Original Article

Lack of association between MTHFR C677T polymorphism and breast cancer risk in Ahvaz, west south-Iran

Ghorban Mohammadzadeh, Maryam Karimi[1], Mohammad Bazyar[1], Seyed-Mohammad Hosseini[2]

Department of Biochemistry, Faculty of Medicine, Hyperlipidemia Research Center, [1]Department of Biochemistry, Faculty of Medicine, [2]Department of Radiation and Clinical Oncology, Faculty of Medicine, Ahvaz Golestan Hospital, Ahvaz Jundishapur University of Medical Sciences, Ahvaz, Iran

Abstract

Background: Association between C677T polymorphism of the methylenetetrahydrofolate reductase (MTHFR), a key enzyme involved in folate metabolism and DNA methylation, and breast cancer risk are inconsistent. We investigated in a case-control study, possible effect of the common MTHFR C677T polymorphism on breast cancer risk in a sample of Iranian patients.

Materials and Methods: The study subjects comprised of 123 breast cancer cases and 110 cancer-free control, who were matched for age and body mass index (BMI). C677T genotypes were determined by polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) assay. Lipid profile was measured in all subjects by standard method.

Results: The genotypes distributions (CC, CT, and TT) were 55.3, 39, and 5.7% in breast cancer cases and 51.8, 44.5, and 3.6% in controls. Chi square analysis revealed that there was no significant association between breast cancer risk and MTHFR genotypes and alleles. Additionally, no significant association was observed between C677T genotypes and biochemistry parameters. A multinomial logistic regression model with MTHFR genotypes, lipid profiles, BMI and age as covariates revealed that there is no significant association between MTHFR genotypes and risk of breast cancer, but higher values of LDL and HDL significantly increase risk of breast cancer.

Conclusions: Our findings do not support the hypothesis that genetic variation in the MTHFR C677T polymorphism is implicated in the breast cancer risk in a sample of Iranian patients.

Key Words: Brest Cancer, MTHFR C677T polymorphism, PCR-RFLP

Address for correspondence:
Dr. Ghorban Mohammadzadeh, Hyperlipidemia Research Center, Department of Clinical Biochemistry, Faculty of Medicine, Ahvaz Jundishapur University of Medical Sciences, Ahvaz, Iran. E-mail: mohammadzadeh@ajums.ac.ir

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INTRODUCTION

Folate, a group of water-soluble B-vitamins, has an important role in DNA methylation, synthesis and repair of DNA, and might protect against cancer. Epidemiological evidence indicating that low intake of folate may increase the risk for neoplasia, including breast cancer.[1,2] The molecular mechanisms linking between folate insufficiency and cancer development could include purine and thymidine depletion and misincorporation of uracil into DNA synthesis, increased DNA strand breaks, aberrations in DNA methylation and disruption of DNA repair.[3,4]

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The N_5, N_{10}–methylenetetrahydrofolate reductase (MTHFR) is a critical enzyme in folate metabolism, which catalyzes irreversible reaction of N_5, N_{10}–methylenetetrahydrofolate (N_5, N_{10}–methylene-THF) to N_5–methyltetrahydrofolate (N_5–methyl_THF), the predominant circulatory form of folate and a one-carbon donor for re-methylation of homocysteine to methionine. Folate that is not converted through this pathway enters another pathway that leads to purine and thymidylate synthesis.[5]

