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The MTHFR A1298C polymorphism and Breast Cancer Susceptibility

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Abstract

Background:Breast cancer (BC) is still a worldwide health problem with a high mortality rate in Iraq and other countries. reductase gene (MTHFR) controls cell DNA methylation, and any defect in this gene may lead to cancer development.
Aim: The goal of this study was to investigate the associations of SNP (rs1801131) A1298C genetic polymorphism of MTHFR gene as well as its haplotypes with breast cancer risk among Iraqi females. Materials and Methods: The study included fifty individuals diagnosed with breast cancer and fifty healthy controls. The ages of the patients ranged between 25 and 67 years. For DNA extraction, 1 ml of peripheral blood from each individual was used. The A1298C polymorphism was detected with a previously prepared kit according to the manufacturer's instructions. Taq-Man allelic discrimination assay was used for genotype identification. Then, p values were calculated via a chi-square test for statistical analysis.
Results: DNA was successfully extracted from all the samples. The breast cancer patients and control groups all had frequency genotypes consistent with Hardy-Weinberg equilibrium (HWE) expectations. Logistic regression was used to assess the etiological risk of A1298C polymorphism in breast cancer cases. Breast cancer cases were more common in people with the heterozygous mutant AC genotype, which was significantly associated ($p = 0.016$; OR = 2.88; 95% CI: 1.29–6.4). The risk factor of the mutant AC genotype increased 2.88 times more than that for the wild type. Conclusions: According to the study results, there is a variation in the distribution of the genetic frequency of the MTHFR polymorphism between patients and control individuals, which is associated with increased etiological risk of the A1298C SNP (rs1801131) in breast cancer patients.

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Introduction

In Iraq, BC is the primary cause of cancer-related mortality among females, representing approximately one-third of all cancer diagnoses nationwide. This may be due to several potential risk factors contributing to this increase, including dietary changes, Overweight, Smoking, Physical inactivity, high marriage age and low birth rates (1). Folate, a water-soluble B vitamin, plays a vital role in important cellular activities like DNA synthesis, repair and methylation reactions. Folate's role as a coenzyme in various enzymatic reactions (2). Imbalances in folate metabolism can contribute to carcinogenesis by disrupting global DNA methylation patterns and altering the methylation status of specific gene promoters (3). Methylation of DNA is identified as a chemical modification in DNA that influences genetic expression without changing the DNA sequence and plays a crucial role in transmitting epigenetic information across cell divisions. Alterations in DNA methylation patterns are strongly correlated with the progression of cancer. Specifically, global DNA hypomethylation, characterized by a decrease in overall DNA methylation levels, has been linked to low folate intake (4, 5).

The MTHFR gene codes for an enzyme named the same gene, MTHFR, which has a critical role in amino acid metabolism and protein synthesis. It is considered the main key involved in folic acid metabolism. It is located on the short arm of chromosome 1 (1p36.3). It consists of 13 exons (6) and spans approximately 20.242 nucleotides. Located within the MTHFR gene's exon 7, we find the MTHFR A1298C polymorphism. The change of the 429th amino acid in the MTHFR enzyme from glutamic acid to alanine is caused by this mutation. This change causes changes in the structure of the gene, resulting in a loss of its enzymatic activity, but this variant does not increase the heat sensitivity of the enzyme (7). The different forms of the MTHFR gene cause the enzyme's catalytic activity to decrease. This enzyme is crucial for the proper formation of DNA and its repair process. MTHFR polymorphisms influence BC risk, but the association remains controversial (8). Breast cancer is a big public health problem around the world; thus, the current study investigates the relationship between changes in folate metabolic pathways due to the A1298C polymorphism of the MTHFR gene and the initiation of BC.

Materials & Methods

Patients

The sample included 50 females diagnosed with BC and 50 others healthy controls. All the chosen participants were referred from Alkarama Hospital in Baghdad from January 2021 to October 2022. The categories of age were between 25 and 67 years, with a mean age of 46 years.

