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Association of rs12108497 (*Caspase 3* Gene) Polymorphism in Gastric Cancer Patients in Sari

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Abstract

Gastric cancer is the fourth most common cause of malignancy death in the world, which also has a high prevalence in Iran. *Caspase 3* gene is considered as one of the major genes related to gastric cancer. The present study aimed to investigate the relationship between rs12108497 polymorphism of *Caspase 3* gene. Due to the fundamental role of caspases, we have considered an SNP of *Caspase 3* (common to two-way joint execution caspases), which has been proven to be related to various cancers. In this case-control study, 95 patients and 90 healthy subjects were enrolled. The patient status was approved, by endoscopy and pathology, to be gastroduodenal adenocarcinoma. Overall, 5 ml of the peripheral blood was collected from all participants, after obtaining an informed consent. Genomic DNA was extracted using the salting-out method and genotypic polymorphism was investigated using the PCR-RFLP method. The frequency of rs12108497 polymorphism was significantly different between patients with gastric adenocarcinoma and healthy subjects ($p = 0.0259$). Also, the difference in the distribution of T/T genotype was significant ($P = 0.0111$) and according to the OR score ((95.1% CI = 1.3539-10.5412) and OR = 3.7778), it is suggested that the T/T genotype increases the risk of disease. Our results confirm the association between the rs12108497 polymorphism of *Caspase 3* gene and the increased risk of gastric adenocarcinoma in individuals referring to Toba Sari Clinic.

Key words: Adenocarcinoma; rs12108497 polymorphism; *Caspase3* gene; PCR-RFLP

Introduction

Gastric cancer is the most common gastrointestinal cancers, which is very common in the northern parts of Iran. In Iran, unlike in Western countries and Japan, the incidence of gastric cancer has been rising over the past two decades. It is suggested that genetic susceptibility plays an important role in the progression of gastric cancer. A defect in the pathway of Apoptosis may lead to the accumulation of immortal cells and can lead to many human disorders including cancer (Hengartner *et al.*, 2000). Caspases are members of the cysteine proteases family and play an important role in the regulation and implementation of apoptosis. In general, there are two distinct but converging pathways for activating caspases (Ashkenazi *et al.*, 1999; Li *et al.*, 1997). An external path or receptor-dependent and intrinsic or mitochondrial pathway, each of which has independent groups of initiator caspases (Kurokawa *et al.*, 1999; Scorrano *et al.*, 2003), but they use the same groups of executable caspases (Loo *et al.*, 2002). Several studies have suggested that caspases have been associated with human cancers, including gastric cancer.

The most common form of human genetic differences is Single Nucleoid Polymorphisms (SNPs). Several studies have been suggested that some differences in the pathways of apoptosis are associated with susceptibility to several different cancers. In a study investigated the association of *caspase 3* and *caspase 7* polymorphisms and the risk

of cancer (Yan *et al.*, 2013). Due to the fundamental role of caspases, we have considered an SNP (rs12108497) of *caspase 3* (related to common two-way executable caspases), which has been proven to be related to various cancers.

Regarding the high incidence of non-surgical gastric adenocarcinomas and the low response of patients to conventional treatments (surgery, radiotherapy, and chemotherapy) in Mazandaran, this study was planned for the first time to determine the caspase 3 polymorphisms in the genomic DNA extracted from peripheral blood leukocytes, in patients with gastric cancer and healthy individuals. Identifying genetic polymorphisms in people with gastric cancer, in addition to helping to recognize the mechanism of the disease, is effective in identifying and screening people who are prone to illness and as a result of their prevention.

Table 1. Characteristics of the primers used and the restriction enzyme

Name	Primer sequence	Tm (°C)	Restriction enzyme
Forward primer	5'-CCAGGCTAGAAATAACCAGG-3'	56	**StuI**
Reverse Primer	5'-GGATCGGTAGGGCAGTGTA-3'		

Materials and methods

Case study and samples

In this case-control study, 95 patients and 90 healthy controls participated. Five ml of peripheral blood of known gastroduodenal adenocarcinoma patients is obtained based on the results of endoscopy and pathology after obtaining informed consent (Shokrzadeh *et al.*, 2017; Saleh Tabari *et al.*, 2018).

