RESEARCH ARTICLE

Interleukin-6 Genetic Variation and Susceptibility to Gastric Cancer in an Iranian Population

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Abstract

Background: Despite recent decrease in the incidence of gastric cancer, it is still a common type of cancer in the north of Iran. Many evaluations have shown that polymorphisms of cytokine genes like that for interleukin 6 (IL-6), which play important roles in regulation of the immune response, can increase the risk of gastric cancer. This study examined the role of the IL-6-174 gene polymorphism in susceptibility in an Iranian population. **Method:** Genomic DNA was extracted from peripheral whole blood of 100 patients and 361 healthy controls. Genotyping was accomplished by the sequence-specific primer-polymerase chain reaction (SSP-PCR) method and statistical analyses were carried out using Fisher's exact test. Frequencies of the IL-6-174 G/C genotypes were determined under co-dominant, dominant, and recessive genetic models. **Results:** An association between the polymorphism of IL-6 -174 G/C and susceptibility to gastric cancer was observed. The frequency of G allele was higher in patients (78%) than in controls (70.5 %) (OR=1.48, 95% CI=1.01-2.20, P=0.04). **Conclusions:** The high G allele and G/G genotype frequency in patients compared to control subjects suggests that the IL-6 -174 G/C polymorphism may influence the susceptibility to gastric cancer. In addition, the demographic information showed that most of the subjects were male (69.0%) that gastric cancer is related to environmental factors.

Keywords: Gastric cancer- Interleukin-6- polymerase chain reaction (PCR)- polymorphism- Iran

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Introduction

Gastric cancer is the fifth most common cancer in the world and the third major cause of cancer-related deaths (Stewart and Wild, 2014). Incidence of gastric cancer in different geographical regions has a wide diversity. Developing countries include more than 70% of incident cases and deaths(Crew and Neugut, 2006).

The most frequent type of cancer during 1980s was the stomach cancer (Wu et al., 2002). Stomach cancer, with approximately 870,000 new patients per year, is the second most common cancer worldwide (Sentani et al., 2008; Ferlay et al., 2010).

At the moment, it has been shown that the progression of gastric cancer is related to underlying gastric diseases (like gastritis, gastrectomy, etc.) (Rugge et al., 2014), Helicobacter pylori (H. pylori) infection (Gatti et al., 2004), and genetic susceptibility factors (Gatti et al., 2004; Xie et al., 2014).

There have been several reports on interleukins in recent years (Cheng et al., 2013; Kim et al., 2014; Kutikhin et al., 2014) that explain gene polymorphisms at many loci can increase the risk of developing gastric cancer. Human interleukin-6 (IL-6) gene, the pro-inflammatory cytokine, is located on chromosome 7p21 and is composed of five exons and four introns. This multifunctional cytokine interferes in the maturation and differentiation of immune system (Kakumu et al., 1993; Galun et al., 2000). IL-6 gene promoter region has three single nucleotide polymorphisms (SNP) at positions -597 G/A, -572 G/A, and -174 G/C. Previous studies indicated a cooperative impact of these SNPs on IL-6 transcription regulation (Fishman et al., 1998; Terry et al., 2000).

Based on prior findings, the polymorphism of IL-6 gene position -174 G/C (rs1800795) is related to some diseases like diabetes (Fernandez-Real et al., 2000; Hamid et al., 2005), Alzheimer (Capurso et al., 2004), Hepatitis B (Attar et al., 2016), and multiple sclerosis (MS) (Shahbazi et al., 2010).

IL-6 is a phosphorylated glycoprotein and composed of 185 amino acids involved in various physiologic and pathophysiologic processes such as inflammation, bone metabolism, and synthesis of C-reactive protein (CRP). It plays a significant role in the development and progression of many forms of cancer (Mantovani et al., 2002; Salgado et al., 2003; Bozcuk et al., 2004), especially gastric cancer (Asschert et al., 1999; Diehl and Rincón, 2002; Lu et al., 2015).

Malekzadeh et al., (2009) in a review article expressed that high-risk areas for gastric cancer in Iran are northern and northwestern regions. The aim of this study was to evaluate whether IL-6 -174 G/C promoter polymorphisms

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are related to gastric cancer resistance in an Iranian population in the north of Iran.

Material and Methods

Samples

Fresh peripheral blood samples of gastric cancer patients (including esophageal, colon, stomach, and rectum) were collected from 100 5FU patients who referred to 5th Azar hospital, Hakim Jorjani hospital, and Moosavi hospital of Golestan province and were treated during 2011. They were also taken from 361 healthy controls without a history of cancers who referred to Gorgan blood donation centers in Golestan province of Iran (5 ml peripheral blood collected in falcon tubes contained EDTA).