Samples and DNA extraction

One milliliter of peripheral blood from each participant was used for DNA extraction via the standard phenol–chloroform method (9). A spectrophotometer from Thermo Scientific (nanodrop model 2000) from the USA was used to determine the concentration and purity of the DNA samples. After the genomic DNA was extracted, 1% agarose gel electrophoresis was performed to the DNA was present and to check the quality. The solutions were made according to standard methods (10).

Genotyping and allelic discrimination

We analyzed the A1298C SNP (rs1801131) in the MTHFR gene via Taq Man allelic discrimination technology with a real-time PCR kit (SNP, Biotechnology). The allelic discrimination procedure was performed according to the qPCR preparation and cycling conditions provided by the manufacturer.

Statistical methods

The DNA concentration and purity variables are expressed as the means with standard deviations. The observed and expected genotype frequencies were assessed via the web tool HWE. The chi-square tests were used to detect allele and genotype differences between the patient and control groups. The etiological risk was detected by odds ratios (ORs) and confidence intervals (CIs) (95% CIs).

Results

DNA was successfully extracted from all the samples via an organic procedure. The mean DNA concentration in the patient group was 436.92130.35± ηg (mean ± SD) and for control 424.77 ± 102.65 ηg (means ± SD) and integrity was assessed via gel electrophoresis (Fig. 1). The mean DNA purities of the patients and controls were 1.650.204± and 1.770.13±, respectively.

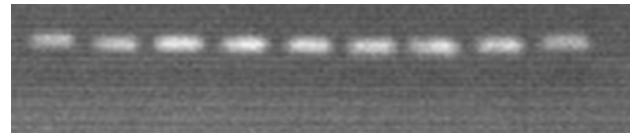

Figure 1: DNA extraction was performed via 1% agarose gel electrophoresis, and red-safe DNA staining visualization was performed for 40 minutes.

MTHFR SNP (rs 1801113)A1298C genotyping

The kit system employs specific primers, probes and internal controls with each included master mix. To ensure accurate genotyping, each isolated DNA sample was analyzed via both proprietary (approved) wild-type and mutant-type real-time PCR master mixes (Fig 2).

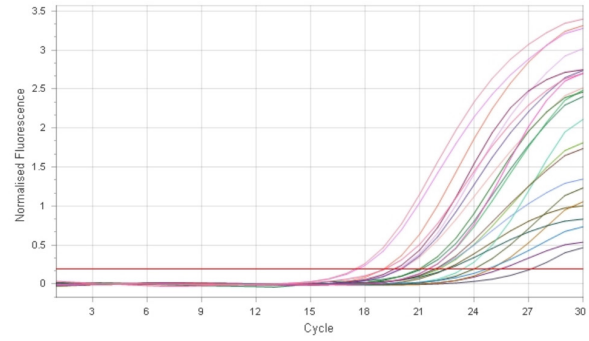

Figure 2: MTHFR gene (rs 1801131) amplification plots generated by the MIC-4 real-time PCR system/ Australia.

The genotype distributions of the control and patient samples were tested for HWE for the MTHFR A1298C (G429A; rs1801131) polymorphism. The expected genotype frequencies under HWE were not significantly different from the observed values in either group (control group P = 0.8; patient group P = 0.1). There was no substantial difference between the observed and expected genotype frequencies, as shown in Table (1).

