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Relationship between MTHFR Gene Polymorphism [C677T] and Risk of Breast Cancer.

Thoria Ahmed Omar[1], Waleed Mohamed Fathy[1], Mohamed Sabry Ammar[2], Alshimaa Mahmoud Alhanafy[3], Doha said sayed[4],

[1](Clinical Pathology Department, faculty of Medicine, Menoufia University, Egypt)
[2](General Surgery Department, faculty of Medicine, Menoufia University, Egypt)
[3](Clinical Oncology and Nuclear Medicine, Faculty of Medicine, Menoufia University, Egypt)
[4](Clinical Pathology Department, Shebin El-Kom, Teaching Hospital, Menoufia, Egypt)

Abstract:
Background*: Breast cancer is a complex disease, constitutes a main community health concern. Mutation in tumor suppressors' genes and oncogenes were involved in its pathogenesis. Methylenetetrahydrofolate reductase helps in the 5, 10-methylenetetrahydrofolate transformation into 5-methyltetrahydrofolate. 5-methyltetrahydrofolate assists in the re-methylation of homocysteine to de novo methionine necessary for DNA production. In this study we analyze the relationship between the MTHFR gene C677T [rs1801133] polymorphism and the risk of breast cancer.*
Materials and Methods*: This case–control study was carried out on 50 women with breast cancer and 50 women who apparently healthy. Real time polymerase chain reaction technique was used to detect MTHFR gene C677T [rs1801133] polymorphism.*
Results*: 677T allele and 677TT genotype were significantly higher in breast cancer patients with (p<0.001) & (OR =3.998 [CI: 2.162 – 7.394])and(p<0.001) (OR=18.462 [CI: [3.699 – 92.141]) respectively than 677C allele or 677CC genotype.*
Conclusion:*MTHFR gene C677T polymorphisms may be a candidate variant in the predisposition of breast cancer.*
Key Word:*Breast cancer, gene polymorphism, Methylenetetrahydrofolate reductase, real-time polymerase chain reaction.*

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I. Introduction

Breast cancer (BC) is a significant cause of mortality and morbidity amongst women[1]. BC is predicted to be the most predominant cancer between Egyptian women, representing about 18.9 % of overall age-adjusted cancer cases with incidence rate 49.6/100,000, according to the Egyptian National Cancer Institute [NCI]. Genetic, hormonal, and environmental factors contribute to the BC pathogenesis [2]. Hormone-dependent and chemotropic-based interventions are the frequently target therapy used for BC. However, it lacks the efficacy to meet patient spectrum and patient outcomes requirements[3]. Folate is an essential water-soluble vitamin that occurs naturally in foods as well as in the synthetic form that is used in supplements and food fortification programs. There are many critical cellular pathways dependent on folate including DNA, RNA, and protein methylation[4]. MTHFR enzyme represent the main enzyme that catalyzes the reduction of 5, 10-methylenetetrahydrofolate to 5-methyltetrahydrofolate which is the carbon donor for homocysteine methylation to methionine. Then, methionine is changed into S-adenosyl methionine (SAM)which is the methyl donor in biological procedures, including proteins and nucleic acids methylation. Besides, MTHFR acting a major role in de novo production of purines and the pyrimidine nucleoside later in DNA biosynthesis, repair, and maintenance of DNA stability [5].Homozygote variants [TT] have 30 percent enzyme activity compared to wild-type C allele [CC] homozygotes, while heterozygotes [CT] retain 65 % of wild-type MTHFR enzyme activity. In addition, 677 TT mutant homozygotes have ~ 25 % higher in the level of homocysteine than the CC homozygotes, especially, when the folate is low [6]. Mutations in the MTHFR gene can take mild or severe forms. Patients with extreme MTHFR deficiency have high levels of homocysteine in their plasma and urine and have a wide variety of clinical symptoms, including developmental delay, vascular, problems and neurodegenerative disorders[7].Several researchers have identified relations between MTHFR polymorphisms and a variety of disorders including autism, schizophrenia, heart disease, fetal neural tube defects, poor pregnancy outcomes and

cancers [e.g. breast, bladder, GIT, lung][8,9]. The present research aimed to study the relationship between the polymorphisms of the MTHFR gene C677T [rs1801133] and the risk of breast cancer.

