Journal of Advances in Medical and Biomedical Research | ISSN:2676-6264

Association between Polymorphisms of X-ray Repair Cross Complementing 5 and 6 Promoter Genes and the Risk of Metastatic Breast Cancer

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Article Info

10.30699/jambs.31.144.46

Received: 2021/08/17;
Accepted: 2022/07/20;
Published Online: 12 Dec 2022;

Use your device to scan and read the article online

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ABSTRACT

Background & Objective: Breast cancer is the second leading cause of cancer-related death in women. Better individualized treatment needs novel prognostic predictors. X-ray repair cross complementing *XRCC5* and *XRCC6* are coding genes of the Ku protein complex (key components of the non-homologous end-joining [NHEJ] pathway), which could serve as prognostic factors in breast cancer. Hence, in this study, the association of *XRCC5* (coding the Ku70 subunit) and *XRCC6* (coding the Ku80 subunit) single polymorphisms with the risk of metastatic breast cancer was assessed.

Materials & Methods: This study included 30 breast cancer patients and 30 age-matched healthy women. Tetra-Arms polymerase chain reaction (PCR) and high-resolution melt (HRM) real-time PCR were performed to determine *XRCC5* (variable number tandem repeat [VNTR] polymorphism, rs6147172) and *XRCC6* (rs132793) polymorphisms, respectively. Demographical and clinical tumor status was recorded for all women. Allele frequencies and related genotypes were identified.

Results: Our results indicated that 34% of patients receiving chemotherapy had metastases in other organs, mostly in the lung. The frequencies of 0R/0R, 1R/1R, 2R/2R, and 1R/R genotypes in the *XRCC5* gene were 6.6%, 63.3%, 6.6%, and 23.3%, respectively. No significant association was found between *XRCC5* and metastatic breast cancer ($P = 0.426$). In addition, the *XRCC5* polymorphism was associated with progesterone ($P = 0.068$), as well as the time interval between chemotherapy and relapse ($P = 0.069$). The frequency of AA, GG, and AG genotypes in *XRCC6* were 0%, 33.3%, and 66.7%, respectively. The XRCC6 polymorphism was associated with cancer metastasis. There was a significant relationship between age ($P = 0.048$) and family history ($P = 0.020$) with cancer incidence. A significant association was observed between the *XRCC6* polymorphism with human epithelial receptor 2 (HER2; $P = 0.070$) and radiotherapy sessions ($P = 0.007$).

Conclusion: We speculate that the genetic variation of the *XRCC6* gene (rs132793 SNP) might be considered as a diagnostic biomarker in breast cancer, but further studies are necessary to confirm the results.

Keywords: Breast cancer, Metastasis, Polymorphism, *XRCC5, XRCC6*

Introduction

Nowadays, cancer has become one of the major health challenges worldwide. Indeed, cancer is mainly associated with a class of pathological conditions, leading to altered cellular processes and subsequently resulting in uncontrolled cellular growth and division. Among various types of cancer, breast cancer is the most common type in women and is known as the second cause of mortality after lung cancer (1). Metastasis is defined as the spread of tumor cells and their movement from the initial site to other tissues and organs in different sites of the body (2, 3). The metastatic property of cancer cells is a major challenge, hindering the efficient treatment of cancer (3). The mechanism of breast cancer metastasis mainly involves metastatic cascade, invasion, migration, motility, and tumor microenvironment adaption (4-10). It has been proved that cancer is mainly related to genetic abnormalities and mutation in certain genes, as well as changes in the expression of cell cycle-related genes, which could principally start tumor generation or metastasis (11). Single nucleotide polymorphisms (SNPs) are considered the most prevalent genetic modification, demonstrating a distinctive change in a single nucleotide. Genetic polymorphism is defined as a difference in DNA sequences, determining the variation of organisms in higher levels of integration (12-14).

DNA double-strand break (DSB) is the most threatening form of genomic integrity, and its inactivation leads to uncontrolled cell growth and cancer progression (15). DSB repair involves non-homologous end-joining (NHEJ) and homologous recombination (HR). The identification of breast cancer gene 1 (BRCA1) and BRCA2 (familial breast cancer susceptibility genes) function in the HR repair mechanism should be investigated. On the other hand, defects of the NHEJ pathway could be considered a risk factor for the formation of breast tumors (16, 17). The NHEJ pathway comprises multiple genes (such as X-ray repair cross complementing 4 [*XRCC4*], *XRCC5*, and *XRCC6*) associated with DSB repair. Ku protein is a heterodimeric DNA-binding complex capable of DSB repair in the process of the NHEJ pathway. This complex comprises Ku70 and Ku86 as 2 main subunits encoded by *XRCC5* and *XRCC6* (18). Ku is well-known as a main DSB repair complex and initiates the repair process through the NHEJ pathway. Ku consists of 2 subunits, Ku70 (encoded by *XRCC6*) and Ku86 (encoded by *XRCC5*). Various studies have investigated the association between various cancers (such as lung, cervical, prostate, and oral cancers) and polymorphisms of *XRCC6* and *XRCC5* genes (19, 20). Some studies have focused on breast cancer and found that the genotypes of *XRCC5* (1R/0R) and *XRCC7* (6721 G/T) significantly increased the risk of breast cancer (21-23). Given the pivotal role of Ku in breast tumorigenesis and tumor progression (24), the evaluation of genetic variants in these subunits encoding genes could be a key issue in breast cancer predisposition. In this regard, the main aim of this study was to investigate the relationship between rs132793 and rs6147172 polymorphisms in *XRCC6* and *XRCC5* genes, respectively. This study was conducted on 30 cases with metastatic breast cancer undergoing chemotherapy.