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.*, 2017).

Genotyping

Genotype determination was performed by the PCR-RFLP method. Amplification was performed by using a specific primer pair and a DNA piece with 619 bp length was produced. The gene (rs12108497) sequences were obtained from the NCBI database and primers were designed using the Gene Runner software (V6.5.46). The sequence of primers is listed in Table 1. Polymerase chain reaction (PCR) mixture consisted of 2 µl of genomic DNA (100ng/µl), 12.5 µl of PCR Master mix and 1 µl of the above-mentioned primers, adjusted with distilled water to final volume of 25 µl.The PCR reaction schedule was as follows: 95 ° C for 5 minutes, then 35 cycles: 95 ° C for 30 seconds, 56 ° C for 30 and 72 ° C for 30 seconds. After completion of 36 cycles, the reaction mixture was left at 72°C for 10 minutes. Then, to evaluate the quality of the PCR product, each of the samples was evaluated by 1.5% agarose gel electrophoresis. The Restriction enzyme for gene polymorphism (rs12108497) *caspase 3* is the StuI enzyme. To determine the genotype, 10 µl of the PCR product was digested with StuI enzyme at 37 ° C for 2 hours. Then the enzyme digestion was electrophoresed on 1.5% agarose gel and photos were taken by GelDoc.

Statistical analysis

In order to interpret the results of laboratory research, quantitative (numerical) parameters are required. In this research, statistical analyses were performed using Medcalc software Ver.21. Chi-square test (X2) and odds ratio (OR), as well as parameters such as Confidence Interval (CI) and P-value, are also determined.

Results

Amplification and Genotyping: Polymerase Chain Reactions (PCR) were performed using specific primers. The PCR reaction produced a single specific band for all samples (both cases and controls). PCR-RFLP was developed using the StuI restriction enzyme to examine the genotype and screen for the alleles of the given SNP in the *Caspase 3* gene. Then PCR-RFLP products were

analyzed by electrophoresis on 1.5% agarose gel (Fig. 1).

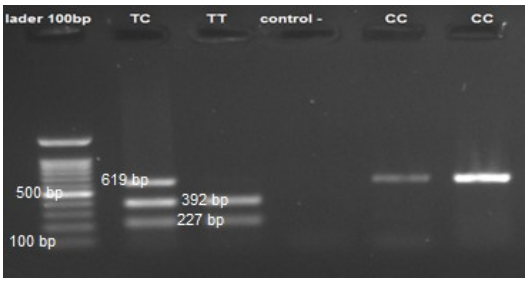

Fig. 1. RFLP results for *Caspase 3* gene rs12108497 polymorphism.

The presence of a 619 bp band is a sign of the CC genotype, the existence of two bands of 392 and 227 base pairs is a sign of the TT genotype and the existence of a triple band of 619, 392, and 227 bp is a sign of the CT genotype that representing wild-type homozygous, mutant homozygous and heterozygotes, respectively.

Genotypic Frequency

In this case-control study, 185 subjects (95 patients and 90 healthy individuals) were studied. The results of the experiments showed that among 90 controls 6 (7%) had TT genotype, 36 (40%) had TC genotype and 48 (53%) had CC genotype. Among the 95 patients, 17 (18%) had TT genotype, 42 (44%) had TC genotype, and 36 (38%) had CC genotype. The observed difference was significant between the healthy subjects and the patient according to the chi-square test ($p = 0.0256$, $\chi2 = 7.307$). In this study, the distribution of T/T genotype was statistically significant ($p = 0/0111$) and according to the OR score (95 % CI =1.3539 to 10.5412 and OR = 3.7778), this results suggest that the genotype (T/T) increases the risk of the disease and is a risk factor (Table 2).

