Original Article

Role of Tumor Necrosis Factor-α -308 G/A Promoter Polymorphism in Gastric Cancer

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ABSTRACT

Background/Aim: Gastric cancer (GC) is the fourth most common cancer and the second most common cause of cancer death world-wide after lung cancer. It is a multifactorial disease with the involvement of both genetic and environmental risk factors. Genetic variation in genes encoding cytokines and their receptors, determine the intensity of the inflammatory response, which may contribute to individual differences in severity of outcome of the disease. Tumor necrosis factor alpha (TNF-α) is a potent pro-inflammatory cytokine and acid inhibitor. A bi allelic G to A polymorphism at -308 upstream from the transcription initiation site of the promoter is associated with elevated TNF levels. The present study is aimed at evaluating the role of TNF-α-308 (G → A) gene polymorphism and susceptibility to GC. **Subjects and Methods:** A case-control study was carried out in 114 GC patients and 229 healthy control subjects. TNF-α genotyping at position-308 (G → A) was carried out by amplification refractory mutation system-polymerase chain reaction (ARMS-PCR) method followed by agarose gel electrophoresis. **Results:** The distribution of TNF-α genotypes at -308 (G → A) were GG 28.07%, GA 66.67% and AA 5.26% in GC patients and GG 33.19%, GA 55.89% and AA 10.92% in control subjects. The frequencies of alleles G and A were 0.614 and 0.386 in GC patients and 0.611 and 0.389 in control subjects respectively. **Conclusion:** The study showed no significant difference in the distribution of genotype and allelic frequencies between GC patients and control subject.

Key Words: Gastric cancer, *Helicobacter pylori*, promoter polymorphism, tumor necrosis factor alpha

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Gastric cancer (GC) is one of the most commonly diagnosed malignancies and remains a considerable public health problem world-wide.[1] The incidence of GC shows marked variation among countries.[2] Incidence rates are especially high in Japan and other East Asian countries, Eastern Europe and parts of Latin America.[3] Marked differences in GC incidence among different ethnic groups living in the same geographical area have been observed, pointing to host genetic factors or socio environmental factors peculiar to a particular racial group. GC remains one of the most common cancers in Asia.[4,5] It is the third most common cancer in India and the second leading site of cancer occurrence world-wide. The incidence rate of GC is 4 times higher in Southern India compared to Northern India.[6]

GC is a multifactorial, multigenetic, and multistage disease. Host genetic and environmental factors are important determinants of increasing risk for many cancers including GC.[6-8] It is known that diet, smoking, alcohol consumption, and low intake of fruits and vegetables can be implicated in disease development.[9,10] *Helicobacter pylori* (*Hp*) infection is the strongest risk factor for non-cardia GC and chronic gastritis. Only < 1% of *Hp* carriers will ever develop GC. *Hp* is responsible for triggering a pathological progression in the gastric mucosa that begins with chronic gastritis and progresses to atrophic gastritis, intestinal metaplasia, dysplasia, and eventually GC.[11]

Cytokines play an important role in the regulation of both humoral and cell-mediated immune responses. Cytokines participate in the inflammatory response associated with

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innate and acquired immune responses. Polymorphisms within regulatory and other functional regions of cytokine and cytokine receptor genes markedly influence cytokine expression and secretion profiles in response to infectious agents. Gene polymorphisms that modify the intensity of the inflammatory response may contribute to GC risk variations. Genetic variants of inflammation-related cytokines are a potential risk factor because *Hp* infection induces chronic inflammation in gastric mucosa, which is a critical step in gastric carcinogenesis. One of the major cytokines associated with *Hp* infection is the tumor necrosis factor (TNF) expressed by TNF-α and TNF-β genes.[12,13]

TNF-α is a cytokine induced by *Hp* and inhibits gastric acid secretion.[14,15] The TNF-A gene on chromosome 6p21.3 encoding TNF-α is known to have five biallelic single-nucleotide polymorphisms in the promoter region; G-238A, G-308A, C-857T, C-863A, and T-1031C.[16] *Hp* is responsible for triggering a pathological progression in the gastric mucosa that begins with chronic gastritis and progresses to atrophic gastritis, intestinal metaplasia, dysplasia, and eventually GC. The TNF-α-308 promoter polymorphism is a bi-allelic G to A polymorphism, and the TNF-α. A allele is associated with increased levels of TNF in plasma.[17] Although studies have reported TNF can modify the risk of GC, the exact role of TNF as a gastric carcinogen is still controversial. In the present study, we investigated the association between the TNF-α-308 G/A polymorphism and susceptibility to GC in south Indian population.

