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Original Research Article

Association of Methylenetetrahydrofolate reductase (MTHFR) C677T polymorphism in breast cancer patients of Bihar: Case-control study

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ABSTRACT

Background: Breast cancer (BC) is the most frequent cancer in women globally and the main cause of cancer-related mortality. MTHFR C677T is a functional polymorphism in the genes encoding a one-carbon metabolism enzyme that affect folate metabolism and may be associated with breast cancer susceptibility.
Method: We have conducted a case-control study taking 100 cases of diagnosed breast cancer and 100 healthy control. PCR-RFLP technique was used to detect the genotype of C677T polymorphism in the study population.
Result: About 35% of cases had normal genotype(C677C), 49% had the genotype C677T & rest 16% had the genotype T677T for MTHFR. Among control the frequency of C677C genotype was 55% C677T was 40% & T677T was 5%. C677T & T677T MTHFR genotype were more common in case as compared to control [$\chi 2$ (2, N = 200) = 11.12, p = 0.004]. T allele frequency was significantly higher in case (40%) as compared to control (5%) [$\chi 2$ (1, N = 400) = 10.91, p = 0.001]. Cases with MTHFR 677TT genotype had a significantly higher risk of BC than 677CC individual [OR=1.64 (1.03 to 2.10)] and increasing T-allele was significant in BC [OR=1.07 (0.71 to 1.33)] (P for trend = 0.04).
Conclusion: C677T of MTHFR gene has a strong correlation with breast cancer in the studied population and can be used as a prognostic marker for the development of breast cancer.

1. Introduction

Breast cancer (BC) is the most frequent cancer in women globally and the main cause of cancer-related mortality.[1] Tumor elimination and recurrence prevention are the treatment goals for persons who do not have metastatic illness.[2] Breast cancer is frequently caused by a combination of hereditary and environmental influences. When genetic variants involved in the encoding of these defensive processes are mutated, cells lose their ability to initiate suicide when they are no longer needed, which contributes to the emergence of cancer.[3] Methylenetetrahydrofolate reductase (MTHFR), one of the atypical prognostic indicators for survival rates in cancer, is a priority of therapeutic intervention in the field of oncology.[4]

MTHFR (C677T), methionine synthase (MTR A2756G), methionine synthase reductase (MTRR A66G), and thymidylate synthase are functional polymorphisms in the genes encoding one-carbon metabolism enzymes that affect folate metabolism.

C677T is associated with reductions in MTHFR activity and an elevation of homocysteine levels, as well as A1298C, which is linked to a decrease in MTHFR activity.

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These polymorphisms, namely the C677T and A1298C polymorphisms of the MTHFR gene may also influence breast cancer survival prognosis.[5]

Based on the premises mentioned above, the current study aimed to evaluate the polymorphism of the Methylenetetrahydrofolate reductase (MTHFR) gene (C677T) in breast cancer patients of Bihar.

2. Methods

2.1. Study population

Women diagnosed with bilateral or unilateral breast cancer were enrolled in the study. Breast cancer patients presenting at the outdoor Department of Surgical Oncology of the Indira Gandhi Institute of Medical Sciences (IGIMS), Patna, Bihar, India, with or without family history were included in the study. Total number of included patients(cases) was 100, who were enrolled in the study only after providing signed written consent. Ethical approval was obtained from the Institutional Ethics Committee of IGIMS, prior to the initiation of the study. (Letter No- 635/IEC/IGIMS/2018 dated December 18, 2018).

Age-matched healthy individuals (number=100) without any type of cancer and systemic illness such as asthma, psoriasis, rheumatoid arthritis, and diabetes were enrolled as controls in the study.

2.2. Sample preparation

Two ml (2 ml) of whole blood was collected in EDTA vials from clinically and histopathologically diagnosed breast cancer (operable or inoperable) patients and healthy age- matched controls. Genomic DNA was immediately extracted from whole blood using DNA extraction kit (QIAmp DNA Blood mini kit; Cat. No. 51104) strictly following the manufacturer's instructions. DNA samples were either stored at +4C° for immediate use in PCR or were stored at -80°C until further analysis.

