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

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Amaç: Dünya çapında en sık görülen 4. malignite olan mide kanseri, Kuzey Batı ‹ran'da bulunan Ardabil bölgesinde en s›k görülen kanserdir. Metilen tetrahidrofolat redüktaz, polimorfizmi belirgin olarak görülen kanser yatk›nl›¤› genlerinden birisidir. Metilen tetrahidrofolat reductase C677T genetik olgularda, hastaların %35.5’inde yaygın tipte, %5.3’ünde intestinal tipte algılanmıştır. 

Gereç ve Yöntem: Daha önce yapılan çalışmalarda, mide kanseri riskini azaltabilecek genetik polymorfizm genetik analizleri ile araştırılmıştır. Bu çalışmadan, 76 gastrik kanserli ve 91 sağlıklı kontrol grubundan alınan kan örneklerinin enzimolojik analizi ile genetik analizleri yapılmıştır. 

Bulgular: 

- Hasta ve kontrol gruplarının yaş ortalamaları 64.2±11.1 ve 62.1±9.8 yıldır.
- Tumörlerin %35.5’inde yaygın tipte, %5.3’ünde intestinal tipte algılanmıştır.
- Hasta grupunda CT heterozigotlar gastrik kanser açıdan daha düşük yatkınlık göstermiş (P=0.02).

Sonuç: Ardabil bölgesinde T alelinin yaş ile koruyucu bir ilişki olduğunu bulmuştur.
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-methylene-tetrahydrofolate 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).

Table 1. General characteristics of participants

<table>
<thead>
<tr>
<th></th>
<th>Cases</th>
<th>Controls</th>
</tr>
</thead>
<tbody>
<tr>
<td>No</td>
<td>76</td>
<td>91</td>
</tr>
<tr>
<td>Age</td>
<td>64.2±11.1</td>
<td>62.1±9.8</td>
</tr>
<tr>
<td>≤50 Years</td>
<td>9 (11.8%)</td>
<td>9 (9.9%)</td>
</tr>
<tr>
<td>&gt;50 Years</td>
<td>67 (88.2%)</td>
<td>82 (90.1%)</td>
</tr>
<tr>
<td>Gender</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>56 (73.7%)</td>
<td>67 (73.6%)</td>
</tr>
<tr>
<td>Female</td>
<td>20 (25.3%)</td>
<td>24 (25.4%)</td>
</tr>
<tr>
<td>Tumor type</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Diffuse</td>
<td>27 (35.5%)</td>
<td></td>
</tr>
<tr>
<td>Intestinal</td>
<td>59 (64.5%)</td>
<td></td>
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</tbody>
</table>

*Age-, sex-, place of residence-adjusted population
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%, 50.7%, and 4.2% 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
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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REFERENCES