Materials and Methods

Study Population

Thirty women aged 25-69 years with metastatic breast cancer diagnosed by clinical and pathological trials were included in this study. Patients were receiving several medical therapeutic procedures, including surgery, radiotherapy, and chemotherapy, adhering to the ethical principles of the Oncology Department of Valiasr Hospital in Zanjan Province (Iran). In addition, 30 healthy women were included as control subjects with negative mammography and ultrasonic results and no breast mass or metastasis. These subjects were selected by the Milad Pathobiology Laboratory in Zanjan Province.

Evaluation of Clinical Features

The frequency of the patient's clinical data included age, body mass index (BMI), menopausal age, age at first pregnancy, age of infection, pre-menopausal infection, familial history, estrogen/progesterone receptor, P53, human epithelial receptor 2 (HER2), tumor degree, contraceptive consumption, stress level, metastasis, diet, activity level, time interval between initial chemotherapy and disease recurrence, and radiotherapy sessions.

Clinical Information

A questionnaire was used to record the demographic, clinical, and pathological information of the subjects. This information included age, height, weight, alcohol consumption, smoking, diet, age at first birth, number of chemotherapy/radiotherapy sessions, metastasis, familial history of cancer, abdominal surgery, and contraceptive consumption. It should be noted that the type of chemotherapeutic agents, types of tumor cells, and grade of cancer were obtained from the patients' records.

This study was approved by the Ethics Committee of Islamic Azad University of Zanjan (code: IR.IAU.REC.1396.59).

Peripheral Blood Sampling and DNA Extraction

Peripheral blood (2 mL) was taken from the axillary vein of each subject. The blood was poured into the vial containing EDTA, followed by a vigorous shake. Then, the samples were stored at -20 °C for further analysis.

DNA was extracted using standard Bio basic and Cinna Gene kits according to the company protocol. The purity of the extracted DNA was measured by gel electrophoresis. DNA concentration and quality were examined by spectrophotometry.

Tetra-ARMS and High-Resolution Melt Real-Time Polymerase Chain Reaction

Tetra-ARMS polymerase chain reaction (PCR) was used to evaluate the expression of the *XRCC6* gene, and high-resolution melt (HRM) real-time PCR was performed to detect variable number tandem repeat (VNTR) in the *XRCC5* gene. A RealQ Plus Master Mix Green Kit was used according to the manufacturer's instructions. The sequences of the used primers for XRCC6 (rs132793) included inner primer (A allele): forward primer 5′- ACTGCCCCTGACTGTAAGGA-CCCGGA-3′, inner primer (G allele): reverse primer 5′-CTTCCATACATGATGCAGAGAAGGTTGAAC -3′, forward outer primer: 5′-AAAAAAACAGAAGAAAG-GCAGGGCAGGA-3′, and reverse outer primer: 5′-ATGGTCATGCTAAAATTGC AGGGTAGCG-3′. The sequences of the used primers for VNTR (rs6147172) included forward 5′-AGGCGGCTCAAA CACCACAC-3′ and reverse 5′- CAAGCGGCAGATAGCGGAAAG-3′.

Statistical Analysis

Allelic frequencies and polymorphisms in all subjects were assessed and analyzed using SPSS version 20 (SPSS Inc, Chicago, Ill, USA). The results were analyzed using the K2 score and Fisher`s method. *P* values less than 0.05 were considered statistically significant.

Results

Thirty patients with metastatic breast cancer and 30 control subjects were enrolled in this study. Control subjects had no mass in their breast tissue, and their mammography or ultrasound findings were negative.

Genotypic Analysis of XRCC5

Eight samples of PCR products were sent for sequencing (Gene Fanavaran Company, Tehran, Iran). The sequencing results are presented in Figure 1. The sequence analysis of the samples revealed that alleles were not presented in the original sequence of the gene and can be referred to as new alleles. The sequence results were analyzed using FinchTV software. The results confirmed the presence of genotypes of 0R/0R (n = 3), 1R/1R (n = 3), 0R/1R (n = 1), and 2R/2R (n = 1). These sequenced samples were then used as positive controls in HRM real-time PCR.

