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ORIGINAL ARTICLE

Association of Methylenetetrahydrofolate Reductase (MTHFR) (C677t) Gene Polymorphism with Breast Cancer in Egyptian Women.

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ABSTRACT

Background: Breast cancer is the most common type of cancer among women. The percentage of patients developing metastasis and progressing to advanced stages remains high. Methylenetetrahydrofolate reductase (MTHFR) is a critical enzyme in folate metabolism and involved in DNA synthesis, DNA repair and DNA methylation. The most common MTHFR gene variant is C677T polymorphism. The aim of work was to investigate the association of the Methylenetetrahydrofolate reductase (MTHFR) gene polymorphism C677T and occurrence of breast cancer in Egyptian women.

Subjects &Methods: This is a case control study. Sixty-four subjects were included in this study divided into case group & control group thirty-two subjects each.

Results: The percentage of TT genotype among case group was found to be significantly higher than that of control group (15.6% versus 3.1% respectively). The results showed that the individual carrying TT genotype suffered from higher risk of cancer breast compared to individual carrying CC genotype (OR=6, CI= 1.63-57.1). There was significant difference between the different genotypes among cases group as regarding estrogen, progesterone receptors and tumor grade. Thus estrogen, progesterone receptors and grade 2 were found to be significantly positive among CT genotypes compared to CC and TT genotypes.

Conclusion: This study shows an association of the TT genotype and the T allele of the MTHFR C677T gene with increased genetic risk for breast cancer among Egyptian females.

Key words: MTHFR; Gene Polymorphism; Breast cancer; PCR-RFLP.

INTRODUCTION

Breast cancer (BC) is by far the world's most common cancer among women, and leading cause of death among women worldwide (1). In Egypt, according to National Cancer Institute (NCI), cancer pathology registry 2012. Breast cancer is the most common female malignancy accounting for 37,7% of all female cancer. Breast cancer in Egypt carriers an unfavorable prognosis with 29% mortality. The median age is 46 years (2).

Development of BC is a multistep process, arising from genetic alterations, and leads to the transformation of normal mammary epithelial cells into highly malignant derivatives. BC originates in the any part of the breast and is caused due to abnormal cell division and growth. Literature reveals that imbalance in folate metabolism may be involved in predisposition to BC (3).

The folate metabolism pathway regulates the intracellular folate pool needed for the synthesis and methylation of DNA. In

the folate metabolism pathway, methylenetetrahydrofolate reductase (MTHFR) is the key enzyme that catalyzes the irreversible reduction of 5,10-methylenetetrahydrofolate to 5-methyltetrahydrofolate, which is the primary circulating form of folate and the methyl donor in DNA methylation. MTHFR, a critical enzyme in one-carbon metabolism, is of interest because aberrations in DNA synthesis, repair, and methylation have been implicated in BC risk (4).

Human MTHFR gene is composed of 11 exons encoding a protein of 656 amino acids. It is located on the short arm of Chromosome 1. Allelic variant of the MTHFR gene [C677T (rs1801133)] have been described for the Ala222Val substitutions, and this plays a role in decreased enzyme activity as well (5). The substitution of cytosine (C) with thymine (T) at nucleotide 677 in the MTHFR gene is a common polymorphism (C677T) and is correlated with increased thermolability and reduced MTHFR activity (6).

Aberrant methylation patterns have been found to be associated with the development of BC (7). It has been shown that the C677T variant increases the plasma homocysteine concentration in humans and reduces DNA methylation in cancer patients. It leads to reduced synthesis of methionine and a more limited availability of the methyl donor (S-adenosyl methionine) in the presence of the low-activity T allele (8)**.**

AIM OF WORK

Investigate the association of the Methylenetetrahydrofolate reductase (MTHFR) C677T gene polymorphism and occurrence of breast cancer in Egyptian women.

