Association of Interleukin-10 Promoter Polymorphism (-1082 G/A) and Gastric Cancer in Andhra Pradesh Population of South India

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Abstract

Background: Gastric Cancer (GC) is one of the most commonly diagnosed malignancies. Genetic variation in genes encoding cytokines and their receptors, determine the intensity of the inflammatory response, which may contribute to individual differences in the outcome and severity of the disease. Interleukin-10 (IL-10) is a multifunctional cytokine with both immunosuppressive and antiangiogenic functions. Polymorphisms in the IL-10 gene promoter genetically determine inter-individual differences in IL-10 production. In the present study, we investigated the association between the IL-10 –1082 G/A polymorphism and the susceptibility to gastric cancer in a South Indian population from Andhra Pradesh.

Methods: We genotyped 100 patients diagnosed with gastric cancer and 132 healthy control subjects for -1082G/A single nucleotide polymorphism by Amplification Refractory Mutation System-Polymerase Chain Reaction (ARMS-PCR) method followed by agarose gel electrophoresis.

Results: The distribution of IL-10 genotypes at -1082 G/A were GG 18 %, GA 35% and AA 47 % in gastric cancer patients and GG 31.82 %, GA 37.88 % and AA 30.3% in control subjects. The allelic frequencies of G and A were 0.355 and 0.645 in GC patients and 0.508 and 0.492 in control subjects respectively. The IL-10 -1082 A allele was associated with risk of gastric cancer (OR=1.873, 95%CI-1.285-2.73and P= 0.001048**).

Conclusion: Our study indicates that allele A of IL-10-1082 G/A polymorphism may be considered as one of the important risk factor in the etiology of gastric cancer.

Keywords: Cytokines; Interleukin-10; Gastric cancer; Polymorphism

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Introduction

Gastric Cancer (GC) is one of the most commonly diagnosed malignancies and remains a considerable public health problem worldwide. Gastric cancer is the fourth most common cancer and the second leading cause of death from cancer, after lung cancer causing nearly one million deaths worldwide per year [1]. Gastric cancer incidence shows considerable variation in geography, though a male preponderance is reported. Age standardized incidence is highest in countries in Eastern Asia, Eastern Europe, and in some countries in central South America (overall age standardized rates 20-35/100,000). The incidence is low in North America, Western Europe (<5), sub-Saharan Africa (5-10) and in Southern Asia (<10). This geographic variation in incidence, points to differential distribution of etiological factors [2]. Annual incidence rate of gastric cancer in India is 10.6 per 100 000 population. The incidence rate of gastric cancer is four times higher in Southern India compared with Northern India. Gastric cancer is the third most common cancer in South India [3].

The etiology of gastric cancer is multifactorial, multigenetic and multistage. Several factors are suspected to play a role in gastric carcinogenesis which includes diet, genetic factors and infectious agents etc [4]. Persistent inflammation caused by Helicobacter Pylori (H.pylori) infection induces hypochlorhydria and gastric atrophy, which are two early precursors of gastric cancer development. However, although the prevalence of H.pylori infection ranges from 40 to 80% in humans, only small proportions (probably < 3%) of infected patients develop gastric cancer. Genetic variation in genes encoding cytokines and their receptors, which determine the intensity of the inflammatory response to the bacteria, may contribute to individual differences in severity of outcome of H.pylori infection and progression of gastric lesions [5, 6].

Cytokines play an important role in regulating both humor and cell-mediated immune responses. Promoter regions of some cytokine genes contain polymorphisms that may directly influence the cytokine transcription or expression. These promoter polymorphisms may lead to either high or low-level production of the given cytokines, cause inter-individual differences in antitumor immune response and subsequently influence the susceptibility to cancers [7, 8].

