IJBPAS, March, 2015, 4(3): 1020-1029

ISSN: 2277–4998

International Journal of Biology, Pharmacy and Allied Sciences (IJBPAS)

'A Bridge Between Laboratory and Reader'

www.ijbpas.com

MTHFR C667T GENE POLYMORPHISM WAS NOT INCREASED RISK FOR BREAST CANCER DISEASE

MILAD ASADI[1], SAID ASLANI[2], MUSTAFA ASADNIA[2], TANIYA BAGHERI[3], ATABAK MOHAMMADI AGHJEH GHALEH[3], DONYA BAZHAN[4], MAJID GHARGHANI[5],* ASGHAR HOSSEINZADEH[6]

1: Department of Medical Genetics, Faculty of Medicine, Tabriz University of Medical Science, Tabriz

2: Department of Biochemistry, School of Medicine, Tehran University of Medical Sciences, Tehran, Iran

3: Department of Biology, College of Science, Tabriz Branch, Islamic Azad University, Tabriz, Iran

4: Department of Molecular Biology, Faculty of Basic Science, Ahar Branch, Islamic Azad University, Ahar, Iran

5: Department of Medical Biochemistry, Faculty of Medicine, Yasuj University of Medical Science, Yasuj, Iran

6: Immunology Research Center, Tabriz University of Medical Sciences, Tabriz, Iran

ABSTRACT

Methylene tetra hydro folate reductase (MTHFR) is an enzyme that catalyzes the conversion of 5, 10-methylenetetrahydrofolate to 5-methyltetrahydrofolate. This enzyme plays an important in controlling folate metabolism. Therefore it is essential for DNA synthesis and DNA repair. It has been shown that C667T polymorphism may compact on susceptibility towards various cancers. This study aimed to investigate the associations between C677T polymorphisms for *MTHFR* gene and breast cancer in East Azerbaijan, Iran. Fifty women patients with breast cancer and 78 healthy women as control group were studied. Fragment Length Polymorphism (RFLP)

polymerase chain reaction (PCR) was carried out for assessment of C677T polymorphism of for *MTHFR* gene in breast cancer cases and control groups.

No obvious difference was found between all genotypes of MTHFR gene of breast cancer women and healthy controls. Frequencies of MTHFR polymorphism were 58%, 35%, 7% for CC, CT, TT genotype in patients and 55%, 37%, 8% in healthy controls respectively.

Our findings seem that there was no association between C677T polymorphism *MTHFR* gene with increased risk of breast cancer in comparison to control group.

Keywords: Breast Cancer, Methylene tetra hydrofolate reductase, MTHFR, RFLP

INTRODUCTION

Breast cancer is a heterogeneous group of malignancies which is originated from the ductal epithelium. Mammary ductal carcinoma is common type of breast cancer **[1]**. Breast cancer is the top cause of cancer-associated mortality and the most common malignancy of women in all over the world **[2]**. Nowadays, the incidence of breast cancer is increasing worldwide, and this increase is high in developing countries of Africa, Southeast Asia, and South America **[3]**. The etiology of breast cancer is not clear and multifactorial, which is the result of the interactions between genetic, environmental, and lifestyle-related risk factors **[4]**. Several mutations especially in *BRCA1* and *BRCA2* as well *P53* and DNA repair-related gens such as *ATM, ATM1*, and *PALB2* have been realized to be associated with breast cancer **[5]**. It has been shown that the incidence distinct of breast cancer is associated with geographical variation which can be pertaining to exposure towards various risk factors. Among these factors are no breast feeding, early menarche, late menopause, late first full-term pregnancy, nulliparity, and family history of breast cancer in two or more first-degree relatives have major attribution. Also some minor risk factors such as obesity in postmenopausal women, hormone, replacement therapy (HRT), smoking, exposure to low-dose radiation, excessive alcohol intake, and so on are correlated with breast cancer **[6, 7]**. Methylenetetrahydrofolatereductase (MTHFR), an enzyme, reduces 5,10-methylenetetrahydrofolate to 5-methyltetrahydrofolate which is the carbon donor for the homocysteine/methionine conversion.As a circulating form of folate, 5-methyltetrahydrofolate plays an important role in DNA synthesis, repair and methylation **[8]**. Several studies have associated polymorphisms for MTHFR gene and cancers such as colorectal and breast cancer **[9]**. Also