Visualization of rs132793 XRCC6 PCR Products

The obtained PCR product (337 base pairs [bp]) was amplified by outer forward and reverse primers through Tetra-ARMS PCR. Thereafter, a 235-bp fragment was amplified by outer forward and inner reverse primers, and a 157-bp fragment was amplified by inner forward and outer reverse primers. Consequently, 1 sample was selected and sent for further sequencing (Figure 1).

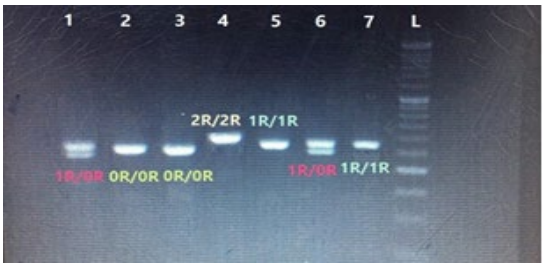

Figure 1. The *XRCC5* gene PCR result for sequencing. Genotypes were identified after sequencing. Sample 1 has a heterozygous 1R/0R genotype, samples 2 and 3 have a 0R/0R genotype, sample 4 has a 2R/2R genotype, sample 5 has a 1R/1R genotype, sample 6 has a 1R/0R genotype, and sample 7 has a 1R/1R genotype. In well L, there is a marker that acts as a ruler; the marker is 50 bp.

Association of Clinical Information and Cancer Recurrent

A significant association was found between breast cancer and familial history (P = 0.001). In addition, a meaningful relationship was observed between the time interval of the first chemotherapy and cancer recurrence (P = 0.052) (Table 1).

Table 1. Association of clinical information and cancer recurrent

Variable		Patient	Control	*P* value
BMI	<16.5	0 (0%)	0 (0%)	0.447
	16.5-18.5	0 (0%)	0 (0%)	
	18.5-25	14 (46.6%)	13 (43.3%)	
	25-30	14 (46.6%)	17 (56.6%)	
	30-35	2 (6.6%)	0 (0%)	
	35-40	0 (0%)	0 (0%)	
	>40	0 (0%)	0 (0%)	
Menstrual age	<11	3 (10%)	1 (3.3%)	0.716
	12	3 (10%)	4 (13.3%)	
	13	7 (23.3%)	6 (20%)	
	14	8 (26.6%)	12 (40%)	
	<15	9 (30%)	7 (23.3%)	
Familial history	All	12 (40%)	0 (0%)	0.001
	None	16 (53.3%)	30 (100%)	
Contraceptive consumption	None	17 (56.6%)	0 (0%)	0.480
	LD	6 (20%)	0 (0%)	
	HD	2 (6.6%)	0 (0%)	
	LD-HD	5 (16.6%)	0 (0%)	
Age at first pregnancy	None	2 (6.6%)	4 (13.3%)	0.454
	<20	18 (60%)	18 (60%)	
	20-24	3 (10%)	2 (6.6%)	

Variable		Patient	Control	*P* value
	25-29	5 (16.6%)	2 (6.6%)	
	<30	1 (3.3%)	0 (0%)	
Time interval between initial chemotherapy and disease recurrence (year)	<1	10 (33.3%)	0 (0%)	0.052
	1-2	1 (3.3%)	0 (0%)	
	2-3	8 (26.6%)	0 (0%)	
	3-4	3 (10%)	0 (0%)	

The genotypic and allelic frequency in both XRCC6 and XRCC5 genes

According to the results of *XRCC5* genotyping, there was no significant difference between genotype frequencies in the patient and control groups ($P = 0.426$; Table 4). However, genotypic frequencies of *XRCC6* demonstrated a significant difference between the patient and control groups ($P = 0.00001$).

There was no significant difference between the XRCC5 alleles in the patient and control groups ($P = 0.548$), while a significant difference was seen in the frequency of *XRCC6* alleles ($P = 0.03$; Table 2).

Table 2. Genotypic and allelic frequency in *XRCC5* and *XRCC6* genes

	Subjects	Genotypes				*P* value
		0R/0R	**0R/1R**	**1R/1R**	**2R/2R**	
XRCC5	Patients	2 (6.6%)	7 (23.3%)	19 (63.3%)	2 (6.6%)	
	Controls	6 (20%)	4 (13.3%)	18 (60%)	2 (6.6%)	
	Total	8 (13.3%)	11 (18.3%)	37 (61.6%)	4 (6.6%)	0.426
		Alleles				*P* value
XRCC5	Subjects	0R		1R	2R	
	Patients	11 (18.3%)		45 (75%)	4 (6.6%)	
	Controls	16 (26.6%)		40 (66.6%)	4 (6.6%)	0.548
	Total	27 (22.5%)		85 (70.8%)	8 (6.6%)	
	Subjects	**Genotypes**				*P* value
		AA		AG	GG	
XRCC6	Patients	0 (0%)		20 (66.7%)	10 (33.3%)	
	Controls	3 (10%)		27 (90%)	0 (0%)	0.00001
	Total	3 (5%)		47 (78.33%)	10 (16.66%)	
		Allele				*P* value
XRCC6	Subjects	A		G		
	Patients	20 (33.3%)		40 (66.67%)		
	Controls	33 (55%)		27 (45%)		0.030
	Total	53 (44.16%)		67 (55.83%)		