II. Subjects and methods

This case-control study was conducted at the clinical pathology department Faculty of Medicine, Menoufia University from July 2018 to December 2019.It included 100 females: **Group 1**: 50 breast cancer patients, selected from the outpatient clinics of the general surgery department and clinical oncology and nuclear medicine department Faculty of Medicine, Menoufia University. Group 2: 50 matched apparently healthy females with no history of BC amongst first and/ or second-degree relatives.

. Informed written consent was attained from all participants. The study was approved by the Ethical Committee of Medical Research, Faculty of Medicine, Menoufia University.

Patients were subjected tofull history taking, clinical examination, radiological investigations, histopathological examination of the breast mass, laboratory investigations and detection of *MTHFR* gene [C677T] single nucleotide polymorphism in extracted DNA from blood samples by real time-PCR using TaqMan allelic discrimination Assay.

Sample collection: 7 ml of venous blood was withdrawn under complete aseptic condition 1.5 ml in K-EDTA tube for PCR, 1.8 ml for ESR tube and the remaining was delivered into a plain tube for other tests.

Laboratory Investigation: ALT, AST, ALP, urea and creatinine tests were done by auto analyzer AU 680 [Beckman coulter, AU chemical analyzer, USA], CBC was measured by Sysmex1 XN-1000 Automated Hematology Analyzer [Sysmex Corporation, Japan], ESR by aspirating specimens into Westergren pipettes till zero mark placing into vertical rack after 60 minutes measure the distance from plasma to the top of the sediment erythrocytes. CA 15-3 by VIDAS® [bioMérieux SA RCS, Lyon, France].

DNA Extraction. Double stranded DNAs were extracted from whole blood by using QIAamp DNA Blood Mini Kit [Qiagen, San Diego, CA]. The purity and concentration of extracted DNAs were observed with a spectrophotometer [Implen NanoPhotometer™ N60 UV/VIS spectrophotometer, Germany]. The extracted DNA was aliquoted and stored at - 80º C.

Genotyping of *MTHFR* [rs 1801133] polymorphism was performed using [Rotor-Gene, QIAGEN Hilden, Germany]. Fluorescent labeled probes [VIC/VAM] GAAAAGCTGCGTGATGATGAAATCG**[C/T]** CTCCCGCAGACACCTTCTCCTTCAA. Taqman master mix [Applied Biosystems, Foster City, CA] The total volume of polymerase chain reactions was 20 µL.

Statistical analysis:IBM SPSS software package version 20.0[Armonk, NY: IBM Corp**].** Chi square testwas used for categorical variables, to compare between different groups,Monte Carlocorrection used for correction of chi-square when more than 20% of the cells have expected count less than 5.ANOVA testwas used for comparison of quantitative variables of normally distributed data. Student t-testfor normally distributed quantitative variables, to compare between two studied groups whileMann Whitney tests were usedforquantitative variables not normally distributed. Odd ratio (OR)used to calculate the ratio of the odds and 95% Confidence Interval of an event occurring in one risk group to the odds of it occurring in the non-risk group. The Significance of the obtained results was judged at the 5% level**.**

III. Result

The groups were age matched. There was a highly statistically significant difference among groups regarding the family history (p<0.001) and age of menopause (p<0.001). While no statistically significant difference regarding marital status, age of menarche, height, weight, and BMI (Table 1).

Table (1): Demographic data of the studied groups.

parameter	Cases (n = 50)	Control (n = 50)	P value
Age (years) Min. – Max. Mean ± SD	30 – 82 47.58 ± 10.13	30 – 72 45.38 ± 9.13	0.257
Family history Negative Positive	35(70%) 15(30%)	50(100%) 0(0%)	<0.001*
Marital status Single Married	3(6%) 47(49%)	5(10%) 45(90%)	0.715
Age of menarche (years) Min. – Max. Mean ± SD.	9 – 16 11.90 ± 1.64	9 – 15 11.80 ± 1.68	0.764

