Original Article

The Role of Interleukin 1β Gene Polymorphism in Gastric Cancer in North-eastern Iran

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ABSTRACT

Background and Objective: Host genetic factors such as cytokine gene polymorphisms as well as *Helicobacter pylori* (*H. pylori*) infection have been found to be associated with gastric cancer risk. Interleukin 1 is a pro-inflammatory cytokine involved in H. pylori-induced gastric inflammation. Therefore, we analyzed the association between IL-1β and IL-1-RN polymorphisms and gastric cancer in Persian residents in north-eastern Iran.

Methods: In a case-control study, the genotyping was carried out by PCR-RFLP in 109 gastric cancer patients and 101 randomly-selected healthy controls. The polymorphic sites include promoter region of IL-1β at 511 (C-T transition) position and IL-RN VNTR *H. pylori* infection was determined by ELISA assay in patients.

Results: No significant differences were observed in the allele and genotype frequency of IL-1β-511 and IL-1RN VNTR between patients and control. Genotype frequencies in healthy controls were not significantly different from gastric cancer cases in separate histological types (intestinal or diffuse). IL-1β-511 CT genotype frequency was significantly higher among healthy subjects than *H. pylori* positive gastric cancer patients (41.6% vs. 20%, p = 0.01, OR 0.30, 95% CI: 0.11-0.76). Meanwhile, relatively higher frequency of IL-1β-511 T genotype was observed among *H. pylori* positive cases as compared to healthy controls (42.9% vs. 26.7%, p = 0.06, OR 2.16, 95% CI: 0.96-4.8)

Conclusion: Our results suggest the association between IL-1β-511 polymorphism and ***H. pylori*** infection and their contribution to the risk of gastric cancer. While IL-1β-511 CT genotype has a protective effect against *H. pylori* associated gastric carcinoma, IL-1β-511 TT may increase the risk.

Key words: Gastric cancer, Genetic polymorphism, Interleukin 1, Receptor, Antagonist, *Helicobacter pylori*

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Introduction

Gastric adenocarcinoma is one of the most prevalent malignancies and ranks only second to lung cancer as the leading cause of cancer-related death worldwide (1). According to the Iran's annual national cancer registration report, gastric cancer in Iran was the second most common diagnosed malignancy after skin cancer in 2004. As a result, gastric adenocarcinoma is a major health problem not only in Iran but also in many other countries.

Gastric cancer has a complex multifactorial etiology. Several case-control studies have shown that the *H. pylori* infection is a predisposing factor for gastric cancer (2-4). This infection produces different clinical manifestations ranging from asymptomatic gastritis to peptic ulcer and cancer. The chronic inflammatory response induced by the *H. pylori* infection is the key pathophysiologic event for subsequent gastroduodenal diseases. The association between *H. pylori* and cancer involves a step by step development of corpus gastritis, gastric atrophy, and intestinal metaplasia which as a consequence progressively leads to gastric cancer (5).

Despite the high prevalence of the *H. pylori* infection, only a minority of infected people (1-2%) develop gastric carcinoma (6). Thus, various factors including environmental cofactors (such as diet and smoking) (7), *H. pylori* strain variation (8-10) , the patient's age at infection (11), and host genetics determine the severity of the inflammatory response and the risk of cancer.

There is also some epidemiologic evidence that indicates the effects of host genetic factors on the development of gastric carcinoma. For example, a three-fold increase in the risk of developing gastric cancer has been found among first degree relatives of patients with this malignancy (12,13). At least a part of familial occurrence can be attributed to family clustering of the *H. Pylori* infection. Even after adjusting for the *H. Pylori* infection, a family history of gastric cancer remains a significant risk factor. Some trials have been conducted to elucidate the importance of host genetic factors on controlling the inflammatory response to *H. pylori* and the role the may play in the progression of gastritis to cancer. The Varying rates of gastric cancer worldwide could be partly attributable to different genetic propensities to gastric carcinoma.