it has been shown that aberrant DNA repair is a contributing factor in tumorogenesis **[10]**. C667T polymorphism for MTHFR gene causes an amino acid change of alanine to valin, resulting in reduced enzyme activity **[11]**. A study to evaluate the association between C667T polymorphism for MTHFR gene and increased risk of breast cancer in Turkish population depicted that C667T polymorphism did not increase breast cancer risk **[12]**.On the other hand a study in England population showed that there was an association between MTHFR C667T polymorphism and breast cancer **[13]**. During a meta-analysis studyit was found that MTHFR C677T polymorphismwas significantly associated with breast cancer risk inoverall and East Asian population while no association wasshown in Caucasian and menopausal status-based population **[14]**. In this study we aimed to find if there is association between C667T polymorphism for MTHFR gene and breast cancer risk in East Azerbaijan population of Iran.

MATERIALS AND METHODS

Patients

In this study which was carried out at Tabriz University of Medical Sciences, patients were chosen from Imam Reza Hospital, Tabriz, Iran. An ethics committee of Human Research of the hospital and Tabriz University of Medical Sciences approved the study. Measurement of biochemical markers to realize the situation of patients is a routine procedure and therefor all patients signed an informed consent, including applying the data for research, when they referred to hospital. Then their breast cancer had been diagnosed histopathologically. Fifty breast cancer affected women (mean age of 44±5) and 78 age matched healthy controls (mean age of 47±3.5) were recruited in our study. All patients and controls had not taken hormone therapy or other drugs. Also they had no malnutrition towards any vitamins. Cancer grade, lymph node metastasis, chemotherapy and radiotherapy-receiving context of the patients had been designated histologically. Characteristics of patients are depicted at the **Table 1** with more details. Blood sample was obtained and PBMCs were collected through Ficoll-Paque procedures Genomic DNA was extracted through Phenol-Chloroform method and qualified using Spectrophotometry.

PCR-RFLP

In order to analyze the MTHFR C677T genotype, PCR-RFLP method was applied using Hinf I digestion as restriction enzyme, according to a previous study by Frosst*et al*. **[11]**. Primers were used from previous study **[11]**. For accuracy and specificity all primers were blasted in NCBI website: http://

www.ncbi.nlm.nih.gov/tools/primer-blast/.

Primers were produced by the custom oligonucleotide synthesis service, Metabion (Martinsried, Germany).

Performing the StepOne RFLP-PCR (Thermo Fisher Scientific Inc.), each reaction mixture contained a total volume of 25µl (master mix 12.5µl, DNA 3µl, primer 3µl, and H_2O 6.5µl). The PCR conditions were: 50°C for 2 minutes, 95°C for 10 minutes, then 30 cycles of 95°C for 30 seconds, and 60°C 30 seconds and 72°C for 30 seconds.

After PCR, the reactions were centrifuged at 1000 x g for 2 minutes and the supernatants were collected for restriction digestion. The digestions were prepared directly in the supernatants with restriction enzyme HinfI (0.4 U/µL) for amplified *MTHFR*. Restriction enzyme processing and choosing procedures was done using website: http://tools.neb.com/NEBcutter2/. Then 10 µL reactions were incubated for one hour at 37°C. The resulting fragments were ran on agarose gel and compared to untreated PCR products.

Statistics

SPSS statistical software package (version18.0) was used to analyze data. Data are shown as mean ± standard deviation (SD) or as proportions. A two-tailed p value of 0.05 was considered statistically significant. Frequencies of genotypes were compared between patient and control group using chi-square test. The odds ratio and 95% confidence intervals were calculated.

RESULTS

Breast cancer women were classified histopathologically according to historical documents and it was found that about 69.8 % of womenhad ductal carcinoma, and the remaining 28.5 % were lobular carcinoma. Using TNM classification principals, approximately 35% of the women were detected to be at stage III, and 45% of them were at the stage II. But less than 20% of the cases were at the stage I. About 36 % of women were in premenopausal status, and 64 % of them were in postmenopausal status.The axillary lymph node status of patients was found to be 95 % negative, and the remaining 5 % were positive with a higher risk of metastasis but lymph node metastasis stage analysis depicted that nobody had definite metastasis manifestation.