Allele Frequency

The results of the experiments shown that the frequency of T and C alleles was respectively 40% and 60%, and among healthy subjects, was 27% and 73%, respectively. Considering the results of chi-square test ($p = 0.0722$, $\chi2 = 3.232$), because P-value is not less than 0.05, there is not a significant difference in the distribution of allele polymorphism rs12108497 of CASPASE3 gene between two groups of patients Control.

Table 2. Genotypic polymorphism rs12108497 of *Caspase 3* gene

Significance level	OR(95%CI)	Healthy(%) 90 person	Patient(%) 95 person	Genotype
-	1	(53)48	(38)36	CC
P=0.1628	1.5556(0.8365 to 2.8929)	(40)36	(44)42	CT
P=0.0111	3.7778(1.3539 to 10.5412)	(7)6	(18)17	TT
P=0.0358	1.8730(1.0425 to 3.3652)	-	-	CT+TT

Discussion and Conclusion

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 (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 (Shokrzadeh *et al.*, 2017).

In a study by Deng *et al.,* on caspase-3 polymorphism, it was reported that rs12104897 SNP is more prevalent among smokers with HCC in Chinese population (Deng *et al.*, 2016). In a similar study, Fang *et al.* (2016) investigated the association between *Caspase 3* polymorphisms and lumbar disc herniation (LDH). They found that rs4647693, rs4647610, rs12108497 alleles increase the susceptibility to LDH (Fang *et al.*, 2016). A little earlier, Li and colleagues revealed that rs4647693, rs12104897, rs4647610 alleles have a statistically significant difference in distribution between healthy and patient with gastric cancer individuals (Bing *et al.*, 2014). On the other hand, in a study on Non-Small-cell lung cancer by Yoo and colleagues, it seemed that Caspase 3 polymorphisms did not have any effect on cancer predisposition (Yoo *et al.*, 2009). Wu *et al.* in an investigation about the association of *caspases 3*, *8*, and *9* genes polymorphisms with colorectal cancer

(CRC) progression also achieved similar results (Wu *et al.*, 2013). In a systematic study, the association of *caspase 3* and *caspase 7* polymorphisms and the risk of cancer were investigated. The results indicated that SNPs rs2705897, rs4647603, rs12415607, rs2227310, rs3124740 have a significant association with the risk of cancer (Yan *et al.*, 2013). Chen and colleagues also found that allele (rs4647603: G>A) CASP3 was not associated with the risk of SCCHN (squamous cell carcinoma of the head and neck) (Chan *et al.*, 2008). In a study by Mittal *et al.* on different caspases in people with prostate cancer, it is showed that rs4647603: G>A polymorphism increases the incidence of prostate cancer and affects its survival. Moreover, polymorphisms in this area may provide useful markers for predicting prognosis in patients undergoing surgery (Mittal *et al.*, 2012). In a study by Jang and his colleagues in 2008 on lung cancer, it was shown that *caspase 3* polymorphism (928A > G, 77G > A, and 17532A>C) reduced the risk of lung cancer (Jang *et al.*, 2008).

The results of the current study indicate that Caspase 3 gene allele rs12108497 is associated with adenocarcinoma. This finding can also be useful for determining the appropriate therapeutic approaches to increase longevity and improve the quality of life of patients with adenocarcinoma. It should be noted that this result is limited to the population studied and more studies are needed on a larger number of individuals and races to confirm the association of rs12108497 polymorphism with adenocarcinoma.

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References

Ashkenazi A, Dixit VM. 1999. Apoptosis control by death and decoy receptors. *Curr Opin Cell Biol* 11(2): 255-260.

Chen K, Zhao H, Hu Z, Wang LE, Zhang W, Sturgis EM, Wei Q. 2008. CASP3 polymorphisms and risk of squamous cell carcinoma of the head and neck. *Clin Cancer Res* 14(19): 6343-6349.

Deng B, Liu F, Luo L, Wei Y, Li B, Yang H. 2016. CASP 3 genetic polymorphisms and risk of Hepatocellular carcinoma: a case-control study in a Chinese population. *Tumour Biol* 37(7): 8985-8991.