Cytokines such as interferone-γ (INF-γ), interleukin-1 (IL-1), and tumor necrosis factor-α (TNF-α) are involved in the immune response to the *H. pylori* infection. The IL-1 gene cluster includes three related gene on chromosome 2: IL-1β, IL-1α, and IL-RN that encode IL-α, IL-1β, and their endogenous receptor antagonist the IL-1 receptor antagonist (IL-1ra) (14). In this regard, IL-1 beta is a pro-inflammatory cytokine and a powerful inhibitor of acid secretion in the stomach (15). Some studies in Korea, Portugal, Poland, and Scotland have found that polymorphisms in genes IL-1β and IL-RN can affect the risk of gastric atrophy and hypochlorhydria as well as gastric cancer (16-18). The IL-1 receptor antagonist is an anti-inflammatory cytokine and competes with IL-1β for binding to the IL-1 receptor (19).

Host genetics accompanied with environmental factors may contribute to the high prevalence of gastric carcinoma in Iran. The main goal of this study was to determine the association between IL-1 loci polymorphisms and the risk of gastric adenocarcinoma in the Persian population of north-eastern Iran. At the same time, the frequency of the different genotypes of IL-1β and IL-RN were investigated. We also examined the correlation between IL-1 loci polymorphisms and histological types, as well as the *H. pylori* infection.

Materials and Methods

Collection of samples

This case-control study was approved by the ethical committee of the Mashhad University of Medical Sciences (MUMS) and was conducted at Omid hospital and Bu-Ali Institute. The study included 109 Persian cases with gastric cancer (median age 63 years, range 29-82 years, male/female ratio as 80/29) treated at Oncology Department of Omid hospital. The diagnosis of all the patients was confirmed pathologically. The control group included 101 randomly-selected healthy Persian individuals (median age 37 years, range 22- 52 years; male/female ratio as 44/57) without a family history of malignancy. Considering that polymorphisms do not change throughout the life, we did not match the case and control groups for age. The anatomical location and pathological features of patients has been shown in Table 1. Written informed consent was obtained from all participants. Genomic DNA was isolated from whole blood collected with EDTA as anticoagulant, using a salting out method with commercial Biogene kit (Mashhad, Iran). *H. pylori* status of patients was determined by an ELIZA assay for anti-*H. pylori* IgG (Pishtaz Teb Diagnostics, Tehran, Iran). Patients were considered to be *H. pylori* positive if titers exceeded 10 U/ml.

Table 1: Anatomical and pathological features of patients with gastric cancer

Anatomical site:	n	(%)	Grade:	n	(%)	Histology:	n	(%)
Cardia	18	(16.5%)	**Good**	15	(13.7%)	**Intestinal-type**	44	(40.3%)
Body	46	(42.2%)	**Moderate**	30	(27.5%)	**Diffuse**	38	(34.8%)
Antrum	41	(37.6%)	**Poor**	43	(39.4%)	**Mixed type**	3	(2.7%)
Linitis plastica	4	(3.6%)	**Undefined**	21	(18.3%)	**Undefined**	24	(22%)

Genotyping of IL-1-511 polymorphisms

IL-1β polymorphic site into the promoter region at position-511 (C-T transition) was genotyped by polymerase chain reaction (PCR). The oligonucleotides 5`-GCCTGA ACC CTGCAT ACC GT-3` and 5`-GCCAAT ACG CCT CCCTGT CT-3` were used as the forward and reverse primers. PCR amplification was conducted at a total volume of 25 µl with 0.5 µmole of each primer; genomic DNA (200-300 ng); 10×buffer (2.5 µl), dNTP (200 µM), 0.5 µM of each primer, and 1.5 mM of MgCl2 and 0.5 units of Taq DNA polymerase (Sinagene, Iran). PCR conditions were as follows: 3 cycles of 95°c for 0.5 min, 65°c for 0.5 min and 72 °C for 0.5 min; after this 15 cycles of 94 °C for 0.5 min, 60 °C for 0.5 min and 72 °C for 0.5 min and finally 5 cycles of 94 °C for 0.5 min, 55 °C for 0.5 min and 72 °C for 0.5 min. PCR products were digested by restriction endonuclease AvaI (MBI Fermentas-Germany) at 37 °C for overnight and then analyzed by 3% agarose (LM) gel electrophoresis. Alleles were coded as follows: 155 bp for T/T; 88 and 67 bp for CC; 155+88 and 67 bp for C/T (Figure 1).