Age of menopause(years) Negative Positive	32(64%) 18(36%)	39(78%) 11(22%)	0.123
Min. – Max. Mean ± SD.	45 – 52 48.33 ± 2.11	49 – 55 51.18 ± 1.83	0.001*
Height (cm) Min. – Max. Mean ± SD.	150 –171 161.70 ± 4.87	150 –171 161.76 ± 4.91	0.951
Weight (kg) Min. – Max. Mean ± SD.	60 –104 77.78 ± 11.77	60 –104 77.26 ± 11.65	0.825
BMI Min. – Max. Mean ± SD.	23 –42 29.50 ± 4.35	23 –42 29.22 ± 4.25	0.746

BMI: body mass index *: Statistically significant at $p \leq 0.05$

There were highly statistically significant differences among groups as regard AST (p= 0.009), Creatinine, ALP, CA15.3, PLT, HB and ESR (p= 0.001), Regarding ALT, urea and TLC there were no statistically significant difference (Table 2).

Table (2): Comparison between the studied groups according to laboratory results.

parameter	Cases (n = 50)	Control (n = 50)	P value
AST (IU/L) Min. – Max. Median (IQR)	10 –80 23 (18 – 30)	11 –33 20 (17.5 – 22.5)	0.009*
ALT (IU/L) Min. – Max. Median (IQR)	10 –80 20 (15 – 25)	12 –33 19 (16 – 28.5)	0.917
Creatinine (mg/dl) Min. – Max. Mean ± SD.	0.60 –1.50 0.91 ± 0.22	0.30 –1.10 0.67 ± 0.21	<0.001*
Urea (mg/dl) Min. – Max. Mean ± SD.	18 –37 278 ± 4.31	15 –47 26.80 ± 7.23	0.815
Alkaline phosphatase (IU/l) Min. – Max. Median (IQR)	90 –1030 130 (117 – 140)	32 –60 47.50 (46 – 53)	<0.001*
CA15.3 (u/ml) Min. – Max. Median (IQR)	13 –210 48 (22 – 48)	10 –30 14.50 (10 – 14)	<0.001*
PLT($\times 10^3$/ul) Min. – Max. Mean ± SD.	102 –313 196.34 ± 36.29	232 –420	<0.001*
HB (g/dl) Min. – Max. Mean ± SD.	8 –13 10.36 ± 1.17	9.70 –12.10 111 ± 0.63	0.001*
TLC ($\times 10^3$/ul) Min. – Max. Mean ± SD.	3.20 –13 7.79 ± 2.43	3.90 –12 7.57 ± 2.34	0.640
ESR Mean ± SD. Min. – Max.	10.36 ±38 5-8	7.92 ±2.73 5-17	<0.001*

AST: aspartate aminotransferase ALT: alanine aminotransferase HB: hemoglobin PLT: platelet TLC: total leucocytic count
ESR: erythrocyte sedimentation rate. *Statistically significant at $p \leq 0.05$

MTHFR [C677T] rs1801133: There was a statistically significant difference between the studied groups in genotyping and alleles distribution. In control group, the genotypes were: wild type CC [60%] [30/50], heterozygousCT [36%] [18/50], and homozygous mutant TT [4%] [2/50]. The allele frequency: C represented 78 [78%] and T was 22 [22 %] of total allele distribution.

In BC group, the genotypes were: wild type CC [26%] [13/50], hetero CT [42%] [21/50] and homozygous mutant TT [32%] [16/50]. The allele frequency: C represented 47 [47%], and T was 53 [53 %] of total allele distribution.

TT genotype was more risk than CC "reference group" with (OR=18.462 [CI: [3.699 – 92.141]) while CT genotype was more risk than CC "reference group" with (p<0.001)&(OR =2.692 [CI: 1.088 – 6.658]) also T allele wasstatistically significant high in BC with (p<0.001) &(OR =3.998 [CI: 2.162 – 7.394])[Table 3].