Interleukin-10 is a pleiotropic and potent immunoregulatory T-helper 2 (Th2) cytokine that inhibits the production of pro-inflammatory cytokines by inhibition of T-helper 1 (Th1) lymphocytes and stimulation of B lymphocytes and Th2 lymphocytes. It down regulates cell-mediated and cytotoxic inflammatory response. The IL-10 gene is comprised of 5 exons, spans approximately 5.2 kb and is localized at 1q31-1q32 region [9]. Three Single Nucleotide Polymorphisms (SNPs) in the IL-10 promoter at positions -1082 (G>A), -819 (C>T), and -592 (C>A) have been reported to influence IL-10 expression and to be associated with increased risk of gastric cancer. It has been reported that IL-10-1082G/A polymorphism is correlated with the expression of IL-10 and accordingly affects the susceptibility to some types of tumors, such as cervical cancer and prostate cancer. The association of -1082 alleles G and A with a low (AA), high (GG) and medium (GA) IL-10 production were shown by in vivo and in vitro studies [10- 12].

In the present study, we investigated the association between the IL-10-1082 G/A (rs1800896) polymorphism and the susceptibility with gastric cancer in Andhra Pradesh population of South India.

Materials and Methods

Subject

A total of 100 endoscopically and histopathologically confirmed gastric cancer patients in the age group of 22-70 years, referred to the Department of Gastroenterology, Osmania General Hospital, Hyderabad and Department of Gastroenterology, Gandhi Hospital, Secunderabad were considered for the present study. One hundred thirty two healthy controls with no family history of gastric ulcer or cancer were selected randomly. A structured questionnaire was used to elicit information on epidemiological factors such as age, sex, dietary habits, weight, addictions; family history of cancer etc. All the patients were tested for Helicobacter pylori infectivity status on antral biopsies by urease test following the method of Vaira et al [14]. The study was approved by the Institutional Ethical Committee and informed consent was obtained from all recruited subjects.

DNA Extraction

Five ml of blood was collected from each subject in vacutainers with anticoagulant Ethylenediamine Tetra Acetic Acid (EDTA). Genomic DNA was isolated from whole-blood samples of all the patients and control subjects, by the salting out procedure of Lahiri and Nurnberger [15].

IL-10-1082G/A Genotyping by ARMS-PCR

IL-10 -1082 G/A polymorphism genotyping was carried out with Allele Refractory Mutation detection System–Polymerase Chain Reaction (ARMS-PCR) [16]. Allele A was amplified with Forward Sense Primer FSP-A (5' -AAC ACT ACT AAG GCT TCT TTG GGT A-3') and allele G was amplified with the primer FSP-G (5'-AAC ACT ACT AAG GCT TCT TTG GGT G -3'). The primer RP-CAS (5'-GTA AGC TTC TGT GGC TGG AGT C-3') was used as reverse primer (common antisense) in both the reactions. These reactions amplify allele specific sequence of 161 bp of the promoter of IL-10 gene. Internal control primers amplifying a 796-bp fragment from the third intron of the HLA-DRB1 gene were included in each reaction. Sequences of internal control primer are as follows: Internal Control 1: 5' TGC CAA GTG GAG CAC CCA A 3' and Internal Control 2: 5' GCA TCT TGC TCT GTG CAG AT 3'. PCR was performed in a total volume of 10µl with 100 ng of total genomic DNA, 1X reaction buffer with1.5 mM MgCl2, 200µM of each dNTPs, and 0.5 µM of each primer and 0.8 IU of Taq polymerase. The cycling conditions were as follows: an initial denaturation at 95^{0}C for 5 minutes, followed by 35 cycles at 95^{0}C