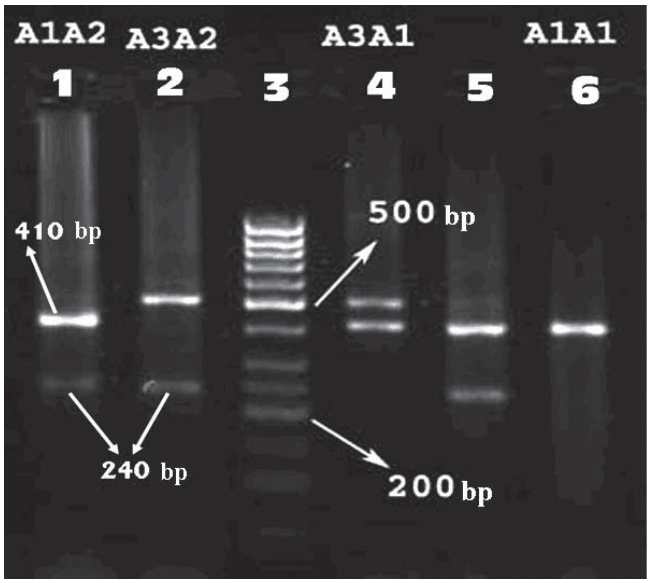

Figure 1. Analysis of the IL-1RN (VNTR) polymorphism.Lanes 1, 2, 4, 5 and 6 show patients with different genotyping, A1 (410 bp), A2 (240 bp), and A3 (500 bp). Lane 3 shows DNA size marker (100 bp).

Genotyping of IL-1RN VNTR

Primers for the IL-1-RN VNTR (88 bp) in intron 2 were 5`-CTCAGCAACACTCCTAT-3` and 5`-TCCTGGTCTGCAGGTAA-3`. Enzymatic amplification of DNA was performed by polymerase chain reaction (PCR). PCR analysis was carried out in a total volume of 20 µl, containing genomic DNA (200-300 ng); 1 µl of each primer; 10× buffer (2 µl); dNTP (200 µM); MgCl2 (1.5 mM), and 1 unit of Taq DNA polymerase (Sinagene, Iran).

PCR amplification was performed under the following conditions: Denaturating step at 94 °C for 3 min, 35 cycles of 94 °C for 1min, 60 °C for 1 min, and 72 °C for 1 min; followed by final extension of 72 °C for 5 min. The PCR products were separated by electrophoresis on 2.5% agarose (LM) gels and stained with ethidium bromide. Gel documentation was done by IMAGO (B & L system, Netherlands). Allele 1 (4 repeats) was 410 bp, allele 2 (2 repeats) 240 bp, allele 3 (5 repeats) 500 bp, allele 4 (3 repeats) 325 bp, and allele 5 (6 repeats) 595 bp (Figure 2).

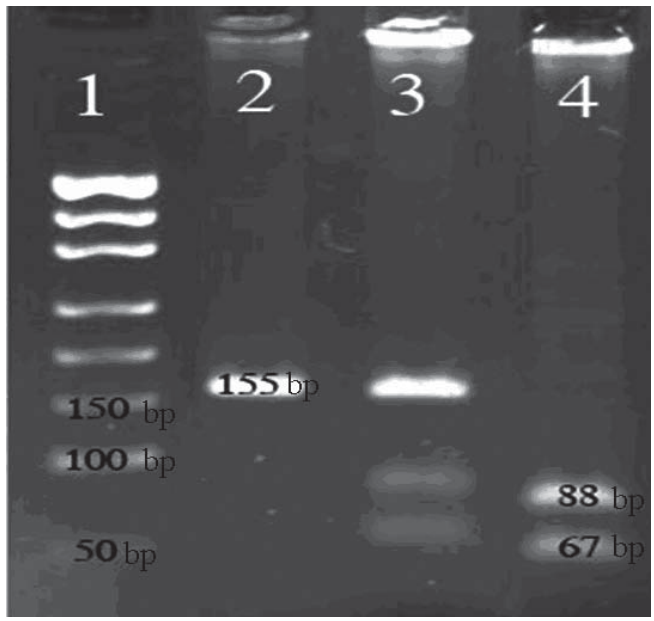

Figure 2: Analysis of IL-1β(-511C/T) polymorphism. Lane1 shows DNA size marker (50 bp), Lane 2 uncut TT product, lane 3 CT heterozygous and lane 4 CC homozygous.

