MMP 9 Gene Promoter Polymorphism in Gastric Cancer

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Abstract Matrix metalloproteinase-9 (gelatinase B) plays a key role in cancer invasion and metastasis by degrading the extracellular matrix and basement membrane barriers. A cytosine (C) > thymidine (T) single nucleotide polymorphism (SNP) at position –1562 in the MMP-9 promoter is reported to influence the expression of the gene. Genotyping of MMP-9 –1562 C→T promoter polymorphism in 140 gastric cancer patients and 132 healthy control subjects was carried out in order to evaluate its association with progression and development of gastric cancer. The SNP was genotyped by tetra-primer amplification refractory mutation system-polymerase chain reaction followed by agarose gel electrophoresis. Statistical methods were adopted to test for the significance of the results. Risk factor profile of the patients revealed age above 50 years, smoking, alcoholism as the factors associated with the disease. The distribution of genotype frequencies in gastric cancer patients were 28.7 % of CC, 45.5 % of CT and 25.7 % of TT, whereas in control subjects 31.8 % of CC, 53.03 % of CT and 15.15 % of TT, respectively. The allelic frequencies were 51.51 % of C and 48.48 % of T in patient group and 58.33 % of C and 41.66 %

of T in controls respectively. The present study shows the possible association of epidemiological risk factors with gastric cancer. There is an increased frequency of T allele in the disease compared to control subjects. However, there is no association of the MMP-9 –1562 C→T promoter polymorphism in the development of gastric cancer.

Keywords Gastric cancer · Polymorphism · Matrix metalloproteinase-9 · Metastasis

Introduction

Gastric cancer (GC), also known as stomach cancer can arise in any part of the stomach. It is the fourth most frequent type of cancer and the second most common cause of death worldwide [1]. On the whole 90 % of all tumors of the stomach are cancerous and 95 % of these malignancies account for gastric adenocarcinoma [2]. Frequency of gastric cancer is higher in patients above the age 50 years and shows unusual pattern [3]. Gastric cancer is a complex disorder with multifactorial inheritance and mediated by multiple genetic, epigenetic and environmental risk factors [4].

The matrix metalloproteinase's (MMPs) are zinc-dependent, extracellular endopeptidases whose substrates being proteins of the extracellular matrix (ECM). MMPs are involved in tumor invasion and cancer metastasis. They are also thought to be involved in angiogenesis essential for tumor growth during carcinogenesis [5]. Matrix metalloproteinase-9 (MMP-9), a known gelatinase B or type IV collagenase of 92 kDa is the major MMP produced by human macrophages and its substrates are basement membrane collagen types IV and V, different types of gelatin, fibronectin (FN), and elastin [6–9]. It is a proteolytic enzyme

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necessarily involved in extravasation, migration and tissue remodeling during chronic inflammation [10–12].

The human MMP-9 gene is located on 20q11.2–20q13.1 and contain 13 exons and 12 introns [13]. A Single nucleotide polymorphism C>T transition at –1562 in promoter region results in C/T polymorphism (rs3918242) and was found to have functional effect on gene expression there by resulting in the loss of binding of a nuclear protein in the promoter region of MMP-9 activity in macrophages. The cells with the C/C genotype showed a low transcriptional activity, whereas those with C/T and T/T genotypes resulted in high MMP-9 transcriptional activity [14].

In view of the genotype influence on gene expression, the present study is aimed to evaluate the role of MMP-9 gene promoter polymorphism (–1562 C>T) in the pathogenesis of gastric cancer.

Materials and Methods

Study Population

The study includes 140 endoscopically and histopathologically confirmed gastric cancer patients and 132 healthy control subjects. Gastric cancer patients referred to the Gastroenterology unit of Gandhi Hospital, Hyderabad were considered while the control subjects were randomly selected among the individuals visiting our Institute for regular health check up. Based on the structured questionnaire the information on demographic features such as age, sex, dietary habits, weight, addictions such as smoking, alcoholism, family history of cancer was elicited along with an informed written consent from all the subjects and the study was approved by Institutional Ethics Committee. All the patients were tested for *Helicobacter pylori* infectivity status in antral biopsies by urease test following the method of Vaira et al. [15].

Biospecimens

5 ml of blood was collected from each subject in vacutainers with and without anticoagulant for separation of plasma and serum respectively. Genomic DNA was isolated from whole blood samples of all the patients and control subjects following the method of Lahiri et al. [16].

