observed in OSCC as compared to OPMDs and healthy individuals. Evaluation of MMP-9 could be of clinical importance in OSCC and can be used as a diagnostic marker of OSCC and OPMDs

Key words: Oral squamous cell carcinoma; Oral Potentially malignant disorders; Matrix Metalloproteinase-9; Immunohistochemistry.

Introduction

Oral cancer is the sixth leading cancer worldwide[1]. The incidence rate of oral cancer varies geographically[2]. The mortality and incidence rate of oral cancer is more in men than women[3]. The commonest epithelial malignancy that occurs in oral cavity is OSCC (90%) [4]. In Pakistan, it is marked third among the male and female population respectively. Amongst adults (> 18 years) it is the second commonest malignancy[5, 6]. The reported male-to-female ratio in Pakistan is 2:1[7] .The average age in Pakistan's population in both male and female is approximately 45-50 years[8]. The common site of OSCC in Pakistan's population is the tongue followed by buccal mucosa[2].

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The five-year survival rate of OSCC is poor and is about 50% worldwide. The survival rate is low in patients with advanced stages of oral cancer, even after treatment like radiotherapy or chemo radiotherapy. It may be a diagnosis of OSCC in the late stage, so there should be an authentic marker that could predict the disease in the early stage.

Mostly the OSCC is preceded by oral potentially malignant disorders. According to WHO oral potentially malignant disorders are introduced as clinical presentations that carry a risk of cancer development in the oral cavity, whether in clinically definable precursor lesion or in the clinically normal oral mucosa. Oral potentially malignant disorders include oral Erythroplakia, leukoplakia (proliferative verrucous, speckled, etc.). Oral submucosal fibrosis, smokeless tobacco keratosis, chronic candidiasis, and lichen planus. The worldwide prevalence rate of OPMD's ranges from 1% to 5%[9].

Matrix Metalloproteinases (MMPs) are important enzymes that are both zinc and calcium-dependent endopeptidases. These are the factors that regulate the cell behavior and enhance the cancer development process by modulation of angiogenesis, proliferation, invasion, and migration. They are required in remodeling of tissue and disintegration of ECM which includes type IV collagen, the main component of the basement membrane.

MMPs, especially MMP-9 belongs to gelatinase group also introduced as 92 KDa type IV collagenase/gelatinase, promote tumor growth by formation of new blood vessel they disturb morphology of tissue that allows the growth of a tumor and they degrade the basement membrane, it enables metastasis and overexpression of MMP-9 in OSCC have prognostic value[10].

Objective:

This study aims to assess the immunohistochemical expression (staining intensity and percentage of positive cells) of MMP-9 in tissue samples of diagnosed cases of oral squamous cell carcinoma, OPMDs, and normal oral mucosa.

Methodology

The study was performed in the oral pathology department of Peshawar Medical College (PMC). The cases were collected from the archives of the PMC histopathology lab and Pakistan Institute of Medical Sciences (PIMS) histopathology lab. The study includes patients of all age groups with histopathological diagnoses of OSCC and OPMDs. Immunohistochemistry was used for the study to evaluate the expression of IHC marker MMP-9 in OSCC cases. A total of 25 histologically diagnosed cases of OSCC (n=25), OPMDs (n=25), and 10 cases of healthy individuals were included in the work. Slides were prepared for MMP-9 monoclonal antibody by immunohistochemical staining. Antigen retrieval was done by inserting in a citrate buffer. Immunoreactivity of MMP-9 was assessed by marking a case as positive and negative on basis of staining intensity and percentage of positive cells. All stained sections were evaluated using 4x, 10x and 40x objectives of a light microscope to demonstrate positivity for MMP-9 expression. Data were analyzed and IHC scoring was performed. Mean, median, and standard deviation for MMP-9 were calculated for OSCC and OPMDs age groups. Data were further examined for statistical significance using SPSS version 20.