Two common single nucleotide polymorphisms (SNPs) in the MTHFR gene that affect the efficiency of folate metabolism have been described as MTHFR C677T (NCBI SNP ID: rs1801133) transition substitution in exon 4 and MTHFR 1298 A > C transversion substitution in exon 7).[6] The C677T SNP of MTHFR is common at the folate binding site of the MTHFR gene which results in alanine to valine substitution at codon 222.[7,8] *In vitro* analysis of the MTHFR activity demonstrated that heterozygous and homozygous bearing of the 677T allele variant have a 30–40% and 60– 70% reduced enzyme activity, respectively.[7,9,10] Many studies have been found that these low-activity genotypes of MTHFR associated with the risk of a variety of cancers, such as colorectal,[11,12] gastric,[13,14] endometrial,[15] lung cancer[16] and acute leukemia.[17] In addition, numerous case-control studies assessed the association between MTHFR C677T SNP and breast cancer risk, but the findings have been controversial.[18-34] Some of them reported a positive association between the 677TT genotype of MTHFR and breast cancer risk,[19,22,29,32] whereas no association was noted in other studies.[18,20,21,23-28,30-34] Moreover, in another study, an increased risk of breast cancer was found in a selected population of BRCA1 mutation carriers with MTHFR 677TT genotype.[35] We conducted a case-control study in a sample of Iranian women in order to investigate the association between MTHFR C677T genotypes and breast cancer risk.

MATERIALS AND METHODS

Study population

The study population consisted of patients (n = 123) with histologically confirmed breast cancer, admitted to the Ahvaz Medical Faculty and the department of radiation and oncology of Golestan University Hospital, Ahvaz, Iran. The control subjects (n = 110) were recruited from the same geographic area during the same period and were matched to the cases by age and BMI. The control subjects were randomly selected among the people admitted to the same hospital. Anthropometric indices and clinical parameters were measurement by standard methods, as previously described.[36]

MTHFR genotyping

In order to DNA extraction, blood samples were collected into K3-EDTA-treated tube from both patients and controls, and were stored at -20°C. Total genomic DNA was extracted from peripheral blood leukocytes and was dissolved in sterile TBE buffer. The variant MTHFR C677T was genotyped by using PCR-RFLP analysis. The PCR primers were synthesized by primer 3 software and their sequences were as follows: Forward, 5'-CCTGACTGTCATCCCTATTGGC–3′ and reverse 5'-GGAGCTTATGGGCTCTCCTG–3′. Conditions for PCR amplification were 12.5 µl commercially available PCR premix (AccuPower PCR PremiX; BIONEER, Daejeon, Korea) containing (dNTP, TaqDNA polymerase, MgCl2, buffer), 2.0 µl (20 pmol/µl) forward and reverse primers, 2.0 µl (50 ng/µl) template DNA, and 6.5 µl sterile nuclease free water. The thermal cycling conditions were as follows: Initial denaturation at 94°C for 5 min, 35 cycles of denaturation at 94°C for 60 s, annealing at 53°C for 45 s, and extension at 72°C for 60 seconds, with a final extension of 5 min at 72°C. The PCR amplified products were scored in 248-bp in a mixture reaction consisting of: PCR products (10 µl), 10 × buffer (2 µl), 10 units *Hin*fI (New England Bio labs, USA) restriction enzyme, and sterile nuclease free water (18 µl). The reaction mixture was kept overnight at 37°C for 1-16 h. The fragments were separated by electrophoresis on 3% agarose gel, stained with ethidium bromide and results were recorded with photographs of gels in UV light. The C677T substation introduces a new *Hin*fI restriction site which results in the digestion of the 248-bp PCR product into 100 and 148-bp fragments. After electrophoresis of digested DNA fragments, homozygous C allele was represented by a DNA band sized at 248, whereas homozygous T allele was represented by a DNA band sized at 100 and 148-bp and heterozygotes sized at 248, 100 and 148-bp [Figure 1].

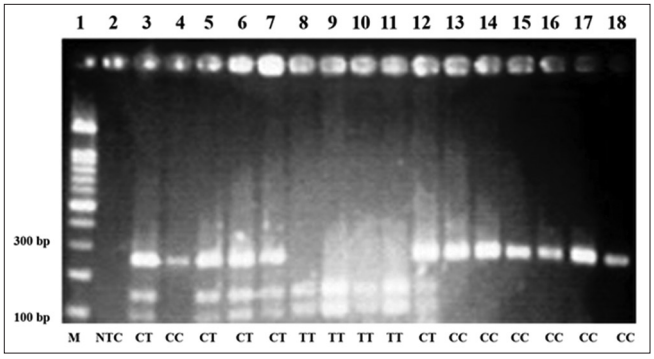

Figure 1: Representative example of MTHFR C677T polymorphism products by PCR-RFLP on agarose gel electrophoresis. Lane 1 shows 100-bp DNA Ladder; Lane 2 shows Non-template control; lanes 3, 5, 6, 7 and 12, show heterozygote CT genotype (248,148 and 100-bp); lanes 4, 13,14,15,16, 17, and 18, show wild type CC genotype (248-bp); lanes 8,9,10 and 11 show mutant TT genotype (148 and 100-bp)

