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Full Length Research Paper

IL 10 promoter polymorphism and gastric cancer risk in A^{+ve} blood group patients in Kashmiri population

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Within the past few years, there has been increasing evidence that the genetic variation in the genes coding pro- and anti-inflammatory markers may play an important role in the pathogenesis of various human cancers, including gastric cancer. The aim of the study was to evaluate the association of Interleukin- 10 (IL-10)-1082 G/A and -592 C/A promoter polymorphism with gastric cancer in a North Indian population from Kashmir valley, using a PCR-RFLP approach. In this study, 102 gastric cancer patients and 156 age, sex, dwelling and blood group matched healthy controls were included. Association between genotypes and gastric cancer was examined by odds ratio (OR) with 95% confidence interval (CI) and Chi-square analysis. In our study, it was observed that significant difference exists between the patients and healthy controls, in genotypic distribution as well as allelic frequency ($p < 0.05$) in case of -1082 and not with -592 polymorphism. We also found significant association of the IL 10-1082 with EGD biopsy, blood group (A+ve), hot salt tea consumption, sex and dwelling. Dwelling showed highest association ($P>0.0001$) with gastric cancer. Our results suggest that A allele of IL-10 gene is an important genetic risk factor for Gastric cancer in the North Indian population from ethnic Kashmiri population. However, this is a preliminary study and the results need to be confirmed in a larger cohort.

Key words: Gastric cancer, inflammation, Kashmir valley, polymorphism.

INTRODUCTION

Chronic inflammation is thought to contribute to the development of cancer (Balkwill and Mantovani, 2001). Progressive inflammation leads to activation of inflammatory cytokines, recruitment of inflammatory cells, generation of free radical species, and subsequent malignant transformation. Individual responses to inflammation, mediated by genetic variation, may influence the degree of inflammation and thus the risk for cancer. Interleukin-10 (IL-10) is an anti-inflammatory cytokine, which is involved in down-regulating cell-mediated and cytotoxic inflammatory responses (Wu et al., 2002). The gene encoding IL-10 is located on chromosome 1 (1q31-1q32). IL-10 has three confirmed biallelic polymorphisms in the gene promoter region: -1082 A/G, -819 C/T, and -592 C/A. Presence of -1082A is associated with lower production of IL-10 *in vitro* and *in vivo*, and an accordingly stronger inflammatory response (Rad et al., 2004). Interleukin-10 (IL-10) is an immunoregulatory Th2 cytokine with a polymorphic promoter (-1082, rs1800896; and -592, rs1800896), with variants correlated with increased IL-10 production (De Vita et al., 2001). These IL-10 polymorphisms have been associated with noncardia gastric cancer risk (Suarez et al., 2003).

In the multistage model of gastric carcinogenesis,

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gastric inflammation is a prerequisite for the development of GC (Correa, 1992). Accordingly, factors involved in initiation and regulation of the inflammatory response may confer susceptibility to or protection against GC. In this respect, cytokines that play a crucial role in regulating inflammation are potential candidates for correlating with such variation. The *in vitro* maximal capacity to produce different cytokines varies among different individuals. Family studies indicate that much of this variability is genetically determined (Westendsop et al., 1997). Such inter individual differences can be attributed to several molecular mechanisms, including single nucleotide polymorphisms (SNPs) in the coding or promoter regions of cytokine or cytokine receptor genes. These polymorphisms may affect the overall expression and secretion of cytokines.

Gastric cancer, the second leading cause of death from cancer throughout the world, is an important health problem. A 2005 analysis of the worldwide incidence of, and mortality from, cancer showed that 934,000 cases of gastric cancer occurred in 2002 and that 700,000 patients die annually of this disease (Parkin et al., 2002). The Kashmir valley (India) which borders the southern part of the high incidence belt represents a moderately high incidence area where incidence rates for gastric cancer were: men 36.70/lack per annum, women 9.9/ lack per annum (Khuroo et al., 1992).

MATERIALS AND METHODS

Patient recruitment

Blood samples from gastric cancer patients were collected in the Gastroenterology ward and clinics in the Sher-I-Kashmir Institute of Medical Sciences; all cases were histologically confirmed to be gastric carcinoma. A pool of control subjects was recruited from the same hospital, from the same geographic area and ethnic background. The controls did not have a previous diagnosis of any type of cancer and were matched as far as possible with cases in terms of age, sex, dwelling and blood group. Written informed consent was obtained from each recruited subject, and the study was approved by the local institutional ethical committee. Cases and controls were interviewed face to face during hospital admission using standard questionnaires.

One hundred and fifty six blood samples of normal controls and one hundred and two Gastric cancer patients were recruited at random among prospective blood donors from the blood transfusion services and gastroenterology department of Sher-i-Kashmir Institute of Medical Sciences (SKIMS), Soura, Srinagar, Jammu and Kashmir (India), respectively. All the donors were related Kashmiri residents. Ethnic bias within the population studied was minimized by excluding non-Kashmiri resident subjects. Informed consent was obtained from all the individuals that participated in the study.