DNA extraction

DNA was extracted using the phenol-chloroform method by modified standard protocol (Shahbazi et al., 2002). The concentration was determined by spectrophotometer at 260 nm (Techne, UK) and DNA samples were maintained in -20˚C.

Genotyping

The SSP-PCR (sequence-specific primer-polymerase chain reaction) method (Mansoori et al., 2015) was used for genotyping of cancer cases (n=100) and the control group (n=361) by primers: Forward1: 5´-CCC CTA GTT GTG TCT TGC C-3´for allele C, Forward2: 5´-CCC CTA GTT GTG TCT TGC G-3´ for allele G and generic primer: 5´-GCC TCA GAG ACA TCA CCA GTC C-3´. Primers used for human growth hormone (HGH) as an internal control were Forward: 5-GCCTTCCCAACCATTCCCTTA-3 and Revers: 5-TCACGGATTTCTGTTGTGTTTC-3. Each PCR reaction included 100–150 ng of DNA and 9.5 ml master mix containing 20 mM dNTP, 1X Ready load PCR buffer, 12% Sucrose (Merck, Germany), Two unit Taq DNA polymerase (GenetBio, Korea), 6 mM HGH primer, and 30 mM of each specific IL-6 primer.

The PCR reaction was carried out in a Thermal Cycler (Techne, UK), with the following program: 1min at 95°C followed by 10 cycles of 15 s at 95˚C, 50 s at 58˚C, 40 s at 72˚C, then, 20 cycles of 20 s at 95˚C, 50 s at 54˚C and 50s at 72˚C, with 5min at 72˚C as the final extension.

PCR product was submitted to electrophoresis in 1.5% agarose gel (Merck, Germany) and stained with Safe Stain (CinaGene, Iran). Using a gel documentation system (UVITEC, UK), DNA bands were visualized under the UV radiation.

Statistical analysis

Web program (http://ihg.gsf.de/cgi-bin/hw/hwa2.pl) was applied for testing the genotype results for deviation from Hardy-Weinberg equilibrium. Statistical analysis was done by the Statistical Program for Social Sciences (SPSS version 17.0) and the means of parametric variables were calculated. For parametric and non-parametric values, data were presented as Mean±SD and percentage, respectively. After calculating Alleles and genotype frequencies, results compared with non-parametric tests followed by Fisher's exact analysis using STATA v-8 (CA, US). P-values were determined and those less than 0.05 were considered to be significant.

Results

In the present study, frequency of the IL-6 -174 G/C genotypes was evaluated in 100 gastric cancer patients and 361 controls under Co-dominant, Dominant and Recessive genetic models. As showed in Table 1, there was an association between polymorphism of IL-6 -174 G/C and susceptibility to gastric cancer in various genetic models. G allele was more frequent in patients (78%) than

Table 1. Frequency of the IL-6 -174 G/C Genotypes among Patients (N=100) and Controls (N=361) Under Co-Dominant, Dominant, and Recessive Models

Alleles and Genotype	Controls n (%)	Case n (%)	OR (95% CI)*	P-value
C/C	13 (3.6 %)	7 (7 %)	1 (-)**	-
C/G	187 (51.8%)	30 (30 %)	0.3 (0.1-0.96)	0.02
G/G	161 (44.6%)	63 (63 %)	0.72 (0.25-2.26)	0.6
C	213(29.5 %)	44 (22 %)	1 (-)	-
G	509 (70.5 %)	156 (78 %)	1.48 (1.01-2.20)	0.04
Model of Inheritance				
Dominant				
C/C	13 (3.6 %)	7 (7 %)	1 (-)	-
C/G + G/G	348 (96.4%)	93 (93%)	0.49 (0.18-1.51)	0.16
Recessive				
C/G + C/C	200 (55.4%)	37 (37%)	1 (-)	-
G/G	161 (44.6%)	63 (63 %)	2.11 (1.31-3.44)	0.0015
Co-dominant				
C/C + G/G	174 (48.2%)	70 (70%)	2.5 (1.5-4.18)	0.0001
C/G	187 (51.8%)	30 (30 %)	1 (-)	-

*, OR, odds ratio; CI, confidence interval; **, The frequency of C allele was higher in the control group, so it was selected as a reference group.