Statistical analysis

Direct gene counting method was used to determine the frequency of genotypes and alleles. The χ^2 test or Fisher's exact test was used to compare genotype frequencies of polymorphisms between different groups. A p value less than 0.05 was considered as statistically significant. We also calculated odds ratio (OR) with 95% confidence interval (CI). We used the SPSS program version 11.5 and the STATA 8 statistical package.

Results

Genotyping of IL-1β-511 and IL-1-RN VNTR were unsuccessful in one and four patients respectively. IL-1-RN VNTR was not genotyped in one healthy control. Serological *H. pylori* test was measured in 99 patients among whom 35 (35.3%) were positive. In 76 patients information regarding both histology and *H. pylori* were available. In comparison with diffuse subtype, the prevalence of *H. pylori* infection was relatively higher in patients with intestinal subtype (18/40 45% vs. 11/36, 30.6%). However, the difference did not reach statistical significance (p = 0.19)

Allele and genotype distributions of IL-1β (-511 C/T) and IL-1RN (-86 bp VNTR) in gastric carcinoma cases and controls in the north-eastern Iran are shown in Table 2. No statistically significant difference in allele and genotype frequencies was observed between gastric cancer patients and controls in Persians in the north-eastern Iran. The comparison of the genotype frequencies between cases in separate histological subtypes and healthy control group is illustrated in Table 3. There were no significant differences in genotype frequencies between intestinal or diffuse histological types and healthy control group. Table 4 compares genotype frequencies between cases sorted by *H. pylori* test results and healthy control group. While there was no significant difference in the frequency of IL-1β -511 CC among *H. pylori* positive cases and control group, IL-1β-511 TT genotype was relatively more common among patients with *H. pylori* positive gastric carcinoma (42.9% vs. 26.7%, P = 0.06). Meanwhile, in comparison with*H. pylori* positive gastric carcinoma cases, IL-1β-511 CT genotype frequency was significantly higher among healthy control group (20% vs. 41.6%, p = 0.01).

Table 2. Allele and genotype distributions of IL-1β (−511 C/T), IL-1RN (−86bp VNTR) gene polymorphisms in gastric cancer cases and healthy controls

Polymorphisms	Cases (n=108)	Controls (n=101)	P value	OR (CI 95%)
IL-1β (−511 C/T)				
Allele frequencies				
Allele C	117 (54.2%)	106 (52.4%)		
Allele T	99 (45.8%)	96 (47.5%)		
Genotype frequencies				
CC	38 (35.1%)	32 (31.7%)	P=0/659	1.13 (0.64-2.02)
CT	41 (37.9%)	42 (41.6%)	P=0/522	0.83 (0.48-1.45)
TT	29 (26.8%)	27 (26.7%)	P=0/952	1.00 (0.54-1.85)
P value = 0.834 (cases and controls)				
IL-1RN VNTR (86 bp)	Cases (n=105)	Controls (n=100)	P value	OR (CI 95%)
Allele frequencies				
Allele 1	155 (73/8%)	150 (74.2%)		
Allele 2	43 (20.4%)	41 (20.3%)		
Allele 3	10 (4.7%)	8 (4.0%)		
Allele 4	1 (0.5%)	1 (0.5%)		
Allele 5	1 (0.5%)	0		
Genotype frequencies				
11	60 (57.1%)	59 (59%)	P=0.57	0.85 (0.49-1.47)
12	28 (26.7%)	26 (26%)	P=0.91	1.03 (0.55 -1.93)
22	6 (5.7%)	7 (7%)	P=0.65	0.77 (0.25-2.38)
P value = 0.958 (cases and controls)				