Fang Y, Qiu J, Jiang ZB, Xu SR, Zhou ZH, He RL. 2016. Associations of Caspase-3 gene polymorphism with lumbar disc herniation. *Kaohsiung J Med Sci* 32(11): 552-558.

Hengartner MO. 2000. The biochemistry of apoptosis. *Nature* 407(6805): 770-776.

Jang JS, Kim KM, Choi JE, Cha SI, Kim CH, Lee WK, Kam S, *et al*. 2008. Identification of polymorphisms in the Caspase-3 gene and their association with lung cancer risk. *Mol Carcinog* 47(5): 383-390.

Kurokawa H, Nishio K, Fukumoto H, Tomonari A, Suzuki T, Saijo N. 1999. Alteration of caspase-3 (CPP32/Yama/apopain) in wild-type MCF-7, breast cancer cells. *Oncol Rep* 6(1): 33-40.

Li P, Nijhawan D, Budihardjo I, Srinivasula SM, Ahmad M, Alnemri ES, Wang X. 1997. Cytochrome c and dATP-dependent formation of Apaf-1/caspase-9 complex initiates an apoptotic protease cascade. *Cell* 91(4): 479-489.

Li B, Liu H, Gong F, Sun P, Yan Y, Jia B. 2014. Molecular epidemiologic correlation analysis between caspase3 gene polymorphism and gastric cancer susceptibility. *Cell Biochem Biophys* 70(3): 1647-1653.

Liu ZF, Aikenmu ALJ, Zhao J, Meng QC, Fang R. 2015. Influence of ERCC2 gene Polymorphisms on the Treatment outcome of osteosarcoma. *Genet Mol Res* 14(4):12967-12972.

Mittal RD, Mittal T, Singh AK, Mandal RK. 2012. Association of caspases with an increased prostate cancer risk in north Indian population. *DNA Cell Biol* 31(1): 67-73.

Saleh Tabari Y, Bakhshi O, Mohammadpour A, Hoseini V, Haghi Aminjan H, Shokrzadeh M. 2018. Evaluation of NQO1 Polymorphism in Gastric Adenocarcinoma. *J Mazandaran Univ Med Sci* 27(157): 230-235.

Scorrano L, Korsmeyer SJ. 2003. Mechanisms of cytochrome c release by

proapoptotic BCL-2 family members. *Biochem Biophys Res Commun* 304(3): 437-444.

Shokrzadeh M, Mohammadpour A, Tabari YS, Parvizi Almani S. 2017. Investigating the Distribution of ERCC2 (rs13181) Gene Polymorphism in Gastric Cancer Patients in Mazandaran: A Case-control Study. *J Genet Resour* 3(1): 54-60.

Shokrzadeh M, Fattahi I, Mohammadpour A, Mashhadban AH. 2017. Presence of CagA Gene and Its Antibiotic Resistance Pattern in Helicobacter Pylori Isolates. *J Mazandaran Univ Med Sci* 27(154): 60-72.

van Loo G, Saelens X, Van Gurp M, MacFarlane M, Martin SJ, Vandenabeele P. 2002. The role of mitochondrial factors in apoptosis: a Russian roulette with more than one bullet. *Cell Death Differ* 9(10): 1031-1042.

Wu Z, Li Y, Li S, Zhu L, Li G, Yu Z, Zhao X, et al. 2013. Association between main Caspase gene polymorphisms and the susceptibility and prognosis of colorectal cancer. *Med Oncol* 30(3): 565.

Yan S, Li YZ, Zhu XW, Liu CL, Wang P, Liu YL. 2013. HuGE systematic review and meta-analysis demonstrate association of CASP-3 and CASP-7 genetic polymorphisms with cancer risk. *Genet Mol Res* 12(2): 1561-1573.

Yoo SS, Choi JE, Lee WK, Choi YY, Kam S, Kim MJ, Jeon HS, *et al*. 2009. Polymorphisms in the CASPASE genes and survival in patients with early-stage non–small-cell lung cancer. *J Clin Oncol* 27(34): 5823-5829.