Table 2. Frequency of Four Different Types of Gastric Cancer within Age

	Age					Total
Type	under 39 years old	40-49 years old	50-59 years old	60-69 years old	upper 70 years old	
Esophagus	1 (2.6%)	2 (5.1%)	8 (20.5%)	12 (30.8%)	16 (41.0%)	39 (100.0%)
Colon	3 (6.4%)	15 (31.9%)	12 (25.5%)	9 (19.1%)	8 (17.0%)	47 (100.0%)
Stomach	0 (.0%)	3 (27.3%)	2 (18.2%)	2 (18.2%)	4 (36.4%)	11 (100.0%)
rectum	0 (.0%)	2 (66.7%)	0 (.0%)	0 (.0%)	1 (33.3%)	3 (100.0%)
Total	4 (4.0%)	22 (22.0%)	22 (22.0%)	23 (23.0%)	29 (29.0%)	100 (100.0%)

Table 3. Frequency of Four Different Types of Gastric Cancer Cases within Sex

	Sex		Total
Type	Man N (%)	Woman N (%)	
Esophagus	29 (74.4%)	10 (25.6%)	39 (100.0%)
Colon	31 (66.0%)	16 (34.0%)	47 (100.0%)
Stomach	8 (72.7%)	3 (27.3%)	11 (100.0%)
rectum	1 (33.3%)	2 (66.7%)	3 (100.0%)
Total	69 (69.0%)	31 (31.0%)	100 (100.0%)

in controls (70.5 %) under co-dominant genetic model (OR=1.48, 95% CI=1.01-2.20, P=0.04).

Patients were classified into five age groups, namely, less than 39 years, 40-49 years, 50-59 years, 60-69 years and, more than 70 years. Frequency of four different types of gastric cancer was analyzed within these age groups which is evaluated and presented in Table 2.

Evaluation of disease prevalence by gender revealed most patients were male (69.0%). Complementary data are provided in Table 3.

Discussion

So far, we have examined the association of several genes with cancer (Abdolmohammadi et al., 2016; Attar et al., 2016; Azar et al., 2016; Mansoori et al., 2016; Shamsabadi et al., 2016). In this evaluation, frequency of the IL-6 -174 G/C genotypes was assayed under Co-dominant, Dominant, and Recessive genetic models in gastric cancer patients compared with healthy population. A highly statistically significant difference in the frequency of the G allele was observed between patients and controls (OR=1.48, 95% CI=1.01-2.20, P=0.04). G allele at the position IL-6 -174 had higher distribution among patients in comparison to controls. Recessive and Co-dominant analysis models of this polymorphism also revealed a significant difference between cases and controls (Table 1).

AM Sampaio et al., (2015) in Portugal stated that IL-6 -174 C/G genotype showed a higher prevalence among gastric cancer cases. Landi et al., (2003) determined the IL-6 -174C genotype is associated with increased risk of colorectal cancer. Ruzzo et al., (2014) demonstrated that IL-6 gene polymorphism can increase the risk of developing gastric cancer. On the contrary, the result of a meta-analysis by Yin et al., (2012) showed that polymorphism of IL-6 -174 C/G is not associated with the risk of gastric cancer.

Some previous studies examined the IL-6 serum level and illustrated the relationship of IL-6 gene overexpression and the increased risk of cancers (Waldner et al., 2012; Woo and Humphries, 2013). Accordingly, Ruzzo et al., (2014) hypothesized that anti-IL-6 compounds can be used as novel therapeutics in treatment of cancer patients (Ruzzo et al., 2014). Belluco et al., (2003) showed that -174 C/G polymorphism of interleukin-6 gene promoter affects interleukin-6 serum level in patients with colorectal cancer.

Although we did not assay the IL-6 serum level in our study, it is estimated that there is an association between genotype and expression level of IL-6 gene.

Evaluation of frequency of four different types of gastric cancer in patients demonstrated that colon cancer was the most common type (47%) of gastric cancer and then esophageal (39%), stomach (11%) and rectum (3%) cancer were more abundant, respectively (Table 2).

Previous studies have shown that the age at disease onset is over 55 and men are more likely to be affected than women (Pinto et al., 1994; Axon, 2006). In a review article, Malekzadeh R. et al. showed that age-standardized incidence rate (ASR) of men and women was 27.8 and 8.3, respectively, in Golestan province of Iran (Malekzadeh et al., 2009). Investigating frequency of four different types of gastric cancer within age in our patients shows that there is a direct relationship between aging and increased incidence of esophagus cancer whereas there is not such a relationship in other types of gastric cancer (Table 2).

Examining frequency of gastric cancer within gender also shows that the prevalence of gastric cancer in men (69.0%) is more than women (31%) (Table 3). These results suggest that polymorphism of IL-6 -174 C/G may influence the susceptibility of women gender to this cancer. On the other hand, high prevalence of the disease in men may be due to environmental factors like smoking or high intake of salt and alcohol and etc.

