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ORIGINAL ARTICLE

Study on association between H-ras gene polymorphism and gastric adenocarcinoma risk

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ABSTRACT

Aim: The aim of this study was to investigate relation between H-ras T81C polymorphism and some of the important risk factors in gastric adenocarcinoma (GA).

Background: GA is one of the leading causes of cancer death in most countries. RAS gene is an important member in the PI3K-AKT signaling and the single nucleotide polymorphism at H-rasc DNA position 81 has been demonstrated has an important role in tumor genesis.

Patients and methods: In this study, we carried out single-nucleotide polymorphism analysis in an Iranian population. A total of 100 patients with gastric adenocarcinoma and 100 controls were examined for the presence of T81C H-ras polymorphism using PCR- RFLP assay.

Results: Statistical analysis revealed no relationship significant between TT, TC, CC and risk of GA, but, there was a poorly relation between male patient with C-carrier genotype and increasing risk of GA (P=0.07). Also, we investigate effect of four important risk factors for GA. There was a statistically significant difference between increasing of age and susceptibility for GA (OR=1.106, 95%CI=1.073-1.139, P<0.001). We observed a statistically significant between smoking and T81C polymorphism C-carrier genotypes (OR=3.98, 95%CI=1.831-8.68, P<0.001) as this individual had three-time risk for GA. We did not show a significant association between three main genotypes and H. pylori infection for risk of GA.

Conclusion: These results suggested that there is no relationship between T81C-HRAS polymorphism and gastric cancer risk in Iranian patients. But, gender (male in our study) and the other risk factor described above have an important role in developing of GA.

Keywords: Gastric adenocarcinoma; H-ras mutations; PCR-RFLP.

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Introduction

Gastric adenocarcinoma (GA) is the fourth most frequent cancer worldwide (1,2). It is a leading cause of death in Asian countries, especially in the Iranian population as the rate of death due to this malignancy has increased nearly 2- fold when compared with the data of 40 years ago (3-5).GA has divided into two major types and the world wide incidence and mortality varies widely based on geographic location, race, and environment factors (6,7). Patients with GA are thought to have a poor prognosis and short survival because of early tumor cell invasion and spread to the neighboring normal tissues (5). The mechanisms of carcinogenesis in GA is still unclear but some

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risk factors that are considered important include *Helicobacter Pylori* infection, smoking and genetic susceptibility (8). The effect of several genes in GA development including four closely related human Ras proto-oncogenes that encode small guanine nucleotide-binding proteins with intrinsic GTPase activity, has been described (9-11). H-ras is a member of the RAS gene family. This family of genes has a key role in the development and progression of many human cancers (12). An important Single Nucleotide Polymorphism (SNP) has been observed in H-ras gene which is caused by point mutations in the coding nucleotide position 81 (13). This SNP may be as a risk factor for some cancers, especially GA as previous described (14). Nitroso-containing compounds derived from tobacco may affect H-ras and an effect has been demonstrated in some cancers (15, 16).In this study, we aimed to evaluate the genetic variation of gastric adenocarcinoma and several important risk factors such as H.pylori infection, smoking, age and gender. We carried out our study on Iranian patients diagnosed with GA and investigated the presence of H-ras gene mutation T81C using restriction fragment length polymorphism.

Patients and Methods

The T81C polymorphism was assessed using a cross-sectional study in healthy individuals without clinical evidence of cancer (n=100) and patients with evident of gastric cancer (n=100). PCR was done according to the standard protocol as described earlier. Finally, a face-to-face interview with several scheduled questions was executed among subjects to gather information including individual's age, gender and cigarette smoking. All samples were obtained with the permissions of the individuals before their inclusion in the study after informed consent.

Sample DNA extraction

Blood samples were collected (5ml) from patients and controls by means of a standard vein puncture technique using EDTA containing tubes. Briefly, genomic DNA was extracted from peripheral blood leukocytes by a standard Salting-out protocol (17). Then, the quantity of DNA samples was assessed spectrophotometrically using nanodrop ND-1000 (Nanodrop Technologies, USA) at 260 and 280 nm and the samples were stored in a -20º C freezer.