Table 1. Demographic Details of Gastric Cancer Patients and Controls

Variable	GC cases (N=100) N (%)	Controls (N=132) N (%)	OR (95%CI)	P-value†
Gender				
Male	68 (68)	96 (72.73)		
Female	32 (32)	36 (27.27)	0.797 (0.451-1.407)	0.433
Age (years)				
≤ 40 Years	18 (18)	72 (54.45)		
>40 Years	82 (82)	60 (45.55)	0.182 (0.099-0.338)	<0.0001**
Addictions				
Smokers	58 (58)	52 (39.39)		
Non-smokers	42 (42)	80 (60.61)	1.532(1.132- 2.070)	0.0049*
Alcoholics	43 (43)	45 (34.09)		
Non-alcoholics	57 (57)	87 (65.90)	1.458 (0.854- 2.49)	0.166
H.pylori Infection status				
Infected	18 (18)	23 (17.42)		
Non-infected	82 (82)	109 (82.58)	0.961 (0.487- 1.896)	0.909
Familial Incidence				
Familial	04 (04)			
Non- familial	96 (96)	132		
Histological type				
Intestinal	84 (84)		-	
Diffuse	16 (16)	-		

† Two-sided $\chi 2$- test; OR: Odds ratio; CI: Confidence Interval; p value ≤ 0.05 *

for 30s, 63^{0}C for 30s and 72^{0}C for 30s. The final extension step was at 72^{0}C for 5 min.

The amplified PCR products (161bp) were separated by electrophoresis on a 1.5% agarose gel stained with ethidium bromide. The gel was visualized under ultraviolet light (Figure 1). All the collected samples were successfully genotyped. Ten percent 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 $\chi 2$ test was used 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 OpenEpi software [17]. A p value ≤ 0.05 was considered as significant.

Results

A total of 100 gastric cancer patients and 132 controls were enrolled in this case-control study. Table 1 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) and smoking (p=0.00494) between GC patients and controls. However, no significant difference was observed between cases and controls with regard to gender, H. pylori infection and alcohol consumption. In GC patients, 84 cases were of intestinal type, and the remaining 16 were of diffuse type.

The distribution of genotypes and allelic frequencies in cases and controls are shown in Table 2. The distribution frequencies of IL-10 -1082 G/A genotypes and alleles in cases (GG, 18%; GA, 35% and AA, 47 %; allele G=0.355 and A=0.645) were significantly different from those in controls (GG 31.82 %, GA 37.88 % and AA 30.3 %; allele G=0.508 and A=0.492) [for GG vs. AA genotype: OR= 2.742 (95% CI :1.369-5.492) ; p =0.0039; GG vs. (GA+AA): OR =2.126 (95% CI : 1.135-3.984) ; p =0.0173 and G allele vs. A allele : OR=1.873 (95% CI =1.285-2.73) ; p=0.00104]. An increase in the frequency of A allele was observed in the patients compared with control subjects, thereby indicating its possible role in the etiology of gastric cancer.

Table 2. Distribution of Genotypes and Allele Frequencies of IL-10 (-1082 G/A) Promoter Polymorphism in Gastric Cancer Patients and Control Subjects

Genotypes	GC Cases (N=100)	Controls (N=132)	OR (95% CI)	P value‡
	N (%)	N (%)		
Genotypes				
GG	18 (18)	42 (31.82)	1.0 (ref)	
GA	35 (35)	50 (37.88)	1.633 (0.810-3.292)	0.169
AA	47 (47)	40 (30.30)	2.742(1.369-5.492)	0.0039**
GA/AA	82 (82)	90 (68.18)	2.126 (1.135-3.984)	0.017**
Alleles				
G	71 (0.355)	134 (0.508)	1.0 (ref)	
A	129 (0.645)	130 (0.492)	1.873(1.285-2.730)	0.001048*

‡Cases vs. controls; OR: Odds Ratio; CI: Confidence Interval; ref: reference

Table 3. Distribution of Genotype and Allele Frequencies of IL-10 (-1082 G/A) Promoter Polymorphism in Gastric Cancer Patients According to Age Groups and Smoking Habits

	<40years	> 40 years	OR(95% CI)	P value‡	Smokers	Non smokers	OR(95% CI)	P value‡
Genotypes								
GG	7 (38.89)	10 (12.19)			11 (18.97)	6 (18.970		
GA	5 (27.77)	28 (34.15)	0.255 (0.066-0.99)	0.09	18 (31.03)	14 (33.33)	1.174(0.422-4.808)	0.788
AA	6 (33.33)	44 (53.66)	0.195 (0.054-0.707)	0.023*	29 (50)	22 (52.8)	1.391(0.445-4.343)	0.776
Alleles								
G	19 (0.528)	48 (0.293)			40 (34.58)	26 (30.95)		
A	17 (0.472)	116 (0.707)	0.3702 (0.177-0.772)	0.012*	76 (65.52)	58 (69.05)	1.174 (0.644-2.14)	0.710

