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Decrease in gastric cancer susceptibility by MTHFR C677T polymorphism in Ardabil Province, Iran

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Background/aims: *Gastric cancer, as the fourth most frequent malignancy worldwide, has the highest rate among cancer-related disorders in Ardabil province, located in North-West Iran. Methylene tetrahydrofolate reductase is one of the cancer susceptibility genes with considerable polymorphisms. Methylene tetrahydrofolate reductase C677T leads to a decrease of about 30% in its product activity and is reported to have an effect on cancer susceptibility.* ***Materials and Methods:*** *Methylene tetrahydrofolate reductase C677T genotyping was carried out by polymerase chain reaction-restriction fragment length polymorphism on peripheral blood DNA from 76 gastric cancer patients and 91 healthy controls. The statistical significance was calculated by logistic regression analysis.* ***Results:*** *The mean age was 64.2±11.1 and 62.1±9.8 years for cases and controls, respectively. Among tumors, 35.5% were diffuse type and others were intestinal. The frequency of genotypes was 61.8%, 32.9%, and 5.3% among cases and 45.1%, 50.6%, and 4.4% among controls for Ala/Ala, Ala/Val, and Val/Val, respectively. CT heterozygotes had lower susceptibility to gastric cancer (p=0.02). This relationship of significance was detected considering gender and age.* ***Conclusions:*** *It was found that T allele has a protective association with age in the Ardabil province.*

Key words: Gastric cancer, methylene tetrahydrofolate reductase, C677T, Ardabil

Ardabil'de MTHFR C677T polimorfizmi ile gastrik kanser yatkınlığının azalması

Amaç: *Dünya çapında en sık görülen 4. malignite olan mide kanseri, Kuzey Batı İran'da bulunan Ardabil bölgesinde en sık görülen kanserdir. Metilen tetrahidrofolat redüktaz, polimorfizmi belirgin olarak görülen kanser yatkınlık genlerinden birisidir. Metilen tetrahidrofolat redüktaz C677T, gen ürününün aktivitesini %30 azaltmakta ve kanser yatkınlığını etkilemektedir.* ***Gereç ve Yöntem:*** *Metilen tetrahidrofolat redüktaz C677T genotiplemesi, 76 gastrik kanserli ve 91 sağlıklı kontrolde polimeraz zincir reaksiyonu-restriksiyon parça uzunluğu polimorfizmi ile periferik kan DNA'sından yapılmıştır. İstatistiksel anlamlılık lojistik regresyon analizi ile hesaplanmıştır.* ***Bulgular:*** *Hastalar ve kontrol grubunda ortalama yaş sırasıyla 64.2±11.1 ve 62.1±9.8 idi. Tümörlerden %35.5'i yaygın tipte diğerleri intestinal tipte idi. Genotip frekansları Ala/Ala, Ala/Val, ve Val/Val genotipleri için hastalar arasında %61.8, %32.9 ve %5.3 ve kontrol grubunda %45.1, %50.6 ve %4.4 idi. CT heterozigotlar, gastrik kanser açısından daha düşük yatkınlığa sahipti (P=0.02). Bu anlamlı ilişki, yaş ve cinsiyet dikkate alınarak bulunmuştu.* ***Sonuç:*** *Ardabil bölgesinde T alelinin yaş ile koruyucu bir ilişkisi olduğu bulunmuştur.*

Anahtar kelimeler: Gastrik kanser, metilen tetrahidrofolat redüktaz, C677T, Ardabil

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INTRODUCTION

Gastric cancer is the fourth most frequent malignancy worldwide and has been known as the second leading cause of cancer-related deaths (1,2). In Iran, there is wide variation in gastric cancer incidence among various areas. Ardabil province is located in North-West Iran, and has been reported to have the highest incidence rate in the country, with an ASR (age-standardized incidence rate) of 49.1 and 25.4 in males and females, respectively (3,4).

The cardia type has been detected in 36% of gastric cancers in Ardabil, which is mentioned as the highest rate recorded anywhere in the world (5). Gastric cancer constituted about 33% of all cancer-related deaths in this area (6,7).

Gastric cancer susceptibility has been proven to be associated with some genes, including TP53, DNA repair system-related genes, interleukins, and methylene tetrahydrofolate reductase (MTHFR). Because of the interaction between genetic and environmental factors and diversities present in different environments, the importance of genetic variations on cancer susceptibility could vary among different populations.

Methylene tetrahydrofolate reductase (MTHFR) is an enzyme involved in the metabolism of folate and methyl groups. Reduction of 5, 10-methylenetetrahydrofolate to 5-methylenetetrahydrofolate is catalyzed by MTHFR and involved in the regeneration of methionine from homocysteine. Therefore, at least two processes are affected by MTHFR activity. First, synthesis of nucleotides for DNA replication, and thus proliferation capacity, and second, DNA methylation associated with SAM, the methyl donor form of methionine as a result of MTHFR activity.