Genotyping

Genomic DNA was extracted from 10 ml EDTA (Ethylene di amine tetracetate) treated venous blood samples using the standard phenol-chloroform extraction protocol. DNA purity was assessed by a UV–Vis spectrophotometer estimating the A260/A280 ratio or by running samples on 1% agarose. IL 10-1082 and -592 promoter polymorphisms were analyzed with polymerase chain reaction (PCR) amplification followed by restriction fragment length polymorphism (RFLP). Primers used were as described by Ja et al. (2005). For IL 10-1082 genotyping, PCR condition was as follows; 94°C for 2 min, then 40 cycles of 94°C for 1 min, 58°C for 1 min, 72°C for 1 min, and finally 72°C for 10 min. The PCR products were digested with MnlI (Fermentas Inc., Hanover, MD) at 37°C for 4 h and separated by electrophoresis on a 3% agarose gel. A fragment containing the MnlI polymorphic site at position -1082 of the IL 10 gene was separated as follows: The A allele was designated if 2 bands of 125 and 65 bp were obtained, and the G allele was designated if 3 bands of 93, 65, and 32 bp were obtained.

The PCR condition for IL 10-592 site polymorphism was as follows; 94°C for 2 min, then 40 cycles of 94°C for 1 min, 59°C for 1 min, 72°C for 1 min, and finally 72°C for 10 min. The PCR products were digested with RsaI (Fermentas Inc., Hanover, MD) at 37°C for 4 h and separated by electrophoresis on a 3% agarose gel. A fragment containing the RsaI polymorphic site at position -592 of the IL 10 gene was separated as follows; The C allele was designated if 2 bands of 302 and 135 bp were obtained, and the A allele was designated if 3 bands of 240, 135, and 62 bp were obtained.

For quality control, distilled water was used instead of DNA as a negative control in each PCR reaction, and more than 20% of the samples were reanalyzed blindly by another co researcher. Results were reproduced up to 98% satisfactorily.

RESULTS

One hundred gastric cancer patients and one hundred fifty six controls were included in the study. The demographic features of the population under study have been listed in Table 1. The risk factor profile revealed that hot salt tea consumption was the most common risk factor in patients followed by smoking and blood group in gastric cancer patients. The genotypic distribution and allelic frequencies of IL-10 gene polymorphism in patients and controls are given in Tables 2 and 3. There were statistically significant differences in the genotypic distribution and allelic frequency between the patients and healthy controls [for AA vs. GG genotype: X^2 = 14.35; OR = 0.2857 (95% CI: 0.157 to 0.555) and $p < 0.001$; for AA vs. (GG + GA): X^2 = 41.83; OR = 0.171 (95% CI: 0.098 to 0.298) and ($p = <0.0001$)]. The X^2 for A vs. G allele was 30.82; OR = 0.3543 (95% CI: 0.2444 to 0.5136) and $p = <0.0001$ (Table 4). There were statistically no significant differences in the genotypic distribution and allelic frequency between the patients and healthy controls in case of IL 10-592 C/A polymorphic study.

The correlation of IL 10-1082 polymorphic status with the clinicopathological characteristics was carefully analyzed. It was found that the IL 10-1082 polymorphism was significantly related to EGD biopsy, salt tea consumption, blood group, sex and dwelling and not to other clinicopathological features (Table 5).

Statistical analysis of allele frequencies was performed using Chi-square statistics (Pearson test using SPSSv10 software). Genotype distribution for polymorphism was first compared to predictable values from Hardy–Weinberg equilibrium. In all cases, P-values less than

Table 1. Clinical characteristics of Gastric cancer patients and controls.

		Cases N=102 (%)	Controls N=156 (%)	P Value (95% CI)
Age				
	<45	24 (23.53)	54 (34.61)	P>0.05
	>45	78 (76.47)	102 (65.38)	
Sex				
	Male	80 (78.43)	112 (71.79)	P>0.05
	Female	22 (21.57)	44 (28.21)	
Dwelling				
	Rural	76 (74.51)	98 (62.82)	P> 0.05
	Urban	26 (25.49)	58 (37.17)	
Hot salt tea consumption				
	<4 cups/day	81 (79.41)	99 (63.46)	P< 0.01
	>4 cups /day	21 (20.59)	57 (36.54)	
Blood group				
	A+ve	62 (60.78)	92 (58.97)	P>0.05
	Others	40 (39.22)	64 (41.03)	
Smoking				
	Ever	79 (77.45)	90 (57.69)	P< 0.01
	Never	23 (22.53)	66 (42.31)	

Table 2. Distribution of IL-10-1082 genotypes and allelic frequencies of the study population.

Study groups	IL 10 1082 genotype				Allele frequency		
	GG	GA	AA	Total	G	A	Total
Controls n (%)	32 (20.51)	20 (12.82)	104 (66.66)	156	84 (0.27)	228 (0.73)	312
Patients n (%)	28 (27.45)	48 (47.06)	26 (25.49)	102	104 (0.51)	100 (0.49)	204

Table 3. Distribution of IL-10-592 genotypes and allelic frequencies of the study population.