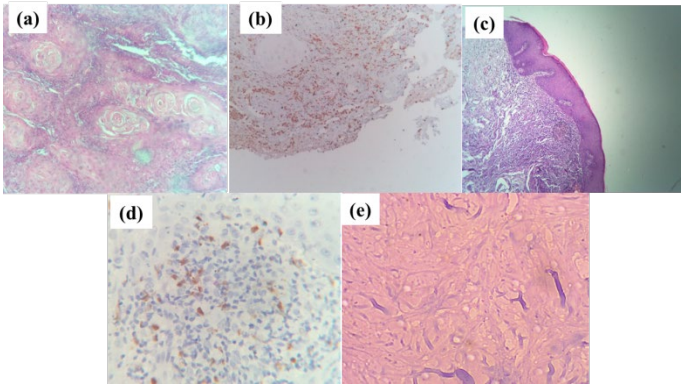

Figure 1: (a) Photomicrograph of H&E staining of OSCC showing well-differentiated squamous cell carcinoma. Numerous keratin pearls (Magnification X10). **(b)** Photomicrograph of IHC staining OSCC shows increase expression of MMP-9 immunoreactivity (Magnification X10). **(c)** Photomicrograph of H&E staining of OPMDs on buccal mucosa show that section lined by stratified squamous keratinized epithelium .Few foci show full thickness atypia and loss of orientation (Magnification X4). **(d)** Photomicrograph of IHC staining of OPMDs shows presence of MMP-9 immunoreactivity (Magnification X40). **(e)** Photomicrograph of IHC staining of normal oral mucosa show no staining of MMP-9 immunoreactivity (Magnification X40).

Result

A total 60 cases were studied using SPSS for analysis. Among them 25 cases of OSCC, 25 cases of OPMDs and 10 cases from healthy individuals.

Table-1: Mean and SD of Age of Study Population

Group	Minimum	Maximum	Mean	SD	p-value
A	23	82	56.52	12.24	P=0.200
B	41	76	61.16	10.7	P=0.00001
C	32	73	49.9	13.46	P=0.021

The age of cases of OPMDs ranged from 41 to 76 years. The mean age of presentation for cases of OSCC was 56.52 (SD± 12.24). Single sample "t" test revealed that the difference in the age of cases of healthy individuals

and OPMDs were found to be statistically significant (< 0.05) and insignificant for cases of OSCC (Table 1)

Table – 2: Description of age & gender of the study population

Study Variables	Group A (OSCC) N=25 (%)	Group B (OPMDs) N=25 (%)	Group C (Healthy individuals) N=10 (%)	p- value (Chi square test)
AGE IN YEARS				
20-40	2(8%)	-	3(30%)	0.04
41-50	3(12%)	5(20%)	3(30%)	
51-60	10(40%)	6(24%)	2(20%)	
>60	10(40%)	14(56%)	2(20%)	
GENDER				
Male	10(40%)	11(44%)	6(60%)	0.55
Female	15(60%)	14(56%)	4(40%)	
M/F	1.6:1	1:1.2	1.5:1	

Most of the cases of OSCC (n=20) and OPMDs (n=20) presented in the age above 50 years. The male to female ratio (M: F) observed for the cases of OSCC was 1.6:1, OPMDs 1:1.2 and healthy control 1.5:1 respectively. A statistically significant (p=0.04) relation was observed while comparing the age in groups among the study participants (Table 2).

Table –3: MMP–9 Expression and Staining Intensity in OSCC Lesions

Grades of Staining Intensity	MMP–9 Expression					p-value
	Absence of staining or no expression	Grade 1= <25 %	Grade 2= 26-50%	Grade 3= >50%	Total	
No staining	2(8%)	-	-	-	2(8%)	**0.001**
Weak staining	-	4(16%)	1(4%)	-	5(20%)	
Moderate staining	-	3(12%)	1(4%)	1(4%)	5(20%)	
Intense staining	-	4(16%)	5(20%)	4(16%)	13(52%)	
TOTAL	**2(8%)**	**11(44%)**	**7(28%)**	**5(20%)**	**25(100%)**	

Among cases of OSCC, only 2 cases (8%) did not express MMP–9 expression while remaining 23 cases (92%) expressed MMP–9 staining. Statistically significant relation (p=0.001) was observed while comparing the grades of staining intensity and MMP–9 expression among OSCC lesions (Table 3)

Table – 4: MMP-9 Immunohistochemical Expression and Staining Intensity among Cases of OPMDs

Grades of Staining Intensity	MMP–9 Expression					p-value
	Absence of staining or no staining	Grade 1= <25%	Grade 2= 26-50%	Grade 3= >50%	Total	
No stain	15(60%)	-	-	-	15(60%)	**≤0.01**
Weak staining	-	-	-	-	-	
Moderate staining	-	5(20%)	-	-	5(20%)	
Intense staining	-	2(8%)	2(8%)	1(4%)	5(20%)	
Total	**15(60%)**	**7(28%)**	**2(8%)**	**1(4%)**	**25(100%)**	

Among cases of OPMDs, 15 cases (60%) did not express MMP–9 expression while 10 cases (40%) showed MMP–9 expression. Statistically, significant relation was observed while comparing the grades of staining intensity and MMP–9 expression (Table 4).Among Healthy individual, MMP-9 was not expressed in normal oral mucosa.