Table 1 shows properties of patients. Most of breast cancer cases in our study had more than 40 years old (P<0.001). According to their area of living, most of cases were found to be from urban population in comparison to controls (P<0.001). However, in terms of occupation, there was no significant difference between the cases and controls.

After treating the amplicons with restricting enzymes and running on the gel electrophoresis, frequencies of *MTHFR* polymorphism were 58%, 35%, 7% for CC, CT, TT genotypes in patients and 55%, 37%, 8% in healthy controls, respectively (Table 2). Statistical analysis resulted that there were no significant correlation between SNPs of *MTHFR* gene and contraction to breast cancer (P > 05).

DISCUSSION

Breast cancer is more striking and common cause of cancer-related death among women in both India and Western countries. The etiology of breast cancer is multifactorial, and several risk factors are linked to. The environmental and lifestyle factors are important determinants of breast cancer risk. In women, the incidence of breast cancer increases with increasing age until 45 to 50 years. Chronological age could be a crude equivalent for breast tissue age, adding to the severity of the disease **[15]**. According to Elizabeth B. claus*et al*. the onset age of the breast cancer was up to 35 years old. On the other hand in our study the onset age of the breast cancer was > 40 and the mean age of distribution of breast cancer patients was 51.4±7.44 years **[16]**.

Folate deficiency could increase risk of malignancy by two mechanisms: (1) by causing DNA hypomethylation and proto-oncogene activation; and/or (2) by inducing uracil misincorporation during DNA synthesis, leading to inappropriate DNA repair, DNA strand breakage and chromosome damage **[17]**.

As an enzyme, 5, 10-Methylenetetrahydrofolate reductase (MTHFR) irreversibly converts 5, 10-methylenetetrahyrdofolate to 5-methylenetetrahydrofolate, the primary circulating form of folate. Two functional polymorphisms in MTHFR have been reported – C677T and A1298C. For C677T, compared to homozygotes for the common variant, heterozygotes have been reported as having 65% of normal enzyme activity and 30% for those who are homozygous variant **[18]**. In this study we aimed to detect if there is relationship between C677T polymorphism for *MTHFR* and breast cancer. Different studies have linked polymorphisms for *MTHFR* gene with various cancers. In one of them it was realized that *MTHFR* genotypes associated with metabolism and serum level of folate, vitamin B12, and vitamin B6 in colorectal adenomas(19).To make this result vigorous, one study supported an important role of folic acid in colon carcinogenesis. In particular, these results suggested that the common C677T mutation in *MTHFR*gene

reduces colon cancer risk, perhaps by increasing 5, 10-methyleneTHF levels for DNA synthesis. They also found that low folate intake or high alcohol consumption appear to reduce the potential benefit **[20]**. Some previous studies have investigated associations between *MTHFR* gene and breast cancer. In one study it was resulted that there was a weak correlation between *MTHFR* 677TT genotype and postmenopausal breast cancer. But they found that HRT users showed an increased risk of breast cancer with high mammary epithelial cell proliferation pertaining to *MTHFR* 677TT genotype **[21]**. **Manel Esteller *et al***. designed a study to assay the polymorphisms for *MTHFR* and *CYP1A1* simultaneously and susceptibility towards breast cancer.Their data were in agreement with a genetic susceptibility to endometrial cancer associated with a *MTHFR* germ line polymorphism and a recently described rare *CYP1A1* variant found by them. In addition to suggest the contribution of polymorphisms in DNA methylation-related genes and carcinogen-metabolism genes to an enhanced cancer risk, the study could provide a relation with the epidemiological association between estrogen exposure, obesity and endometrial cancer **[22]**.

Also a study to detect an association of the C677T polymorphism in the MTHFR gene with breast and/or ovarian cancer risk in Jewish women was in accord with the idea that disruption in folate metabolism increase breast/ovarian tumorogenesis. However, they indicate that breast/ovarian cancer patients homozygous for the 677T mutation would be at higher risk for acquiring a second primary tumor **[23]**.In another study of this type in Shanghai breast cancer women population, they found that, although there was no overall association between MTHFR genotype and breast cancer risk, women who had low intake of folate and who are homozygous for the MTHFR 677T polymorphism may be at potentially increased risk of breast cancer. They also suggest that this relationship may be further modified by vitamin B12, vitamin B6, and methionine intake. Therefor it can be inferred that one-carbon metabolism and MTHFR polymorphisms have a role in carcinogenesis and may be important in breast carcinogenesis **[24]**. On the other side some investigations could not associate MTHFR genotypes and susceptibility to breast cancer. A study was performed to realize if there was a link between MTHFR, MTR, and TYMS genotypes and breast cancer in Caucasian women, and the study did not support this idea and they could not find any associations

[25].Also an investigation in breast cancer patients in Turkish population did not support the idea that C677T polymorphism for MTHFR gene enhances the risk of breast cancer **[12]**.