SUBJECTS AND METHODS

Subjects

A total of 114 endoscopically and histopathologically confirmed GC patients in the age group of 20-75 years, referred to the Department of Gastroenterology, Gandhi Hospital and the Department of Gastroenterology, Osmania General Hospital, Hyderabad from Oct. 2009 to Dec. 2012, were considered for the present study. Total 229 healthy controls with no family history of gastric ulcer or cancer were selected randomly amongst the persons having normal upper gastro-intestinal endoscopy (UGIE) report. Any control subject with present or past history of gastritis, gastric ulcer, GC and related problems were not considered for the study. A structured questionnaire was adopted to elicit information on epidemiological factors such as age, sex, dietary habits, weight, addictions, family history of cancer etc. *Hp* infection status of all the subjects was determined from plasma by sandwich ELISA method using anti-Immunoglobulin G (IgG) as described by the manufacturer (IBL, Hamburg, Germany). The study was approved by the Institutional Ethical Committee and informed consent was obtained from all recruited subjects.

Deoxyribonucleic acid (DNA) extraction

A total of 5 ml of blood was collected from each subject in vacutainers with anticoagulant ethylenediaminetetraacetic acid (EDTA). Genomic DNA was isolated from whole-blood samples of all patients and control subjects by the salting out procedure.[18]

Genotyping of TNF-α -308 G/A polymorphism

Genotyping of TNF-α-308 G/A polymorphism was carried by tetra-primer amplification refractory mutation system-polymerase chain reaction (ARMS-PCR) method as described by Shu *et al*.[19] PCR primers sequences are given in Table 1. Each PCR reaction was carried out in a total volume of 10 μl, containing 30 ng of genomic DNA, 10 pmol of each inner primer, 1 pmol of each outer primer, 200 μM of each deoxynucleotide triphosphstes dNTPs, 1X reaction buffer, 2 mM $MgCl_2$ and 0.5 U Taq polymerase. The cycling conditions were as follows: An initial denaturation at 95°C for 4 min, followed by 35 cycles at 95°C for 30 s, 60°C for 30 s and 74°C for 30 s. The final extension step was at 74°C for 6 min. After amplification, the PCR products were separated by electrophoresis on an agarose gel (1.5%) stained with ethidium bromide. The gel was visualized under ultraviolet light with a 100-bp ladder. All the collected samples were successfully genotyped. 10% of the samples were randomly taken and the assay was repeated and found no bias in the genotyping. The findings were similar on replicative study with the results being 100% concordant.

Statistical analysis

The two sided Pearson's χ^2 test was adopted to examine the differences between the cases and the control group with respect to sex, age, smoking, alcoholism, and family history. Odds ratio (OR) and corresponding 95% confidence intervals (CI) were calculated by Open Epi software.[20] A P value ≤ 0.05 was considered as significant.[20]

RESULTS

A total of 114 GC patients and 229 controls were enrolled in this case-control study. Table 2 shows the distribution of gender, age, smoking, alcoholism, and family history between cases and controls. Results showed statistically significant difference with respect to age (P = 0.00001), gender (0.0188) smoking (P = 0.00494) and *Hp* infection status (P = 0.0329) between GC patients and controls. However, no significant difference was observed between cases and controls with regard to alcohol consumption.

In GC patients, 84 cases were of intestinal type and the remaining 30 were of diffuse type. The distribution of genotypes and allelic frequencies are shown in Table 3. The distribution of TNF-α genotypes at -308 (G → A) were

Table 1: PCR primers and conditions for TNF-α (-308 G/A) polymorphism

Primers	Primer sequence	Tm	Amplicon size
Forward inner primer (A allele)	5'TGGAGGCAATAGGTTTTGAGGGGCAGGA-3'	68°C	154 bp (A allele)
Reverse inner primer (G allele)	5'TAGGACCCTGGAGGCTGAACCCCGTACC-3'	72°C	224 bp (G allele)
Forward outer primer	5'ACCCAAACACAGGCCTCAGGACTCAACA-3'	68°C	323 bp
Reverse outer primer	5'AGTTGGGGACACGCAAGCATGAAGGATA-3'	65°C	(from two outer primers)

TNF-α: Tumor necrosis factor-alpha, PCR: Polymerase chain reaction

Table 2: Demographic variables in gastric cancer patients and controls

Variable	Controls (*N*=229) *N* (%)	GC cases (*N*=114) *N* (%)	OR (95%CI)	*P* value[a]
Gender				
Male	170 (74.24)	78 (68.42)		
Female	59 (25.76)	36 (31.58)	1.48 (1.09-2.70)	0.0188*
Age (year)				
≤50 years	93 (40.62)	24 (21.05)		
>50 years	136 (59.38)	90 (78.95)	0.288 (0.17-0.48)	<0.00001**
Smoking status				
Smokers	88 (38.43)	48 (42.11)		
Non-smokers	141 (61.57)	66 (57.89)	0.486 (0.28-0.76)	0.00051**
Drinking status				
Alcoholics	120 (52.4)	52 (45.61)		
Non-alcoholics	109 (47.6)	62 (54.39)	0.672 (0.43-1.04)	0.07440
Helicobacter pylori infection status				
Positive	145 (63.31)	86 (75.44)		
Negative	84 (36.69)	28 (24.56)	1.779 (1.075-2.945)	0.03296*
Family history				
Familial	0	2 (1.75)		
Non-familial	229	112 (98.25)	-	-

