The Role of Tumor Necrosis Factor-α Promoter Polymorphisms in Gastric Carcinoma in Iranians: A Case-Control Study in the North-East of Iran

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ABSTRACT

Background

Host genetic and environmental factors are involved in development of gastric cancer. Tumor necrosis factor (TNF)-α has a key role in *Helicobacter pylori*-induced gastritis. We analyzed the association between TNF-α polymorphism and the risk of gastric cancer in an Iranian population residing in northeastern Iran.

Materials and Methods

In a case-control study, the genotyping was carried out by PCR-RFLP in 108 patients with gastric cancer and 100 randomly-selected healthy individuals. The polymorphic sites studied include promoter region of TNF-α at position 308 (G-A transition). *H. pylori* infection was determined by ELISA assay in 100 patients.

Results

The frequencies of TNF-α 308 GG, AG and AA genotypes were 67%, 29% and 4% for controls and 75.9%, 13.2% and 10.2% for patients. AG genotype significantly reduced the risk of gastric carcinoma (p=0.008). There was no association between TNF-α 308 polymorphisms and the risk of diffuse type gastric carcinoma. Carriers of AG genotype were associated with a decreased risk of intestinal type gastric carcinoma (OR=0.25, 95% CI: 0.08-0.7). With stratification of patients according to *H. pylori* infection status, there were no significant differences in the frequencies of genotypes between those with *H. pylori*-negative gastric carcinoma and controls. However, the frequency of AG genotype was significantly higher among controls than *H. pylori*-positive cases (OR=0.230, 95% CI: 0.06-0.8). In contrast, AA genotype was significantly more frequent among *H. pylori*-positive cases compared to the controls (OR=4.1, CI: 1.04-16.4).

Conclusions

Our results suggest an association between TNF-α 308 polymorphism and the risk of gastric cancer in Iranians. The effect is prominent among *H. pylori*-infected patients and intestinal type of gastric carcinoma.

Keywords: Gastric carcinoma, Cytokine, Polymorphism, Tumor necrosis factor-α, *Helicobacter pylori*
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INTRODUCTION

Gastric adenocarcinoma is the second cause of cancer-related death worldwide.(1), According to the annual report of the Iranian National Cancer

Registry, gastric carcinoma was the second most diagnosed malignancy after skin cancer in 2004. Therefore, Iran can be regarded as a high-incidence region.

Based on Lauren classification, gastric adenocarcinoma has been classified into the intestinal (well-differentiated) and the diffuse type (undifferentiated) which differ in their etiology, epidemiology and pathogenesis. While the intestinal type is considered to be more attributed to environmental factors, the diffuse type is caused to a larger extent by genetic factors.(2), Helicobacter pylori infection is considered as the main environmental cause of gastric carcinoma.(3-5), Chronic inflammatory response induced by H. pylori infection is the main pathophysiologic event for subsequent gastrodoudenal diseases which may range from asymptomatic gastritis to peptic ulcer and cancer. H. pylori infection creates acid-secreting corpus gastritis, gastric atrophy and intestinal metaplasia which may ultimately progress to gastric cancer.(6), However, gastric carcinoma is developed in a minority of infected people (1%-2%) (7) and seems to depend on a number of factors including diverse bacterial strains (8), environmental co-factors (diet and smoking) and host genetics.(9), Considering that the first step in pathogenesis of both histologic subtypes of gastric carcinoma is chronic gastritis, one could hypothesize that polymorphism of pro-inflammatory cytokine genes which regulates the quality of inflammatory response, may have a pivotal role in the susceptibility of a person to different histologic types of gastric carcinoma.(10), Moreover, the significant variation in the prevalence of gastric carcinoma among different nations might be explained by the difference in the genotype of cytokine genes.(11)

