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ASSOCIATION BETWEEN MTHFR (C677T) GENE POLYMORPHISM WITH BREAST CANCER IN NORTHERN IRAN

A. HEDAYATIZADEH-OMRAN, R. ALIZADEH-NAVAEI, F. TOGHANI-HULARI, O. AMJADI

Gastrointestinal Cancer Research Center, Mazandaran University of Medical Sciences, Sari, Iran.

Abstract – *Objective:* *Breast cancer is considered as the most common malignancies in women worldwide. Genetic polymorphisms of methylenetetrahydrofolate reductase (MTHFR) may affect the breast cancer risk by involving in folate metabolism and DNA methylation. This study was designed to examine the possible association of MTHFR gene polymorphism (SNP) in breast cancer susceptibility among the North Iranian women population.*

Patients and Methods: *We genotyped 677 C > T locus in 114 individuals that were classified into cases (N=54) and controls (N=60). Genotyping for MTHFR C677T was performed by polymerase chain reaction- restriction fragment length polymorphism (PCR- RFLP) using genomic DNA extracted from the peripheral blood of participant.*

Results: *The distribution of MTHFR (C677T) genotype frequencies, CC, CT, and TT among patients was 70.4%, 24.1%, and 5.6%, respectively. In the healthy control group, the CC, CT, and TT frequencies were 63.3%, 30%, and 6.7%, respectively. X^2 analysis revealed that there was no significant association between breast cancer risk and MTHFR genotypes and alleles. Furthermore, no significant association was observed between C677T genotypes, estrogen, Her2 receptor, tumor stage, and grade.*

Conclusions: *The findings do not suggest that genetic variation in the MTHFR C677T polymorphism is involved in the breast cancer risk in a population of North Iranian patients.*

KEYWORDS: *Brest Cancer, MTHFR C677T polymorphism, PCR-RFLP.*

INTRODUCTION

Breast cancer is the most common cause of mortality among women. The prevalence of breast cancer is about one-third of all cancers that represent a significant burden to women's health worldwide. In Iran, cancer has become a major health crisis and studies[1] have shown an increase in the incidence of breast cancer-associated mortality. Development of breast cancer is a multistep process, arising from genetic alterations that leads to the transformation of normal mammary epithelial cells into highly malignant derivatives[2]. The folate metabolism pathway that regulates DNA methylation and its aberrant patterns has been found to be associated with the development of breast cancer. MTHFR is the key enzyme in DNA biosynthesis and repair[3]. Human MTHFR gene is located on short arm of chromosome 1 and has two promoters and isoforms (70 kDa and 77 kDa). It is composed of 11 exons encoding a protein of 656 amino acids. The substitution of cytosine (C) to thymine (T) at nucleotide 677 in the *MTHFR* gene is a common polymorphism (C677T) and is correlated with reduced MTHFR activity[4]. It leads to aberrant DNA synthesis and repair and decreases DNA methylation, which is implicated in breast cancer risk[5]. Geographical and racial/ethnicity differences affect folate metabolism and also take part in breast cancer incidence and mortality rates[6]. The present research has focused on the possible association of C677T polymorphism and breast cancer.

Corresponding Author: Reza Alizadeh-Navaei, MD, Ph.D; e-mail: reza_nava@yahoo.com

PATIENTS AND METHODS

Patients

In the present case-controlled study, 54 breast cancer patients who referred to Tuba Clinic (academic referral center) in Mazandaran Province and 60 healthy controls were analyzed. All breast cancer cases diagnosed were histopathologically confirmed. Different characteristics, such as age and gender, were compared between cases and controls. All data for the study population, including age, gender, hormone status, clinical and laboratory diagnoses were collected based on related checklists. This study was approved by the Mazandaran University of Medical Sciences Ethics Committee. Informed consent form containing full disclosure of the study objectives and procedures was obtained from all volunteers. Progesterone receptor, Estrogen receptor, and HER2 receptor status were extracted from the immunohistochemical result in patient medical records.

DNA extraction

Whole blood samples from patients and controls were stored with EDTA at -20°C. The genomic DNA was isolated through a column-based method adopted from (DynaBio, blood/tissue DNA extraction mini kit, Cat#KI0015, Takapouzist, Iran). At first, 20 µl proteinase K were transferred to a microtube containing 500 µl sample of whole blood and were incubated 30 minutes at room temperature. Columns were prepared by adding 100 µl binding buffer and 10 minutes incubation. Samples were transferred to a column and centrifuged; the flow-through was discarded. DNA eluted by pre-heated elution buffer following two times column washing using wash buffer 1 and 2. Concentration and quality of extracted DNA were assessed by the absorbance values at 260 nm, $A_{260/280}$ and $A_{260/230}$ ratio using Nanodrop spectrophotometer (WPA, UK).