[a]Two-sided χ^2-test, OR: Odd ratio, CI: Confidence interval, GC: Gastric cancer, **P* value ≤0.05, ***P* value ≤0.001

Table 3: Genotype distribution and allelic frequency of TNF-α (-308 G/A) promoter polymorphism in control subjects and gastric cancer patients

	Controls (*N*=229) *N* (%)	GC cases (*N*=114) *N* (%)	OR (95% CI)	*P* value[a]
Genotypes				
GG	76 (33.19)	32 (28.07)	1.0 (ref)	
GA	128 (55.89)	76 (66.67)	1.41 (0.8543-2.328)	0.222
AA	25 (10.92)	6 (5.26)	0.57 (0.2135-1.522)	0.366
Alleles				
G	280 (0.611)	140 (0.614)	1.0 (ref)	
A	178 (0.389)	88 (0.386)	0.988 (0.713-1.37)	0.987

TNF-α: Tumor necrosis factor-alpha, OR: Odds ratio, GC: Gastric cancer, CI: Confidence interval **P* value ≤0.05, ***P* value ≤0.001

GG 28.07%, GA 66.67% and AA 5.26% in GC patients and GG 33.19%, GA 55.89% and AA 10.92% in control subjects. The frequencies of alleles G and A were 0.614 and 0.386 in GC patients and 0.611 and 0.389 in control subjects respectively. The distribution frequencies of TNFα-308 G/A genotypes and alleles in cases were not significantly different from those in controls (for GG vs. AA genotype: OR = 0.57 [95% CI: 0.2135-1.522; *P* = 0.366] and G allele vs. A allele: OR = 0.988 [95% CI = 0.713-1.37]; *P* = 0.987). Comparison was also made with respect to *Hp* infection status in patients and control subjects and found no significant difference with respect to the TNF genotype distribution.

DISCUSSION

The critical roles of different cytokines in regulating antimicrobial immunity and inflammation make them attractive candidates for being genetic host markers in evaluating individual susceptibility to GC development. They may influence the risk of developing GC by altering the quality and vigor of inflammatory responses produced by the host after exposure to various environmental or infectious triggers.[21,22] TNF-α is a pro-inflammatory cytokine with a wide range of activities. It has been proposed that TNF-α polymorphisms may determine the quality of the inflammatory response to infection and ultimately affect the risk of progression to GC. In the present study, we investigated the association between TNF-α-308 G/A polymorphism and risk of GC in a south Indian population from Andhra Pradesh.

Genetic pre-disposition is an important contributor in the pathogenesis of GC. Genetic factors determining cancer risk have been postulated for the last decades and seem to be more apparent for GC.[23-26] A study by Wilson *et al.*, suggests that the less common allele of TNF-α-308 A induces a more efficient transcription of TNF-α and consequently intensifies the inflammatory response against the infection.[27] High TNF-α production inhibit gastric acid secretion which may cause the spread of the organism into the corpus. However, the correlation between TNF-α 308 A allele and higher production of TNF-α has not been shown in other studies.[28,29]

Some case-control studies have been conducted to elucidate the correlation between TNF-α polymorphisms and the risk of gastric carcinoma. Machado, *et al.*, in a case-control study on an *Hp*-infected population including 306 controls and 286 patients with gastric carcinoma found that carriers of TNF-α-308 A allele are at increased risk for developing gastric carcinoma (OR = 1.9; 95% CI: 1.3-2.7).[30] In another case-control study by El-Omar, *et al*. TNF-α-308 AA genotype was associated with an increased risk of non-cardia gastric carcinoma especially in *Hp* seropositive individuals (OR = 2.6).[25] A significant increased risk of GC was observed among TNF-α-308 GA heterozygotes compared to TNF-α-308 GG homozygotes (OR = 1.81; 95% CI: 1.04-3.14) in a study by Lu *et al*.[31] Some other case-control studies, which were conducted in China,[32] Japan,[33] Taiwan,[34] Italy,[35] Korea,[36,37] and Mexico,[38] did not find any significant association between TNF-α-308 polymorphism and the risk of GC. Our results are also in agreement with the latter reports showing no association between TNF-α-308 G/A polymorphism and GC and indicate that the -308 polymorphism is either generally functionally silent or does not play a functional role in TNF-α expression.

The discrepancy between the results of these studies may be explained by the effects of genetic heterogeneity and varied gene-environment interactions in the development of GC in different populations. Inflammation is a highly complex process that involves several genes. The fact that numerous gene polymorphisms contribute to the inflammatory response adds further complexity to the analysis of a specific polymorphism because each individual gene is likely to contribute only modestly to the risk. Thus, TNF-α may interact in important ways with multiple cytokine/chemokines involved in inflammation pathways that also display genetic variation. GC has a complex and multifactorial etiology, which might explain discrepancies in the attribution to GC risk to a single SNP. Furthermore, discrepant results might be influenced by differences in ethnic background, diet, life-style, and other factors between the study populations.

CONCLUSION

In conclusion, the results of the present study indicate that TNF-α-308 promoter polymorphism is not associated with GC in south Indian population from Andhra Pradesh.

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