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Investigating the Distribution of *ERCC2* (rs13181) Gene Polymorphism in Gastric Cancer Patients in Mazandaran: A Case-control Study

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Abstract

Gastric cancer is one of the most common cancers of the gastrointestinal tract in the world, which also has a high prevalence in Iran. *ERCC2* gene is considered one of the major genes related to gastric cancer. The present study aimed to investigate the relationship between rs13181 polymorphism of *ERCC2* gene. The polymorphism in the promoter region of *ERCC2* gene can affect the activity of this gene and thus the susceptibility to gastric cancer. In this case-control study, 81 patients and 75 healthy individuals were recruited. Five ml of the peripheral blood of individuals were collected in EDTA-containing tubes. Genomic DNA was extracted using the Salting-out method and polymorphism was genotyped using the PCR-RFLP method. The prevalence of rs13181 (G>T) genotype and polymorphism allele were significantly different between patients with gastric cancer and control group (both p <0.0001). The frequency of T allele in the gastric cancer group was 54% and in the control group was 18%, and this allele was significantly associated with the risk of gastric cancer (OR= 5, CI 95%= -0.9611 to 0.5138). Regarding the association of TT genotype with the studied disease, the results of this study introduce TT genotype as a protective factor in the development of disease (OR= 114, 95% CI= 6.7909 to 1926.2942).Our results confirmed the association between rs13181 polymorphism of ERCC2 gene and the increased risk of gastric cancer in Mazandaran province.

Key words: Polymorphism; Adenocarcinoma; *ERCC2* gene; PCR-RFLP

Introduction

Gastric cancer is the second most common cause of mortality due to malignancy worldwide, which has an epithelial origin and is caused by genetic and environmental factors (Oshima *et al.*, 2014; Liu *et al.*, 2015). Gastric cancer is currently one of the lethal cancers in the world due to the high incidence, poor prognosis, and restriction therapies (Colagar *et al*., 2011). Most patients are diagnosed in advanced stages, and despite the decreasing incidence of mortality and progress in the treatment of diseases, surgery is the only promising treatment. However, even after surgery, 5-year-old survival is about 30%, and most patients die of metastasis (Dicken *et al.*, 2005; Dragovich and Campen, 2009). The incidence of gastric cancer is very diverse throughout the world, with more than 10 in 100,000 in Japanese men; higher prevalence is observed in countries such as China, Japan, Southern and Eastern Europe, Southern and Central America and lower prevalence in South Asia, North and East Africa and North America (Li *et al.*, 2012). As with other cancers with the epithelial origin, the risk of gastric cancer increases with age, with the highest incidence of gastric cancer at age 75 (Siadati *et al.*, 2012).
ERCC2 protein is an important part of the TFIIH transcription factor, which plays a crucial role in

NER nuclear elimination, located on the 19q13.32 chromosome (Yu-Zhe *et al*., 2015; Zheng *et al*., 2015). Among the well-known DNA repair pathways, the nucleotide excision repair (NER) pathway is an important mechanism that maintains genomic integrity by removing DNA bulky lesions or interstrand adducts induced by exogenous and/or endogenous factors (Wu *et al*., 2005; Neumann et al., 2005; Damandan and Moradpour Hesari, 2016). SNPs in exons of DNA repair genes may influence their protein activity, resulting in differences of individual NER and DNA repair capacity that may affect the susceptibility of diseases. The common in exons of ERCC2 gene is at codon 751 (G> T substitution at nucleotide position 35931, exon 23, Lys> Gln, rs13181) and codon 312 (G> A substitution at position 23951, exon 10, Asp> Asn, rs1799793 (Majumder *et al*., 2007; Kiettubthew *et al*., 2006).