Table (3): distribution of *MTHFR* [C677T] rs1801133 in the studied groups

parameters	Cases (n = 50)	Control (n = 50)	OR	p	95% C. I
MTHFR polymorphism					
CC®	13(26%)	30(60%)	1.00	-	-
CT	21(42%)	18(36%)	2.692*	0.032*	1.088–6.658
TT	16(32%)	2(4%)	18.462*	<0.001*	3.699–92.141
Allele frequency					
C®	47(47%)	78(78%)	1.00	-	-
T	53(53%)	22(22%)	3.998*	<0.001*	2.162 – 7.394

OR: Odd`s ratioC.I: Confidence interval *: Statistically significant at $p \leq 0.05$

Univariate and multivariate logistic regression analysis among patent and control [Table 4]. Breast cancer is dependable and multifactorial disease.

Table (4): Univariate and multivariate logistic regression analysis among patent and control

Parameters	Univariate		#Multivariate	
	p	OR (95%C. I)	p	OR (95%C. I)
Age (years)	0.257	1.025(0.982 – 1.069)		
Family history	**0.998**	–		
Marital status (Single)	**0.465**	1.741(0.393 – 7.713)		
Age of menarche (years)	**0.761**	1.038(0.817 – 1.317)		
Menopause	**0.126**	1.994(0.824 – 4.827)		
Height (cm)	**0.951**	0.997(0.920 – 1.082)		
Weight (kg)	**0.823**	1.004(0.970 – 1.038)		
BMI	**0.743**	1.016(0.926 – 1.114)		
HB	**0.002***	0.465(0.286 – 0.755)	**0.020***	0.228(0.065 – 0.791)
TLC	**0.636**	1.041(0.882 – 1.229)		
PLT	**0.331**	3.128(0.314 – 31.142)		
AST	**0.004***	1.082(1.025 – 1.142)	0.152	1.094(0.967 – 1.238)
ALT	**0.327**	1.021(0.980 – 1.064)		
Urea	**0.812**	1.008(0.943 – 1.078)		
Alkaline phosphatase (IU/l)	**0.983**	2.610(0.0 – 1.044103960143e+038)		
CA 15.3 (u/ml)	**<0.001***	1.350(1.181 – 1.545)	<0.001*	1.330(1.142 – 1.549)
MTHFR polymorphism				
CT	**0.032***	2.692(1.089 – 6.658)	0.702	1.501(0.187 – 12.040)
TT	**<0.001***	18.462(3.699 – 92.138)	0.040*	13.500(1.122 – 162.468)

BMI: body mass index. HB: hemoglobin TLC: total leucocytic countPLT: platelet AST: aspartate aminotransferase
ALT: alanine aminotransferase *Statistically significant at $p \leq 0.05$OR: Odd`s ratioC.I: ConfidenceInterval

IV. Discussion

BC is a relatively rapid disorder; most patients may develop progression or recurrence of the disease. Hidden micro-metastasis can lead to failure of primary treatment their detection can have a significant impact on those patients' prognosis and treatment choices.[8] *MTHFR* is a polymorphic gene. In actively dividing cells, it participated in folate metabolism, DNA biosynthesis, methylation, and genomic integrity.[10]Mutations in *MTHFR*lead to high homocysteine that play as pro-oxidant, generating free radicals through auto-oxidation, prompting lipid peroxidation, and initiating damage to endothelial cells.[11]

Rs1801133 is the most predominant mutation which reduces the efficacy of *MTHFR***.** C to T missense mutation at nucleotide 677 [C677T] creates a thermo-labile type of the enzyme linked to reducing activity due to alanine being converted to valine**.**The alanine to valine missense mutation at rs1801133 located in the exon eight of *MTHFR* caused 30% [T;T] to 65% [C;T] reduction in *MTHFR* enzyme activity in contrast with the normal homozygous C;C.[12]Researches have documented a relation between *MTHFR* gene polymorphismsand higher incidence of multiple cancers such as BC, head and neck cancer, leukemia, pulmonary carcinoma, gastrointestinal cancer,skin cancer and pancreatic and.[13,14] This study aimed to assess the association of *MTHFR* [C677T] gene polymorphism with the risk of BC.