2.3. Genotype assessment

MTHFR C677T polymorphism was analyzed using PCR-RFLP methods. After checking the quality of DNA on Nano drop, PCR was performed for C677T. PCR amplification of exon 4 of the MTHFR gene was done using a specific set of forward and reverse primers. Forward primer: 5'- TGAAGGAGAAGGT GTCTGCGGGA -3' Reverse primer: 5'- AGGACGGTGC GGTGAGAGTG – 3'[6] and the 198 base pair (bp) were amplified. The following composition of 1X PCR(25μl) for C677T was used. Buffer-1 μl, $Mgcl_2$-1.5μl, dNTP-2μl, FP-1μl, RP-1μl, Taq DNA polymerase-0.5μl, Template DNA- 2μl.

Thermal Cycling Conditions: Initial denaturation at 95ºC for 7 minutes followed by denaturation at 94ºC for 45 seconds, annealing of primer at 64ºC for 30seconds, and extension at 72ºC for 35 seconds. This was repeated for 32 cycles. This was followed by a final extension for 7 minutes at 72ºC.

The PCR products were analyzed on a 2% agarose gel containing ethidium bromide (0.5μg/ml). The PCR product was digested with HinfI in the proper composition of the reaction mixture. The reaction was allowed to incubate at 37°C for 2-3 hours, then stop reaction by placing it at 62°C for 20 minutes following the manufacturer's instructions. The RFLP products (15μl) were loaded in 2% agarose gel for 90 minutes at 50 volts followed by visualization of the gels under gel doc (Vilber). GeneRuler DNA Ladder Mix was used as a marker

When MTHFR C677T polymorphism is present, the substitution of a C with a T creates a restriction site for HinfI and the DNA- fragment of 198 bp, previously amplified in PCR, is cut in two pieces, one of 175 bp and the other of 23 bp (TT), three pieces of 198bp,175bp, and 23bp (CT), and one piece of 198bp (CC).

2.4. Statistical analysis

Data were analyzed by SPSS version 21 and presented in Mean (SD) and frequency (%). Categorical variables were compared by χ^2 /Fisher's exact test. p value less than 0.05 was considered statistically significant.

3. Results

The mean age of the cases and control were 47.31±14.24 and 47.15±11.72 respectively. There was no statistically significant difference in the age of the cases and controls (p=0.45) (Table 1) About 35% of the cases had a normal genotype (C677C), 49% had the genotype C677T, and the rest 16% had the genotype T677T for MTHFR. Among the controls, the frequency of the C677C genotype was 55%, C677T was 40%, and T677T was 5%. The C677T and T677T MTHFR genotypes were more common in cases as compared to the controls [χ^2 (2, N = 200) = 11.12, p = 0.004] (Table 2). The T allele frequency was significantly [χ^2 (1, N = 400) = 10.91, p = 0.001] higher in cases (40.5%) as compared to controls (5%). This signifies that the T allele is associated with breast cancer (Table 3). Cases with the MTHFR 677TT genotype had a significantly higher risk of breast cancer [OR=1.64 (1.03 to 2.10)] than 677CC individuals (Table 3). The test for increasing breast cancer risk with the increasing number of variant T alleles was significant (P for trend = 0.04), although there was no clear increased risk for the heterozygous MTHFR 677CT genotype [OR=1.07 (0.71 to 1.33)] (Table 4).

Tabular data is represented as frequency (n), %. Chi-Square test was performed to evaluate the association. P value less than 0.05 was considered to be significant.