SUBJECTS AND METHODS

The study was done in Clinical Pathology and General Surgery Departments, Zagazig University Hospitals. The patients' samples collected from General surgery clinics of Zagazig University Hospitals in the period from December 2017 to December 2018. The study included the following. Formal consent was obtained from all individual and the study protocol was approved by the Zagazig medical research ethical committee. The work has been carried out in accordance with The Code of Ethics of the World Medical Association (Declaration of Helsinki) for studies involving humans.

Sixty-four subjects were included in this study, age ranged from (21-63 years); they were classified into two groups as follows:

Group I: included 32 healthy females with no risk factor or medical complaint serve as (control group). Age ranged (21-62 years old).

Group II: included 32 females proven to have breast cancer recently diagnosed (Case group). Age ranged (35-63 years old).

Inclusion criteria: *Female patients proven to have breast cancer & didn't take chemotherapy or radiotherapy.*

Exclusion criteria: *Patient on radiotherapy, chemotherapy or with metastatic breast cancer.*

All groups were subjected to: *Full history taking, body mass index (BMI), laboratory investigations (Collected from patients' sheet) including Complete blood count (CBC), Liver and Kidney function tests, Estrogen receptor, Progesterone receptor & Human epidermal growth 2 receptor. Radiological investigations include (breast ultrasound & mammography). Histopathology from Tru-cut biopsy, fine needle biopsy or from surgical removal. Specific investigation: Detect MTHFR gene polymorphism using Polymerase chain reaction- restriction fragment length of polymorphism (PCR-RFLP).*

The procedure for genotyping MTHFR gene polymorphism

Specimen collection. *Two ml of venous blood was obtained from each subject under complete aseptic condition and was delivered into Ethylenediaminetetraacetic acid (EDTA) vacutainer tube as anticoagulant and stored frozen at - 80ºC.*

DNA extraction. Genomic DNA extraction from venous blood samples collected from 32 cases and 32 healthy controls. The quality of DNA were checked by agarose gel

electrophoresis. Then DNA was stored at -80°C.

MTHFR gene C677T polymorphism. The MTHFR C677T polymorphism was analyzed by polymerase chain reaction (PCR) followed by restriction fragment length polymorphism. Genomic DNA was amplified using the following PCR conditions: 94°C for 4 minutes, then 34 cycles at 94°C for 30 seconds, 60.7°C for 45 seconds, 72°C for 45 seconds, and finally 72°C for 12 minutes. The primers (AmpliTaq Gold 360 master mix) used for amplification of the MTHFR C677T gene polymorphisms were as follows: forward primer 5′-TGAA GGAGAAGGTGTCTGCGGGA-3′; and reverse primer 5′-AGGACGGTGCGGTGAGAGTG-3′. Amplification was performed with 12.5 µl Ready-to-use PCR Master Mix, 1 µl Primer mix reverse, 1 µl Primer mix follower, 6 µl Genomic extracted DNA & 4.5 µl Distilled water. Then visualization of the amplified PCR products by ethidium bromide stained agarosed gel electrophoresis and the PCR-amplified product was 198 base pair (bp) long.

Digestion with restriction enzyme. The PCR products were further digested using Hinf1 enzyme provided from (iNtRON Biotechnology) to screen for C677T polymorphism. The enzymatic mixture contained 1 µL restriction enzyme (RE) (Hinf1), 1 µL 10× buffer, 6 µL PCR products (DNA), and 2 µL distilled water; the mixture was incubated overnight at 37°C for digestion. The digested product was run on 3% agarose gel. The gel was electrophoresed in 0.5x TBE buffer, After electrophoresis (100 volts for 20 minutes) DNA bands were visualized by ultraviolet transillumination and photographed using Wealtec Dolphin-Doc imaging system (Wealtec Corp, USA) and then we observed two bands of 175 bp and 23 bp for homozygous TT, three bands of 198 bp, 175 bp, and 23 bp for heterozygous CT, and one fragment of 198 bp for homozygous CC.

