Original Article

Association of Matrix Metalloproteinase-7 (−181A>G) Polymorphism with Risk of Esophageal Squamous Cell Carcinoma in Kashmir Valley

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ABSTRACT

Background/Aim: Degradation of the basement membrane and extracellular matrix by matrix metalloproteinases (MMPs) is believed to be an essential step in the complicated process of hematogenous metastasis. Matrix metalloproteinase-7 (MMP-7) is a small secreted proteolytic enzyme with a broad substrate specificity, and its expression has been shown to be associated with tumor invasion and metastasis for various cancers. **Patients and Methods:** To document the role of MMP-7 polymorphism in esophageal carcinogenesis, a case-control study was performed comprising 135 patients with esophageal cancer (EC) and 195 healthy controls. Genotyping was done by the polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) method. Data were statistically analyzed using χ^2- test and logistic regression models. **Results:** Carriers for the MMP-7 (-181A>G) GG were associated with an increased risk for EC [odds ratio (OR = 2.17; 95% confidence interval (CI) = 1.21-3.92; $P = 0.010$; $P_{\text{-trend}} = 0.04$]. Also, in a recessive model, our results showed that MMP-7 (-181A>G) GG allele conferred significantly higher risk for EC (OR =2.16; 95% CI = 1.31-3.54; $P = 0.003$). The high risk due to MMP-7 (-181GG) genotype was limited to squamous cell histology of EC (OR = 2.41; 95% CI = 1.27-4.56; $P = 0.007$). Although smoking (*Hukka*) and high consumption of salted tea are independent risk factors for EC, the interaction of MMP-7 (-181A>G) genotypes with these factors did not further modulate the risk of EC. **Conclusions:** In conclusion, our results show that MMP-7 (-181A>G) GG carriers are at a higher risk of esophageal squamous cell carcinoma in Kashmir valley.

Key Words: Esophageal cancer, Kashmir valley, MMP-7 (−181A>G) polymorphism

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Esophageal cancer (EC) is the most aggressive malignant tumor of the gastrointestinal tract. EC is the eighth most commonly occurring cancer in the world.[1] Within the Indian subcontinent, the valley of Kashmir presents a strikingly different picture where the incidence of EC has been reported to exceed 40% of all cancers and is 3-6 times higher than various metropolis cancer registries in India.[2] Some of the genetic and environment factors have been reported to be associated with an increased risk of EC in Kashmir valley.[3-5]

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Matrix metalloproteinases (MMPs) are a family of enzymes responsible for the breakdown of connective tissue proteins. These enzymes play an important role in tissue remodelling associated with growth, development, and repair. In these physiological processes, MMP activity is tightly regulated. However, it is clear that aberrant MMP expression can contribute to the pathogenesis of several diseases including rheumatoid arthritis, multiple sclerosis, cerebral hemorrhage, and inflammatory bowel disease. Matrix metalloproteinase-7 (MMP-7) is one of the MMP family members and consists of a primordial form of these members.[6] It can degrade laminin, type IV collagen, and entactin,[7-9] which are the main components of the basement membrane, and activate other important MMPs (eg, MMP-1, MMP-2, and MMP-9).[10,11] It can also inactivate α1-antitrypsin, which augments the serine protease activity, and thus indirectly activates MMPs.[12]

The gene encoding MMP-7 is localized on chromosome 11q21–q22. Two polymorphisms exist in the MMP-7 promoter region, -181A>G and −153C>T, which are known to modify the gene transcription activity.[13,14] Previous studies have shown association of MMP-7 (-181A>G) (rs11568818) polymorphisms with esophageal gastric and other malignancies.[15-21] However, till date, no study has been carried out to evaluate the role of MMP7 -181A>G polymorphism in relation to higher prevalence of EC in Kashmir valley, a high-risk population bordering the EC belt, known for high incidence of EC. Therefore, the aim of the present study was to investigate the role of MMP-7 (−181A>G) (rs11568818) in conferring genetic susceptibility to EC in the Kashmir valley.

