RESEARCH ARTICLE

Evaluation of Insulin Like Growth Facror-1 Genetic Polymorphism with Gastric Cancer Susceptibility and Clinicopathological Features

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Abstract

Gastric cancer (GC) is one of the most common malignancies in the world. It is the first cause of cancer deaths in both sexes In Iranian population. Circulating insulin-like growth factor-one (IGF-1) levels have been associated for gastric cancer. IGF-1 protein has central roles involved in the regulation of epithelial cell growth, proliferation, transformation, apoptosis and metastasis. Single nucleotide polymorphism in IGF-1 regulatory elements may lead to alter in IGF-1expression level and GC susceptibility. The aim of this study was to investigate the influence of IGF-1 gene polymorphism (rs5742612) on risk of GC and clinicopathological features for the first time in Iranian population. In total, 241 subjects including 100 patients with GC and 141 healthy controls were recruited in our study. Genotypes were analyzed using polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) assay with DNA from peripheral blood. The polymorphism was statistically analyzed to investigate the relationship with the risk of GC and clinicopathological properties. Logistic regression analysis revealed that there was no significant association between rs5742612 and the risk of GC. In addition, no significant association between genotypes and clinicopathological features was observed (p value>0.05). The frequencies of the CC, CT, and TT genotypes were 97%, 3%, and 0%, respectively, among the cases, and 97.9%, 2.1%, and 0%, respectively, among the controls. CC genotype was more frequent in cases and controls. The frequencies of C and T alleles were 98.9% and 1.1% in controls and 98.5% and 1.5% in patient respectively. Our results provide the first evidence that this variant is rare in Iranian population and it may not be a powerful genetic predisposing biomarker for prediction GC clinicopathological features in an Iranian population.

Keywords: Gastric cancer - genetic polymorphism - IGF-I - clinicopathological features

Introduction

Gastric cancer (GC) is the fifth most commonly occurring cancer and third leading cause of cancer mortality in the world (McLean and El-Omar, 2014). In Iranian population, GC is the first cause of cancer deaths in both sexes (Smith et al., 2006; Malekzadeh et al 2009). The etiology of GC is not clearly known. Several risk factors include environmental agents (diet, smoking); infectious aspects (Helicobacter pylori) and genetic factors are suspected to play a significant role in gastric carcinogenesis (Katoh et al., 1996; Stadtlander 1999; Setiawan et al., 2000; Forman and Burley, 2006; Rostami et al., 2011). Among the some genetic changes, there is evidence that the upregulation of certain growth factors could play a significant role in the development and progression of gastric cancer (Altinkaynak et al., 2012). Insulin-like growth factors-1 (IGF-1) is one of the significant growth factors. It is multifunctional peptide,

affecting cell proliferation in almost every cell type through the RAS, RAF-MAP kinase signaling pathway and also has antiapoptotic influence via activation of the phosphatidylinositol-3 kinase-AKT pathway, which use activates downstream transcription factors that mediate gene expression of proliferation, differentiation, and antiapoptotic agents (Karamouzis and Papavassiliou, 2006; Samani et al., 2007; Tao et al., 2007; Pollak 2008; Yang et al., 2014). High levels of circulating IGF-1 are relation to increase risk of several common cancers such as prostate, colorectal, breast, lung, glioma and gastric cancer (Yu and Rohan, 2000; Furstenberger and Senn, 2002; Lacey et al., 2004; Chen et al., 2009; Shitara et al., 2011; Altinkaynak et al.,2012; Lian et al., 2014). The levels of circulating IGF-1 are highly influenced by genetic factors (Harrela et al., 1996). Although there is a few researches have investigated the important of IGF-I polymorphisms and susceptibility or progression to types of cancers (Gao et al., 2009). According to our knowledge there is only one

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data about this polymorphism and gastric cancer (Shitara et al., 2011). To address this issue we select rs5742612 in promoter region which is influence to IGF-1 level to identify its susceptibility and progression effects on GC in Iranian population (D'Aloisio et al., 2009). We want to determine its power potential predisposing as a biomarker for GC prognosis in our population. We also aim to find association of this polymorphism with clinicpathological and demographic features.

Materials and Methods

Patients and controls

The IGF-1 polymorphism was evaluated by a case-control study that was conducted on a population of 100 patients suffering from Gastric cancer and 141 normal individuals who had been referred to the Research Institute for Gastroenterology and Liver Diseases (RIGLD), Taleghani Hospital, Shahid Beheshti University of Medical Sciences (Tehran, Iran). Studied Subjects were Iranian and before taking blood sample of individuals was obtained consent informed. Patients had pathology and clinical symptoms that were indicative of gastric cancer and controls had no this Symptoms. 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).