STATISTICAL ANALYSIS

All data were collected, tabulated and statistically analyzed using SPSS version 19**.** Continuous Quantitative variables was expressed as the mean ± SD & median (range), and categorical qualitative variables were expressed as absolute frequencies (number) & relative frequencies (percentage). Independent student's t test was used to compare between two groups of normally distributed data. Categorical data were compared using chi square test. Odds ratio was used for risk quantification. The tests were two sided with p-value < 0.05 was considered statistically significant (S), p-value < 0.001 was considered highly statistically significant (HS), and p-value ≥ 0.05 was considered statistically insignificant (NS).

RESULTS

As regarding age there was significant difference between the control and case groups with mean age are 41.8 ± 12.03 & 52.06 ± 8.25, respectively (Table 1).

The laboratory data collected from patients' sheet including CBC, liver function tests & kidney function tests are tabulated as Mean ± SD & range for each control & case group (Table 2).

The percentage of TT genotype among case group was found to be significantly higher than that of control group (15.6% versus 3.1% respectively). The results showed that the individual with TT suffered from higher risk of cancer breast compared to CC genotype (OR=6, CI= 1.63-57.1). The frequency of CT genotype among case group was found to be non-significantly lower than that of control group (37.5% versus 40.6% respectively) (Table 3).

No significant differences between the studied groups as regarding body mass index (BMI) which was found to be higher among CC genotype compared to other genotypes in case group. Also, the difference between them was non-significant as regarding family history which was highly positive among those with TT genotype compared to others (Table 4).

Among the case group 50% (16/32), 43.8% (14/32) and 25% (8/32) of the studied participants were positive as regarding estrogen, progesterone and HER2 positive respectively. More than half of cases were of grade 2 (56.3%) & (43.8%) were of grade 3

and as regarding pathology, 81.3% of cases had invasive ductal carcinoma, while the remaining 18.7% of them had invasive lobular carcinoma (Table 5).

There was significant difference between the different genotypes among cases group as regarding estrogen, progesterone receptors and grade. However, the difference between them was non-significant as regarding HER2 and pathology. Positive estrogen and progesterone receptors and grade 2 were found to be significantly higher among CT genotypes compared to CC and TT genotypes (Table 6).

Table (1): Studying mean age in control & cases:

Variable	Control group (n=32)	Cases group (n=32)
Age: (years)		
Mean ± SD	41.8 ± 12.03	52.06 ± 8.25
Range	21 - 62	35 - 63

SD: Standard deviation

Table (2): Studying mean & range of CBC, liver function & kidney functions tests among control & case group:

Variable	Control group (n=32)	Cases group (n=32)
CBC		
Hemoglobin		
Mean ± SD	12.2±0.83	11.2 ±1.05
Range	10.8 – 13.7	9.2 – 13.1
Platelets:		
Mean ± SD	320 ±35.1	286.5 ±56.6
Range	249 - 371	204 - 425
LFT		
ALT:		
Mean ± SD	23.4 ± 4.35	19.9±4.87
Range	16 - 29	8 - 34
AST:		
Mean ± SD	22.6 ±5.00	22.3 ±5.4
Range	15 - 32	9 - 52
Albumin:		
Mean ± SD	4.01 ±0.27	3.93 ±0.41
Range	3.7 – 4.6	3.1 – 4.7
KFT		
Creatinine:		
Median	0.60	0.59
Range	0.2 – 0.7	0.3 – 1.4

CBC: Complete blood count RBCs: Red blood cells WBCs: White blood cells LSF: Liver function tests ALT: Alanine aminotransferase AST: Aspartate aminotransferase KFT: kidney function tests SD: Standard deviation

Table (3): Comparative Study of MTHFR genotype between the control and cases:

Variable	Control group (n=32)		Cases group (n=32)		OR (95% CI)	p
	No.	%	No.	%		
MTHFR:						
CC:	18	56.3	15	34.4	Reference	
CT:	13	40.6	12	37.5	1.10 (0.39-3.13)	0.05
TT:	1	3.1	5	15.6	**6 (1.63-57.1)**	**0.004**
Alleles:						
C:	49	76.6	42	65.6	Reference	0.17
T:	15	23.4	22	34.4	1.71 (0.78-3.71)	(NS)

P < 0.05, significant. NS; Non significant

Table (4): Studying of relation between BMI as well as family history and genotypes among case group:

Variable	CC group (n=15)		CT group (n=12)		TT group (n=5)		F	p
BMI:								
Mean ± SD	28.03 ±3.84		26.8 ± 3.01		26.7 ±2.40		0.823	0.449
Range	24.2 - 35		22.9 - 30		26.7 – 28.4			(NS)
	No	**%**	**No**	**%**	**No**	**%**	**χ^2**	**P**
Family history:								
Positive:	2	13.3	5	41.7	3	60	4.771	0.092
Negative:	13	86.7	7	58.3	2	40		(NS)

BMI: Body mass index. SD: Standard deviation N: Number NS: Non significant

Table (5): Percent of disease characteristics as regards estrogen receptors (ER), progesterone receptors (PR), Human epidermal growth 2 (HER2), histopathological tumor grade and pathology among the case group:

Variables	Cases group (n=32)	
	No.	%
ER		
Negative	16	50
Positive	16	50
PR		
Negative	18	56.3
Positive	14	43.8

Variables	Cases group (n=32)	
	No.	%
HER2		
Negative	24	75
Positive	8	25
Tumor grade		
2	18	56.3
3	14	43.8
Pathology		
(IDC)	26	81.3
(ILC)	6	18.7

IDC: Invasive ductal carcinoma ILC: Invasive lobular carcinoma

Table (6): Frequency distribution of disease characteristics among different genotypes in case group:

Variable	CC group (n=15)		CT group (n=12)		TT group (n=5)		χ^2	P
	No.	%	No.	%	No.	%		
ER								
Negative	8	53.5	0	0	3	60	**10.13**	**0.006**
Positive	7	46.7	12	100	2	40		**(S)**
PR								
Negative	10	66.7	0	0	3	60	**11.81**	**0.002**
Positive	5	33.3	12	100	2	40		**(S)**
HER2								
Negative	10	66.7	12	100	4	80	4.86	0.087
Positive	5	33.3	0	0	1	20		(NS)
Tumor Grade								
2	8	53.5	12	100	2	40	**9.03**	**0.01**
3	7	46.7	0	0	3	60		**(S)**
Pathology								
IDC	11	73.3	10	83.3	5	100	1.805	0.405
ILC	4	26.7	2	16.7	0	0		(NS)

P < 0.05, significant ER: Estrogen receptor PR: Progesterone receptor HER2: Human epidermal growth receptor 2 IDC: Invasive ductal carcinoma ILC: Invasive lobular carcinoma

DISCUSSION

Breast cancer (BC) is the most common cancer among women worldwide [International Agency for Research on Cancer (IARC)] (1).

In Egypt Ibrahim reported also that it is the most common cancer in females with its high consideration among urban than rural community across women older than 45 years old (9).

Polymorphism of the gene encoding methylene-tetrahydrofolate reductase (MTHFR) have been studied in several types of cancer including breast (10). The studies evaluated the effect of mutations in MTHFR gene (which plays a role in DNA methylation) on breast cancer pathogenesis (11).

So, the aim of this study was to evaluate the association between the MTFHR gene C677T polymorphism and occurrence of breast cancer.

C677T polymorphism is a point mutation at the position 677 on MTHFR gene with the substitution of cysteine to thymine nucleotide at that position. This point mutation causes the substitution of alanine to valine in MTHFR enzyme (12).

Several studies have been reported that, there is an association between the MTHFR gene C677T polymorphism and BC with conflicting results (13, 14).