Table 3. The comparison of genotype frequencies between healthy controls and gastric cancer cases sorted by histological type of tumor

Polymorphisms	Controls	Gastric carcinoma					
		Intestinal			Diffuse		
		Cases (n = 44)	p value	OR(95% CI)	Cases (n = 38)	p value	OR(95% CI)
IL-1β (−511 C/T)							
CC	32	16 (36.4%)	0.58	1.23(0.58-2.59)	12 (31.6%)	0.59	0.8(0.36-1.77)
CT	42	14 (31.8%)	0.26	0.65(0.31-1.38)	15 (39.5%)	0.85	1.06(0.52-2.18)
TT	27	14 (31.8%)	0.53	1.27(0.59-2.76)	11 (28.9%)	0.82	0.91(0.40-2.05)
IL-1RN VNTR (86bp)							
11	59	23 (53.5%)	0.49	0.77(038-1.58)	24 (63.2%)	0.94	1.02(0.50-2.11)
12	26	14 (32.6%)	0.45	1.34(0.61-2.93)	9 (23.7%)	0.69	0.84(0.36-1.95)

Table 4. The comparison of genotype frequencies between healthy controls and gastric cancer cases sorted by *H. pylori* test result

Polymorphisms	Controls	Gastric carcinoma					
		H. pylori positive			*H. pylori* negative		
		Cases (n = 35)	p value, OR (95% CI)		Cases (n = 64)	p value, OR (95% CI)	
IL-1β (−511 C/T)							
CC	32	13 (37.1%)	0.48	1.33(0.59-2.99)	21 (32.8%)	0.98	1.00(0.51-1.95)
CT	42	7 (20%)	0.01,	0.3(0.11-0.76)	30 (46.9%)	0.49	1.24(0.46-2.32)
TT	27	15 (42.9%)	0.06,	2.16(0.96-4.8)	13 (20.3%)	0.29	0.67(0.31-1.42)
IL-1RN VNTR (86bp)							
11	59	18 (52.9%)	0.39	0.71(0.32-1.55)	37 (59.7%)	0.91	0.96(0.51-1.81)
12	26	12 (32.4%)	0.45	1.37(0.59-3.21)	13 (21.0%)	0.36	0.70(0.33-1.50)

Discussion

According to the Lauren classification, gastric adenocarcinoma is divided into two histological types: intestinal and diffuse (20). In comparing the two, the intestinal type is more common in countries with a high incidence of gastric carcinoma, observed among older ages, and occurs predominantly in males. In addition, gastric atrophy is mostly associated with an intestinal histological type. Considering these differences, it

is supposed that these two histological entities have different carcinogenesis pathways. Assuming that chronic gastritis is the first step in pathogenesis of both histological subtypes, one could hypothesize that the polymorphisms of pro-inflammatory genes which determine the quality of the inflammatory response play a part in the susceptibility to different histological types of gastric carcinoma (21). Furthermore, the significant variation in the prevalence of gastric carcinoma among different nations might be explained by the difference in the genotype frequency of cytokine genes (22).

In this study, we investigated the association between pre-inflammatory IL-1β and IL-1RN gene polymorphism and gastric cancer in Persian ethnic group in the north-eastern Iran. To our knowledge, the effect of these gene polymorphisms on susceptibility to gastric cancer in Iran had not yet been studied. We have not observed any significant difference in the frequency of IL-1β genotypes as well as IL-1RN genotypes, among patients with gastric cancer, and the healthy control group. However, when we divided patients into *H. pylori* positive and negative infection groups, the IL-1β-511 TT genotype was detected more frequently in *H. pylori* positive patients as compared to the healthy controls. However, this occurrence did not reach a statistical significance ($p = 0.06$). Meanwhile, the IL-1 β-511 CT genotype was significantly more common in the healthy control group as compared to *H. pylori* positive cases ($p = 0.01$).