Study groups	IL 10 592 Genotypes				Allele Frequency		
	CC	CA	AA	Total	C	A	Total
Controls n (%)	36 (23.07)	98 (62.82)	22 (14.10)	156	170 (0.54)	142 (0.45)	312
Patients n (%)	20 (19.61)	70 (68.63)	12 11.76)	102	110 (0.54)	94 (0.46)	204

0.05 were considered to be statistically significant.

DISCUSSION

The incidences of polymorphism in genomic DNA, their susceptibility to genetic alterations, and the risk of tumor progression in patients with cancer can vary substantially between different racial groups (Perez et al., 2006; Bojesen and Nordestgaard, 2008; Katkoori et al., 2009). Although, most polymorphisms are functionally neutral, some affect regulation of gene expression or the function of the coded protein (Costa et al., 2008). The objective of this study was to find any association between IL 10 polymorphism and gastric cancer from Kashmir valley. The meta analysis performed by Yong Zhou suggests that the IL 10-1082 promoter polymorphism may be associated with gastric cancer among Asians, and that

Table 4. Odds ratio and P values.

Patients vs. control	Chi square	odds ratio	95% CI	P value
AA vs. GG	14.35	0.2857	0.157-0.555	0.00015
AA vs. GG +GA	41.83	0.171	0.098-0.298	<0.0001
A vs. G	30.82	0.3543	0.2444-0.5136	<0.0001
AA vs. CC	0	1.0185	0.4029-2.3928	1
AA vs. CC+CA	0.29	0.8121	0.3827-1.723	0.590221
A vs. C	0.02	1.023	0.7179-1.4579	0.887537

Table 5. Association between IL-10-1082 phenotypes and clinicopathologic characteristics.

Variables	IL 10 1082 genotype				Chi square	P value
	GG	GA	AA	Total		
Site of growth						
GE junction	8	11	3	22		
Others	20	37	23	80	0.241	0.2996
EGD biopsy						
Adenocarcinoma	15	40	21	76	8.97	0.011
Intestinal carcinoma	13	8	5	26		
Blood group						
A+ve	18	36	8	62	14.04	0.0009
Others	10	12	18	40		
Hot Salt tea consumption						
<4 cups/day	20	43	18	81	5.78	0.05
>4 cups /day	8	5	8	21		
Smoking						
Ever	23	39	17	79	2.92	0.2322
Never	5	9	9	23		
Age						
<45	4	10	10	24		
>45	24	38	16	78	4.75	0.093
Sex						
Male	17	45	18	80		
Female	11	3	8	22	13.15	0.0014
Dwelling						
Rural	11	44	21	76		
Urban	17	4	5	26	26.34	0.000002

differences in genotype distribution may be associated with the location of gastric cancer (Yong et al., 2008). Interleukin-10 is a potent anti-inflammatory cytokine which has multiple functions. It is secreted by many cell types and a number of cell types are responsive to it (Moore et al., 1993). The IL 10 gene is located on chromosome 1 at q 31–32 and is highly polymorphic (Eskdale et al., 1999). Endogenously produced IL-10 is a potent immunosuppressant and important modulator of acute and chronic inflammation (Goldman et al., 1997; Rennick et al., 1997). It limits the production of pro-inflammatory cytokines by providing a negative feedback

mechanism and therefore down-regulates the deleterious action of pro-inflammatory cytokines (Spera et al., 1998; Ooboshi et al., 2002; Dietrich et al., 1999; Grilli et al., 2000; Yang et al., 2000).

In this study, we assessed the polymorphism of two Interleukins, IL 10-1082 and IL 10-592 polymorphism and found a significant association of IL 10 1082 allele frequency of patients with controls. We also found that the IL 10-1082 polymorphism was significantly related to EGD biopsy, salt tea consumption, blood group, sex and dwelling and not to other clinicopathological features.

IL 10 is also an important regulatory cytokine that is involved in many aspects of the immune response, and it seems to dysregulate in human autoimmune (Llorente et al., 1995; Mekala et al., 2005; Duan et al., 2005; Chai et al., 2005), malignant (Luscher et al., 1994; Kamiya et al., 2003; Guo et al., 2005; Chan et al., 2005; Azar et al., 2004; Murta et al., 2004) and infectious (Chung et al., 2005; Stoycheva et al., 2005; Migita et al., 2005) disease. IL-10 production has also been implicated in the tumorigenesis of various types of cancers (Pisa et al., 1992; Huang et al., 1995). IL 10 polymorphism has also been extensively studied and reported to be associated with other cancers and diseases. Stratifying for race, patients with gastric cancer had a significantly lower frequency of AA and higher frequency AG than non cancer patients among Asians (Yong et al., 2008).

In conclusion, the present study carried out for the first time in Kashmir suggests that IL 10-1082 polymorphism is associated with risk of gastric cancer.

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