Although the results have not been consistent, some studies in different countries supported the association between interleukin-1β (IL-1β) (12-14), interleukin-1 receptor antagonist (IL-1 RN) (8,15), interleukin-8 (IL-8), (16) interleulin-10 (IL-10) (17) and tumor necrosis factor-α (TNF-α) polymorphisms and the risk of gastric cancer. TNF-α which is a pro-inflammatory cytokine, has the capability to inhibit gastric acid secretion.(18), A polymorphism in the promoter region at position 308 that present as "G-A transition" has been reported to be effective on the risk of gastric carcinoma.(17,19), The main objective of these trials was to identify the host genetic risk factors. Therefore, it might be possible to recognize those people who are genetically more susceptible for developing gastric carcinoma.

This study was designed to investigate the effect of TNF-α 308 polymorphism on gastric carcinoma in a population of Iranians residing in the North-east of Iran. The correlation with H. pylori infection and pathologic subtype (intestinal or diffuse) was also evaluated.

PATIENTS AND METHODS

Conduction of this case-control study was approved by the Ethics Committee of Mashhad University of Medical Sciences (MUMS). It was conducted at Omid Hospital and Bu-Ali Institute. Informed written consents were taken from cases and controls. The study included 108 Iranian patients with gastric cancer (median age: 63 years; range: 29-82 years; male/female: 80/29) treated at Department of Oncology of Omid Hospital. The control group included 100 randomly-selected healthy Iranian individuals without any family history of cancer (median age: 37 years; range: 22-52 years; male/female: 44/57). Considering that polymorphisms do not change throughout the life, we did not match the case and control groups for age.

Genomic DNA was isolated from whole blood collected in EDTA tubes, using a "salting out" method with commercial Biogene kit (Mashad, Iran). Anti-H. pylori IgG antibody was checked by ELISA method (Pishtaz Teb diagnostics, Tehran, Iran) for serologic detection of infection in all patients. Antibody titers >10 U/mL were

considered positive.

Genotyping of TNF-α polymorphism

A polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) assay using for detection and amplification of a 117-bp fragment containing the polymorphism 308 G/A of the TNF-α was used. The PCR was performed with an initial denaturation at 96°C for three min followed by 40 cycles of 95°C for one min, 62°C for one min, 72°C for one min and a final extension step for one min at 72°C.

The forward primer used was 5`-AGGCAATAGGTTTTGAGGGCCAT-3`. The reverse primer used was 5`-A C A C T C C C C A T C C T C C C G G C T - 3 ` (Fazapajouh, Tehran, Iran). The amplicons were electrophoresed in 1.5% agarose gel.

The restriction digestion was carried out in 20 µL reaction containing 1000 ng PCR product and one U of NCOI enzyme in the two µL NCOI buffer supplied by the Sinagene at 37°C overnight. The reaction was resolved on 2.5% agarose gel (LM) which was scored under a UV illuminator after staining with ethidium bromide. The wild type (A at nucleotide 308) was not cut by NCOI and was isolated as a larger 117-bp fragment, while the variant (G at nucleotide 308) was cut by NCOI to produce two smaller fragments of 97-bp and 20-bp (Fig 1).

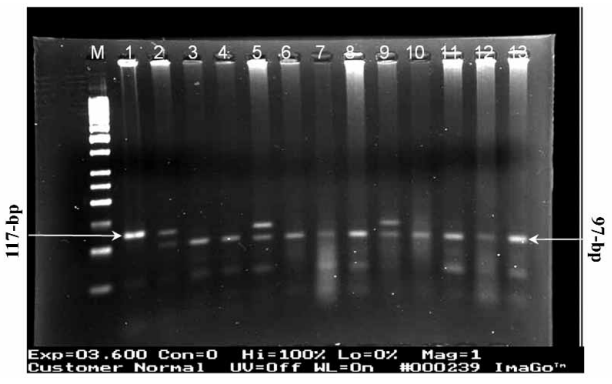

Figure 1. Analysis of TNF-α 308A/G polymorphism. Lanes M shows DNA size marker (50-bp). Lane 1 uncut AA product, lanes 2, 5, 9 AG heterozygous and lanes 3,4,6,7,8,10,11,12,13 GG homozygous