Statistical analyses

Data are expressed as mean ± standard deviation, and all statistical analyses were performed using SPSS software for Windows version 20.0 (IBM Corporation New York, USA). Anthropometric indices and biochemical parameters were compared between breast cancer cases and controls using independent sample *t*- test, and one way analysis of variance (ANOVA) were used to compare those variables between MTHFR genotypes. All frequencies were estimated by gene counting. The observed genotype frequencies in the breast cancer cases and controls were tested for the Hardy-Weinberg equilibrium (HWE). The statistical significance of the C677T genotype distributions between cases and controls was determined by Chi square analysis. In order to estimate odds ratios (ORs) for breast cancer risk and the corresponding 95% confidence intervals (CI) logistic regression model was used. Multinomial logistic regression analysis was also determined, and results were expressed as *P*- value, odds ratio (OR) and 95% confidence intervals (95% CI). A $P < 0.05$ was considered as the criterion for statistical significance.

RESULTS

Comparisons of anthropometric indices and biochemical characteristics between breast cancer cases and controls.

Anthropometric indices and biochemical characteristics of breast cancer cases and controls are summarized in Table 1. There were no statistically significant differences between the breast cancer cases and controls for age and BMI ($P = 0.755$; $P = 0.218$, respectively). In addition, there were no statistically significant differences between two groups for the means of biochemical characteristics including total cholesterol, triglyceride. However, there was a statistically significant difference between two groups for the means of HDL ($P < 0.001$) and LDL ($P = 0.017$).

MTHFR C677T genotype analysis

Genotype and allele frequencies of MTHFR C677T polymorphism were compared between breast cancer cases and controls [Table 2]. The observed allele and genotype frequencies in the both breast cancer cases and controls for MTHFR C677T were in accordance with the Hardy-Weinberg lows of equilibrium. The frequencies of the CC, CT, and TT genotypes were 55.3%, 39%, and 5.7% in breast cancer cases and 51.8%, 44.5%, and 3.6% in controls, respectively. Between two study groups, the frequency of genotypes were not different [Table 2, $\chi^2 = 1.075$, $P = 0.584$]. Likewise, the allele frequency, which for C and T alleles were 77.6% 22.4% in cases, and 75.9 and 24.1 in controls, respectively, no significant difference between two groups was observed [Table 2, $\chi^2 = 0.196$, $P = 0.658$]. Overall, there was no association between breast cancer risk and MTHFR C677T genotype and alleles. Conversely, there was an association between CC genotype of this polymorphism and higher mean total cholesterol level [Table 3].

Risk factors for breast cancer

In order to determine predictors of breast cancer we used multinomial logistic regression model, with the dependent variable being breast cancer, and the independent potentially confounding variables being age, BMI, LDL-C, HDL-C, total cholesterol and triglyceride levels and MTHFR C677T genotypes

Table 1: Comparison the means of age, BMI and lipid profile between breast cancer cases and controls

Variables	Breast cancer cases (*n*=123)	Controls (*n*=110)	*P*
Age (years)	48.56±11.32	48.96±781	0.755
BMI (Kg/m²)	27.69±4.08	28.41±4.82	0.218
Total Cholesterol (mg/dl)	198.30±36.29	200.88±56.59	0.677
LDL-C (mg/dl)	96.01±17.78	92.29±12.82	0.017
HDL-C (mg/dl)	53.82±10.13	46.93±11.50	<0.000
Triglyceride (mg/dl)	117.13±55.12	128.69±62.82	0.136

