

Study the Effect of RAGE and HMGB1 Genes Polymorphism on Breast Cancer Susceptibility in Iraqi Female

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Abstract

Background: The most frequent malignancy among women globally is breast cancer (BC). Breast lobules or ducts are the genesis of breast cancer, which is an uncontrolled development of epithelial cells. High mobility group protein box 1 (HMGB1) possesses both pro- and anti-tumorigenic bioactivities, which contribute to its complicated function in carcinogenesis. Damage associated Molecular Pattern molecules (DAMPs) generated following tissue injury include HMGB1, many S100 proteins, and others. RAGE is thought to be a receptor for these molecules.

Aim of the study: The study was aimed to find the High mobility group protein box 1 (HMGB1) HMGB1 (rs1412125) and Receptor for Advanced Glycation End Products RAGE (rs1800624) genotypic and allelic frequency. Using Allele Specific polymerase chain reaction AC-PCR and Polymerase Chain Reaction Restriction Fragment Length Polymorphism RFLP-PCR in BC patients and healthy controls, as well as their correlation with breast cancer susceptibility.

Material and methods ∴. Samples were taken at Marjan Cancer Hospital, Babylon. HMGB1 rs1412125 and RAGE rs18400624 polymorphisms were evaluated for patients and controls using the PCR-RFLP and allele-specific polymerase chain reaction (AS-PCR) method. SPSS software was used to perform the statistical analysis.

Results ∴ There is non-significant difference between patients and control in TT, AA, and TA genotyping of (rs1800624) in RAGE gene ($p=0.22$), non-significant difference between patients and control in TT, TC, and CC genotyping of (rs1412125) in HMGB1 gene ($p=0.09$).

Conclusion ∴ Our results showed no association between HMGB1 polymorphisms and RAGE and breast cancer risk.

Keywords: HMGB1 polymorphisms, Breast cancer, , Receptor for Advanced Glycation End Products (RAGE), (DAMPs).

Introduction

The most frequent malignancy among women worldwide is breast cancer (BC), comprising 23% of the 1.1 million female cancers that are newly diagnosed each year [1]. Breast lobules or ducts are the genesis of breast cancer, which is an uncontrolled development of epithelial cells. Breast cancer that has invaded the surrounding breast stroma (primary invasive breast cancer), spread to distant lymph nodes, or metastasized to distant organs is included in this disorder. Early, noninvasive breast cancers like ductal carcinoma in situ (DCIS) and lobular carcinoma in situ (LCIS) are also included [2]. The incidence of breast cancer increased in Iraq in recent years, causing a significant health problem [3]. Due to the strong correlation between the incidence of breast cancer and advancing age, age is the most significant recognized risk factor for breast cancer. Therefore, women 40 years of age or older must undergo a mammography screening in advance [4]. Numerous studies show that high grade malignancy, minimal expression of hormonal receptors, frequent overexpression of the HER-2 receptor, or the occurrence of a molecular biological subtype called "basal-like" ("triple negative") breast cancer are characteristics of the disease in young women [5]. The genetic predisposition accounts for 5–10% of instances of breast cancer. BRCA1 and BRCA2 gene mutations are among the most well-known genetic abnormalities linked to this malignancy [6]. As a member of the high mobility group protein superfamily with secretory and intracellular activity, high mobility group protein box 1 (HMGB1) has both secretory and intracellular activity [7]. The human HMGB1 gene (located at 13q12) encodes HMGB1 [8]. (HMGB1) is a factor that controls the proliferation and malignant tumorigenesis [9]. In HMGB1, two nuclear localizing sequences (NLSs), one in Box-A and the other in Box-B, are present. There are lysine AA residues in both NLSs. These lysine residues can be hyperacetylated, which allows HMGB1 to move from the nucleus to the cytoplasm [10]. The total level of

acetylation influenced how quickly HMGB1 protein moved between the nucleus and cytoplasm. Active secretion or passive secretion are the two potential ways by which immune cells release nuclear HMGB1 protein [11]. (HMGB1) possesses both pro- and anti-tumorigenic bioactivities, which contribute to its complicated function in carcinogenesis. The degree of HMGB1 up- or down-regulation varies depending on the kind of cancer [12]. Advanced glycosylation end-products (AGEs) and numerous S100 family members are among the active ligands that are expressed in conjunction with RAGE, a cell surface protein that is a multi-ligand receptor of the immunoglobulin superfamily [13]. Damage-Associated Molecular Pattern (DAMP) molecules, including as HMGB1 and numerous S100 proteins, are thought to be receptors for Receptor for Advanced Glycation End Products RAGE [14]. By interacting with AGEs, HMGB1, or the S100 group of proteins many of which are expressed frequently during pathological conditions of glycation and inflammation RAGE functions as a master regulator of the origin, invasion, and metastasis of tumors. Regardless of the cancer's place of genesis, molecular subtype, or disease stage, the receptor-ligand pair is the primary target for cancer prevention and effective therapy. When found, these medications might be utilized in conjunction with conventional chemotherapy to effectively control the growth and spread of malignancies without having any negative effects on normal cells since they give specific cytotoxicity to therapies targeting RAGE and its ligands.[15].

