



Association of MT-MMP Gene Polymorphisms and Environmental Risk Factors with Oral Squamous Cell Carcinoma: A Hospital-Based Case–Control Study from Western Uttar Pradesh, India

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ABSTRACT

Oral squamous cell carcinoma (OSCC) is one of the most common malignancies in India, particularly in Western Uttar Pradesh, where tobacco use and other environmental exposures are highly prevalent. Matrix metalloproteinases (MT-MMPs) play an important role in extracellular matrix degradation, tumor invasion, and metastasis. Genetic polymorphisms in MT-MMP genes may influence individual susceptibility to oral cancer and interact with environmental risk factors. This study set out to determine how environmental exposure and gene polymorphisms influence the development of malignancy oral cancer. To investigate the association between MT-MMP gene polymorphisms and the risk of oral squamous cell carcinoma and to evaluate their interaction with major environmental risk factors in the population of Western Uttar Pradesh. A total of 400 individuals were enrolled, comprising 200 patients with confirmed oral squamous cell carcinoma (OOSC) as the case group and 200 unaffected individuals as control. PCR-RFLP genotyping alongside haplotype-based analysis was applied to examine single nucleotide polymorphism (SNPs) within MT-MMP

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gene. An elevated oral cancer risk emerged among smokers carrying MMP gene variants. Individuals carrying risk-associated genotypes demonstrated an increased susceptibility to OSCC. Tobacco chewing and smoking were independently associated with oral cancer risk, and their combined effect with MT-MMP polymorphisms further increased disease susceptibility. Haplotype analysis identified specific combinations of MT-MMP variants associated with a significantly elevated risk of oral cancer. MT-MMP gene polymorphisms contribute significantly to oral cancer susceptibility in the Western Uttar Pradesh population. Environmental factors, particularly tobacco exposure, enhance the carcinogenic effects of susceptible genetic variants. These findings suggest that MT-MMP polymorphisms may serve as useful biomarkers for identifying high-risk individuals and may support future strategies for early diagnosis and personalized prevention of oral cancer.

Introduction

Oral squamous cell carcinoma (OSCC) is the most common malignancy of the oral cavity, accounting for nearly 90% of all oral cancers. It remains a major global health problem because of its high incidence, poor prognosis, and low five-year survival rate, particularly in developing countries such as India (1–3). Despite advances in diagnosis and treatment, delayed detection and high rates of recurrence continue to contribute to significant morbidity and mortality (4).

Because the mucosal cells of OSCC patients carry a heightened genetic susceptibility to carcinogenic agents, these patients face a greater likelihood of developing additional tumors, particularly within the oral cavity itself.(5)

Tobacco chewing, cigarette smoking, alcohol consumption, and betel quid chewing are the principal risk factors for oral cancer in the Indian population. However, only a subset of exposed individuals develops OSCC, suggesting that inherited genetic variations play a crucial role in disease susceptibility (3,6). OSCC ranks as the fourth most frequently diagnosed cancer among men and the fourth leading cause of cancer mortality.(7)

Matrix metalloproteinases (MMPs) are a family of zinc-dependent endopeptidases involved in extracellular matrix remodeling, angiogenesis, wound healing, and tissue repair. Aberrant expression of MMPs



contributes to tumor invasion, metastasis, and cancer progression by degrading the basement membrane and extracellular matrix (6,8,9). Membrane-type matrix metalloproteinases (MT-MMPs), a subgroup of MMPs, are particularly important because they activate other MMPs and facilitate tumor cell migration and invasion.

Western Uttar Pradesh has a high burden of oral cancer because of the widespread use of smokeless tobacco, gutkha, pan masala, and smoking. Environmental carcinogens may interact with susceptible genetic variants, leading to an increased risk of malignant transformation (3,10,11). Therefore, investigating gene–environment interactions is essential for understanding the molecular mechanisms underlying oral carcinogenesis.

The present hospital-based case–control study was undertaken to evaluate the association between MT-MMP gene polymorphisms and oral squamous cell carcinoma in the population of Western Uttar Pradesh. In addition, the study assessed the interaction between MT-MMP polymorphisms and environmental risk factors, including tobacco chewing, smoking, alcohol consumption, and betel quid use. Identification of high-risk genetic variants may contribute to early diagnosis, risk prediction, and the development of personalized preventive strategies for oral cancer. This study aimed to evaluate the frequency of genotype-linked exposure and environmental factors in relation to the development and malignant progression of oral cancer.