The result of this study found that the percentage of TT genotype among case groups was 15.6 % with high value in relation to control group (3.1 %). This results was in agreement with the result of Kono (15) & Zhang (16) who reported in meta-analysis that homozygote genotype of TT had elevated level of BC risk and T allele could be a risk factor for developing BC. Also Weiwei (17) who reported that TT genotype and T allele in C677T position was observed among Chinese females had a significant increase risk for BC. The same result was in accordance with that of kaya (18) who found a significant increase in the frequency of TT genotype in patients than in control groups.

The result of current study also agree with Rahimi (19) who reported that, the frequency of T allele of MTHFR was found in 30% of patients compared to 27.6% of healthy control and this may enhanced the susceptibility to BC. They also reported that frequency of MTHFR C677T among patients were CC 50.7%, CT 43.7% & TT 56%.

In contrast to these results, Prasad (10) found that, there is no link between the occurrence of MTHFR variants and BC this may be due to large number of people he studied.

Another study done by Hedayatizadeh-Omran (20) concluded that, their findings suggest that MTHFR C677T gene polymorphism may not be a risk factor for BC.

The current study revealed that, the frequency of CT genotype among case group was insignificant lower than that of control group. This result was in agreement with that of Hosseini (21) who concluded that, there was a significant association between BC and C677T polymorphism, whereas, there was a decrease frequency for MTHFR 677 CT, TT in case compared with control.

A study done by Papandreou (22) involve 300 females with BC and 283 healthy women, they revealed that, there was an associated only for BC and C677T gene polymorphism, but adjustment for age diminished this association.

As regard estrogen receptor (ER) and progesterone receptor (PR), the present study revealed that, in case group was positive in 50% for ER & 43.8% for PR, while human epidermal growth receptor was +ve in 25% of patients.

This results was in agreement with that of Wassem (23) who reported that about 54% of studied patients have +ve ER. Also matched with results of Hedayatizadeh-Omran (20) reported +ve ER in 59% of patients.

The same result was obtained by Suner (11) except in PR was +ve in 70% of patients, this may be due to large number of cases.

The result was disagreed with that of Rahimi (19) who reported only 28.8% +ve for ER, 27.7% for PR among patients group.

As regard, Histopathology study & grade of disease the present study showed that 56.3% of cases were of grade 2 and 43.8% were of grade 3.

The invasive ductal carcinoma (IDC) was found in 81.3% of cases while 18.7% have invasive lobular carcinoma (ILC). These

results were matched with that of Hedayatizadeh-Omran(20) who reported that grade 2 was present in 44.4% and grade 3 was present in 40.7% of cases.

Also, result agree that of Papandreou (22) who reported that most case had grade 2 was 56% & most of them with IDC was 84%.

The result also agree that of Vivien (24) who found that IDC 87% and ILC 11% and others 2% in breast cancer patients. Kaya (18) disagree this result, they reported that IDC was 88.9% and ILC was 5.8%.

The National cancer Registry program of Egypt (2011) reported that IDC was the most predominant histopathological type for BC, it constituting 81.3% of studied cases (25).

In present study, the frequency distribution of ER, PR, HER2, Grades and histopathology among genotypes in case group revealed that, the +ve ER, PR and grade 2 were significantly higher among CT genotype compared to CC and TT genotype.

This is not matched with study of Kaya (18) that was done on Turkish population & Hedayatizadeh-Omran (20) that was done on Iranian population in which both stated that the estrogen and progesterone receptors & grade 2 are significantly higher in CC genotype compared to CT & TT. This difference may be due to different population.

CONCLUSION

The C677T gene polymorphism of MTHFR was considered as a risk factor for developing breast cancer. Women with MTHFR 677 TT genotype and T allele has a significantly increased risk to BC. Increased BMI may lead to high risk for develop of BC & weight control in obese women may be an effective measure of BC prevention.

- Conflict of Interest: No.
- Financial Disclosures: No.

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