H-ras gene polymorphism

Based on information from publicly available databases, such as dbSNP (http://www.ncbi.nlm.nih.gov/SNP/)we collected data on H-ras polymorphisms. Consequently, the exon 1 of H-ras was amplified by polymerase chain reaction as previous described (17). The sequence of forward and reverse primers which were used in order to amplification of a 200 bp DNA segment were 5'-CTTGGCAGGTGGGGCAGGAGA-3' and 5'-GGCACCTGGACGGCGGCGCTAG-3'respectively. Briefly, PCR conditions were as follows: an initial denaturation step at 94º C for 5 min, followed by 35 cycles of denaturing at 94º C for 1 min, annealing at 59º C for1 min, and extension at 72º C for 1 min. The final extension step was continued at 72º C for 10 min. After PCR amplification, reaction products were digested using RFLP technique, with *Drau* restriction endonuclease (Fermentas, Lithuania) for 6 h at 37º C. Results were observed in 3% agarose gel stained with ethidium bromide (Figure 1). Three fragments were observed including: 145, 55 and 200 bp. In some samples, experiments were performed two times to confirm our experiment.

Statistical analysis

In this present study, the data analysis was performed by SPSS software (Version16.0, USA). Chi-square analysis was used to compare categorical variables, a two-sided P value of less than 0.05 was considered significant and 0.05< P >0.1 shown as poorly significant. Variables were

age, gender, H.pylori infection, smoking and H-ras (T, C) alleles. Multivariate logistic regression analysis was used to identify odds ratio (OR) and its 95% confidence interval (CI) as a measure of the association between genotypes and risk for development of gastric cancer. Gender and age were included in regression analysis. Hardy–Weinberg equilibrium was assessed by Chi-square –test analysis in cases and healthy controls.

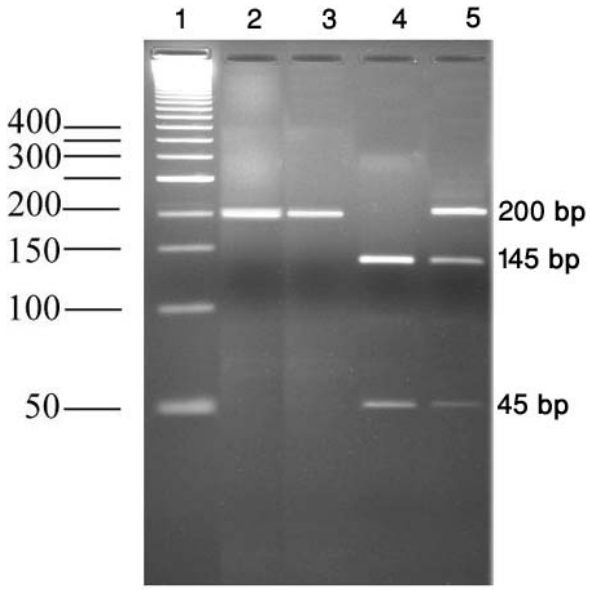

Figure 1. PCR-RFLP analysis of –T81C H-ras polymorphism; Lane 1: 50 bp DNA ladder; Lane2: PCR product (undigested); Lane 3: Homozygous -81TT genotype; Lane 4: Homozygous -81CC genotype; Lane5: Heterozygous -81TC genotype.

Results

Allelic distribution of H-ras T81C polymorphism

Risk of gastric cancer in carriers of the homozygous mutant C-allele (145 and 55bp), heterozygote CT (145, 55 and 200 bp) and wild-type carriers TT (200bp) were detected. The genotype prevalence and allele frequency of -81T>C H-ras are shown in Table 1.

Table 1. Allelic distribution of H-ras polymorphism in gastric cancer

Genotypes	Gastric cancer (n=100)	Control (n=100)
TT	69(69%)	60(60%)
TC	29(29%)	33(33%)
CC	2(2%)	7(7%)
C-Carrier	31(31%)	40(40%)

The frequency of CC, TC and TT genotypes were 7%, 33% and 60% in healthy controls and 2%, 29% and 69% in cases. Furthermore, C-carrier genotype in case and control was respectively 31% and 40%. Statistical analysis revealed no significant relation between three genotypes (TT, TC and CC) and risk of GA (P=0.160, $\chi 2$= 3.66).Generally, we did not observe a statistically significant relation between subjects that carried C- allele and risk of gastric cancer (P=0.184). However, differences could consider poorly significant after consideration of gender, as male patients with C- carrier genotypes (OR=0.159, 95%CI=0.020-1.231, P=0.07) had an increased risk for GA when compared with control groups. Hardy–Weinberg equilibrium was (P=0.59, $\chi 2$= 0.27) and (P=0.41, $\chi 2$= 0.67) in cases and controls respectively.