Table 1: Allele frequencies of the A1298C polymorphism: Genotypic and haplotype frequency distributions and Hardy–Weinberg equilibrium (HWE) in the patient and control groups

Genotypes	Control (No=50)		*P* –value	Patients No-50		*P* –value
	Observed	**Expected**		**Observed**	**Expected**	
AA	34(0.67)	34.17(0.67)		21(0.41)	(24.99)0.49	
AC	16(0.31)	13.26(0.26)		29(0.56)	(21.42)0.42	
CC	1(0.02)	1.27(0.025)		1(0.02)	(4.59)0.09	
			0.8			0.1
Haplotype	**Observed**	**Allele frequency**		**Observed**	**Allele frequency**	
A	84	41.82(0.82)		71	(35.7)0.7	
C	18	8.16(0.16)		31	(15.3)0.3	

p value was calculated via the chi-square test. (A1298C: AA wild type (homozygous), AC heterozygous mutant, CC homozygous mutant, significant if p ≤0.05.

Compliance with HWE guarantees the validity of genetic association studies. The pattern of genotypes for each was consistent with that expected under HWE, and the results are shown in Table (2).

Table 2: Allele frequency of the A1298C polymorphism (rs1801131) and risk of breast cancer

Genotype	Controls (No=50)	Patients (No=50)	*P* value	Odd ratio	CI 95 % Confidence interval
AA	34	21	0.017	1.71	(0.63-4.69)
AC	16	29	0.016	2.88	(1.29-6.43)
CC	1	1	1.00	1	(0.06-15.99)

The CC allele was not detected in the study samples but for statistical reasons the number 1 was assigned

The patients in this study had AC allele frequency that was higher than the controls (56% vs. 31%, p = 0.016). The specific score for the difference between the two groups was 2.88 (95% CI: 1.29–6.43). In the model for the homozygous mutant genotype, the CC genotype frequency did not differ between the patients and the controls (OR = 1; 95% CI: 0.06–15.99; p = 1).

Discussion

Breast cancer is a multi-faceted and diversified disease that is the number one cause of cancer mortality among women (11). This study revealed that there was a correlation between the heterozygous AC genotype and an increase in the incidence of breast cancer compared with the control group. An investigation of the genetic variations in the folate metabolic pathway might assist in the identification of individuals with additional BC susceptibility. The genotypic frequencies of the A1298C polymorphism are presented in Tables 1 and 2, thus critical insight into the Iraqi women population. The high odds ratio of the heterozygous AC genotype of 2.88 with 95% CI= 1.29--6.43 shows a connection between the MTHFR A1298C polymorphism and a high risk of BC. According to the evidence that the heterozygous AC genotype is associated with etiological risk, a considerable contributor to risk is the environment rather than genetics.

The folate enzyme produced by the mutant allele leads to a reduction in its activity. The effect may be direct to the function of the DNA repair system, inactivation of tumor suppressor genes and activation of oncogenes (12). Many studies, such as Omran et al. (2021), reported a strong

correlation between the MTHFR polymorphism A1289C and an increased incidence of BC among Egyptian females (13). Another analysis revealed that the MTHFR A1298C alteration might increase the occurrence of breast and ovarian cancer in the Caucasian population (14). Conversely, a prior large meta-analysis reviewing data from 33 different case–control investigations reported that there is no correlation between the A1298C polymorphism and the incidence of breast cancer (15). A research with Northern Brazilian women also found A1298G polymorphism was not significantly associated with BC (16). Limitations of the study include the small sample size and inability to measure the impact of folate and vitamin B dietary intake on both the control and patient groups since these two elements play a role in modulating the effect of this polymorphism.

Conclusion

The current research reveals an important connection between breast cancer in Iraqi females and the genetic variant MTHFR A1298C. These findings show that the heterozygous AC genotype is associated with genetic risk in the studied Iraqi females. However, the allele appears to have a greater effect because of the presence of environmental agents. These findings suggest that polymorphisms may act as molecular markers for the early detection, prediction and prognosis of breast cancer.

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Ethical approval

Ethical approval was obtained from the Institutional Ethics Committee of the Higher Institute of Forensic Science at Al-Nahrain University. The official authorization approval came on 11 September 2023, with reference number (33) associated with it.

Ethical clearance

The informed consent was provided to all participants.

Conflict of interest

No conflicts related to this study.

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