PATIENTS AND METHODS

Study population

The present case/control study comprised untreated histopathologically confirmed cases with EC (135) and healthy controls (195). The sample size of the present study was adequate to provide 80% power. All subjects were unrelated permanent residents of Kashmir and were referred from the departments of gastroenterology, Sher-i-Kashmir Institute of Medical Sciences, Srinagar, from May 2006 to December 2008. Patients and controls were matched by ethnicity, mean age, and gender. Patients were excluded if they had non-malignant conditions such as corrosive esophageal injury, achalasia injury, Barrett's esophagus, gastro-esophageal reflux disease (GERD), and non-ulcer dyspepsia. Controls were also recruited from Sher-i-Kashmir Institute of Medical Sciences, Srinagar. Healthy controls were individuals who came for their routine health checkups or minor illness such as fever, common headache, or minor surgery. They were ethnicity matched with cases, free from any chronic disease, unrelated to patients, and having similar socioeconomic background. All individuals were personally interviewed about their age, occupational history, medical history of other diseases, demographic features, family history of cancer, use of hot noon *chai* (salted tea), drinking alcohol, and smoking habits. Tobacco use included smoking cigarettes or *Hukka* (water pipe). Written informed consent was obtained from all study participants. The research protocol was approved by the ethics committee of Sanjay Gandhi Postgraduate Institute of Medical Sciences, Lucknow (project number: 5/13/48/2002-NCDIII).

Sample collection and preparation

Sample collection, storage, and transport were in compliance with committee guidelines. Blood samples were collected in ethylenediaminetetraacetic acid (EDTA) and genomic DNA was extracted from peripheral blood leukocyte pellet using the standard salting-out method.[22] The quality and quantity of DNA were checked by gel electrophoresis and spectrophotometry using Nanodrop Analyser (ND-1000) spectrophotometer (Nano Drop Technologies, Inc., Wilmington, DE, USA). The ratio of absorbance at 260 and 280 nm of DNA was approximately 1.7-1.9. The isolated DNA was stored at −70°C.

Genotyping

The MMP-7 polymorphism (−181A>G) was genotyped in subjects by polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP). Primers for amplification of MMP-7 (−181A>G) were taken as described.[14] PCR products were digested by the restriction endonuclease *EcoR1* (Bangalore Genei) at 37°C overnight. The amplified fragments were separated on 15% polyacrylamide gel and visualized by ethidium bromide staining. Briefly, genotypes of MMP-7 were assigned as follows: 120 bp and 30 bp for −181GG; 150 bp, 120 bp, and 30 bp for −181AG; and 150 bp for −181AA genotype. More than 15% of the samples from patients and controls including samples of each genotype were re-genotyped by other laboratory personnel, but no discrepancy was found and the results were 100% concordant.

Statistical analysis

Demographic characteristics of patients and controls were described as frequencies and percentages, whereas descriptive statistics of patients and controls were presented as mean and standard deviations for continuous measures. Statistical significance of frequency differences between patients and control groups was evaluated using the χ^2 test. Deviation from the Hardy–Weinberg equilibrium (HWE) in controls was assessed using the χ^2 test; *P* value was considered significant at <0.05 level. Risk estimates were calculated for dominant and recessive genetic models using the most common homozygous genotype as reference. Observed genotype frequencies for MMP-7 (-181A>G) polymorphism (rs17878362) in controls were examined for deviation from HWE by using a goodness-of-fit χ^2-test with one degree of freedom. The single control group was used for analyzing two sets of cancer cases, ie, esophageal and gastric cancer. Binary logistic regression analysis was used to fit statistical models to predict the association of MMP-7 (-181A>G) genotypes with susceptibility to EC. Association was expressed as odds ratio (OR) for risk estimation with 95% confidence interval (95% CI). Bonferroni correction was applied in case of multiple comparisons using the formula $pc = p \times n$ (where, pc represents corrected value, and n is the number of comparisons performed). All analyses were performed using the SPSS statistical analysis software, version 15.0 (SPSS, Chicago, IL, USA).