Table 1: Comparison of ages of case- control study

Age (years)	Mean±SD	Minimum	Maximum
Case	47.31±14.24	18.0	77.0
Control	47.15±11.72	20.0	75.0

Table 2: Frequency of MTHFR genotype

Genotype	Cases (n, %)	Controls (n, %)	$\chi^{2,}$ p value
CC	35 (35)	55 (55)	
CT	49 (49)	40 (40)	11.12, 0.004
TT	16 (16)	5 (5)	

Table 3: C and T allele frequencies in cases and controls

Allele	Cases (Frequency)	Controls (Frequency)	$\chi^{2,}$ p value
C	119/200 (59.5%)	150/200 (75%)	10.91, 0.001
T	81/200 (40.5%)	250/200 (5%)	

Table 4: Odds ratios (OR) for breast cancer by MTHFR genotype

Genotype	Cases, n	Controls, n	OR (95% CI)	p value
CC	35	55	1.00	
CT	49	40	1.07 (0.71 to 1.33)	<0.0001
TT	16	5	1.64 (1.03 to 2.10)	
P for trend			1.65 (1.03 to 2.33)	

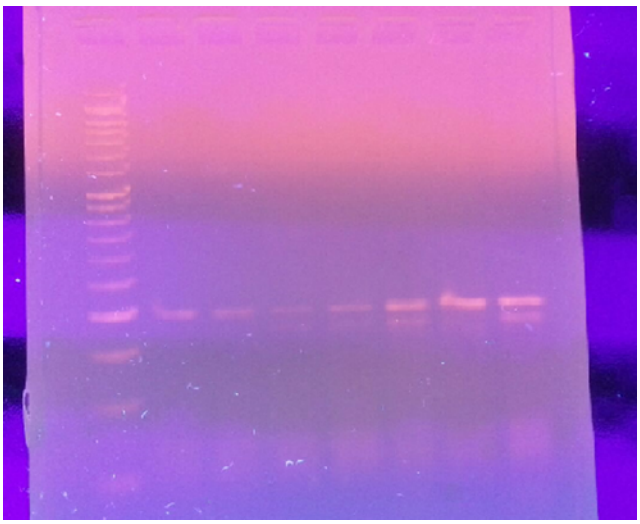

Fig. 1: Representative examples of Genotyping of MTHFR C677T Polymorphism on 2% agarose gel Lane 1- Marker (200bp), Lane 1, 2, 6-CC(198bp), Lane 3,4,5,7-CT(198+175+23bp)

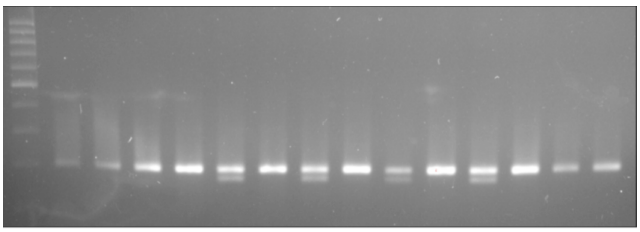

Fig. 2: Lane 1-Marker(100bp), Lane 2-5,7,9,11,13-15-CC(198bp), Lane 6,8,10,12-CT(198+175+23bp)

4. Discussion

Breast cancer is a leading cancer in women around the world, with a high fatality rate. It is distinguished by the expression of abnormal genes that give the tumor a diverse shape and aggressiveness, as well as a variety of clinical symptoms.[7] Genomic variables, such as tumor suppressor and oncogene mutations, copy number diversity, and epigenetic changes, are anticipated to have a role in the onset and progression of cancer in young women.

MTHFR is involved in the biosynthesis of S-adenosyl-methionine, the methyl donor necessary for all methylation events in cells, folate (FA), or vitamin B9, is crucial for the process of cell division and homeostasis.[8] Inadequate folate level or inadequate consumption has been linked to a greater prevalence of cancer risk.[9]

Folate promotes normal cells to become malignant, and nutritional folate consumption has been linked to DNA methylation in patients with breast cancer.[10] MTHFR is reported to be a folate-dependent enzyme and the MTHFR gene regulates intracellular folate amounts, DNA biosynthesis, methylation patterns, and a variety of other biochemical functions including tumorigenesis.