Number of individuals presented in parentheses. Continuous variables are presented as mean±SD and were compared by independent sample *t*-test. BMI: Body mass index, LDL-C: Low-density lipoprotein cholesterol, HDL-C: High-density lipoprotein cholesterol

Table 2: Genotype distribution of MTHFR C677T polymorphism in breast cancer cases and controls

	Breast cancer cases	Controls	χ^2	*P*
Genotypes				
CC	68 (55.3)	57 (51.8)		
CT	48 (39.0)	49 (44.5)		
TT	7 (5.7)	4 (3.6)	1.075	0.584
Alleles				
C	191 (77.6)	167 (75.9)		
T	55 (22.4)	53 (24.1)	0.196	0.658

The χ^2 test was used to determine whether significant differences (*P* value) were observed when the breast cancer cases were compared with the controls. MTHFR: Methylenetetrahydrofolate reductase

Table 3: Comparison the means of age, BMI and lipid profile according to the MTHFR C677T genotypes

Characteristics	Genotype			*P*
	CC (*n*=125)	CT (*n*=97)	TT (*n*=11)	
Age (year)	48.32±9.62	49.38±9.77	48.00±12.56	0.707
BMI (kg/m²)	28.11±4.28	27.62±4.43	30.79±5.83	0.079
Total cholesterol (mg/dl)	206.66±48.41	190.01±44.30	202.27±39.95	0.031
LDL-C (mg/dl)	94.11±15.23	94.22±16.37	96.18±16.72	0.916
HDL-C (mg/dl)	50.16±11.84	51.17±10.63	49.63±11.76	0.777
Triglyceride (mg/dl)	126.47±46.42	119.27±49.81	107.72±38.11	0.464

BMI: Body mass index; MTHFR: Methylenetetrahydrofolate reductase; CC: Wild type genotype; CT: Heterozygote genotype; TT: Mutant genotype; HDL: High- density lipoprotein; LDL: Low-density lipoprotein cholesterol

[Table 4]. Among the inherited risk factors, neither homozygosity for MTHFR C677T (OR = 0.603; 95% CI = 0.152-2.388, P = 0.471) nor heterozygosity for MTHFR C677T (OR = 0.414; 95% CI = 0.101-1.680, P = 0.218) were not associated with having breast cancer [Table 4]. Among the non-inherited risk factors, HDL-C (OR = 1.069; 95% CI = 1.039-1.099, $P < 0.001$), and LDL-C (OR = 1.032; 95% CI = 1.012-1.052, P = 0.002) were associated with having breast cancer [Table 4].

DISCUSSION

In this case-control study we found no association between a commonly occurring polymorphism of MTHFR C677T and breast cancer risk in a sample of Iranian women. The frequency of the T allele in the cancer-free control of Iranian women (22.4%) seemed to be slightly lower than the reports on other populations, including 35% in Greece,[37] 39% in Korea,[25] 30% Sothern England[38] or 27-29% in the USA.[38-41] However, the frequency of the T allele in the whole population (unselected for sex) shows considerable differences in its distribution model in the worldwide, ranging from 10% in African American[42] to 63% in northern China.[43] This variation may account for the basis of the differences observed regarding the association of the C677T polymorphism with cancer risk in studies from different geographical regions.

Previous efforts to investigate the relationship between the common polymorphism of MTHFR C677T and breast cancer have yielded conflicting results.[18-34] Although, the results obtained from two meta-analyses, with large sample size of breast cancer cases and controls, showed that the MTHFR C677T polymorphism had low effect on the development of breast cancer.[44,45] However, a strong inverse association has been constantly observed between the MTHFR 677TT genotype and colorectal cancer, particularly in subjects with high levels of folate intake and low levels of alcohol conception.[12]

Table 4: Results of multinomial logistic regression analysis

Genotype	OR	95% confidence interval	*P*
Age (year)	0.991	0.963-1.020	0.550
BMI (kg/m^2)	0.957	0.897-1.020	0.176
Total cholesterol (mg/dl)	0.998	0.992-1.005	0.613
Triglyceride (mg/dl)	0.997	0.992-1.002	0.296
HDL-C (mg/dl)	1.069	1.039-1.099	<0.001
LDL-C (mg/dl)	1.032	1.012-1.052	0.002
MTHFR677T/T	0.603	0.152-2.388	0.471
MTHFR677C/T	0.414	0.101-1.685	0.218