RESULTS

Population characteristics

The mean age of healthy subjects (controls) and patients with EC was 57.98 ± 12.67 and 60.38 ± 8.41 years,

respectively (*t* test *P* = ns). EC was highly prevalent in males (68.1%) than in females. In patients with EC, squamous cell carcinoma (SCC) histopathology was common (76.3%). Smoking habit (*Hukka*) showed a significantly higher risk in EC (OR =21.45; 95% CI =11.63-39.55; *P* = 0.0001) patients. Individuals consumed salted-tea in a range of 2-8 cups per day; and median consumption of tea was 4 cups per day. So, we grouped individuals in to ≤4 cups or >4 cups per day and individuals consumed salted tea >4 cups per day were regarded as high salted tea consumers. Higher consumption of salted tea was also found to be associated with increased risk of EC (OR =14.86; 95% CI =8.42-26.25; *P* = 0.0001) [Table 1]. None of the patients or controls reported consumption of alcohol, so interaction of alcohol intake with genetic variations could not be analyzed.

Association of genetic variant of MMP-7 (-181A>G) polymorphism with susceptibility to EC

The genotype frequencies of the MMP-7 (-181A>G) polymorphism among cases and controls are shown in Table 2. The observed genotype frequencies among the control subjects were in agreement with the HWE (*P* = 0.55; χ^2 = 0.37). In the present study, when we used the MMP 7 (-181A>G) AA genotype as reference, we found that individuals with MMP-7 (-181A>G) GG genotype were significantly associated with more than twofold increased risk of EC (OR =2.17; 95% CI =1.21-3.92; *P* = 0.010; $P_{\text{-trend}}$ = 0.04) as compared with the control group. Moreover, in the recessive model, our results showed that MMP-7 (-181A>G) GG genotype conferred significantly increased risk for EC (OR =2.16; 95% CI =1.31-3.54; *P* = 0.003) [Table 2].

Association of MMP-7 (-181A>G) genotypes with tumor histopathology

When tumor histopathologies were analyzed, MMP-7 (-181A>G) GG genotype was found to be significantly associated with an increased risk for esophageal SCC (ESCC) (OR =2.41; 95% CI =1.27-4.56; *P* = 0.007) [Table 3]. However, we did not find a significant association in adenocarcinomas of esophagus cancer.

Interaction of MMP-7 (-181A>G) genotypes with environmental factors

Our results show significant association of high consumption of salted tea and smoking with EC [Table 1]. However, in gene–environment interaction, we did not find any

Table 1: Demographic characteristics of study subjects

Variables	Healthy controls *n*=195	Esophageal cancer patients *n*=135	OR* (95%CI)*P*
Mean Age± SD	57.98 yrs ± 12.67	60.38 yrs ± 8.41	
Sex			
Male	139 (71.3)	92 (68.1)	
Female	56 (28.7)	43 (31.9)	
Histology			
Adenocarcinoma		32 (23.7)	
Squamous cell carcinoma		103 (76.3)	
Smoking#			
Smokers (*Hukka*)	38 (20.5)	106 (84.1)	**21.45 (11.63-39.55) 0.0001**
Salted tea intake#			
(≤4 cups daily)	159 (85.9)	36 (28.6)	**14.86 (8.42-26.25) 0.0001**
(>4 cups daily)	26 (14.1)	90 (71.4)	

*Age and gender adjusted odds ratio; #Data missing in some subjects. Significant values shown in bold. Figures in parenthesis are in percentage

Table 2: Frequency distribution of genotypes and of MMP-7 (−181A>G) genotypes with risk of esophageal cancer

Genotypes	Healthy controls		Esophageal cancer		OR* (95%CI)
	n=195	(%)	*n*=135	(%)	
MMP-7 (−181A>G) (rs11568818)					
AA	63	32.3	36	26.7	1 (Reference)
AG	92	47.2	51	37.8	1.02 (0.60-1.74) 0.97
GG	40	20.5	48	35.6	**2.17 (1.21-3.92) 0.010**
Dominant model					
AA	63	32.3	36	26.7	1 (Reference)
AG+GG	132	67.7	99	73.3	0.74 (0.45-1.20) 0.22
Recessive model					
AA+AG	155	79.5	87	64.4	1 (Reference)
GG	40	20.5	48	35.6	**2.16 (1.31-3.54) 0.003**
$P_{\text{-trend}}$					**0.04**

*Age and gender adjusted odds ratio; significant values shown in bold

significant modulation of cancer risk by MMP-7 (-181A>G) genotypes with salted tea and smoking [Tables 4 and 5].