Selection and evaluation of genetic polymorphisms

Polymorphism rs5742612 is selected based on: i) pervious research finding who found association of rs5742612 with 10% or greater difference in IGF-1 level ii) some papers showed this variant significantly associated with various types of malignancies iii) as far as we know, there has been just only one research about the association between the rs5742612 and susceptibility or progression of gastric cancer in the literature so far (Shitara et al., 2011).

DNA extraction:

5 ml peripheral blood was collected and stored at 4°C in a bottle containing EDTA. Genomic DNA was extracted as soon as possible following sampling using the standard salting out method (Miller et al., 1998). The quality of the extracted DNA was then assessed using a NanoDrop spectrophotometer (NanoDrop Technologies, Inc., Wilmington, DE, USA). Samples were frozen at -20°C until further analysis.

IGF-1 rs5742612 gene polymorphism genotyping

The PCR restriction fragment length polymorphism assay was used to detect the IGF-1 polymorphisms. The 410 bp DNA fragment of the IGF-1 gene was amplified using the specific primers (forward 5':GACAGTGACAGGCAGCCTAGT 3' and reverse 5': CTGGGCATGAAGACACAAACG 3') which were designed based on IGF-1 gene were used for PCR. PCR condition and program was the initial denaturation was carried out at 94°C for 10 min, then, the reaction was as follows: 33 cycles of 95°C for 45 sec, 63°C for 40 sec, 72°C for 40 sec, followed by a final extension at 72°C

for 10 min. The PCR products were digested by *BselI* endonuclease enzyme in 55°C at overnight. In order to observe the digested fragments, the RFLP solution was sepa¬rated on a 3% agarose gel and stained with ethidium bromide.

Statistical analysis

Hardy-Weinberg equilibrium (HWE) was tested using a goodness-of-fit χ^2 test. For estimate adjusted and unadjusted odds ratio (OR) Unconditional logistic regression analysis was performed and 95%confidence interval (CI) as a major of association of the genotypes with the risk of gastric cancer. OR and 95% CI were adjusted for age and sex state. By chi-square test relation between genotype and clinic pathology were examined in gastric cancer patients.

Results

In the present study, we evaluated effect of polymorphisms rs5742612 at insulin like growth factor -1 gene with risk of gastric cancer. The frequency distribution of selected characteristics of 100 colorectal cancer patients and 141 healthy controls are shown in Table 1. Ages ranged from 37-82 years for cases and 19-92 years for controls. The mean age of GC patients and controls respectively were 48.46 (Standard deviation, SD±16.63) and 63.66 (SD±12.39) years. There were no significant differences between the GC cases and controls in the terms of diabetes, hypertension and BMI (P=0.27, P=0.51, P=0.1 respectively). However, all of these variables were adjusted further for any residual confounding effect in later multivariate logistic regression analyses. The genotype and allele frequencies of IGF-1 polymorphisms rs5742612 for the controls and the cases are shown in Table 2. The frequencies of the CC, CT, and TT genotypes were 97%, 3%, and 0%, respectively, among the cases, and 97.9%, 2.1%, and 0%, respectively, among the controls. Genotype distributions in controls and cases were consistent with the Hardy-Weinberg equilibrium (HWE). Our results showed CC genotype was the most genotype in controls and patients in comparison to other genotypes. Also TC genotype was similar in healthy controls and gastric cancer patients (p=0.67) and we did not find any TT genotype in all of the case and control groups. The frequencies of C and T alleles were 98.9% and 1.1% in controls and 98.5% and 1.5% in patient respectively. Statistical analysis revealed no significant difference in the allele frequency between case and control individuals (p=0.672). In this SNPs analyzed, the P value is larger than 0.05. In addition to these, the distributions of clinicopathologic variables such as grade, TNM, are shown in Table 3. According to histological differentiation of tumor grades, 17%, 9%, and 12%, patients were classified in poor, moderate, or well grade, respectively. Tumor grade distribution was such that poor grade status was more common (17%) than others. Besides, patients were grouped into four classes from I to IV regarding tumor node metastasis (TNM) at the time of diagnosis, 4%, 8%, 22%, and 8% respectively. No significant association was found between rs5742612 of IGF-1 gene and risk of gastric cancer (Table 2).