Statistical Analysis

Direct gene counting method was used to determine the frequency of genotypes and alleles. χ^2 or Fisher's exact test was used to compare the frequencies of polymorphisms between different groups. $p<0.05$ was considered statistically significant. The risk was reported as odds ratio (OR) and its 95% confidence interval (CI). SPSS v 11.5 (SPSS Science, Chicago, IL) and STATA 8 statistical package (STATA Corp) were used for statistical analyses.

RESULTS

Serologic H. pylori test was done for 100 patients among whom 34 were found positive. The histologic subtype could be identified in 87 patients among whom 43 had intestinal type, 39 had diffuse and five mixed subtypes.

The distribution of genotypes of TNF-α (308 G/A) gene polymorphisms in patients with gastric carcinoma and the control group are shown in Table 1. The most common genotype was GG in both case and control groups followed by AG and AA. However, a statistically significant difference in genotype frequencies was observed between cases and controls. AG genotype was significantly more frequent among controls compared to cases (p=0.008). In comparison with controls, the AA genotype was more frequent among cases, though the difference was not statistically significant (p=0.086).

The comparison of the genotype frequencies between cases in separate histologic subtypes and controls is illustrated in Table 2. There were no significant differences in genotype frequencies between cases with diffuse type of gastric carcinoma and healthy controls. However, in comparison to those with intestinal type histology, AG genotype was significantly more common in controls (OR=0.21; 95% CI: 0.25-0.7).

The risk of gastric carcinoma related to TNF-α G/A genotypes was further examined with

Table 1. The frequency of alleles and distribution of genotype of TNF-α 308 G/A gene polymorphisms in patients with gastric cancer and healthy controls.

TNF-α (-308 G/A) Genotypes/ Allele carrier frequencies	Controls n=100	Cases n=108	p-value	OR* (95% CI)
GG	67 (67%)	82 (75.9%)	0.15	1.55 (0.84-2.85)
AG	29 (29%)	15 (13.2%)	0.008 [a]	0.39 (0.19-0.79)
AA	4 (4%)	11 (10.2%)	0.086	2.72 (0.83-8.84)
G allele carrier	96 (96%)	97 (89.8%)	0.15	0.64 (0.35-1.18)
A allele carrier	33 (33%)	26 (24.0%)	0.085	0.36 (0.11-1.19)

*Odd Ratio

a: Control $_{\text{AG genotype}}$ vs Case $_{\text{AG genotype}}$

Table 2. Comparison of genotype and frequency of alleles between healthy controls and cases with gastric cancer in different histologic subtypes

TNF-α (-308 G/A) Genotypes/ Allele carrier frequencies	Controls n=100	Gastric carcinoma					
		Intestinal			Diffuse		
		Cases n=43	OR (95%CI)	p-value	Cases n=39	OR (95%CI)	p-value
GG	67 (67%)	34 (79.1%)	1.8 (0.8-4.3)	0.14	30 (79.6%)	1.64 (0.69-3.85)	0.25
AG	29 (29%)	4 (9.3%)	0.25 (0.08-0.7)	0.01[a]	6 (15.4%)	0.44 (0.16-1.17)	0.09
AA	4 (4%)	5 (11.6%)	3.15 (0.8-12.4)	0.08	3 (7.7%)	2.00 (0.42-9.30)	0.37
G allele carrier	96 (96%)	38 (88.3%)	0.31 (0.81-1.24)	0.08	36 (92.3%)	0.50 (0.10-2.34)	0.37
A allele carrier	33 (33%)	9 (20.9%)	0.53 (0.23-1.25)	0.14	9 (23.0%)	0.60 (0.25-1.43)	0.25

a: Control $_{\text{AG genotype}}$ vs Case $_{\text{Intestinal AG genotype}}$

stratification of cases according to their H. pylori infection status. As shown in Table 3, there were no significant differences in genotype frequencies between H. pylori-negative cases and controls. AG genotype significantly reduced the risk of gastric carcinoma (OR=0.23; 95% CI: 0.06-0.8). Moreover, TNF-α 308 G carriers were associated with a reduced risk of H. pylori-positive gastric carcinoma. Furthermore, AA genotype was detected more frequently among H. pylori-positive cases compared to controls (OR=4.1; 95% CI: 1.04-16.4).