DISCUSSION

MMPs are a class of proteases that contribute significantly and uniquely to the tumor microenvironment, which provides the elements needed for advanced tumor growth (i.e., cytokines, loss of contact inhibition, angiogenesis, and invasion). Overexpression of matrilysin (MMP-7) is predominantly associated with epithelial pre-malignant cells.[23]

In the present study, we looked for the effect of MMP-7 (-181A>G) promoter polymorphism on the genetic susceptibility to EC. Based on the presented results, we report an association of MMP-7 (-181A>G) GG genotype with increased EC risk at genotype level as well as in the recessive model, suggesting that this polymorphism contributes to enhanced susceptibility for EC in Kashmir valley. Our results are supported by previously reported studies suggesting an association of MMP-7 (-181A>G) GG genotypes with an increased risk of various cancers with controversies.[17-21,24-32] i.e. in some populations GG genotypes are associated with the diease while in others no associations was found. The association of MMP-7 (-181A>G) polymorphism with cancer has been observed in Asians and not in Europeans.[19] Thus, there may be ethnic and geographical differences in the influence of MMP-7 (-181A>G) on the susceptibility of various cancers.

Previous reports have shown different etiologies and genetic risk factors for two histological types of EC. In the present study, MMP-7 (-181A>G) GG genotype was significantly associated with an increased risk for only squamous-type EC. Some other studies have also shown positive association of MMP-7 (-181A>G) GG with SCC.[15-17,24] Wu *et al.*[33] have reported that MMP-7 is highly expressed in metastatic cervical SCC, and may serve as a marker in estimating the invasive and metastatic potential of cervical SCC.

The underlying mechanism of this association may be related to the promoter activity variation of the −181G alleles. Functional analysis has shown that MMP-7-181G alleles can increase gene transcription activity.[14] The expression

Table 3: Association of MMP-7 (−181A>G) genotypes with tumor histopathology and risk of esophageal cancer

Genotypes	Controls 195 (100%)	ESCC[1] 103 (76.3%)	OR* (95% CI)☼*P*	EADC[2] 32 (23.7%)	OR*(95% CI) *P*
MMP-7 (−181A>G) (rs11568818)					
AA	63 (32.3)	27 (26.2%)	1 (Reference)	9 (28.1%)	1 (Reference)
AG	92 (47.2)	37 (35.9%)	0.99 (0.55-1.82) 0.99	14 (43.8%)	1.09 (0.45-2.71) 0.84
GG	40 (20.5)	39 (37.9%)	**2.41 (1.27-4.56) 0.007**	9 (28.1%)	1.64 (0.59-4.49) 0.35

[1]Esophageal squamous cell carcinoma; [2]Esophageal adenocarcinoma; *Age and gender adjusted odds ratio; ☼Bonferroni corrected *P* values; Significant values shown in bold

Table 4: Interaction of MMP-7 (−181A>G) genotypes with salted tea

Genotypes	Controls		Esophageal cancer#		
	Tea (Cups)/day <4 159 (88.2%)	Tea (Cups)/day >4 26 (11.76%)	Tea (Cups)/day <4 36 (28.5%)	Tea (Cups)/day >4 90 (71.4%)	OR*(95%CI)☼*P*
MMP-7 (−181A>G) (rs11568818)					
AA	51 (32.1)	8 (30.8)	15 (41.7)	18 (20.0)	1 (Reference)
AG	74 (46.5)	13 (50.0)	17 (47.2)	29 (32.2)	1.14 (0.46-2.87) 0 .79
GG	34 (21.4)	5 (19.2)	4 (11.1)	43 (47.8)	4.22 (1.38- 12.86) 0 .06