Genotype Analysis

Analysis of C –1562 T MMP-9 gene polymorphism of the MMP-9 gene was carried out by using tetra-primer amplification refractory mutation system PCR wherein incubation buffer (50 mM KCl, 10 mM Tris–HCl, pH 8)

with 2 mM MgCl₂, 250 μM of each dNTP, 200 ng of template DNA and 1 U *Taq* polymerase (Gene i, Bangalore) in the final reaction volume of 20 μl with 5 pmol of outer primers, 3 pmol of T-allele-specific primer (forward inner), and 15 pmol of C-allele-specific primer (reverse inner) were added. Reactions were based on the following conditions: initial denaturation at 95 °C for 3 min, 35 cycles at 95 °C for 30 s, 68 °C for 30 s, 72 °C for 30 s, and final extension at 72 °C for 2 min. The amplified products were run on agarose gel and the genotypes were determined based on the bands in respective lanes.

Statistical Analyses

Hardy–Weinberg equilibrium was tested for MMP-9 gene polymorphism in patients and controls and found to be in agreement with the equilibrium ($p > 0.05$ for both groups). The evaluation of case control differences in the distribution of alleles and genotypes was carried out by χ^2 test of association. Odds ratios (OR) and their 95 % confidence intervals were analysed to measure the strength of association between MMP-9 gene polymorphism and gastric cancer.

Results

A total of 140 gastric cancer patients and 132 control subjects were included in the present study. The demographic features of the population under study had been listed in Table 1. All the patients belonged to a South Indian population from A.P. The patients were categorized based on sex, age, addictions, family history, *H. pylori* infection etc. It is evident that most of the patients belong to age above 50 years group. The risk factor profile of the patients revealed that male sex, age above 50 years, smoking, alcoholism etc. were the most common risk factors in patients compared to the control subjects ($p < 0.01$). Among the gastric cancer patients 2.85 % of them showed familial incidence of gastric cancer. No significant difference was observed between cases and controls with regard to *H. pylori* infection ($p = 0.869$). Among gastric cancer patients 132 cases (94.28 %) were of intestinal type and the remaining (5.71 %) were of diffuse type.

The genotyping analysis was carried out for all the patients and control subjects. But the comparison of genotypes was done only among the intestinal type of gastric cancer patients and control subjects as the diffuse type of cancer were very few in number (5.71 %). The distribution of genotypic and allelic frequencies of MMP-9 gene promoter polymorphism in patients and controls are given in Table 2. The frequencies of CC, CT and TT

Table 1 Demographic features in gastric cancer patients and control subjects

	Controls/cases	Odd's ratio	95 % CI	<i>p</i> Value
Gender				
Male	94/98			
Female	38/42	1.060	0.608–1.848	0.894
Age				
≥50 years	26/108			
<50 years	106/32	0.073	0.039–0.135	>0.000*
Addictions				
Smokers	34/68			
Non-smokers	98/72	0.367	0.213–0.632	>0.000*
Alcoholics	60/110			
Non alcoholics	72/30	0.227	0.129–0.399	>0.000*
Familial incidence				
Familial	0/4			
Non-familial	132/136	0.0	0–1.611	0.123
<i>H. pylori</i> infection				
Positive	14/14			
Negative	118/126	1.068	0.457–2.494	0.869
Tumour type				
Intestinal	–	132 (94.28 %)	–	
Diffuse	–	8 (5.71 %)	–	

* $p < 0.01$ **Table 2** Distribution of MMP-9 (–1562 C>T) genotypes and allelic frequencies of the study population

	Patients		Controls	
	<i>n</i>	%	<i>n</i>	%
Genotypes				
CC	38	28.78	42	31.81
CT	60	45.45	70	53.03
TT	34	25.75	20	15.15
Allele frequency				
C	136	51.51	154	58.33
T	128	48.48	110	41.66

genotypes in gastric cancer patients were 28.7, 45.45 and 25.7 %, respectively whereas in control subjects 31.8, 53.03 and 15.15 %, respectively. The allelic frequencies were 51.51 % of C and 48.48 % of T in patient group while 58.33 % and 41.66 % in controls respectively. There is a significant difference in the distribution of TT genotype in patients compared to control subjects [TT vs. CT + CC, $\chi^2 = 4.563$; $p = 0.047$; odds ratio = 1.943 (95 % CI; 1.007–3.766)]. However, no significant difference was observed in the allele frequency of C and T alleles in patients and controls [$\chi^2 = 2.479$; $p = 0.137$; odds ratio = 1.318 (95 % CI; 0.921–1.886)] (Table 3).

Comparison of genotypes was also made in GC patients and control subjects with respect to the epidemiological factors and presented in Table 4. There was no significant

difference of MMP-9 gene C/T polymorphism with respect to sex, age and alcoholism. However, a significant association of MMP-9 C/T polymorphism with smokers and nonsmokers of GC group compared to control subjects was observed irrespective of the genotype. An interesting observation made was that smoking has a 12 fold risk with respect to TT genotype individuals compared to CC and CT genotypes.