Amplification of the MTHFR (C677T) region

The region of the *MTHFR* (C677T) gene was amplified by polymerase chain reaction (PCR). The primers used for amplification were as follows: forward primer 5′-TGAA GGAGA-AGGTGTCTGCGG GA-3′; and reverse primer 5′-AGGA CGGTGCGGTGAGAGTG-3′. The final volume of PCR reaction mixture was 20 µl which contains: 10 µl Mastermix (Parstous, Mashhad, Iran), 10 pmol of forward and reverse primers, 50 ng genomic DNA, and distilled water. The reaction mixtures were pre-incubated for 10 minutes at 94°C in a thermal cycler (Applied Biosystem, Foster City, CA, USA). The PCR conditions were 94°C for 30 s and 55°C for 1 min, followed by 72°C for 1 min for 40 rounds. Amplification success of samples was monitored on 2% agarose gel by electrophoresis and the PCR-amplified product was 198 bp long. The PCR products were digested with 2 units of Hinf I restriction enzyme (Fermentase, Germany). The enzymatic mixture contained 1 µL restriction enzyme (RE) (Hinf I), 1 µL 10 × R buffer, 6 µL PCR products, and 2 µL distilled water; the mixture was incubated 16 h at 37°C for digestion. The digested product was run on 3% agarose gel with voltage 60 V around 1 h.

Determining genotype

We used the restriction fragment length polymorphism (RFLP) method to determine *MTHFR* (C677T) genotypes. Digested fragments were separated by running them on a 2% agarose gel containing Green Viewer (Parstous, Mashhad, Iran). Digested products were visualized under a UV light transilluminator device (Kimia Gene, Iran).

Statistical Analysis

Totally, the sample size of the study was estimated at least 114 subjects (case group 54 and control group 60). Data were entered into SPSS statistical software 20 (SPSS Inc., Chicago, IL, USA), and analyzed by using *t*-test and X^2 tests. The odds ratio and its confidence interval (95%) were calculated with logistic regression. *p*-value less than 0.05 were considered as a significant level.

RESULTS

This study was performed on 54 patients with breast cancer and 60 subjects as a control group. The distribution of the tumor characteristics of breast cancer patients is shown in Table I. The breast cancer metastasis was shown at 35.2%. The frequencies of MTHFR genotypes in breast cancer patients and control subjects are shown in Table II. Association between tumor characteristics and MTHFR genotypes in breast cancer patients was shown in Table III and there was not a significant association between MTHFR genotype and progesterone receptor (p=0.875), estrogen receptor (p=0.652), HER2 receptor (p=0.635), metastasis (p=0.96), tumor stage (p=0.176), and tumor grade (p=0.52) in these cases. Agarose gel electrophoresis results demonstrated one single band of 198 bp for CC genotype, TT genotype appears as two bands of 175 bp and 23 bp, TC heterozygote has all three bands (198 bp, 175 bp, and 23 bp) (Figure 1).

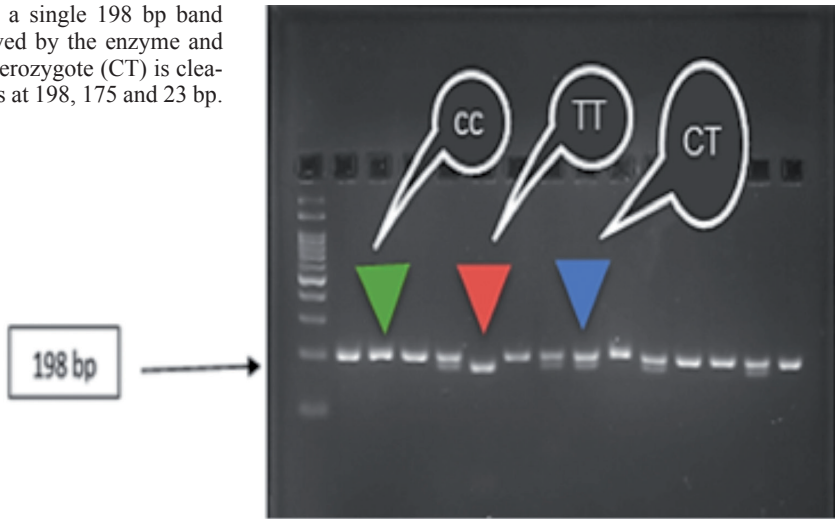

Fig. 1. Homozygote genotype to show a single 198 bp band (CC) and the homozygote (TT) is cleaved by the enzyme and generates 2 bands at 175 and 23 bp. Heterozygote (CT) is cleaved by the enzyme and generates 3 bands at 198, 175 and 23 bp.