*Age and gender adjusted odds ratio. #Data missing in some subjects. *P* was calculated only with Tea (Cups)/day >4. ☼Bonferroni corrected *P* values

Table 5: Interaction of MMP-7 (−181A>G) genotypes with smoking

Genotypes	Controls		Esophageal cancer#		
	Non-smokers 147 (79.4%)	Smokers 38 (20.5%)	Non-smokers 20	Smokers 106	OR*(95%CI)*P*
MMP-7 (−181A>G) (rs11568818)					
AA	51 (34.7)	10 (26.3)	4 (20.0)	30 (28.3)	1 (Reference)
AG	69 (46.9)	17 (44.7)	12 (60.0)	38 (35.8)	0 .626 (0 .265- 1.483) 0 .287
GG	27 (18.4)	11 (28.9)	4 (20.0)	38 (35.8)	1.254 (0.496- 3.169) 0.633

*Age and gender adjusted odds ratio; #Data missing in some subjects. *P* was calculated only with smokers

and promoter activity of the MMP-7-181G allele is twofold to threefold higher than the −181A allele due to presence of a putative binding site (NGAAN) for a heat-shock transcription factor.[14] The presence of high-expression MMP-7-181G allele may alter the cell surface signaling including cellular proliferation, invasion, and apoptosis processes.[34] Therefore, individuals with excess MMP-7 activity by harboring the −181G allele may be predisposed to malignant transformation. Although *in vitro* studies have suggested that the −153C/T polymorphism (another polymorphism in the promoter region) may also modify promoter activity of the MMP-7 gene, we did not evaluate its role in EC development because of low frequency of the −153T allele in the study population. The higher promoter activity of the −181G allele may induce elevation of the MMP-7 mRNA and, subsequently, increase protein expression. Individuals with excess MMP-7 activity by harboring the −181G allele may predispose to malignant transformation through the 'sheddase' activity of MMP-7 protein via recently described substrates such as tumor necrosis factor A, E-cadherin, and Fas ligands. These substrates have been known to play important roles in signal transduction, cell-cell adhesion, and apoptosis[35-38] In addition, elevated expression of MMP-7 induced by the −181G allele may lead to increased activation of other members of the MMP family such as MMP-2.[39] The latter may therefore modulate tumor development via regulating cancer cell growth, angiogenesis, and immune surveillance.[40]

Gu *et al.*[41] reported that the high expression of MMPs in the invasive margin may help degrade the extracellular matrix surrounding ESCC cells, thereby facilitating the invasion or metastasis of this malignancy.

The people residing in the Kashmir valley have several unique dietary features, which are different from the rest of the world. Salted tea used by people is prepared by using baking soda (sodium bicarbonate) along with common salt (sodium chloride) and boiled for few hours before consuming. It is suspected that the salts might cause thermal injury to the esophageal and gastric epithelia.[2] In the present study, high consumption of salted tea (>4 cups a day) was independently associated with an increased risk for EC (OR =14.86; $P = 0.0001$). Similarly, significant association of smoking (*Hukka*) with EC (OR =21.45; $P = 0.0001$). An association between drinking large amounts of hot salted tea and enhanced risk of EC has been reported in other studies, which have been attributed to thermal irritation of the oesophageal and gastric mucosa by the hot drink.[42,43] However, based on our gene-environment interactions, after Bonferroni correction, we did not find any significant modulation of cancer risk due to interaction of MMP-7 (-181A>G) genotypes with smoking or salted tea consumption.

The sample size of the present study was adequate to provide 80% power for overall association. Because the sample size became smaller in subgroup analyses, we applied Bonferroni correction for multiple comparisons. Moreover, this is the first report of genetic susceptibility of EC due to MMP-7 (-181A>G) gene polymorphism in the Kashmir valley. In conclusion, our data suggest that the MMP-7 (-181A>G) gene polymorphism may influence the susceptibility to ESCC. Determination of MMP-7 (−181A>G) genotype may provide a useful genetic marker in predicating high-risk individuals for the development of EC. However, it may be worthwhile to conduct additional population-based studies including a large subject group before its clinical application.

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