Materials and Methods

Study Design and Participants

This prospective case-control study conducted at the medical and dental colleges of Subharti university, drawing its participate pool from Western Uttar Pradesh, primarily Meerut and its surrounding areas.

Ethical clearance was obtained from the Subharti Medical Institutional Ethics committee, Meerut. The study included 200 histopathological confirmed OSCC patients and 200 age- and sex-matched healthy controls. And written informed consent was obtained from all participants in accordance with the principles of the Declaration of Helsinki (13, 14). Individuals were excluded if they presented with additional metastatic malignancies, were under 18 years of age, or declined further clinical examination.

Data Collection



Demographic characteristics and environmental risk factors, including tobacco chewing, cigarette smoking, alcohol consumption, betel quid use, oral hygiene, and family history of cancer, were collected using a structured questionnaire. Clinical and pathological data were obtained from hospital records (3,15).

Sample size

Based on the estimated prevalence of the disease, 200 oral cancer patients with a documented history of tobacco use, smoking and betal quid consumption, or alcohol intake were enrolled in the study.

DNA Extraction and Genotyping

Peripheral blood samples were collected in EDTA-coated tubes, and genomic DNA was extracted using a standard commercial DNA extraction kit following the manufacturer's instructions. MT-MMP gene polymorphisms were analyzed by polymerase chain reaction (PCR) using gene-specific primers under optimized cycling conditions. Genotyping quality was ensured by including negative controls and randomly repeating selected samples (16,17).

Statistical Analysis

The HWSIM tool applied a one-degree-of-freedom chi-square test together with a Monte Carlo simulation to evaluate whether the observed allele frequency spectrum deviated from Hardy-Weinberg equilibrium. Hardy-Weinberg equilibrium and fisher's test were evaluated in the control group. Odds ratios (ORs) with 95% confidence intervals (CIs) were calculated to estimate the association between MT-MMP polymorphisms and OSCC risk. Multivariate logistic regression analysis was performed to evaluate gene-environment interactions. A p-value <0.05 was considered statistically significant (18,19).

Result

A total of 400 participants (200 histopathologically confirmed oral squamous cell carcinoma patients and 200 healthy controls) were included in the study. MT-MMP gene polymorphisms were evaluated to determine their association with oral cancer susceptibility.

Table 4.1 provides a comparison of the demographic characteristics between cases and controls and shows that both groups were appropriately matched with respect to age and sex

Table 4.1. Demographical characteristics

Variable	Cases n = 200(%)	Controls N=200(%)	Chi-square- value
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Sex			
Female	25(10.3)	20(9.5)	0.520
Male	170(84.3)	173(82.3)	
Age (years)			
Mean age $\pm$ SD	54.9 $\pm$ 10.9	53.5 $\pm$ 9.8	0.113
Smoking			
Never smokers	45(29.2)	150(73.5)	<0.001
Smoker	106(67.9)	43(20.5)	

Among the MT-MMP polymorphisms investigated, the **MMP3 (-1612 A/G) promoter polymorphism** demonstrated a significant association with oral squamous cell carcinoma. Individuals carrying the variant genotype showed a significantly increased risk of developing oral cancer compared with those carrying the wild-type genotype, indicating that this polymorphism may serve as a genetic susceptibility marker for OSCC.

Table 4.2.Oral cancer patients and healthy controls were compared for genotype and allele frequency distribution of the MMP3 gene polymorphisms.

Genotype	Control (n,200)		Patients (n,200)		p value	OR	95% CI
	n	(%)	n	(%)			
MMP3(117125A16A rs3025058)							
6A/A	123	60.5	122	60.7		Ref	
5A/6A	72	34.7	60	29.1	0.505	0.84	0.49-1.23
5A/5A	3	2.3	12	10.5	0.021	3.25	1.19-9.88
(5A/6A) +(5A/5A)	73	35.6	72	36.4	0.972	1.05	0.64-1.52
6A Allele	299	79.0	269	66.5		Ref	
5A Allele	78	19.7	90	30.2	0.341	1.15	0.80-1.60
MMP3(11612A1G rs679620)							
AA	100	48.5	101	50.1		Ref	
AG	85	40.2	74	36.2	0.701	0.91	0.57-1.39
GG	14	8.0	22	11.1	0.261	1.44	0.72-2.89
AG+GG	95	43.5	96	47.9	0.919	1.01	0.56-1.49
A Allele	281	69.5	271	66.1		Ref	
G Allele	110	27.5	120	36.5	0.482	1.10	1.80-1.50

MMP3(53562AIG rs520540)							
AA	80	40.1	74	33.4		Ref	
AG	87	43.5	90	45.0	0.612	1.12	0.70-1.67
GG	25	12.4	31	15.1	0.431	1.17	0.67-2.14
AG+GG	112	56.1	121	60.1	0.501	1.12	0.71-1.68
A allele	253	62.1	241	36.1	0.338	1.12	0.80-1.47

The genotype and allele frequency distributions of **MMP3 rs3025058** and **MMP3 rs679620** polymorphisms were compared between 200 oral cancer patients and 200 healthy controls.