In conclusion, these findings suggest that IL-6 -174 G/C polymorphism may influence the susceptibility to gastric cancer.

Conflict of interest

The Authors declare that there is no conflict of interest.

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References

Abdolmohammadi R, Azar SS, Khosravi A, et al (2016). CCR5 Polymorphism as a protective factor for hepatocellular carcinoma in hepatitis B virus-infected Iranian patients. *Asian Pac J Cancer Prev*, **17**, 4643-6.

Asschert JG, Vellenga E, Ruiters MH, et al (1999). Regulation of spontaneous and TNF/IFN-induced IL-6 expression in two human ovarian-carcinoma cell lines. *Int J Cancer*, **82**, 244-9.

Attar M, Azar SS, Shahbazi M (2016). Interleukin-6-174 promoter polymorphism and susceptibility to Hepatitis B virus infection as a risk factor for hepatocellular carcinoma in Iran. *Asian Pac J Cancer Prev*, **17**, 2395-9.

Axon A (2006). Symptoms and diagnosis of gastric cancer at early curable stage. *Best Pract Res Clin Gastroenterol*, **20**, 697-708.

Azar SS, Mansoori M, Attar M, et al (2016). Tumor necrosis factor alpha-308 G/A single nucleotide polymorphism and susceptibility to hepatocellular carcinoma via hepatitis B infection. *Asian Pac J Cancer Prev*, **17**, 3381-4.

Belluco C, Olivieri F, Bonafè M, et al (2003). − 174 G> C polymorphism of interleukin 6 gene promoter affects interleukin 6 serum level in patients with colorectal cancer. *Clin Cancer Res*, **9**, 2173-6.

Bozcuk H, Uslu G, Samur M, et al (2004). Tumour necrosis factor-alpha, interleukin-6, and fasting serum insulin correlate with clinical outcome in metastatic breast cancer patients treated with chemotherapy. *Cytokine*, **27**, 58-65.

Capurso C, Solfrizzi V, D'Introno A, et al (2004). Interleukin 6-174 G/C promoter gene polymorphism and sporadic Alzheimer's disease: geographic allele and genotype variations in Europe. *Exp Gerontol*, **39**, 1567-73.

Cheng D, Hao Y, Zhou W, et al (2013). Positive association between Interleukin-8-251A> T polymorphism and susceptibility to gastric carcinogenesis: a meta-analysis. *Cancer Cell Int*, **13**, 1.

Crew KD, Neugut AI (2006). Epidemiology of gastric cancer. *World J Gastroenterol*, **12**, 354.

Diehl S, Rincón M (2002). The two faces of IL-6 on Th1/Th2 differentiation. *Mol Immunol*, **39**, 531-6.

Ferlay J, Shin HR, Bray F, et al (2010). Estimates of worldwide burden of cancer in 2008: Globocan 2008. *Int J Cancer*, **127**, 2893-917.

Fernandez-Real JM, Broch M, Vendrell J, et al (2000). Interleukin-6 gene polymorphism and insulin sensitivity. *Diabetes*, **49**, 517-20.

Fishman D, Faulds G, Jeffery R, et al (1998). The effect of novel polymorphisms in the interleukin-6 (IL-6) gene on IL-6 transcription and plasma IL-6 levels, and an association with systemic-onset juvenile chronic arthritis. *J Clin Invest*, **102**, 1369-76.

Galun E, Nahor O, Eid A, et al (2000). Human interleukin-6 facilitates hepatitis B virus infection in vitro and in vivo. *Virol J*, **270**, 299-309.

Gatti LL, Burbano RR, de Assumpção PP, et al (2004). Interleukin-1β polymorphisms, Helicobacter pyloriinfection in individuals from Northern Brazil with gastric adenocarcinoma. *Clin Experimental Med*, **4**, 93-8.

Hamid YH, Rose CS, Urhammer SA, et al (2005). Variations of the interleukin-6 promoter are associated with features of the metabolic syndrome in Caucasian Danes. *Diabetologia*, **48**, 251-60.

Kakumu S, Shinagawa T, Ishikawa T, et al (1993). Interleukin 6 production by peripheral blood mononuclear cells in patients with chronic hepatitis B virus infection and primary biliary cirrhosis. *Gastroenterol Jpn*, **28**, 18-24.

Kim J, Kim Y, Lee K-A (2014). Ethnic differences in gastric cancer genetic susceptibility: Allele flips of interleukin gene. *World J Gastroenterol*, **20**, 4558.