More than 29 mutations resulting in very low MTHFR activity have been described in homocystinuric patients (8). Indeed, two common polymorphisms, including C677T and A1298C, are related to MTHFR low activity. However, they were seen in healthy individuals, and the reduced activity was not demonstrated in homocystinuric patients. Replacement of C by T in exon 4 at nucleotide 677 leads to Ala222Val. Homozygous individuals for T nucleotide have only 30% activity compared to CC individuals, and this level was 65% for CT heterozygotes (9).

MTHFR C677T variation results in reduction in enzyme activity (10,11). Therefore, conversion of homocysteine to methionine is decreased and has been shown by elevated levels of plasma homocysteine (12,13). This change tends to DNA hypomethylation, and thus affects regulation of some genes. On the other hand, reductions in nucleotide synthesis could have effects on decreasing cell proliferation, which could be one of the mechanisms of presenting a low risk for cancer susceptibility, as shown in some previous reports (14-19).

The present report describes a case-control study aimed to assay the effect of MTHFR C677T polymorphism on gastric cancer susceptibility in Ardabil province.

MATERIALS AND METHODS

Samples

Institutional guidelines including ethical and informed consent were followed. Peripheral blood samples from 76 patients with pathologically confirmed primary gastric cancer, resident in Ardabil province, and 91 age-, sex-, and residency-matched healthy controls, without any cancer type-related family history, were selected and studied.

The assumable potential impacts of population stratification bias in the studied participants were estimated as previously described (13).

Genotyping

Genomic DNA from peripheral blood collected in EDTA-coated tubes was extracted using QIAamp Blood Mini Kit (Qiagen Co.). Genotyping was carried out by polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP).

The primers designed to amplify a fragment located on exon 4 of the MTHFR gene were 5'-AGG ACG GTG CGG TGA GAG TG-3' and 5'-TGA AGG AGA AGG TGT CTG GGG GA-3'. The 198 bp amp-

Table 1. General characteristics of participants

	Cases	Controls*
No	76	91
Age	64.2±11.1	62.1±9.8
≤50 Years	9 (11.8%)	9 (9.9%)
>50 Years	67 (88.2%)	82 (90.1%)
Gender		
Male	56 (73.7%)	67 (73.6%)
Female	20 (25.3%)	24 (25.4%)
Tumor type		
Diffuse	27 (35.5%)	
Intestinal	59 (64.5%)	

*Age-, sex-, place of residence-adjusted population

Table 2. Risk of gastric cancer by MTHFR C677T polymorphism

	Males						Females						Young participants (≤50 Yrs.)			
	Frequency		Odds ratio (95% CI)	P value	Frequency		Odds ratio (95% CI)	P value	Frequency		Odds ratio (95% CI)	P value	Frequency		Odds ratio (95% CI)	P value
	Cases (%)	Controls (%)			Cases	Controls			Cases	Controls			Cases	Controls		
Val/Val (TT)	4 (5.3)	4 (4.4)	0.89 (0.20 -3.92)	0.88	1 (1.8)	2 (3.0)	0.52 (0.05 – 5.97)	0.6	3 (15.0)	2 (8.3)	0.68 (0.09 – 5.43)	0.72	0	0		
Ala/Val (CT)	25 (32.9)	46 (50.6)	**0.47 (0.25-0.90)**	**0.02**	20 (35.7)	31 (46.3)	0.6 (0.28-1.26)		5 (25.0)	15 (62.5)	**0.17 (0.04-0.72)**	**0.02**	4 (44.4)	5 (55.6)	0.64 (0.1-4.11)	0.64
Val/Val +Ala/Val	29 (61.8)	50 (45.1)	**0.5 (0.27-0.93)**	**0.03**	21 (62.5)	33 (50.7)	0.59 (0.29-1.23)	0.16	8 (60.0)	17 (29.2)	**0.23 (0.06-0.87)**	**0.03**	4 (55.6)	5 (44.4)	0.64 (0.1-4.11)	0.64
Ala/Ala (CC) (Ref)	47	41	1.00		35	34	1.00		12	7	1.00		5	4	1.00	
	Older participants (>50 Yrs.)															
	Frequency		Odds ratio (95% CI)	P value	Frequency		Odds ratio (95% CI)	P value	Frequency		Odds ratio (95% CI)	P value				
	Cases (%)	Controls (%)			Cases	Controls			Cases	Controls						
Val/Val (TT)	4 (6.0)	4 (4.9)	088 (0.21-3.77)	0.86	1 (3.7)	4 (4.4)	0.54 (0.06-5.13)	0.59	3 (6.1)	4 (4.4)	1.16 (0.24-5.64)	0.85				
Ala/Val (CT)	21 (31.3)	41 (50.0)	**0.45 (0.23-0.90)**	**0.02**	7 (25.9)	46 (50.6)	**0.33 (0.13-0.87)**	**0.03**	18 (36.7)	46 (50.6)	0.58 (0.28-1.20)	0.14				
Val/Val +Ala/Val	25 (29.6)	45 (54.9)	**0.47 (0.25-0.9)**	**0.02**	8 (29.6)	50 (54.9)	**0.32 (0.13-0.79)**	**0.01**	31 (42.8)	50	0.63 (0.31-1.26)	0.19				
Ala/Ala (CC) (Ref)	42 (62.7)	37 (45.1)	1.00		19 (70.4)	41 (45.1)	1.00		28 (57.1)	41 (45.1)	1.00					