CONCLUSION

To sum up all the facts, three possible genotypes of MTHFR gene in breast cancer women of east Azerbaijan, Iran did not differ in comparison to healthy controls. Our findings were at contrary to some previous studies. However the results support other studies to show no significant difference of genotypes between patients and controls. We did not measure serum level of folate in patients and controls. Therefore it's premature to infer a vigorous result if there was a significant consequence regarding to different genotypes and susceptibility to breast cancer.

ACKNOWLEDGEMENT

We are thankful of the kind collaboration of personnel of the Genetic department of Tabriz University of Medical Sciences and Imam Reza Hospital, Tabriz, Iran.

Disclosure of conflict of interest

There is no conflict of interest to declare.

REFERENCES

[1] Poller D, Barth A, Slamon D, Silverstein M, Gierson E, Coburn W, et al. Prognostic classification of breast ductal carcinoma-in-situ. The Lancet. 1995;345(8958):1154-7.

[2] Jemal A, Siegel R, Ward E, Hao Y, Xu J, Murray T, et al. Cancer statistics, 2008. CA: a cancer journal for clinicians. 2008;58(2):71-96.

[3] Agarwal G, Ramakant P, Forgach ERS, Rendón JC, Chaparro JM, Basurto CS, et al. Breast cancer care in developing countries. World journal of surgery. 2009;33(10):2069-76.

[4] Byrne C, Brinton LA, Haile RW, Schairer C. Heterogeneity of the effect of family history on breast cancer risk. Epidemiology. 1991:276-84.

[5] Easton DF, Pooley KA, Dunning AM, Pharoah PD, Thompson D, Ballinger DG, et al. Genome-wide association study identifies novel breast cancer susceptibility loci. Nature. 2007;447(7148):1087-93.

[6] Pakseresht S, Ingle G, Bahadur A, Ramteke V, Singh M, Garg S, et al. Risk factors with breast cancer among women in Delhi. Indian journal of cancer. 2009;46(2).

[7] Porter PL. Global trends in breast cancer incidence and mortality. salud pública de méxico. 2009;51:s141-s6.

[8] Ames BN. DNA damage from micronutrient deficiencies is likely to

be a major cause of cancer. Mutation Research/Fundamental and Molecular Mechanisms of Mutagenesis. 2001;475(1):7-20.

[9] Toffoli G, Veronesi A, Boiocchi M, Crivellari D. MTHFR gene polymorphism and severe toxicity during adjuvant treatment of early breast cancer with cyclophosphamide, methotrexate, and fluorouracil (CMF). Annals of Oncology. 2000;11(3):373-4.

[10] Cheng P, Schmutte C, Cofer K, Felix J, Yu M, Dubeau L. Alterations in DNA methylation are early, but not initial, events in ovarian tumorigenesis. British journal of cancer. 1997;75(3):396.

[11] Frosst P, Blom HJ, Milos R, Goyette P, Sheppard CA, Matthews RG, et al. A candidate genetic risk factor for vascular disease: a common mutation in methylenetetrahydrofolate reductase. 1995.

[12] Hekim N, Ergen A, Yaylım İ, Yılmaz H, Zeybek Ü, Öztürk O, et al. No association between methylenetetrahydrofolate reductase C677T polymorphism and breast cancer. Cell biochemistry and function. 2007;25(1):115-7.

[13] Campbell IG, Baxter SW, Eccles DM, Choong D. Methylenetetrahydrofolate reductase polymorphism and susceptibility to breast cancer. Breast Cancer Res. 2002;4(6):R14.

[14] Qi X, Ma X, Yang X, Fan L, Zhang Y, Zhang F, et al. Methylenetetrahydrofolate reductase polymorphisms and breast cancer risk: a meta-analysis from 41 studies with 16,480 cases and 22,388 controls. Breast cancer research and treatment. 2010;123(2):499-506.