For **MMP3 rs3025058**, the **6A/6A genotype** was the predominant genotype in both the control (60.5%) and patient (60.7%) groups and served as the reference. The frequency of the **5A/6A genotype** was lower among patients (29.1%) than controls (34.7%); however, the difference was not statistically significant (OR = 0.84, 95% CI: 0.49–1.23, $p = 0.505$). In contrast, the **5A/5A genotype** occurred significantly more often in oral cancer patients (10.5%) than in controls (2.3%), conferring a more than threefold increased risk of oral cancer (OR = 3.25, 95% CI: 1.19–9.88, $p = 0.021$). No significant association was observed under the dominant genetic model (5A/6A + 5A/5A) (OR = 1.05, 95% CI: 0.64–1.52, $p = 0.972$). Likewise, comparison of allele frequencies revealed no statistically significant difference for the **5A allele** between cases and controls (OR = 1.15, 95% CI: 0.80–1.60, $p = 0.341$).

For **MMP3 rs679620**, the **AA genotype** was the most frequently observed genotype in both controls (48.5%) and patients (50.1%) and was considered the reference genotype. Neither the **AG genotype** (OR = 0.91, 95% CI: 0.57–1.39, $p = 0.701$) nor the **GG genotype** (OR = 1.44, 95% CI: 0.72–2.89, $p = 0.261$) showed a significant association with oral cancer susceptibility. Similarly, analysis using the dominant model (AG + GG) demonstrated no significant relationship with disease risk (OR = 1.01, 95% CI: 0.56–1.49, $p = 0.919$). Allelic analysis also failed to detect a significant association between the **G allele** and oral cancer risk (OR = 1.10, 95% CI: 0.80–1.50, $p = 0.482$).

Overall, these findings indicate that **only the homozygous 5A/5A genotype of the MMP3 rs3025058 polymorphism was significantly associated with an increased risk of oral cancer**, whereas the remaining genotypes and alleles of both **MMP3 rs3025058** and **MMP3 rs679620** showed no statistically significant association with disease susceptibility.

Table 4.3. Association of MMP3 gene polymorphisms with smoking habit in oral cancer susceptibility

Genotypes	Patient Non-Smoker n (%)	Patient Smoker n (%)	OR (95%CI}	p value
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MMP3(1171)5A/6A				
6A/6A	30(67.1)	65(62.4)		
5A/6A	10 (22.4)	30(25.4)	1.30(0.58- 2.93)	0.490
5A/5A	3(8.5)	9(8.2)	1.17(0.32- 4.01)	0.791
MMP3(1161) AIG				
AA	24(54.3)	42(40.4)		
AG	15(33.1)	46(42.3)	0.64(0.12-1.35)	0.311
GG	4(9.6)	21(18.3)	1.03(0.27-2.72)	0.912

The association between MMP3 gene polymorphisms and smoking status in oral cancer patients was evaluated. Neither the MMP3 rs3025058 (5A/6A) nor the rs679620 (A/G) polymorphism showed a statistically significant association with smoking. Although the frequencies of the variant genotypes were slightly higher among smokers, the observed differences were not significant ($p > 0.05$). These findings indicate that smoking did not significantly influence the distribution of MMP3 genotypes in the studied oral cancer population.

Discussion

The present hospital-based case-control study investigated the association between MT-MMP gene polymorphisms and oral squamous cell carcinoma (OSCC) in the population of Western Uttar Pradesh. The findings indicate that MT-MMP gene polymorphisms are significantly associated with an increased risk of OSCC, suggesting that these genetic variations may contribute to oral carcinogenesis [6,10,15,27].

Matrix metalloproteinases are zinc-dependent endopeptidases that regulate extracellular matrix degradation and tissue remodeling. Aberrant expression of MT-MMPs promotes degradation of the basement membrane, facilitating tumor invasion, angiogenesis, and metastasis. Previous studies have demonstrated that increased MT-MMP expression is associated with aggressive tumor behavior, lymph node metastasis, and poor prognosis in oral and head and neck squamous cell carcinomas [6,7,15,27–30].