Regarding the high prevalence of advanced non-operable gastric adenocarcinoma and low response of patients to conventional treatments (surgery, and radiotherapy and chemotherapy) in Mazandaran, this study was conducted for the first time to determine ERCC2 polymorphisms in genomic DNA extracted from peripheral blood leukocytes of patients with gastric cancer and healthy patients. Identifying the genetic polymorphisms of gastric cancer patients, in Addition, to help to recognizing the mechanism of the disease, is effective on diagnosis and screening patients who are prone to the disease and, therefore, preventing them.

Cancer is currently spreading around the world, and considering the role of genetic factors in the development of gastric cancer, examination of the genes associated with this disease is necessary. Therefore, the present study aimed to investigate the distribution of ERCC2 (rs-13181) gene polymorphism in patients with gastric cancer who referred to Mazandaran province clinics during 2016.

Materials and methods

Study samples

In this case-control study, 156 subjects including 81 patients and 75 healthy subjects (controls) participated. Five milliliter peripheral blood of patients with known gastric adenocarcinoma, based on the results of endoscopy and pathology, were taken after obtaining informed consent.

DNA extraction

DNA was extracted from blood samples by the salting-out method. After extraction, the quality and quantity of the extracted DNA were measured by the spectrophotometer. Then, DNA samples were stored at -20°C (Shokrzadeh *et al.*, 2017a; 2017b).

Genotyping

The genotype was determined by polymerase chain reaction enzymatic digestion by PCR-RFLP. Amplification was performed using a specific primer pair and a piece of 267 bp. Primers were designed using Gene Runner software. The sequence of primers is listed in table 1. 25 µl Polymerase Chain Reaction (PCR) containing 2 µl genomic DNA (100ng/µl), 12.5 µl Master mix PCR, and 1 µl (10 picomol) of each primer (Table 1) which ultimately reached 25 µl with distilled water. The PCR reaction program was as follows: 95°C for 5 minutes, then 36 cycles: 95°C for 30 seconds to split two DNA strands, 57.5°C for 30 seconds to coupling the primer to the DNA, and 72°C for 20 seconds to elongate. After completing 36 cycles, the reaction mixture was kept for 10 minutes at 72°C until final elongation. Then, to evaluate the quality of the PCR product, each sample was evaluated by 2% agarose gel electrophoresis. The restriction enzyme for rs13181 polymorphism of *ERCC*2 gene was Earl Enzyme. To determine the genotype, 10 µl of the PCR product was digested with Earl Enzyme at 37°C for 16 hours. Then the product of enzyme digestion was electrophoresed on 2% agarose gel and photos were taken by Gel Doc (Tafrihi *et al.*, 2014).

Statistical analysis

In order to interpret the results of laboratory tests, quantitative (numerical) parameters are required. In this research, statistical analyses were performed using Medcalc software ver. 21.

Table 1. Characteristics of the primer used and restriction enzyme

Name	Primer sequence (5'→3')	Tm(°C)	PCR product (bp)
F primer *ERCC* R primer *ERCC*	5'-CCTGGTGGATAGCT6CCT-3' 5'-TGTTCTCTGCAGGAGGATCA-3'	57	267

Results

Amplification and genotyping

PCR was performed using specific primers for Amplification of *ERCC2* gene parts. All extracted DNAs from both case and control groups produced a single-bonded PCR product without any other non-specific bands. To investigate the genotype and screening for the *ERCC2* allele, PCR-RFLP method was developed using the Earl restriction enzyme. Then PCR-RFLP products were analyzed by electrophoresis on 2% agarose gel.

Frequency of Genotypes

In this case-control study, 156 patients (81 patients and 75 healthy individuals) were studied. The results of the experiments showed that among 81 patients, 33 (41%) had GG genotype, 9 (11%) had TG genotype, and 39 (48%) had TT genotype. Among 75 healthy controls (control group), 48 (64%) had GG genotype, 27 (36%) had TG genotype, and 0 (0%) had TT genotype. The observed difference was significant between the healthy and the patient groups according to the results of chi-square test (p= 0.0001, $\chi 2$= 50262). In this study, the distribution of T/T genotype was significantly different (p= 0.0010) according to OR (OR= 11.3731, CI 95% 6.7909-19.26.2942). These results suggest that this genotype (T/T) increases the risk of disease and is considered a risk factor (Table 2).