‡ Two-sided $\chi 2$- test; OR: Odds Ratio; CI: Confidence Interval; p value ≤ 0.05

The distribution of genotypes and allelic frequencies within the disease group with regard to age and smoking habit are shown in Table 3. A significant difference in genotype and allelic frequencies between > 40 years comparable to < 40 years age groups was observed [for GG vs. AA genotype: OR= 0.194 (95% CI =0.054-0.707)); P =0.023; G allele vs. A allele: OR=0.3702 (95% CI =0.177-0.772); p=0. 0.012]. However, smokers and nonsmoker GC patients do not reveal any significant difference in the distribution of the genotypes.

Discussion

Cytokines play a crucial role in the regulation of key pathway of immunity, the balance between cell-mediated (Th1) and humoral (Th2) responses. IL-10 is an important anti-inflammatory and immunosuppressive cytokine, it inhibits various immune functions such as the Th1 type pathway activation, macrophage activation, and antigen-specific T-cell proliferation, prevents Antigen Presenting Cells (APC) from obtaining access to tumor antigen, and down

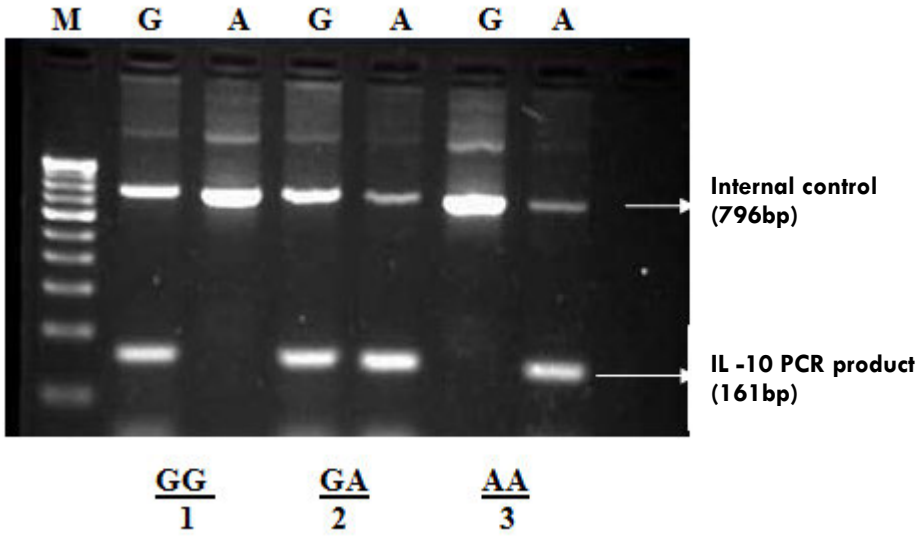

Figure 1. A representative agarose (1.5%) gel electrophoresis of ARM-PCR products for IL-10 -1082 G/A SNP. M: 100bp size DNA marker

regulates surface expression of co-stimulatory molecules CD80 or CD86 on tumor cells. Due to its immunosuppressive and anti-inflammatory properties, it has been hypothesized that IL-10 may contribute to escape of tumor cells from immune surveillance and favor tumor growth [18 - 20]. On the other hand, IL-10 may also play a protective and preventive role against tumors through its ability to downregulate synthesis of Vascular Endothelial Growth Factor (VEGF)—one of the most potent angiogenic factors—along with IL-1β, Tumor Necrosis Factor α (TNFα), IL-6 and Matrix Metalloproteinase-9 (MMP-9) in tumor-associated macrophages, which also play crucial roles in tumor angiogenesis [21]. In addition, IL-10 may also directly affect the secretion of angiogenic molecules from the tumor. However, other studies have shown that IL-10 inhibits tumor metastasis via a natural killer cell-dependent mechanism [22, 23].

