Gastroenterology and Hepatology From Bed to Bench.

ORIGINAL ARTICLE

Programmed death-1 gene polymorphism (PD-1.5 C/T) is associated with gastric cancer

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ABSTRACT

Aim: This study aimed to determine the association between PD-1.5C/T (rs2227981, +7785) and the risk of gastric cancer (GC) in an Iranian population.

Background: Gastric cancer is the fourth most common cancer in the world. The programmed death 1 (PD-1) is a member of the CD28 super family. PD-1 is a negative regulator of T-cell effector mechanisms which decrease immune responses against cancer.

Patients and methods: we conducted case- control study to investigate the association of PD-1.5 C/T polymorphism in 122 GC patients and 166 control individuals. DNA was extracted from blood specimens. Genotypes were analyzed using polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) assay.

Results: The frequency of CC, CT and TT genotypes was 53.6%, 42.2% and 4.2% in control group and 41%, 54.1% and 4.9% in gastric cancer patients respectively. CC genotype was more frequent in control individuals than in patients but we found no statically significant association. The frequencies of PD-1.5CT genotypes were significantly higher in GC patient compared with control individuals (OR= 1.77, 95% CI= 1.077-2.931; *P*=0.026). Allele distribution was similar in patients and healthy individuals (p=0.061).Frequency of C and T alleles was 74.7%, 25.3% in control individuals and 68.03% and 31.97% in gastric cancer patients respectively.

Conclusion: These results suggest that PD-1.5 C/T polymorphism may affect the GC risk and prognosis in an Iranian population.

Keywords: Gastric cancer, PD-1, Single nucleotide polymorphism.

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Introduction

Gastric cancer (GC) is one of the most common malignancies worldwide, with more than 930, 000 new cases every year. Mortality data was obtained from Iran show that, GC is the first cause of death due to cancer in both sexes (1-2).

Although, cause of GC not clearly identified but it is generally accepted that, gastric tumor genesis is a multi factorial. Several factors are suspected to play a key role in gastric carcinogenesis including environmental factors (diet, exogenous chemicals, smoking), intragastric synthesis of carcinogens, infectious aspects (*Helicobacter pylori*), pathological changes in the stomach and genetic factors (3-7). *Helicobacter pylori* stimulates immune cells such as B and T cell that play an important role in the elimination of infection (8). Whereas, T cell have been shown to play the major role in Anti-tumor immune response (9). Programmed cell deaths 1

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(PCD1) gene is located on chromosome 2q37.3 and encode a 50–55 kD a type 1 transmembranous glycoprotein PD-1 protein (10-12) In cancer, immune cells, such as B cell and T cell, play important roles in antitumor immune response (7-8). Programmed cell death protein 1 also known as PD-1 is a protein that is expressed on the surface of activated T cells and B cells and lead to apoptosis (13). PD-L/PD1 interaction between PD1 and its ligand (PD-L) can activate the specific cytoplasmic tail such as immune receptor tyrosine based inhibitory motif (ITIM) that begins intracellular signal transduction pathways which mediate exhausted T cell and reduce activation and proliferation of T cell (11, 12, 14-17). Previous Studies demonstrated that PD1blocking antibodies increase immune mediated antitumor responses(18-19). the most common type of genetic variation is Single nucleotide polymorphisms (SNPs) and they may contribute to an individual's susceptibility to cancer (20). Several single nucleotide polymorphism (SNPs) that may contribute to an individual's susceptibility to cancer ,have been identified in PD1 gene (12). Some functional polymorphism in PD1 gene may be affected on transcriptional and expression gene (21-22). One of the important polymorphisms is PD-1.5C/T that is located in exone 5 (position 7785) (17).There are many studies in case of PD1 polymorphism and autoimmune disease (17, 23-24) but a few published articles investigated the association between PD-1.5C/T polymorphism and cancer (9-10, 25). According to our knowledge there is no data about these polymorphisms and gastric cancer. In our study association between polymorphism PD-1.5C/T and gastric cancer in an Iranian population for the first time was investigated.

Patients and Methods

The PD-1.5C/T polymorphism was evaluated by a case- control study in 122 patients with gastric cancer and 166 controls recruited from 2005 to 2006 at Research Center for Gastroenterology and Liver Disease in Taleghani Hospital, Tehran, Iran. Studied Subjects were Iranian and before taking blood sample consent informed was obtained from each individual. Patients who had pathology and clinical symptoms that were indicative of gastric cancer were taken in as the study group while those who were clear of these symptoms were considered as the control group. This study was conducted under the approval of the ethics committee of the gastroenterology and liver disease research center, Shahid Beheshti University of medical sciences (Tehran, Iran).

DNA extraction

Peripheral blood samples were extracted using salting out standard method (26). Quality and quantity of DNA was evaluated by Nanodrop Spectrophotometer. Samples were frozen at –20˚C until further analysis. Polymorphism PD-1.5 C/T was chosen according to previous publications (9,25). Demographic characteristics were used as a determinant for patients and control individuals.

Genotyping

Genotyping of PD-1.5 C/T polymorphism was determined by polymerase chain reaction restriction fragment length polymorphisms (PCR-RFLP) analysis. A set of primers (forward 5': GGACAGCTCAGGTAAGCAG 3' and reverse 5':AAGAGCAGTGTCCATCCTCAG3') which were designed based on PD1 gene were used for PCR (27).