Risk estimate factors for gastric cancer

Gender and age were included in multivariate analysis and data were adjusted according to age and gender presented in Table 2. Overall, 100 gastric cancer patients including 50female and 50 male (mean age: 61.01±14.9) and 100 control normal 50 female and 50 male (mean age: 51.6±12.4) were enrolled. There was a statistically significant difference between increasing of age and gastric cancer development as the risk of gastric cancer has been increased nearly 1.1-fold instead of every year increasing of age (OR=1.106, 95%CI=1.073-1.139, P<0.001). Moreover, we observed a nearly significant difference between male cases and C-carrier genotype (P=0.07).

Table 2. Risk factors for H-ras polymorphism in gastric adenocarcinoma

Risk factors	Gastric cancer (n=100)	Control (n=100)	Odds Ratio (95% CI)	P -value
C-career (%)	31	40	0.020-1.231	0.07
Age (year)	61.01±14	51.6±12.4	1.073-1.139	<0.001
Male/ Female	50/50	50/50		
H.Pylori Infection (%)	74	46	.017- 1.18	0.066
Smoking (%)	62	30	1.37-6.61	0.006

In order to investigate influence of tobacco smoking factor on progression of GA the logistic regression analyses according to smoking status were carried out and result was adjusted (Table 2).We observed a statistically significant between smoking and T81C polymorphism in C-carrier genotypes(OR=3.98, 95%CI=1.831-8.68, P<0.001). Also subjects who smoked had an increased risk (3 times) for gastric cancer in comparison with non-smokers. Statistical analysis did not show a significant association between three main genotypes (TT, TC and CC) and H.pylori infection for risk of GA in cases (P=0.167) but interestingly P was 0.066 in C-carrier cases. Furthermore, 81% C-allele carrier patients were *H.pylori* positive in our study

Discussion

Despite a decreasing in incidence and mortality of gastric cancer for the past few decades, GA remains a major public health issue in developing countries (3). Iran has a high-incidence of gastric cancer (4, 5).Previous studies have suggested that both genetics and lifestyle factors are major risk factors for GA (1, 8).The rasproto-oncogene family (H-ras, K-ras and N-ras) play a fundamental role in cellular growth and differentiation (9). Several molecular alternations in RAS gene such as mini-satellites and mutations produce the activate ras proto-oncogenes (10, 11). An increased expression of the H-ras oncogene product was found in gastric cancer (12). It has been shown that H-ras 81 C- allele is a dominant genetic susceptibility factor for the development of gastric cancer but the mechanism by which H-rasT81C polymorphism modifies gastric cancer risk is not clear and a coherent model of GA carcinogensis is awaited (13, 14).

In this study, we focused on H-rasT81C polymorphism and investigated one frequent variation in codon 27 of exon 1 at cDNA position 81. Based on our results no significant relation was seen between three main H-ras genotypes and the risk of gastric adenocarcinoma. The frequency of C- allele in our healthy group 40%, which is higher than frequencies reported in studies from China (18), Germany (19), India (20) and Korea (21). The increased risk associated with H-ras 81 C- allele was more pronounced in male patients (P=0.07). The adjusted OR achieved 0.159, which indicated that H-ras T81C polymorphism may be vulnerability factor for the development of gastric cancer in our male population.

In our study%74 cases were *H.Pylori* positive. Generally, we did not show a significant relationship between the three main genotypes, H.pylori infection and risk of gastric adenocarcinoma (P=0.167). Data adjusting revealed a weak association between C-carrier cases including male and female (P=0.066) and *H. pylori* infection. Whereas 81% C-allele carrier in patients group were positive for *H. pylori*, it was 46.2%in our control healthy group with C-allele carriers.

The other predominantly risk factor is tobacco consumption. It has been shown that the risk for gastric cancer in smokers is nearly 2-fold in

compare of non-smokers (16). Based on Derakhshan's report a closely relation has been confirmed between tobacco consumption and gastric cancer risk in Iranian population (22).We found a statistically significant difference between smoking and C-carrier genotype ($P<0.001$). A logistic regression was performed between age, smoking and gastric adenocarcinoma and an increased risk (nearly three-fold) for gastric cancer in smokers was observed. Finally, age and T81C polymorphism was analyzed via multivariate regression. We did not found any relation between any genotypes and age. Based on our results it can be concluded that individuals with the C-allele genotype may be at increased risk of developing gastric cancer in Iran.

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