Kutikhin AG, Yuzhalin AE, Volkov AN, et al (2014). Correlation between genetic polymorphisms within IL-1B and TLR4 genes and cancer risk in a Russian population: a case-control study. *Tumor Biol*, **35**, 4821-30.

Landi S, Moreno V, Gioia-Patricola L, et al (2003). Association of common polymorphisms in inflammatory genes interleukin (IL) 6, IL8, tumor necrosis factor α, NFKB1, and peroxisome proliferator-activated receptor γ with colorectal cancer. *Cancer Res*, **63**, 3560-6.

Lu Y, Lu F, Zeng S, et al (2015). Genetics and gastric cancer susceptibility. *Int J Clin Experimental Med*, **8**, 8377.

Malekzadeh R, Derakhshan MH, Malekzadeh Z (2009). Gastric cancer in Iran: epidemiology and risk factors. *Arch Iran Med*, **12**, 576-83.

Mansoori M, Golalipour M, Alizadeh S, et al (2015). Genetic Variation in the ABCB1 gene may lead to mRNA level chabge: Application to gastric cancer cases. *Asian Pac J Cancer Prev*, **16**, 8467-71.

Mansoori M, Golalipour M, Alizadeh S, et al (2016). Genetic variation in the ABCB1 gene may lead to mRNA level change: Application to gastric cancer cases. *Asian Pac J Cancer Prev*, **16**, 8467-71.

Mantovani G, Macciò A, Madeddu C, et al (2002). Quantitative evaluation of oxidative stress, chronic inflammatory indices and leptin in cancer patients: correlation with stage and performance status. *Int J Cancer*, **98**, 84-91.

Pinto E, Roviello F, De Stefano A, et al (1994). Early gastric cancer: report on 142 patients observed over 13 years. *Jpn J Clin Oncol*, **24**, 12-9.

Rugge M, Capelle LG, Fassan M (2014). Individual risk stratification of gastric cancer: evolving concepts and their impact on clinical practice. *Best Pract Res Clin Gastroenterol*, **28**, 1043-53.

Ruzzo A, Catalano V, Canestrari E, et al (2014). Genetic modulation of the interleukin 6 (IL-6) system in patients with advanced gastric cancer: a background for an alternative target therapy. *BMC Cancer*, **14**, 1.

Salgado R, Junius S, Benoy I, et al (2003). Circulating interleukin-6 predicts survival in patients with metastatic breast cancer. *Int J Cancer*, **103**, 642-6.

Sampaio AM, Balseiro SC, Silva MR, et al (2015). Association between IL-4 and IL-6 expression variants and gastric cancer among portuguese population. *GE Portuguese J Gastroenterol*, **22**, 143-52.

Sentani K, Oue N, Sakamoto N, et al (2008). Gene expression profiling with microarray and SAGE identifies PLUNC as a marker for hepatoid adenocarcinoma of the stomach. *Mod Pathol*, **21**, 464-75.

Shahbazi M, Ebadi H, Fathi D, et al (2010). HLA-DRB1*1501 intensifies the impact of IL-6 promoter polymorphism on the susceptibility to multiple sclerosis in an Iranian population. *Mult Scler*, **16**, 1173-7.

Shahbazi M, Pravica V, Nasreen N, et al (2002). Association between functional polymorphism in EGF gene and malignant melanoma. *Lancet*, **359**, 397-401.

Shamsabadi FT, Eidgahi MRA, Mehrbod P, et al (2016).

Survivin, a promising gene for targeted cancer treatment. *Asian Pac J Cancer Prev*, **17**, 3711-9.
Terry CF, Loukaci V, Green FR (2000). Cooperative influence of genetic polymorphisms on interleukin 6 transcriptional regulation. *J Biol Chem*, **275**, 18138-44.
Waldner MJ, Foersch S, Neurath MF (2012). Interleukin-6–a key regulator of colorectal cancer development. *Int J Biol Sci*, **8**, 1248-53.
Woo P, Humphries SE (2013). IL-6 polymorphisms: a useful genetic tool for inflammation research?. *J Clin Invest*, **123**, 1413-4.
Wu C-W, Chi C-W, Lin W-c (2002). Gastric cancer: prognostic and diagnostic advances. *Expert Rev Mol Med*, **4**, 1-12.
Xie W-Q, Tan S-Y, Wang X-F (2014). MiR-146a rs2910164 polymorphism increases risk of gastric cancer: A meta-analysis. *World J Gastroenterol*, **20**, 15440-7.
Yin YW, Sun QQ, Hu AM, et al (2012). Associations between interleukin-6 gene− 174 C/G and− 572 C/G polymorphisms and the risk of gastric cancer: A meta-analysis. *J Surg Oncol*, **106**, 987-93.