DISCUSSION

The results of the present study suggested that TNF-α 308 polymorphisms affect the risk of

Table 3. The frequency of alleles and distribution of genotype of TNF-α 308 G/A gene polymorphisms in patients with gastric cancer and healthy controls.

TNF-α (-308 G/A) Genotypes/ Allele carrier frequencies	Controls n=100	Gastric carcinoma					
		H. pylori-positive			*H. pylori*-negative		
		Cases n=34	OR (95%CI)	p-value	Cases n=64	OR (95%CI)	p-value
GG	67 (67%)	26(76.5%)	1.6 (0.65-3.9)	0.30	50 (78.1%)	1.75 (0.85-3.63)	0.12
AG	29 (29%)	3 (8.8%)	0.230 (0.06-0.8)	0.01[a]	11 (17.2%)	0.5 (0.2-1.1)	0.08
AA	4 (4%)	5 (14.7%)	4.1 (1.04-16.4)	0.03[b]	3 (4.7%)	1.18 (0.25-5.45)	0.83
G allele carrier	96 (96%)	29 (85.2%)	0.24 (0.06-0.95)	0.03[c]	61 (95.3%)	0.84 (0.18-3.91)	0.83
A allele carrier	33 (33%)	8 (23.5%)	0.62 (0.25-1.52)	0.30	14 (21.8%)	0.56 (0.27-1.17)	0.12

a: Control $_{\text{AG genotype}}$ vs Case $_{\text{H. pylori-positive AG genotype}}$

b: Control $_{\text{AA genotype}}$ vs Case $_{\text{H. pylori-positive AA genotype}}$

c: Control $_{\text{G allele carrier}}$ vs Case $_{\text{H. pylori-positive G allele carrier}}$

gastric carcinoma. The variant genotype AA was associated with an increased risk of gastric carcinoma especially in H. pylori-positive cases. Those who carry the AG genotype had a lower risk of gastric carcinoma. The effect was more prominent for intestinal histologic subtype and H. pylori-positive cases.

TNF-α is a pro-inflammatory cytokine with a wide range of activities. H. pylori-induced gastritis which may lead to step-wise development of gastric carcinoma is mediated by a variety of cytokines including TNF-α. It has been proposed that TNF-α polymorphisms may determine the quality of inflammatory response to the infection and ultimately affect the risk of progression to gastric carcinoma. The results of a study by Wilson, *et al.*, suggest 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.(20), High TNF-α production inhibits 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.(21, 22) The study by Rad, *et al.*, did not show that TNF-α G/A polymorphisms influence mucosal cytokine expression or inflammatory response to H. pylori.(23)

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 H. pylori-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).(19), In another case-control study by El Omar, *et al.*, TNF-α 308*A genotype was associated with an increased risk of non-cardia gastric carcinoma especially in H. pylori seropositive individuals (OR=2.6).(17), In another study including 250 incident cases with gastric carcinoma and 300 controls which is conducted by Lu, *et al.*, in China, a significant increased risk of gastric cancer was seen among TNF-α 308 AG

heterozygotes compared to TNF-α 308 GG homozygotes (OR=1.81; 95% CI: 1.04-3.14). In that study TNF-α 308 AA genotype was very rare (0.0% in cases and 0.7% in controls).(16)