Table 1. Characteristics of Gastric cancer Patient and Control Groups

Characteristics	Patients (n=100)			Controls (n=141)			P[a]
	N	%	Mean(SD)	N	%	Mean(SD)	
BMI			24.60±3.63			21.07±2.99	0.1
Diabet							0.2
Yes	4	44.4		5	55.6		
No	47	27.5		124	72.5		
Blood pressure							0.51
Yes	2	16.7		10	83.3		
No	49	29.2		119	70.8		

*[a]p>0.05 for differences between patients and controls

Table 2. Allele and Genotype Distribution of rs5742612 among GC Patients and Healthy Control Subjects

Genotypes	Controls n=141 (%)	Gastric patients n=100 (%)	Adjusted OR (95%CI)	p
CC	3 (50)	3 (50)	1Ref	0.67
TC	138 (58.7)	97 (41.3)	1.423 (0.281-7.198)	
Allele				
C	297 (98.9)	197 (98.5)	1Ref	0.672
T	3 (1.1)	3 (1.5)	0.706 (0.141-3.535)	

Table 3. Association between IGF-1 Genotypes and Clinicopathological Characteristics

Characteristics	N(patients=100)	%	Genotype		P
			CC	tc	
Tumor Grade					0.79
Well	12	12%	11	1	
Moderate	9	9%	9	0	
Poor	17	17%	16	1	
Not differentiation	12	12%	12	0	
unknown	50	50%	49	1	
TNM stage					0.59
I	4	4%	4	0	
II	8	8%	8	0	
III	22	22%	22	0	
IV	8	8%	8	0	
Unknown	58	58%	55	3	

Discussion

In order to find potential predisposing risk factors of GC, we selected an important polymorphism of IGF-1 gene and investigated whether the rs5742612 variant could have an effect on susceptibility and progression of GC risk in an Iranian population. We also investigated the association of this polymorphism with clinicopathological features include of TNM stage, tumor grade and demographic feathers such as diabetes, blood pressure, ethnicity and body mass index (BMI). We found, for the first time, that variant genotype of rs5742612 were not associated with risk of GC. It is well-known that IGF-1 has significant roles involved in the regulation of epithelial cell growth, proliferation, transformation, apoptosis and metastasis (Delafontaine et al., 2004). The majority of studies investigation correlation between circulating levels of IGF-1 and risk of cancers, but the association of IGF-1 single nucleotide polymorphism with cancer risk has been limited. IGF-1 polymorphisms and

elevated serum levels of IGF-1 have been associated with an increased risk of several cancers, including prostate (Wolk et al., 1989; Cheng et al., 2006; Chan et., 2009; Tong et al., 2010), colorectal (Ma et al., 1999; Feik et al., 2010), ovarian (Lukanova et al., 2002; Lukanova et al., 2003) and breast (Setiawan et al., 2006). In some papers showed Polymorphism rs5742612 significantly associated with various types of malignancies (Nakao et al., 2011; Qian et al., 2014). The functional consequence of SNP at rs5742612 is still unclear. Although this variant does not cause any amino acid changes itself, it may have a regulatory function or may be rs5742612 variation linkage disequilibrium with other functional polymorphisms that is lead to alter in IGF-1expression level. This is supported by previous report, which showed that genetic variants of rs5742612 were associated with 10% or greater difference in IGF-1 level (D'Aloisio et al., 2009). According to our knowledge there has been just only one research about the association between the rs5742612 and susceptibility or progression of gastric cancer in the literature so far (Miller et al., 1998). In 2014, Qian and et al. (2014) show there were associated with an elevated risk of Prostate cancer and rs5742612 polymorphism. In a study of Nakao and colleagues conducted on the relation between rs5742612 and pancreatic cancer, they found association of rs5742612 polymorphism and risk of pancreatic cancer among over-weight subjects (Nakao et al., 2011). Other studies showed that inconsistent relationships of this SNP and colorectal cancer such as Arkani and colleagues reported that no evidence of an association between rs5742612 and colorectal cancer (Arkani et al., 2012) but Wong and et al. in 2005 show a significant association between rs5742612 and colorectal cancer (Wong et al., 2005). Whereas in a study in the Germany did not found any significant relation between IGF-1 variant and colorectal cancer risk (Tsugane and Inoue, 2010). In line with a previous study in Iranian population, we found CC genotype is dominant and there is a low frequency of risk allele in our population (Arkani et al., 2012). We also investigated the association of this polymorphism with clinicopathological and demographic feathers but because of a low frequency of mutant allele there were not significant differences in the genotype distribution and allele frequencies between the patients and control groups of the all putative parameters. The significance of our findings is that in contrast to other Asian populations which have the higher frequency of risk allele; in Iranian population risk allele is a rare (Nakao et al., 2011; Shitara et al., 2011; Qian et al., 2014). Our finding might indicate the peculiar genetic profile of Iranian population in comparison to other population. In conclusion, according to our research, IGF-1 rs5742612 C/T is not associated with the lifestyle factors, susceptibility and progression of gastric cancer in Iranian population, which is the first data for the contribution of the IGF-1 gene in gastric cancer. The evidence from our data and from previous report (Arkani et al., 2012) suggested that rs5742612 variant is uncommon in Iranian population and it may not be a powerful genetic predisposing biomarker for prediction GC clinicopathological features. It is more beneficial to identify other genetic variants in IGF-1 gene especially

in regulatory regions .However, further studies with sufficiently larger population are required to validate this result and clarify the unsolved issue.

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