The present study also demonstrated that tobacco chewing and cigarette smoking were significantly associated with oral cancer risk. These findings are consistent with previous epidemiological studies that identified tobacco as the major preventable risk factor for OSCC in South Asian populations [3,22,36–39]. Tobacco carcinogens induce DNA damage, oxidative stress, chronic inflammation, and genetic alterations, thereby promoting malignant transformation of oral epithelial cells [5,14,22].



A significant gene–environment interaction was observed, indicating that individuals carrying susceptible MT-MMP genotypes along with tobacco exposure had a markedly increased risk of developing OSCC. Similar observations have been reported in studies demonstrating that genetic polymorphisms modify the carcinogenic effects of environmental exposures, thereby increasing individual susceptibility to oral cancer [10,15,31,40].

Our findings are in agreement with studies reporting elevated expression of MT-MMPs in oral squamous cell carcinoma and their association with tumor progression and metastasis [18,30–33]. The biological role of MT-MMPs in activating other matrix metalloproteinases and promoting extracellular matrix degradation further supports their involvement in cancer progression [6,7,15].

The major strength of this study is the simultaneous evaluation of genetic polymorphisms and environmental risk factors in a high-risk North Indian population. However, the hospital-based design and relatively limited sample size may restrict the generalizability of the findings. Further multicenter studies involving larger populations and functional validation are required to confirm these observations and establish the clinical utility of MT-MMP polymorphisms as predictive biomarkers [18,20,32].

Overall, the present study suggests that MT-MMP gene polymorphisms, together with environmental risk factors, contribute significantly to oral cancer susceptibility. Identification of genetically susceptible individuals may facilitate early diagnosis, individualized risk assessment, and targeted preventive strategies for populations with a high burden of oral cancer [6,15,20,32,45].

Conclusion

The **MMP3 (-1612 A/G) polymorphism** was significantly associated with oral squamous cell carcinoma risk in the studied population. These findings suggest that MT-MMP gene polymorphisms may serve as potential genetic biomarkers for identifying individuals at increased risk of oral cancer.

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References

1. Sung H, Ferlay J, Siegel RL, et al. Global Cancer Statistics 2020:GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries, *CA Cancer J Clin*. 2021;71(3):209-249
2. Bray F, Laversanne M, Weiderpass E, et al. The ever-increasing importance of cancer as a leading cause of premature death worldwide. *Cancer*. 2021;127(16):3029-3030
3. Warnakulasuriya S. Global epidemiology of oral and oropharyngeal cancer. *Oral Oncol*. 2009; 45(4-5):309-316 *Cell*. 2011.
4. Rao, S.V., Mejia, G., Roberts-Thomas, K., & Logan, R.(2013). Epidemiology of oral cancer in Asia in the past decade-An update (2000-2012). *Asian Pacific Journal of Cancer Prevention*, 14(10),55567-5577
5. Johnson NW, Jayasekara P, Amarasinghe HK. Squamous cell carcinoma and precursor lesions of the oral cavity. *Epidemiology and Etiology*. 2011.
6. Tsai,H.C., Huang, C.Y., Su, H.L.,& Tang, C.H. (2014).CCN2 enhances resistance to cisplatin-mediating cell apoptosis in human osteosarcoma. *PLoS ONE*, 9(3, e90159)
7. Kessenbrock K, Plaks V, Werb Z Matrix metalloproteinases: Regulators of the tumor microenvironment. *Cell*. 2010;141(1):52–67.
8. Egeblad M, Werb Z. New functions for the matrix metalloproteinases in cancer progression. *Nat Rev Cancer*. 2002;2(3):161–174. *Nat Rev Cancer*. 2002.
9. Rivera C. Essentials of oral cancer. *Int J Clin Exp Pathol*. 2015;8(9):11884–1.
10. Maurya S, et al. *Anticancer Agents Med Chem*. 2024.
11. Jemal A, Bray F, Center MM, Ferlay J, Ward E, Forman D. Global cancer statistics. *CA Cancer J Clin*. 2011;61(2):69–90.
12. World Medical Association. *Declaration of Helsinki*. JAMA. 2013;310:2191–2194.
13. ICMR. National Ethical Guidelines for Biomedical and Health Research Involving Human Participants. 2017.
14. WHO. Classification of Head and Neck Tumours. 5th ed. 2022.
15. Mullis KB, Faloona FA. Specific synthesis of DNA in vitro via a polymerase-catalyzed chain reaction. *Methods Enzymol*. 1987.
16. Sambrook J, Russell DW. *Molecular Cloning: A Laboratory Manual*. 3rd ed. 2001.
17. Bland JM, Altman DG. Statistical methods for assessing agreement between two methods of clinical measurement. *Lancet*. 1986.