DISCUSSION

In the present study, 54 patients with breast cancer and 60 healthy controls were analyzed. In this study, we tried to determine the correlation between MTHFR (C667T) genotype/allele frequency and breast cancer in subjects from the North Iranian population and the mean age of breast cancer patients and controls at the time of diagnosis was 47.9 ± 11.4 and 35.8 ± 12.9 years, respectively (p=0.000). Our results demonstrated that the tumor size in 42.6% was less than 2 cm and 24.1% of patients had tumor stage IV. The tumor in 40.7% was grade III. Progesterone receptor, estrogen receptor, and HER2 receptor were positive in 27.2, 59.3, and 77.8% of breast cancer patients, respectively. Our finding showed that the frequencies of the CC, TT, and CT genotypes of MTHFR were 70.4%, 24.1%, and 5.6% of the patients, and 63.3%, 30%, and 6.7%, in the control group, respectively. These differences were not significant between two groups (p=0.728). This finding is in agreement with the study by Ajai et al[7] as opposed to the study by Waseem et al[2] and Sihua et al[8]. In this study, there weren't a significant differences between MTHFR (C677T) gene polymorphism with tumor grade, stage, and tumor metastasis in breast cancer patients. Previous investigations[9-12] on the association between the common polymorphism of MTHFR C677T and breast cancer showed conflicting results. Kumar et al[13] revealed the modest relations between MTHFR C677T polymorphism with breast cancer risk in a meta-analysis study. They grouped their study population into two Asian and Caucasian subgroups; TT genotype was highly associated with breast cancer risk among Asian population[13]. A study performed on 318 cases and 647 controls showed that TT genotype and T allele of MTHFR C677T polymorphism was highly associated with the risk of breast cancer[14]. Zhang et al[15] investigated the involvement of MTHFR C677T polymorphism and breast cancer susceptibility by

TABLE 1. Basic details of breast cancer patients.

Variable		*N (%)*
Size	≤ 2	23 (42.6)
	3-4	24 (44.4)
	≥ 5	7 (13)
Tumor stage	I	14 (25.9)
	II	20 (37)
	III	7 (13)
	IV	13 (24.1)
Tumor grade	I	8 (14.8)
	II	24 (44.4)
	III	22 (40.7)
Progesterone receptor	No	23 (42.6)
	Yes	31 (27.2)
Estrogen receptor	No	22 (40.7)
	Yes	32 (59.3)
HER2 receptor	No	12 (22.2)
	Yes	42 (77.8)
Metastasis	No	35 (64.8)
	Yes	19 (35.2)

TABLE 2. Genotype of *MTHFR* gene polymorphism in patients with breast cancer in comparison with control.

Genotype	*Case N (%)*	*Control N (%)*	*Odds ratio (CI 95%)*	*p-value*
CC	38 (70.4)	38 (63.3)	-	
CT	13 (24.1)	18 (30)	1.33 (0.27-6.36)	0.728
TT	3 (5.6)	4 (6.7)	0.96 (0.18-5.1)	

TABLE 3. Association between tumor characteristics and genotype of *MTHFR* gene polymorphism in patients with breast cancer.

Variable		*Genotype*			*p-value*
		CC N (%)	*N (%) TT*	*CT N (%)*	
Progesterone receptor	No	17 (73.9)	5 (21.7)	1 (4.3)	0.875
	Yes	21 (67.7)	8 (25.8)	2 (6.5)	
Estrogen receptor	No	17 (77.3)	4 (18.2)	1 (4.5)	0.652
	Yes	21 (65.6)	9 (28.1)	2 (6.2)	
HER2 receptor	No	9 (75)	3 (25)	-	0.635
	Yes	29 (69)	10 (23.8)	3 (7.1)	
Metastasis	No	25 (71.4)	8 (22.9)	2 (5.7)	0.96
	Yes	13 (68.4)	5 (26.3)	1 (5.3)	
Tumor stage	I/II	25 (73.5)	6 (17.6)	3 (8.8)	0.176
	III/IV	13 (65)	7 (35)	-	
Tumor grade	I	5 (62.5)	3 (37.5)	-	0.52
	II/III	33 (71.7)	10 (21.7)	3 (5.6)	

including 15,260 breast cancer patients and 20,411 healthy controls in a meta-analysis study. They concluded that homozygote genotype of TT had elevated level of breast cancer risk and T allele could be a risk factor for developing breast cancer. Previously published pooled data on 28031 cases and 31880 controls demonstrated that MTHFR 677 C > T polymorphism does not effect on breast cancer risk[16]. In another study on 233 women from West-South of Iran, no association was found between MTHFR C677T variant and breast cancer risk[17]. The same results were published by Kaya et al[18] that performed a study on Turkish population. They reported no association between MTHFR C677T polymorphism and breast cancer.

CONCLUSIONS

Totally, our findings suggest that MTHFR C677T polymorphism may not be a risk factor for breast cancer. However, further studies considering different factors such as menopausal status, folate intake, familial genetic history of breast cancer, reproductive history, body mass index (BMI), ethnicity, and large sample size from different provinces, will strengthen these results.

Conflict of interests

The Authors declare that they have no conflict of interests.

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