Frequency of Alleles

The results of the experiments showed that among the patients, the frequency of G and T alleles were 46.5% and 53.5%, respectively, and in healthy subjects, 82% and 18%, respectively. Considering the result of the chi-square test (p = 0.001, 584/26$\chi 2$ =), since the P-value is less than 0.05, there is a significant difference in the allelic distribution of rs13181 polymorphism of ERCC2 gene between the patient and the control groups (Table 2).

In this study, patients with gastric cancer were between the ages 22-90 years and their sex distribution is described in the table below (Table 3).

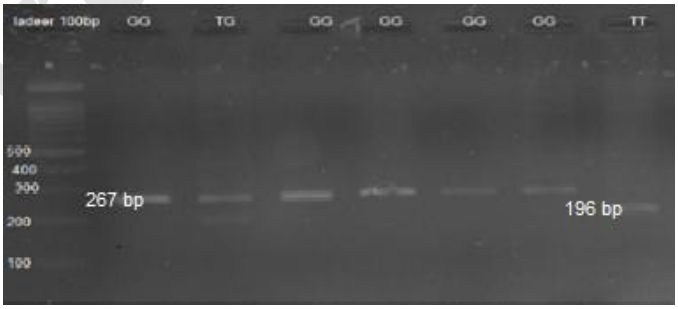

Fig. 1. RFLP results of rs13181 polymorphism of *ERCC2* gene. The presence of two 70 and 196 bands represents TT genotype, the presence of triple bands of 196, 267, and 70 represent TG genotype, and the presence of a 267 bp band represents GG genotype, which in turn represent homozygous, mutant, homozygote, heterozygote, and Wild-type respectively.

Table 2. Genotypic and allelic frequency of rs13181 gene ERCC2

Genotype	Patient (%) N=95	Healthy (%) N=90	OR (95% CI)	P-value
GG	33 (41)	48 (64)	1	-
TG	9 (11)	27 (36)	0.4848 (0.2021-1.1631)	0.01049
TT	39 (48)	0 (0)	114 (6.7909-19.26.2942)	0.0010
TT + TG	-	-	2.5859 (1.3538-4.9391)	0.0040
G	46.5%	% 82	1	-
T	53.5%	% 18	0.2222 (0.09611-0.5138)	0.0004

Chi-square test (X^2) and Odds Ratio (OR) were used, when indicated, as well as Confidence Interval (CI), and P-value.

Table 3. The demographic information of gastric cancer patients.

City (No.)	Male (No.)	Female (No.)
Sari (N=44)	28	16
Ghaemshahr (N=13)	10	3
Behshahr (N=3)	3	-
Neka (N=13)	11	2
Juybar (N=3)	1	2
Mahmoud Abad (N=1)	1	-
Galugah (N=1)	1	-
Surak (N=2)	1	1
Savadkuh (N=1)	1	-
Total (N=81)	57	24

Discussion

Gastric cancer is the most common type of gastrointestinal cancer with a high incidence in the north of the country. In Iran, unlike western countries and Japan, the incidence of gastric cancer has increased over the past two decades. It is suggested that genetic susceptibility plays an important role in the development of gastric cancer (Hengartner 2000).DNA repair systems are important for protecting against mutations and are necessary for maintaining the integrity of the genome. Many identified DNA repair genes are recognized to have genetic variations in humans (Debniak *et al.*, 2006; Mirmohammadrezaei, 2015).

DNA repair gene polymorphisms may alter the protein function. They can also cause reduction in DNA repair capacity, which may result in genetic instability and carcinogenesis (Berwick and Vineis, 2000; de Boer, 2002). DNA damage influences mitosis and the isolation of chromosome, which can be solved by homologous recombination repair (HRR) (Thacker, 1999).