lified fragment was digested by HinfI (Fermentas Co.). An uncut fragment indicates allele C (coding for Ala amino acid). However, substitution of C by T tends to create a HinfI restriction site. Therefore, alleles T (coding for Val) are observed by 175 and 23 bp digested products. If the quantity of restriction enzyme used is inadequate, the homozygous digested results may be detected as heterozygote. Therefore, some sequenced samples were chosen for evaluating the accuracy of digestion.

RESULTS

The mean age of the patient group was 64.2 years (range, 40-84 years), and there were no statistically significant differences in the distributions of age and gender between cases and controls. Participants of the two groups had the same residency and were born and resided in Ardabil. The mean age of controls was 62.1 years (range, 44-81 years). The characteristics of the participants are presented in Table 1.

Table 2 shows the distribution of MTHFR C677T genotypes and their statistical relationships with sex, age, and tumor type among the case and control groups. The distribution of this polymorphism in the control group was in Hardy-Weinberg equilibrium. The frequency of genotypes for Ala/Ala, Ala/Val, and Val/Val was 61.8%, 32.9%, and 5.3% among gastric cancer cases and 45.1%, 50.6%, and 4.4% among healthy controls, respectively.

Compared with CC homozygotes, CT heterozygotes had lower susceptibility to develop cancer (odds ratio [OR]=0.47; 95% confidence interval [CI]=0.25-0.90). The CT was significantly correlated with a reduced risk in females (OR=0.17; 95%CI=0.04-0.72) and older participants (OR=0.45; 95%CI=0.23-0.90). Among 27 cases with diffuse type tumor, CT genotype conferred a lower risk of

cancer (OR=0.33; 95%CI=0.13-0.87). However, distribution of genotypes among male and young participants and patients with intestinal type tumor showed no significant association (Table 2).

DISCUSSION

The high incidence of gastric cancer in Ardabil province, Iran, encouraged us to follow the predisposition and susceptibility factors, including gene polymorphisms. The polymorphism C677T in the MTHFR gene was one of the attractive assays.

Genetic polymorphisms leading to folate deficiency seem to have effects on the tumorigenesis of some types of cancers. Folate, as a part of DNA methylation, has several functions including controlling expression of some genes and chromatin and genome stabilization. The polymorphism MTHFR C677T results in a reduction in enzyme activity. Considering the low capacity of DNA replication offered by substituting C by T, this polymorphism should effect a reduction in cancer risk. However, focusing on the effects of this reduced activity of the enzyme on uncontrolled gene expression and genome stability, an increase in the risk of cancer could be expected.

There is considerable geographic and ethnic variation in the distribution of this polymorphism. The genotype TT distribution ranges from 1% in blacks to over 20% in Europeans, Colombians, and American Indians (20,21).

The results of an association between this polymorphism and gastric cancer have been quite inconsistent. Some results including three meta-analyses showed TT genotype as a risk factor for gastric cancer (15,16,19,22-27). Despite finding an association in some studies (28-30), others have emphasized the decreased risk caused by the C677T polymorphism (31-33).

Our results showed a significant association between allele T and decreased risk of gastric cancer. In spite of no statistically significant association for T homozygotes (TT vs. CC), CT heterozygotes revealed this association (OR=0.47; 95%CI= 0.25-0.90), which was consistent with a valuable study in the Korean population (32), as did patients carrying allele T (CT and TT vs. CC: OR=0.5; 95%CI= 0.27-0.93), as support for the previous finding (33).

The higher frequency of older patients determined in the present investigation (88.2%) was considerable. It could suggest that genetic changes are less important than environmental factors in gastric tumorigenesis in Ardabil, as described previously (7).

As well as the low number of participants, other factors affecting on our results are some nutrients involved in the folate metabolic pathway, alcohol and smoking. Alcohol is a folate antagonist and smoking impairs the folate level. They could interact with folate levels, and therefore, have effects on cancer risk induced by the MTHFR polymorphism (34,35). The inverse association between folate intake and plasma homocysteine levels can be modified by alcohol intake and by MTHFR polymorphism (22,36). Inclusion of data regarding dietary habits, alcohol intake (which is not common among our population) and smoking could increase the accuracy of our results.

The inconsistency observed among different populations could result from different ethics, dietary habits and environmental factors.

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