OR: Odds ratio, CI: Confidence interval, BMI: Body mass index, MTHFR: Methylenetetrahydrofolate reductase, LDL-C: Low-density lipoprotein cholesterol, HDL-C: High-density lipoprotein cholesterol

A study conducted by Marchand *et al*. reported a significant inverse association between MTHFR 677TT genotype and breast cancer risk among postmenopausal women who were on hormone replacement therapy (HRT) at baseline.[28] They suggested that the MTHFR 677TT genotype may confer a 40% decreased breast cancer risk in postmenopausal women using HRT. And this result is consistent with the role of MTHFR in facilitating the flow of folate for thymidylate and purine synthesis and with the increased nucleic acid need resulting from the hyper proliferative effect of HRT on mammary epithelial cells.

Another study reported opposite effects of these two SNPs on the risk of breast cancer. Results from Long Island Breast Cancer Study demonstrated that the 677T mutant allele was associated with an increased risk of breast cancer and the 1298C mutant allele was associated with a decreased risk of breast cancer.[29] The authors hypothesized that the inverse correlations were caused by the linkage disequilibrium between C677T and A1298C that links a low activity genotype at one locus to a high activity genotype at the other locus.[12,45]

There are many factors that could explain the conflicting results from different studies, including different population characteristics (sample size and ethnic differences), different familial genetic background that may modify breast cancer risk such as BRCA1/2 mutations,[35] steroid hormone administrations, reproductive history, and more critically, menopausal status and folate intake.

Some studies[20,25,28,29] which stratified the population by menopausal status found with various results. A study conducted by Semenza *et al*.[18] reported a significantly increased risk for breast cancer in premenopausal women with the MTHFR 677 TT genotype, also Chen *et al*.,[29] observed a higher MTHFR 677 TT frequency in breast cancer cases than in controls, though this difference was borderline significant. Moreover, Ergul *et al*.[20] investigated a population of premenopausal women and reported a significant positive association (P = 0.016) between MTHFR 677TT genotype and breast cancer risk, although Forsti *et al*.[24] evaluated a postmenopausal women population with no confirmation of a significant difference between two groups. In addition, Lee *et al*.[25] Marchand *et al*.,[28] and chen *et al*.[29] showed no statistical significant association based on menopausal status, even though the two latter studies were weak powered to detect a difference, due to the small sample of premenopausal compared with postmenopausal women.

The critical function of folate metabolism in breast cancer risk is also supported by several studies which pointed the influence of folate intake in the assessment of MTHFR C677T SNP and breast cancer risk.[18,23,29,30] Although in the first published paper assessment the interactive effect between MTHFR C677T genotypes and folate intake, Sharp *et al*. did not detected any statistically significant association, probably due to the small sample size,[18] but, Shrubsole *et al*. reported that low intake of folate was significantly associated with an increased risk of breast cancer among all MTHFR C677T genotypes and particularly in subjects with the TT genotype.[23] Chen *et al*. similarly found an increased but not significant risk of breast cancer in 677TT subjects with the lowest levels of folate intake in comparison to 677 wild type subjects with high levels of folate intake. They also found a significantly increased risk for breast cancer among non-supplement users with MTHFR 677TT genotype.[29] Finally, Chou *et al*.[34] evaluated the interaction between plasma folate levels and combined genotypes in MTHFR gene. In particular, they analyzed A1298C SNP other than C677T and reported a more pronounced reduction in breast cancer risk among women with lower plasma folate levels and the compound heterozygote and homozygote variants (677CT/TT and 1298AC/CC).

In conclusion our findings showed that higher serum levels of HDL and LDL are significantly associated with breast cancer risk but MTHFR C677T genotypes and alleles did not associate with breast cancer.

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