Materials and methods :

The participants in this study were divided into two groups. The first group included 50 women who had received a clinical diagnosis of breast cancer at the Babylon Oncology Center in Medical City of Margan; their ages varied from 30 to 69 years, with an average age of 45.11 ± 6.66 . The healthy control group was made up of (50) females, whose ages varied from 30 to 69 with an average age of 42.2 ± 7.02 . A syringe (2 ml) of was used to obtain venous blood samples was taken from each individual in control and patients

groups, then control and placed into EDTA tubes and preserved at - 20 degrees Celsius (deep freeze) for use in the study's genetic component.

Identification the genotypic and allelic frequency of HMGB1 (rs1412125) by AS-PCR technique was performed to detect and genotype the HMGB1 gene polymorphism (rs1412125) from breast cancer patients and healthy blood samples. This technique is based on allele-specific primers, which can be used for single nucleotide polymorphism (SNP) analysis and RAGE (rs1800624) genes. This method began with the amplification of DNA from the gene target area using a PCR machine and then moved on to the RFLP method of cutting DNA amplicons using restriction enzyme and their association with breast cancer susceptibility.

Inclusion criteria:

1. Female subjects were over 20 years of age.
2. Patients include females premenopausal and postmenopausal.
3. Women primarily new diagnosed with breast cancer by physicians and confirmed through screening mammography (digital or film).
4. The patients did not receive any chemotherapy or radiation prior to taking the blood sample.

Exclusion criteria

1. DiscuPatients who received chemotherapy or radiation.
2. Patients less than 20 years of age.
3. Patients with benign breast tumor.
4. Diabetic patients.

Ethical Issues

Before collecting samples, all study participants were informed and verbal consent was acquired from each of them. According to document number 14, on August 29, 2022, a local ethics committee evaluated and approved the research protocol, as well as the permission form and subject information. obtain this endorsement.

Statistical analysis

In order to do the statistical analysis, SPSS version 20.0 was used. Mean and standard deviation are used to represent continuous variables. The analysis of variance student t test was used to compare the patient and control groups, and a change was judged significant if P 0.05. The ANOVA test was used to compare the means of three or more groups.

Results

Demographic and biochemical characteristics of patients and control

Table (1) lists the demographics of the 100 study participants—50 breast cancer patients, and 50 healthy controls. Patients in this research ranged in age from 30 to 69, with a mean age of 49.6 10.3 years standard deviation. Ages in the control group ranged from 30 to 69, and their mean age was 33.0 9.4 years.

Table 1: Means \pm standard deviation of age and BMI in patients and control.

Parameter	Patient (50) Mean \pm SD	Control (50) Mean \pm SD	P-value
Age	45.11 \pm 6.66	42.2 \pm 7.02	0.26
BMI	28.4 \pm 4.23	27.23 \pm 2.33	0.3

*P < 0.05 is consider significant, SD = standard deviation, NS = not significant

These findings were significantly associated with the risk of breast cancer when compared to the healthy control (p 0.001), as shown in Table 2. This study revealed that 21 (42%) of breast cancer patients have a positive family history, and 29 (58%) of breast cancer patients have a negative family history.

Table 2: Distribution of patients and control subjects according to family history.

Family History	Patient (50)		Control (50)		P-value
	N	%	N	%	
Positive	21	42	0	0	0.001
Negative	29	58	50	100	

n: number of cases; S: significant at $P < 0.05$.

Detection of Genes Polymorphisms

Distribution of Allele Frequency of (rs1800624) Single Nucleotide Polymorphisms SNP in breast cancer Patients and Control Group.

According to Table 3, there is no statistically significant difference in the frequency of the A allele and the T allele between the patient and the healthy control. This suggests that none of the two alleles represents a breast cancer risk factor.

Table 3: Allele Distribution of (rs1800624) SNP of RAGE gene Polymorphism.

RAGE	Patient N=50	Control N= 50	P-value	OR	%95CI
T	74	62	0.06	1.194	1.447-0.985
A	26	38		0.684	1.036-0.45

OR: odds ratio, CI: confidence interval.

Genotype Distribution and Allele Frequency of rs1800624

In TT, AA, and TA genotyping of (rs1800624) in RAGE gene, there is no statistically significant difference between patients and controls ($p=0.22$), as shown in Table 4. This shows that SNP genotyping and breast cancer do not have any associations. This might be as a result of the study's limited sample size or the fact that the chosen gene

(rs1800624) or SNP (rs1800624) may not be directly connected to the disease. A bigger sample size might result in a discernible difference.

Table 4: The Genotype Distribution of (rs1800624) SNP in RAGE Gene.

RAGE	Patient N=50	Control N= 50	P-value	OR	%95CI
TT	27	20	0.22		
TA	20	22		1.186	1.55-0.907
AA	3	8		0.489	1.64-0.146

Detection of HMGB1 rs1412125 Polymorphism.