PCR condition and program as an initial denaturation was carried out at 94°C for 10 min, then, the reaction was as follows: 32 cycles of 95°C for 45 sec, 64°C for 40 sec, 72°C for 40 sec, followed by a final extension at 72°C for 10 min. The restriction enzyme for PD1 genotyping was AluI (fermentas, lithuania).PCR products were incubated in 37 c° overnight. The digested PCR products were determined on a 3% agarose

gel and stained with green viewer for visualization under UV light.

Statistical analysis

Hardy–Weinberg equilibrium (HWE) was tested using a goodness-of-fit χ^2 test. To estimate adjusted and unadjusted odds ratio (OR), unconditional logistic regression analysis was performed and 95% confidence interval (CI), as a measure of association of the genotypes with the risk of gastric cancer. Chi-square was used to evaluate association between genotype and clinic pathology in gastric cancer patients.

Results

In this study we examined 122 gastric cancer patients with age average 65.44±12.718 and 166controls with age average 62.87±15.958. Genotype of PD-1.5 polymorphism was in accordance with the HWE in control group. Length of the PCR product was 340 base pair (bp) and digested by AluI restriction enzyme. The sizes of CC, CT and TT genotypes were 181bp+159bp, 181bp+159bp+127bp+54bp and 181bp+159bp respectively. The frequencies of PD-1.5C/T genotypes; CC, CT and TT were 53.6%, 42.2% and4.2% in control individuals and 41%, 54.1%and 4.9% in patients respectively. Also results were showing significant association between CT genotype and risk of gastric cancer (odds ratio (OR), 1.77; 95% confidence interval (CI), 1.077-2.931, p=0.026). Also CC genotype was more in healthy controls than in patients.TT genotype was similar in healthy controls and gastric cancer patients (p= 0.438).The frequencies of C and T alleles were 74.7% and 25.3% in controls and 68.03% and 31.97% in patient respectively. Statistical analysis revealed no significant differences in the allele frequency between case and control individuals (p=0.079) (table1).The demographic characteristics of the patients and controls revealed no significant association between gender and genotype with gastric cancer.

Table 1. Genotypes and alleles frequencies of PD1 gene polymorphism (PD-1.5 C/T) among gastric cancer patients (n=122) and control (n=166)

Genotypes	Control	Gastric cancer patients	Adjusted Odds ratio (95%CI)	P-value
CC	89(53.6%)	50(41.0%)		1.00(Ref)
CT	70(42.2%)	66(54.1%)	1.77(1.077-2.931)	0.026
TT	7(4.2%)	6(4.9%)	1.59(0.489-5.212)	0.438
Alleles				
C	248(74.7%)	166(68.03%)	1.00(Ref)	
T	84(25.3%)	78(31.97%)	1.44(0.948-2.109)	0.061

Discussion

Gastric cancer is one of the widespread cancers in the world (28). The etiology of gastric cancer is very complex , but it has been suggested that genetic variation is a key factor for the etiology of gastric cancer (2). Recent Studies documented that, PD-1 negatively regulates anti-tumor CD8 T cell responses, and also the interaction of PDL and PD-1 is involved in decreasing tumor immunity (13). Previous study had investigated the relation between PD-1.5C/T polymorphism and several diseases including breast cancer (25), type 1 diabetes (29), colon cancer (9) and rheumatoid arthritis(RA) (30). In this research, for the first time, the relationship between PD-1.5C/T polymorphism and the risk of gastric cancer was investigated in an Iranian population. According to our data, CT genotype in patients was more than in control individuals. Statiscal analysis showed significant association in CT genotype and risk of gastric cancer (p=0.026). In line of our observation Mojtahedi et al found significant association between CT genotype and Iranian patients with colorectal cancer (CRC) and suggested that CT genotype is probably a risk factor in CRC(9). In other study Lin and colleagues reported the association of CT genotype of the PD-1.5C/T polymorphism and the risk of rheumatoid arthritis (30).However, Cooper

et al reported no statistical association between CT genotype PD1.5C/T polymorphism and type 1 diabetes (29).

It is notable that, PD-1.5C/T polymorphism is a synonymous variation that dose not change final amino acid sequence of the protein, thus, this significant association may be PD-1.5C/T variation linkage disequilibrium with other PD-1 gene polymorphisms that may lead to alter the PD-1 expression level (30). A recent study by Mojtahedi et al, revealed that CC genotype was shown to be more frequent in healthy controls (9).

Our results show no association between allele frequency and gastric cancer (9). However Hau et al reported that the C allele frequency was more in breast cancer patients than those in control individuals in Chinese population (10). On the other hand, valuable paper documented that, C allele distribution in Vogt–Koyanagi–Harada patients (VKH) syndrome, which is an uncommon multisystem disease of presumed autoimmune etiology, was less than that found in control individuals (31). While, Lin and colleagues showed that T allele is associated with developed rheumatoid arthritis (30).

Studies show that there is a large variety in the PD1.5 genotype and allele frequency that get affected with various ethnic groups, tumor location , kind of disease and other clinical factors (9, 10, 25, 29, 30).

In summary, according to our research, PD-1.5 C/T polymorphism is associated with the risk of gastric cancer in Iranian population, which is the first data for the contribution of the human PD-1 gene in gastric cancer. Further studies with larger sample sizes are required to assess the impact of PD-1.5 C/T polymorphism on disease prognosis.

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