The effect of the IL-1β-511 T allele combined with *H. pylori* infection on the risk of gastric carcinoma has been presented in some other studies. The association between IL-1β and IL-1RN polymorphism and gastric carcinoma has been investigated in low and high incidence regions in China. While the *H. pylori* infection alone had a modest effect on gastric carcinoma, when combined with the IL-1β-511 TT genotype, the risk was significantly elevated (Odd Ratio 17.1, 95% CI 3.8-76.4). However, the effect of IL-1β polymorphism was less prominent in high prevalence regions (23). These results suggest a rise in the gastric carcinoma risk among carriers of the IL-1β-511 TT genotype, especially *H. pylori* positive individuals. In 2001, a case–control study by Machado et al documented the impact of IL-1β-511 T and IL-1RN2 alleles on a Portuguese population of 152 gastric cancer cases and 220 controls with unknown *H. pylori* infection. In this study, the IL-1β-511 T allele frequency in patients and controls was 67% and 53% respectively. In addition, 15% of the patients compared with 9% of the controls were homozygous carriers of the IL-1RN2 allele. The IL-1β-511 T allele and the homozygous IL-1RN2 genotype posed a significant risk of developing intestinal-type gastric carcinoma (18). A case control study by Ruzzo et al on *H. pylori* negative Italian subjects suggested that IL-1β-511 T and IL-1RN2 may contribute to intestinal gastric cancer risk in the absence of the concomitant *H. pylori* infection. It was recommended that future epidemiologic studies consider dietary habits and exposure to carcinogens interacting with pro-inflammatory host genotypes (24).

Our study did not identify any role for IL-1RN polymorphisms on the risk of gastric carcinoma. However, some other studies have suggested the involvement of the IL-1RN (10, 18, 24). The association between IL-1RN and IL-1β polymorphisms and gastric cancer was investigated in 2000 by El-Omar et al. A population of 429 gastric cancer cases and 366 controls in Scotland and Poland were studied. An increased risk of gastric cancer was revealed in carriers of IL-1β-31 C genotype (65% in patients and 49% in controls) and the carriers of the homozygous IL-1RN2 genotype (25% in cases and 9% in controls). The increased risk was similar in subgroups defined by age, sex, and histological type. They also found the effect of these genotypes on the likelihood of a chronic hypochlorhydric response to *H. pylori* infection, which indicated the contribution of genes and environmental factors to the development of early stage gastric carcinomas (17).

The relation between the Il-1β polymorphism and gastric cancer risk has been less significant in other researches. In a study by Ryo et al in Korea, no association between IL-1 β polymorphism at loci -511 and -31 and risk of gastric cancer was detected (25). The association between the polymorphisms of IL-1β, IL-1RN, and tumor necrosis factor-α was examined in two regions of high and low gastric cancer prevalence in Italy. There was no significant correlation between these cytokine genotypes and gastric carcinoma in both regions (21). A meta-analysis was conducted by Camargo et al to examine the association between IL-1β and/or IL1RN gene polymorphisms and gastric cancer. They analyzed 14 studies of IL1β-511, 14 studies of IL-1β-31, 8 studies of IL-1β+3954, and 23 studies of IL1RN. The results revealed that IL0-1β-511 T and IL1RN2 were associated with the gastric cancer risk in Caucasians, but not in Asians. As for IL-1β-511T, its relationship to gastric carcinoma in Caucasians was stronger among intestinal-subtype and non-cardia cases (22). In contrast to the results of the meta-analysis by Camargo, we did not find any correlation between

histological subtypes and cytokine genotypes.

In this study, we detected a higher frequency of IL-1β-511 CT in the healthy control group than among *H. pylori* positive patients. This suggests a probable protective effect of IL-1β-511 CT genotype against the development of *H. pylori* related gastric carcinoma. Meanwhile, considering its relatively higher frequency among *H. pylori* positive gastric carcinoma patients, the IL-1β-511 TT genotype may increase the risk.

Conclusion

Taken together, the results of different trials examining the association of cytokine polymorphisms with gastric carcinoma vary among nations. However, the findings of many studies suggest that cytokine polymorphisms play a part in the development of gastric carcinoma. The effects of multiple environmental factors, the diversity of cytokine genotype frequencies among different nations, and the probable effect of other host genetic factors may explain the contradictory results documented. As for Iran, larger case-control studies which evaluate various host and environmental factors are recommended.

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