Table – 5: MMP-9 Immunoreactivity and Clinico-Pathological Parameters of OSCC

Clinico-pathological Features		MMP-9 Immunoreactivity		Statistics	
ICD-10 Code	**Site of development of OSCC lesions**	**Negative**	**Positive**	**Total**	**p-value**
C00	Malignant neoplasm of Lip Lip (External and inner aspects of lip, commissure of lip)	-	1(4%)	1(4%)	0.675
C01	Base of tongue (Posterior third, dorsal surface of base of tongue)	1(4%)	3(12%)	4(16%)	
C02	Specified parts of tongue (Borders, anterior-two-thirds, dorsal and ventral surfaces of tongue)	1(4%)	6(24%)	7(28%)	
C03	Gum (Upper and lower gum)	-	2(8%)	2(8%)	
C04	Floor of mouth (Anterior and lateral floor of mouth)	-	1(4%)	1(4%)	
C06	Other and unspecified parts of mouth (Cheek mucosa, vestibule of mouth, retromolar area)	-	10(40%)	10(40%)	
WHO GRADING SYSTEM					
WDSCC		2(8%)	13(52%)	15(66%)	0.485
MDSCC		-	7(28%)	7(28%)	
PDSCC		-	3(12%)	3(12%)	

Statistically insignificant relation was observed between the MMP–9 tissue immunoreactivity and site of OSCC lesion (p=0.675), WHO grading system (p=0.485 (Table 5).

Table – 6: MMP-9Immunoreactivity and Clinico-Pathological Parameters Among OPMDs

Clinico-pathological features	MMP-9 immunoreactivity		Statistics	
Site of development of OPMDs	**Negative**	**Positive**	**Total**	**p-value**
Lip (External and inner aspects of lip, commissure of lip)	1(4%)	-	1(4%)	0.433
Tongue	5(20%)	1(4%)	6(24%)	
Gum (Upper and lower gum)	1(4%)	1(4%)	2(8%)	
Floor of mouth (Anterior and lateral floor of mouth)	1(4%)	-	1(4%)	
Palate (Hard and soft palate, uvula)	-	1(4%)	1(4%)	
Other and unspecified parts of mouth (buccal mucosa, vestibule of mouth, retromolar area)	7(28%)	7(28%)	14(56%)	
Histological description of OPMDs				
Squamous cell hyperplasia	7(28%)	6(24%)	13(52%)	0.259
Mild dysplasia	2(8%)	1(4%)	3(12%)	
Moderate dysplasia	5(20%)	1(4%)	6(24%)	
Severe dysplasia	-	2(8%)	2(8%)	
CIS	1(4%)	-	1(4%)	

Statistically, insignificant relation was observed between the MMP-9 tissue immunoreactivity and sites of OPMDs (p=0.433) and binary grading system (p=0.259). Among all cases (n=7; 28%) of OPMDs that developed on the buccal mucosa, vestibule of mouth, retro molar area expressed MMP-9 expression in tissue samples. Among the cases of OPMDs all the lesions diagnosed as squamous hyperplasia (n=6; 24%), mild dysplasia (n=1; 4%), moderate dysplasia (n=1; 4%), severe dysplasia (n=2; 8%) expressed MMP-9 expression (Table 6).