Some other case-control studies which were conducted in Korea,(24, 25) Japan, (13) Taiwan, (26) China, (27) Italy (10) and Mexico (14) did not find any significant associations between TNF-α 308 polymorphisms and the risk of gastric carcinoma even after stratification for H. pylori infection. Table 4 shows the results of different studies on the role of TNF-α 308 G/A polymorphisms on the risk of gastric carcinoma. The discrepancy between the results of these studies could be in part due to the contribution of other genetic and environmental factors in the development of gastric carcinoma in different populations. In a case-control study by Guo, *et al.*, in China, no significant differences in the genotype frequencies of the TNF-α 308 G/A and TNF-β +252 G/A were found between patients with gastric cardiac adenocarcinoma or esophageal squamous cell carcinoma and healthy controls. However, when they analyzed the combined effects of TNF-α and TNF-β genotypes, a significant increase in the risk of developing gastric cardiac carcinoma and esophageal squamous cell carcinoma was shown in the carriers of TNF-β B2/B2 and TNF-α 1/1 compared to those with TNF-β B1/B2 and TNF-α 1/2 or 2/2 genotypes.(28), The frequency of TNF-α 308 polymorphisms especially TNF-α 308 AA has been very low in some nations, particularly Asians.(13, 27), In a case-control study on a Latino population from Honduras TNF-α 308 polymorphisms were nearly absent.(29), Therefore, it is very unlikely to find a strong association between these variant genotypes and the risk of cancer.

The association of other TNF-α polymorphisms and risk of gastric cancer has been investigated in some studies. In the study conducted by Sugimoto, *et al.*, in Japan, the alleles of TNF-α 875 T, TNF-α 863 A and TNF-α 1031 C increased the risk of both gastric cancer and gastric ulcer.(13), Lu, *et al.*, found a non-significant elevated risk for gastric carcinoma in carriers of TNF-α 238 AG.(16),

Table 4. Results of various studies on the role of TNF-α -308 G/A polymorphisms in gastric carcinoma

Study	Country	Cases	Controls	The role of TNF-α -308 G/A polymorphisms
Machado, *et al.* [19]	Portugal	287	306	Increased risk of GC for TNF-α -308 A allele carriers
El-Omar, *et al.* [17]	Scotland	314	212	Increased risk of non-cardiac GC for TNF-α -308 A allele carriers
Lu, *et al.* [16]	China	250	300	Increased risk of GC for TNF-α -308 AG carriers
Kim, *et al.* [25]	Korea	237	474	ns
Lee, *et al.* [24]	Korea	341	261	ns
Wu, *et al.* [26]	Taiwan	204	210	ns
Li, *et al.* [27]	China	59	264	ns
Perri, *et al.* [10]	Italy	164	362	ns
Sugimotto, *et al.* [13]	Japan	105	172	ns
Gonzalez, *et al.* [14]	Mexico	H.pylori + 25	H.pylori - 25	ns

GC: gastric carcinoma
ns: not significant

However, in another case-control study in Korea, a lower risk of gastric carcinoma was found in people with TNF-α 238 A.(30)

In conclusion, the results of our study suggested that TNF-α 308 polymorphisms are associated with the risk of gastric carcinoma. The rare TNF-α 308 AA genotype was significantly more common among H. pylori-positive cases compared to the controls. Carriers of TNF-α 308 G were associated with a lower risk of gastric carcinoma in H. pylori-positive patients. The unexpected finding was the protective effect of TNF-α 308 AG genotype against the development of H. pylori-associated gastric carcinoma. These results should be re-evaluated by larger case-control studies in Iran. Considering the multi-factorial nature of the disease, we suggest that the combined effect of other cytokine polymorphisms be evaluated with stratification of other environmental co-factors (such as diet and smoking) and cag A genotype status in H. pylori strains. Hopefully, the results of these studies will help to recognize people who are genetically at high risk for the development of gastric carcinoma and may benefit from eradication of H. pylori infection or even from screening programs.

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