IL-10 genotype may influence predisposition to a number of solid tumors, but studies on IL-10 gene polymorphisms and current disease association data, are conflicting or contradictory, due to this cytokine having both immunosuppressive (potentially cancer promoting) and anti-angiogenic (potentially cancer inhibiting) properties [24]. The IL-10-1082 G allele has been considered to be associated with higher production of IL-10 from peripheral mononuclear cells [25- 27]. The association of the A to G substitution at position -1082 of the IL-10 gene promoter with increased transcription of IL-10 has been shown in vitro [28]. Levels of IL10 mRNA expression were significantly increased for individuals carrying the GCC/GCC genotype, compared to ATA/ATA or ATA/ACC in a study in Caucasian individuals from Northern Spain [29]. Study by Rad et al [30] also revealed that carriers of the IL-10 -1082G allele had higher mucosal IL-10 mRNA than -1082A allele carriers.

Polymorphisms that resulted in reduced expression of IL-10 have been reported to be associated with a higher risk of gastric cancer in some case- control studies. Subjects with the IL-10-1082 AA genotype were at a two-fold higher risk of gastric cancer compared to those with an IL-10-1082 GG genotype [31]. Zambon et al [32] also reported that the IL-10-1082 A/A, IL-10-819 T/T or IL-10-592 A/A genotype or the ATA/ATA haplotype of IL-10-1082/-819/-592 polymorphisms was related to an increased risk of H. pylori infection-related gastric cancer development. Furthermore, Kato et al found a 60% increase in the risk of intestinal metaplasia and dysplasia subsequent to H. pylori infection, among the carriers of the IL-10-1082 low activity allele [33].

In contrast, others have reported that polymorphisms resulting in increased IL-10 expression are associated with the increased risk of H. pylori-mediated diseases. Deans et al [34] found that GG for IL-10-1082 was associated with reduced survival of gastric cancer patients, but it was not an independent prognosis factor for gastric cancer. Study by Wu et al [35] and Lu et al [36] also showed that the high producer IL-10-1082 G allele or the GCC haplotype are associated with higher risks for gastric cancer development. Although it is

difficult to determine the reasons behind the contradictory results in these studies, the varied genetic background of various ethnic groups may be one of the main factors.

In the present study, we investigated the association between IL-10 -1082 G/A polymorphism and risk of gastric cancer in a south Indian population. We found that subjects with low expresser IL-10 -1082 AA genotype and allele A had an increased risk for gastric cancer. Comparison of genotypes within the patient group with respect to age and smoking habit, revealed a significant association of AA genotype in patents above age of 40 years compared to those below 40 years. Decreased IL-10 expression would de-repress angiogenic activity and promote cancer progression. IL-10 generally protects the host against inflammation after toxin induced injury, but inadequate responses related to IL-10 after systemic injury or inflammation, may render the host susceptible to malignant changes.

In conclusion, this case-control study reports, for the first time, an association between the IL-10 AA genotype and increased risk of gastric cancer in Andhra Pradesh population of South India.

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Conflict of Interest

The authors declare that they have no conflict of interest in this article.

Authors' Contribution

Amar chand Bhayal conceived, designed the study, interpreted the results, drafted the manuscript, and carried out data analyses. Devulapalli Krishnaveni contributed to sample and data collection, participated in writing and revising the manuscript. Kondadasula Pandu Ranga Rao, Boddu Prabhakar, Abbagani Vidyasagar, Bal Murali Krishna and Penchikala Anita helped in providing samples and in confirming the diagnosis (endoscopical and histopathological evaluation) of gastric cancer patients. Akka Jyothy, Pratibha Nallari and Ananthapur Venkateshwari revised and approved the final manuscript. All authors read and approved the final manuscript.

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