Table-7: MMP–9 Immunoreactivity in Tissue Samples of OSCC, OPMDs and Healthy Individuals

MMP–9 Immunoreactivity	OSCC	OPMDs	Healthy individual
Positive	23(92%)	10(40%)	-
Negative	2(8%)	15(60%)	10(100%)
Total	**25(100%)**	**25(100%)**	**10(100%)**

Among cases of OSCC, only 2 cases (8%) did not express MMP–9 expression while remaining 23 cases (92%) expressed MMP–9 staining.Among cases of OPMDs, 15 cases (60%) did not express MMP–9 expression while 10 cases (40%) showed MMP–9 expression.Among Healthy individual, MMP-9 was not expressed in normal oral mucosa (Table 7)

Discussion

Globally, Oral cancer is one of the common malignant tumors[11]. The common histological variant of oral cancer is oral squamous cell carcinoma[12]. Mostly the OSCC is preceded by an oral potentially malignant lesion[13]. Biomarkers can play important role in the diagnosis of OSCC. MMP-9 is a key hallmark in oral cancer that can be used in the early prediction of the disease[14]. In the present study evaluation of MMP-9 in tissue samples was performed among cases of OSCC, OPMDs, and healthy individuals to assess its predictive value in early diagnosis of OSCC and OPMDs.

The present study observed mean age of 56.52 of OSCC cases and with a male-female ratio of 1.6:1 was observed. This finding is in accordance with studies reported by Mirza et al., and Gul et al., who observed a male-female ratio of 1.6:1[4, 15] while this finding is contrary to Beena et al., who observed that most cases were reported in a younger age group (77%) [16]. This finding is contrary to Hosagadde et al., observed that young age (21-30 year) group most commonly affected and also observed that males were more affected than females (2.5:1)[13]The present study showed a mean age of 61.16 among cases of OPMDs with a male-female ratio of 1:1.2 and most cases presented in the old age group i.e.>50 years. This finding is in accordance with the study reported by Hadzic et al., in relation to age and gender in the development of disease[17]. This finding is contrary to Hosagadde et al., observed that young age (21-30 year) group most commonly affected[13].According to the studies reported by Balsaraf et al., and Aroquiadasse et al. observed that most cases of OPMDs presented in the younger age group i.e. < 50 years[18,19].

Our study observed a statistically significant relation while comparing the grades of staining intensity and MMP-9 immunoreactivity among cases of OSCC and OPMDs. These finding are in accordance to Ren et al., and Jose et al., [20, 21]

Regarding the site distribution, commonly involved site by OSCC and OPMDs was buccal mucosa, vestibule of mouth and retromolar area followed by tongue. Our study showed results (Table 5,6) which is consistent with the studies reported by Majumdar et al., Siriwardena et al., and Kumar et al., observed buccal mucosa was commonly affected [22-24] and contrary to Beena et al., and Mello et al., observed that tongue was more commonly affected site (65%)[16]

The most common histopathological variant of OSCC reported by our study was WDSCC (40%) followed by MDSCC (28%) and PDSCC (12%). Our study showed a result (Table 5) which is in accordance with other studies reported such as WDSCC (35%) by Siriwardena et al and Gul et al (42.5%) of WDSCC[23, 24]. These findings were different from some previous studies reported by Remin et al., who showed increased cases among moderately (42%) and poorly differentiated squamous cell carcinoma(15%) [25]Among cases of OPMDs in our study common histopathological variant was squamous cell hyperplasia (52%) which was a commonly occurring epithelial precancerous lesion followed by mild, moderate, and severe dysplasia. Our study revealed the results (Table 6) which is consistent with other studies reported by Kumar et al., [26]. This finding is contrary to Chandolia et al., who observed that varying degrees of epithelial dysplasia from mild to severe dysplasia (55%) [27].

Our study showed that MMP-9 was not expressed in normal mucosa among healthy individuals. The present study revealed a result that is consistent with another study reported by Gkouveris et al[11].

There was statistically significant infiltration of MMP-9 in OSCC cases as compared to OPMDs and normal oral mucosa group. The inconsistent results of present study may be due to sampling technique, methodology used and small sample size. There are some other methods used for MMP-9 expression detection which are more specific (ELISA and PCR). Immunohistochemistry has been used to avoid false-positive results as it is technique sensitive. Thus further research should be done on large sample size along with other methods to confirm the expression of MMP-9 in OSCC and OPMDs cases and their expression should be correlated with the IHC technique.

Conclusions

Evaluation of MMP-9 could be of clinical importance in OSCC and can be used as a diagnostic predictive marker of OSCC and OPMDs. These findings will improve the treatment protocol and prognosis of the patients in the future.

Conflict of Interest: The authors declare no conflict of interests

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