By using a specific allele-PCR approach, the distribution of the HMGB1 rs1412125 polymorphism was discovered. There are three genotypes at this locus: TC, TT, and CC. The sole allele amplified by the wild type homozygote genotype at a 209bp product size was the T allele. Only the C allele was amplified at a 209bp product size in the mutant type homozygote genotype. As shown in figure1, the heterozygote genotype displayed T and C allele amplification at 209bp product size, respectively.

In all research groups, there was no genotype distribution that deviated from Hardy-Weinberg equilibrium.

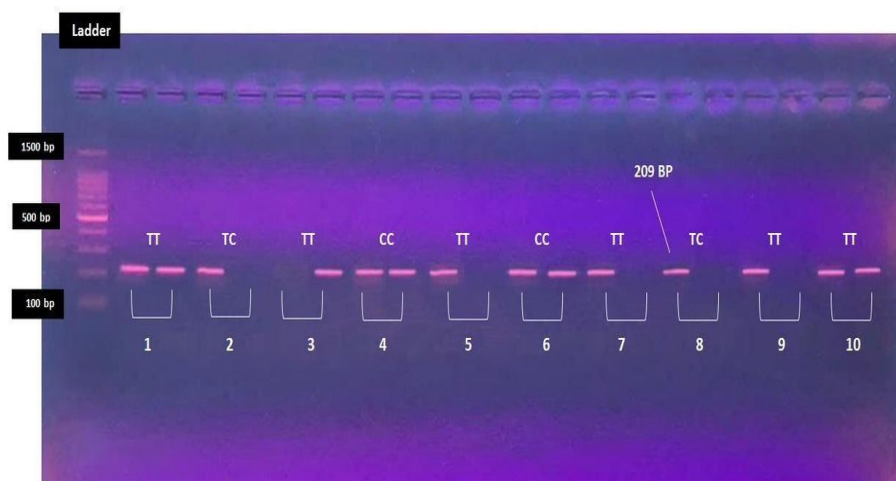


Figure: Agarose gel (1.5-2%) electrophoresis of Allele Specific PCR

Distribution of Allele Frequency of (rs1412125) SNP in breast cancer Patients and Control Group.

According to Table 5, there is no statistically significant difference in the frequency of the T and C alleles between the patient and the healthy control. This suggests that none of the two alleles represents a breast cancer risk factor.

Table 5: Allele Distribution of (rs1412125) SNP of HMGB1 gene Polymorphism.

HMGB1	Patient N=50	Control N= 50	P-value	OR	%95CI
T	60	72	0.237	0.828	1.136-0.6
C	40	28		1.455	2.732-0.774

OR: odds ratio, CI: confidence interval.

Genotype Distribution and Allele Frequency of (rs1412125)

There is non-significant difference between patients and control in TT, TC, and CC genotyping of (rs1412125) in HMGB1 gene (p=0.09) as in Table 6.

Table 6: The Genotype Distribution of (rs1412125) SNP in HMGB1 Gene.

HMGB1	Patient N=50	Control N= 50	P-value	OR	%95CI
TT	25	27	0.09		
TC	10	18		0.514	1.186-0.223
CC	15	5		2.7	10.14-0.72

Discussion

Regarding age , there was non-significant association between the patients group and control group (P >0.05). These results agreement with other studies done by Dodova .et al, 2015 and Andrew et al, 2015 [18],[19]. In both breast cancer patients and healthy controls, the genotype distributions of the RAGE gene's studied polymorphisms were in agreement with the Hardy-Weinberg equilibrium (P > 0.05). These findings concur with

those of previous research like Pan H *et al.*, 2014, 509 breast cancer patients and 504 controls were included in a case-control study in Han-China [16]. Hashemi *et al.*, 2012, In Iran which is case control study included 71 patients with breast cancer and 93 control group there was no difference between RAGE (-374T/A) bp polymorphisms among patients with breast cancer and healthy controls, suggesting that larger studies are necessary to demonstrate any putative relation between RAGE gene polymorphisms and susceptibility to breast cancer [20]. This result was disagreed with study done by Yue L *et al.*, 2016, In Han- China, which is case control study included 524 patients with breast cancer and 518 control group, the frequency of rs1800624 polymorphism mutant A allele in RAGE gene was significantly higher in patients than in controls (24.52% versus 19.50%, $P = 0.006$) [21]. This study differs with the current result may be due to the low sample size and inclusion criteria chosen for the study.

HMGB1 has a variety of functions both inside and outside of cells, including immunological response, chromatin stability, DNA repair, gene transcription, and program cell death control. The HMGB1 gene has been linked to the development of tumors in several cancer types, including colon, liver, breast, oral, and lung cancer [22]. all genotypic frequencies were in Hardy-Weinberg equilibrium ($p > 0.05$), this is consistent with the findings of Yue *et al.* (2016) [17].

This results similar with other studies such as B- Huang *et al.*, 2018, The prevalence of the rs1412125 polymorphism in the HMGB1 gene was non-significantly different between patients and controls in a case-control study conducted in China with 313 breast cancer patients and 217 healthy controls [22], Yue. L *et al.*, 2016, [21]. This result was disagreed with study done by Hantoosh MH *et al.*, 2022 [23].

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