In this study, no statistically significant difference between cases and controls regarding marital status, age of menarche, and height, weight, and BMI. These results disagreed with Aizer et al.,& Alwan et al., who recognized that BC occurrence increased by age with high frequency among the middle-aged and single females. These females are more susceptible to be diagnosed with more progressive disease and less likely to seek conclusive therapy, respectively.[15,16]

Gallegos et al., reported that there was a correlation between obesity and BC which may be due to the effect of leptin, insulin, adipocytes, and other molecules that facilitate the inflammatory process, increase

peripheral circulating estrogens, and metabolic syndromes these can, lead to initiate molecular processes that are mitogenic in breast epithelial cells and hence induce neoplasia.[17]

There was a highly statistically significant difference between groups regarding family history. Inherited genetic mutations could link to the family history associated with the occurrence of BC, also lifestyle shared with increased risk. Parkinson et al., linked family history with a raised incidence of BC in certain families,[18] whileFerlay et al., reported no association between family history and BC.[19] The age of menopause was a highly significant in BC patients however, no significant in the age of menarche. These results agreed withWindrichova et al., found that BC risk not increased for every year younger at menarche but increased for every year older at menopause.[20] WhileFerlay et al., found no relation of BC and age of menopause.[19]Sabatier et al.,reported a statistically significant difference between BC patients and controls as regards age of menarche.[21]AST, Creatinine, ALP, CA15.3, PLT, HB, and ESR were highly significant in patients. The relationship between AST & creatinne and BC are complicated, raised AST and creatinine have been related to many conditions as metastasis, diabetes, myocardial infarction, diet, and chemotherapy. This agreed withTsai et al., while Ferlay et al., reported no statistical differences.[22,19]BC patients had low hemoglobin concentration and platelet count Saito et al. reported thatBC patients have anemia and thrombocytopenia as it is a common complication with cancers.[23]found that BC patients were found to have anemia and thrombocytopenia as it is a common complication in patients with cancers. Different factors are known to be involved in this disorder that directly related to the tumor itself (hemorrhage, bone marrow infiltration, or nutritional insufficiencies) or to anti-cancer therapy which can cause suppression of bone marrow, accentuating pancytopenia and can even lead to dangerous bleeding complications.[23] High level of CA15-3 was noticed in BC patients. CA 15-3 is a consistent tumor marker in patients with relapse and detached metastases. Although Daniele et al., found that the raise of CA15-3 is not a reliable index in the identification of BC as some women's with BC have normal levels CA15-3.[24]Shao et al.,detect that elevated serum CA15-3 may be valuable in defining the risk of relapse and metastases of BC after surgery.[25]ALP was significant higher in BC. This raise because acceleration de novo production of the enzyme and consequent regurgitation into the serum also BC tends to metastasize to the bones this specificity appears to be facilitated by soluble signal molecules as chemokine and transforming growth factor-beta. Another explanation was because breast cancer cells, which collect calcium ions from breast milk, metastasize to bone tissue, where they can gather calcium ions from the bone. This coincided with the results of Singh et al. while Mayne et al., revealed only 20% of BC patients with bone metastases have an increase in ALP activity.[26,27]

MTHFR **rs 1801133** genotyping results were, 26% had C/C genotype in BC, 42% had C/T genotype, remaining 32% had T/T genotype compared to controls 60%, 36%, and 4% respectively. The difference was highly statistically significant. Concerning C allele incidence, it was significantly higher in controls [78%] than in BC [47%] [P= 0.001]. The T allele frequency is significantly higher in BC group [53%] than in controls [22 %].He and Shen,reported, *MTHFR* gene polymorphism C677T associated with the risk of BC,indicating that the T allele is more frequent in these patients.[28]

Ge et al., &Chittiboyina et al., reported that *MTHFR* C677T highly related to the incidence of different types of cancers including BC.[29,30]On the other hand,Chaturvedi et al., reported, *MTHFR* gene polymorphism is not related to BC pathophysiology, so this polymorphism is not a risk factor for BC.[31] AlsoSingh et al., reported that no significant distribution of C677T genotypes between BC patients and controls and not associated with BC risk.[26]

Conclusion: the presence of *MTHFR* [C677T] rs1801133 polymorphism play a role in the susceptibility of BC. Patients with TT genotype and carriers the T allele at significant risk for developing BC. Other studies including larger sample size and the inclusion of more *MTHFR* gene SNPs are recommended.

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