The two well-researched MTHFR polymorphisms are known to be MTHFR 677C> T and 1298A> C. It is a well-known fact that the MTHFR 677C> T polymorphism has been involved in the development of breast cancer in major

clinical investigations.[11]

Polymorphisms may thus have a role in the predisposition to BC in diverse communities, and further research is needed to help comprehend the pathophysiology of the tumor, generate new diagnostic tools, and find more effective therapies.

The C677T and the A1298C variant are the two most researched MTHFR polymorphisms, both decreasing enzyme activity by manifolds.[12] Although there has been a lot of data for investigations on the link between these polymorphisms and breast cancer, genome-wide association conclusions have been conflicting in a lot of situations.

Hence, the present study an attempt to explore a possible interaction between polymorphisms and chromosomal instability, to explore the possibility of using them as a biomarker for response to therapy/ prognosis of breast cancer.

In the present study, it was noted that the C677T and T677T MTHFR genotypes were more common in cases as compared to the controls. (p=0.004) (Table 1). A handful of meta-analyses have found a link between the 677 C>T polymorphism and the risk of BC.[13–15] The 677C>T substitution, according to a reported publication, is a significant risk factor for BC in Indian females of Dravidian origin.[16] However, no significant link between the MTHFR 677 C>T gene polymorphism and BC was noted in Indian women reported in a case-control study involving 588 BC patients and 508 healthy individuals.[17] Mir et al. in a research investigation found that in a north Indian Caucasian community, those harboring the 677 C>T substitution had a 3.5 times lower incidence of breast cancer (P<0.02) than those without the said polymorphism.[18]

In the present study, it was also found that the T allele frequency was significantly [χ^2 (1, N = 400) = 10.91, p = 0.001] higher in cases (40.5%) as compared to controls (5%). This signifies that the T allele is associated with breast cancer (Table 2). The T allele had a greater occurrence in patients (p= 0.0001), according to examinations of peripheral blood samples taken from 100 females based out of Iran with BC and 142 healthy females. Similar findings were further confirmed by another group of researcher who laid upon the function of the single-nucleotide polymorphism (SNP) T of the C677T polymorphism in folate metabolism in vitro as a potential cause for BC and indicated that SNPs might be used as pharmacogenetics for chemotherapeutic intervention.[19]

In the present study, it was also observed that cases with MTHFR 677T genotype had significantly higher risk of Breast cancer [OR=1.64(1.03 to 2.10)] (P <0.0001) than 677CC individuals (Table 3).

According to certain research, the MTHFR 677TT genotype increases the risk of breast cancer, specifically in pre-menopausal women.[20,21] Another nested control study found that post-menopausal women with the TT genotype had a 62% higher risk of breast cancer incidence.[22] Many researchers have examined the link between MTHFR 677TT and the risk of breast cancer in patients.[23,24]

However, results have been inconsistent among populations. Disparities in outcomes could be owing to population discrepancy in the incidence of polymorphisms in genes associated to one-carbon metabolism, dietary consumption, and/or breast cancer risk markers

5. Conclusion

Polymorphisms in the MTHFR gene increase susceptibility to breast cancer. C677T of the MTHFR gene has strong correlation with breast cancer in the studied population. Polymorphisms in the MTHFR gene and chromosomal aberrations in preoperative peripheral blood samples can be a biomarker for the aggressiveness of cancer and response to therapy in breast cancer patients. MTHFR C677T SNP carriers can be identified by genetic testing and this can help in providing molecular detection as a predictive and prognostic marker for the development of breast cancer. One of the most essential steps females can take to minimize their risk of breast cancer is to consume more folate-rich foods including leafy green vegetables, legumes, asparagus, Brussels sprouts, broccoli, and orange juice. Additional folate that is converted to its active form can be obtained by eating more folate-rich foods. It is a perceived notion that dietary folate intake is inversely related to breast cancer risk, regardless of MTHFR status.

6. Source of Funding

None.

7. Conflict of Interest

None.

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