Discussion

Gastric cancer, the fourth most common cancer in the world is characterized as a multifactorial disease that results from individual genetic predisposition and influence

Table 3 Odd's risk estimates of the genotypes in GC patients compared to control subjects

Patients versus controls	χ^2	Odd's ratio	95 % CI	<i>p</i> Value
TT versus CC	3.101	1.879	0.876–4.051	0.112
TT versus CC + CT	4.563	1.943	1.007–3.766	0.047
T versus C	2.479	1.318	0.921–1.886	0.137

Table 4 Distribution of genotype frequencies in patients and control subjects with regard to epidemiological factors

	CC		Odd's ratio	<i>p</i> Value	CT		Odd's ratio	<i>p</i> Value	TT		Odd's ratio	<i>p</i> Value
	Cases	Control			Cases	Control			Cases	Control		
Sex												
Males	28	32	0.875	0.803	46	46	1.64	0.247	18	16	0.286	0.074
Females	10	10			14	24			14	04		
Age												
<50 years	10	10	1.231	0.796	22	14	2.316	0.049	06	02	2.077	0.463
≥50 years	28	32			38	56			28	18		
Smoking												
Smokers	26	12	5.00	0.001	36	18	4.093	0.000	20	04	12.0	0.001
Nonsmokers	12	30			24	52			14	16		
Alcoholism												
Alcoholics	14	18	0.933	0.882	34	28	0.073	2.0	16	14	0.381	0.156
Non-alcoholics	24	24			26	42			18	06		

of environmental risk factors [17]. In the present study a significant association of advanced age, smoking, alcoholism was observed in the patient group compared to the control subjects. Previous studies have also shown that smoking and alcohol use were associated with increased gastric cancer risk which corroborates with the present findings, which can be explained on the basis of the fact that ingestion of cigarette smoke increase the risk of stomach cancer via nitrosoamines whereas consumption of alcohol influences tumor volume doubling time (TVDT) stimulating tumor growth by angiogenesis [22–24]. It is observed that males are at an increased risk to the disease compared to females as reported by Parkin et al. [21]. This may be attributed to environmental triggering factors observed more common in males.

MMP-9 is produced by mesenchymal, epithelial, and hematopoietic cells and also by distinct tumor cell types [18]. MMP-9 gene localized on to the region 20q11.2–q13.1 and polymorphisms in the promoter of MMP-9 have been implicated in the regulation of gene expression and susceptibility to various diseases [13]. A single nucleotide polymorphism (SNP) at –1562 bp due to C to T transition (–1562 C>T) leads to the loss of binding of a nuclear protein and variation in gene expression in macrophages. The C/C genotype leads to a low promoter activity whereas the C/T and T/T genotypes result in high transcriptional

activity of MMP-9 gene [14]. The harboring of the T allele of the –1562 C/T MMP-9 gene polymorphism is related to an increased risk to specific cancers and tumorigenesis [19, 20].

Earlier studies have revealed the implication MMP-9 gene polymorphism in the pathophysiology of various cancers [25–27]. However, there is an apparent discrepancy among results of association studies in cohorts of different ethnicities. This is the first study to examine the role of MMP-9 gene promoter polymorphism in gastric cancer onset of south Indian origin. It is hypothesized that the MMP-9 gene polymorphism may alter the expression and activity of the enzyme, increasing ECM degradation and invasion, leading to cancer progression. A case–control study by Matsumura et al. [28] in 177 gastric cancer patients and 224 healthy subjects revealed no significant variation in genotype distribution between the two groups. Similarly, another study by Kubben et al. [29] with 79 gastric cancer patients and 169 control subjects indicated the presence of the T allele variant in the MMP-9 promoter (–1562 C>T) was not associated with clinical outcome in gastric cancer patients. Zhang et al. [30] carried out MMP-2 –1306 T/C and MMP-9 –1562 C/T polymorphic studies in 228 gastric cancer patients and 774 healthy controls to evaluate the gene interactions of the genotypes and the genetic susceptibility of gastric cancer, but the findings

revealed no significant associations. In the present study no such association of MMP-9 polymorphism with gastric cancer disease was observed. Stratification of results based on epidemiological factors demonstrated a significant association of smoking with the disease irrespective of the genotype thereby suggesting smoking as one of the important risk factor in the etiology of the disease.

Thus, the present study highlights the role of epidemiological risk factors in the etiology of disease. However, the genotype association of MMP-9 does not reveal any significant difference between patients and control subjects. The MMP-9 promoter polymorphism may have an effect on the development of gastric cancer, but the influences may be slight and perhaps restricted to some specific environment–genotype effect. Thus, the environmental triggering factors seem to act as modifiers influencing MMP-9 gene expression and progression of the disease.

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