HRR is a pivotal pathway to repair the DSBs and maintain the genetic stability (Tambini *et al*., 2010). *XRCC2* is involved in the HRR pathway and associated with DNA DSB repair and genomic stability (Tambini *et al*., 2010; Thacker, 2005). ERCC2 is one of the seven nucleotide excision repair enzymes. *ERCC2* could cause Xeroderma pigmentosum when mutated in germ line. *ERCC2* is involved in DNA repair, specifically in nucleotide excision repair. It functions in various types of DNA lesions (Monteiro *et al*., 2014). *ERCC2* gene is located on the 19q13.32. Patients with XP syndrome have a higher susceptibility to a series of cancers, including gastric cancer. The mutation in this gene results in damage to the DNA in human cells due to a defect in the nucleic excision repair enzyme (NER).

In this study, distribution of *ERCC2* (rs13181) gene polymorphism in patients with gastric cancer in Mazandaran province was investigated by the PCR-RFLP method using restriction enzyme activity. In a study by Chu et al., they found that *ERCC1* and *ERCC2* are two important proteins on NER pathway. The results showed that subjects with *ERCC2* TG/G (rs13181) genotype have reduced mortality risk compared with those of TT genotype, and this protective effect was more pronounced among the non-cardia subgroup of patients with gastric cancer and tumor size of ≥5 cm. In general, *ERCC2* polymorphism (rs13181) can play different roles in the survival of gastric cancer (Chu, et al., 2013).

In another study by Zhang *et al*., they determined by PCR reaction that *ERCC1* (rs3212986) and *ERCC2* (rs3212986) genotype has a low speed regarding the complete and partial improvement of patients with gastric cancer through chemotherapy (OR=0.19. 95%

CI=0.06-0.60). They also found that AA genotype in *ERCC1* is associated with high risk of mortality in patients with gastric cancer (HR= 1.60, 95% CI: 0.81-3.16) (Zhang *et al*., 2016).

Joe and Ing studied the role of *ERCC1* rs3212986 and *ERCC*2n rs13181 gene polymorphisms in the development of breast cancer. Unconditional logistic regression analyses showed that TT genotype in rs3212986 is associated with a higher risk of breast cancer compared to GG type (Zhao and Ying, 2015).

Salong *et al*. investigated the genotype for *ERCC2* and *ERCC1* polymorphisms and observed that TT genotype and T allele of *ERCC1* increased pancreatic cancer, compared with GG genotype. In addition, GG genotype and G allele of ERCC2 increased the risk of pancreatic cancer compared with TT genotype (Sileng *et al*., 2016).

Lee *et al.* expressed the relationship between rs13181 and smoking while studying *ERCC2* polymorphisms, ESCC risk, and the effect of double gene smoking on ESCC (esophageal squamous cell carcinoma) risk on a Chinese population, using a 2-position model (P = 0.001) containing rs13181 and smoking. Totally, the model has a cross-validation compatibility of 10/10 and reliability of 62.17%. It was also found that smokers with AC or CC genotype have a higher risk of ESCC compared with non-smokers with AA genotype (OR = 3.16) (Li *et al*., 2017).

Zhang *et al.* examined the role of SNPs in ERCC2 and ERCC1 genes in patients with osteosarcoma treated with cisplatin. PCR-RFLP was performed to evaluate polymorphisms of rs3212986, rs11615 ERCC1, rs13181 and rs1799793 ERCC2 genes. Eventually, they found that CC genotype in rs11615 ERCC1 had a better response to chemotherapy (OR= 2.87) (Zhang *et al.*, 2015).

Conclusion

The results of this study indicate that ERCC2 (rs-13181) gene polymorphism is associated with adenocarcinoma disease, and screening for ERCC2 (rs-13181) polymorphism can be used to prognosticate disease, prevent disease progression, and to use appropriate therapeutic approaches to increase longevity and improve quality of life in patients. It can help with stomach adenocarcinoma.

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