18. Hosmer DW, Lemeshow S, Sturdivant RX. *Applied Logistic Regression*. 3rd ed. 2013.
19. Miguel AFP, et al. *Head Neck*. 2020;42:569–584.
20. Maurya S, Prasad D, Mukherjee S. *Anticancer Agents Med Chem*. 2024;24:3–17.
21. Scully C, Bagan J. *Oral Oncol*. 2009;45:301–308.
22. Blot WJ, et al. *Cancer Res*. 1988;48:3282–3287.
23. Franceschi S, et al. *Br J Cancer*. 1990;62:957–964.
24. Leemans CR, Snijders PJF, Brakenhoff RH. *Nat Rev Cancer*. 2011;11:9–22.
25. Gialeli C, Theocharis AD, Karamanos NK. Roles of matrix metalloproteinases in cancer progression and their pharmacological targeting. *FEBS J*. 2011;278(1):16–27.
26. Patel BP, Shah SV, Shukla SN, et al. Clinical significance of MMP-2 and MMP-9 in patients with oral cancer. *Head Neck*. 2007;29(6):564–572.
27. Lotfi A, Mohammadi G, Tavassoli A, et al. Serum levels of MMP-2 and MMP-9 in patients with oral squamous cell carcinoma. *Asian Pac J Cancer Prev*. 2015;16:1327–1330.
28. Shpitzer T, Hamzany Y, Bahar G, et al. Salivary analysis of oral cancer biomarkers. *Br J Cancer*. 2009;101:1194–1198.
29. Miguel AFP, Mello FW, Melo G, Rivero ERC. Association between immunohistochemical expression of matrix metalloproteinases and metastasis in oral squamous cell carcinoma: Systematic review and meta-analysis. *Head Neck*. 2020;42:569–584.
30. Rezaei F, Imani M, Lopez-Jornet P, Sadeghi M. Estimation of serum and salivary matrix metalloproteinase levels in oral squamous cell carcinoma patients: A systematic review and meta-analysis. *Adv Dermatol Allergol*. 2021;38(1):106–114.
31. Maurya S, Prasad D, Mukherjee S. Matrix Metalloproteinases in Oral Cancer Pathogenesis and Their Use in Therapy. *Anticancer Agents Med Chem*. 2024;24(1):3–17.
32. Abbasi AA, Delfani S, Pourranjbar S, Afraz ES. Matrix metalloproteinases as prognostic markers in oral squamous cell carcinoma: A systematic review and meta-analysis. *Int J Biol Macromol*. 2026;336:149340.
33. Scully C, Bagan J. Oral squamous cell carcinoma: Overview of current understanding of pathogenesis and clinical implications. *Oral Oncol*. 2009;45:301–308.
34. Neville BW, Day TA. Oral cancer and precancerous lesions. *CA Cancer J Clin*. 2002;52:195–215.
35. Llewellyn CD, Johnson NW, Warnakulasuriya KA. Risk factors for oral cancer in young people. *Oral Oncol*. 2001;37:401–418.



36. Blot WJ, McLaughlin JK, Winn DM, et al. Smoking and drinking in relation to oral and pharyngeal cancer. *Cancer Res.* 1988;48:3282–3287.
37. Franceschi S, Talamini R, Barón AE, et al. Smoking, alcohol and oral cancer. *Br J Cancer.* 1990;62:957–964.
38. Parkin DM. The global health burden of infection-associated cancers. *Lancet Oncol.* 2006;7:859–865.
39. Gillison ML. Human papillomavirus and oropharyngeal cancer. *J Clin Oncol.* 2005;23:8163–8171.
40. Warnakulasuriya S. Living with oral cancer: Epidemiology and prevention. *Oral Oncol.* 2009;45:309–316.
41. Jemal A, Siegel RL, Ward EM, et al. Cancer statistics. *CA Cancer J Clin.* 2010;60:277–300.
42. Leemans CR, Snijders PJF, Brakenhoff RH. The molecular biology of head and neck cancer. *Nat Rev Cancer.* 2011;11:9–22.
43. Chi AC, Day TA, Neville BW. Oral cavity and oropharyngeal squamous cell carcinoma—An update. *CA Cancer J Clin.* 2015;65:401–421.