[15] McCawley LJ, Matrisian LM. Matrix metalloproteinases: multifunctional contributors to tumor progression. Molecular medicine today. 2000;6(4):149-56.

[16] Claus EB, Risch NJ, THOMPSON WD. Age at onset as an indicator of familial risk of breast cancer. American journal of epidemiology. 1990;131(6):961-72.

[17] Sharp L, Little J, Schofield A, Pavlidou E, Cotton S, Miedzybrodzka Z, et al. Folate and breast cancer: the role of polymorphisms in methylenetetrahydrofolate reductase (MTHFR). Cancer letters. 2002;181(1):65-71.

[18] Brattström L, Wilcken DE, Öhrvik J, Brudin L. Common methylenetetrahydrofolate reductase gene mutation leads to hyperhomocysteinemia but not to vascular disease the result of a meta-analysis. Circulation. 1998;98(23):2520-6.

[19] Ulrich CM, Kampman E, Bigler J, Schwartz SM, Chen C, Bostick R, et al. Colorectal adenomas and the C677T MTHFR polymorphism: evidence for gene-environment interaction? Cancer Epidemiology Biomarkers & Prevention. 1999;8(8):659-68.

[20] Ma J, Stampfer MJ, Giovannucci E, Artigas C, Hunter DJ, Fuchs C, et al. Methylenetetrahydrofolate reductase polymorphism, dietary interactions, and risk of colorectal cancer. Cancer Research. 1997;57(6):1098-102.

[21] Le Marchand L, Haiman CA, Wilkens LR, Kolonel LN, Henderson BE. MTHFR polymorphisms, diet, HRT, and breast cancer risk: the multiethnic cohort study. Cancer Epidemiology Biomarkers & Prevention. 2004;13(12):2071-7.

[22] Esteller M, Garcia A, Martinez-Palones JM, Xercavins J, Reventos J. Germ line polymorphisms in cytochrome-P450 1A1 (C4887 CYP1A1) and methylenetetrahydrofolate reductase (MTHFR) genes and endometrial cancer susceptibility. Carcinogenesis. 1997;18(12):2307-11.

[23] Gershoni-Baruch R, Dagan E, Israeli D, Kasinetz L, Kadouri E, Friedman E. Association of the C677T polymorphism in the< i> MTHFR</i> gene with breast and/or ovarian cancer risk in Jewish women. European Journal of Cancer. 2000;36(18):2313-6.

[24] Shrubsole MJ, Gao Y-T, Cai Q, Shu XO, Dai Q, Hébert JR, et al. MTHFR polymorphisms, dietary folate intake, and breast cancer risk results from the Shanghai Breast Cancer Study. Cancer Epidemiology Biomarkers & Prevention. 2004;13(2):190-6.

[25] Justenhoven C, Hamann U, Pierl CB, Rabstein S, Pesch B, Harth V, et al. One-carbon metabolism and breast cancer risk: no association of MTHFR, MTR, and TYMS polymorphisms in the GENICA study from Germany. Cancer Epidemiology Biomarkers & Prevention. 2005;14(12):3015-8.

Table 1: Clinical features in woman with breast cancer

Characteristic	No (%)
Total number	50
Mean age	44±5
Mean weight	73
Delivery times	0-3
Hormonal disorders	18 (36%)
Menopausal status	
Premenopausal	12 (24%)
Postmenopausal	38 (76%)
Lymph nodes	
+	8 (16%)
-	42 (84%)
Tumor grade	
I	2 (4%)
II	30 (60%)
III	18 (36%)
Chemotherapy	
Yes	4 (8%)
No	46 (92%)
Radiotherapy	
Yes	5 (10%)
No	45 (90%)
Metastasis	
Yes	0 (0%)
No	50 (100%)

Table 2: MTHFR genotype frequencies of breast cancer patients in comparison with normal controls

Genotypes	Patients % (n=50)	Controls % (n=78)	Odds ratio (95% Confidence interval)
CC	28 (56%)	43 (55%)	0.933 (0.831-2.02)
CT	14 (28%)	21 (27%)	0.54 (0.327-1.852)
TT	8 (16%)	14 (18%)	0.029 (0.17-5.361)