Correlation of the Clinical Information of Metastatic Breast Cancer With XRCC5 Genotypes

There was a significant relationship between *XRCC5* genotypes and the expression of progesterone receptor ($P = 0.068$). Moreover, a significant relationship was detected between *XRCC5* genotypes and the time interval between the first chemotherapy and cancer recurrence ($P = 0.069$; Table 3).

Table 3. **Relationship between clinical characteristics and *XRCC5* genotypes**

Factor		Genotype frequency				*P* value
		0R/0R	1R/0R	1R/1R	2R/2R	
Age	<40	1 (3.3%)	0 (0%)	4 (13.3%)	1 (3.3%)	0.602
	40-49	1 (3.3%)	2 (6.6%)	3 (1%)	1 (3.3%)	
	50-59	0 (0%)	4 (13.3%)	7 (23.3%)	0 (0%)	
	60-69	0 (0%)	1 (3.3%)	4 (13.3%)	0 (0%)	
	>70	0 (0%)	0 (0%)	1 (3.3%)	0 (0%)	
BMI	<16.5	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0.614
	16.5-18.5	0 (0%)	0 (0%)	0 (0%)	0 (0%)	
	18.5-25	1 (3.3%)	5 (16.6%)	8 (26.6%)	0 (0%)	
	25-30	1 (3.3%)	2 (6.6%)	9 (30%)	2 (6.6%)	
	30-35	0 (0%)	0 (0%)	2 (6.6%)	0 (0%)	
	35-40	0 (0%)	0 (0%)	0 (0%)	0 (0%)	
	>40	0 (0%)	0 (0%)	0 (0%)	0 (0%)	
Menstrual age	<11	0 (0%)	1 (3.3%)	1 (3.3%)	1 (3.3%)	0.162
	12	0 (0%)	0 (0%)	2 (6.6%)	1 (3.3%)	
	13	0 (0%)	3 (10%)	4 (13.3%)	0 (0%)	
	14	2 (6.6%)	2 (6.6%)	4 (13.3%)	0 (0%)	
	<15	0 (0%)	1 (3.3%)	8 (26.6%)	0 (0%)	
Radiotherapy sessions	0	0 (0%)	2 (6.6%)	5 (16.6%)	1 (3.3%)	0.692
	<15	0 (0%)	0 (0%)	4 (13.3%)	1 (3.3%)	
	16-25	1 (3.3%)	1 (3.3%)	3 (10%)	0 (0%)	
	<26	1 (3.3%)	4 (13.3%)	7 (23.3%)	0 (0%)	
Contraceptive consumption	None	1 (3.3%)	3 (10%)	12 (40%)	1 (3.3%)	0.668
	LD	1 (3.3%)	2 (6.6%)	3 (10%)	0 (0%)	
	HD	0 (0%)	0 (0%)	2 (6.6%)	0 (0%)	
	LD-HD	0 (0%)	2 (6.6%)	2 (6.6%)	1 (3.3%)	
Age at first pregnancy	None	0 (0%)	0 (0%)	2 (6.6%)	0 (0%)	1.000
	<20	2 (6.6%)	4 (13.3%)	10 (33.3%)	2 (6.6%)	
	20-24	0 (0%)	1 (3.3%)	2 (6.6%)	0 (0%)	
	25-29	0 (0%)	2 (6.6%)	3 (10%)	0 (0%)	
	<30	0 (0%)	0 (0%)	1 (3.3%)	0 (0%)	
Age of infection	40	1 (3.3%)	2 (6.6%)	6 (20%)	2 (6.6%)	0.306
	<40	1 (3.3%)	5 (16.6%)	13 (43.3%)	0 (0%)	
Familial history	All	1 (3.3%)	3 (10%)	7 (23.3%)	1 (3.3%)	0.914
	None	1 (3.3%)	3 (10%)	11 (36.6%)	1 (3.3%)	
Estrogen receptor	-	0 (0%)	2 (6.6%)	7 (23.3%)	0 (0%)	0.586
	+	2 (6.6%)	5 (16.6%)	10 (33.3%)	2 (6.6%)	

Factor		Genotype frequency				*P* value
		0R/0R	1R/0R	1R/1R	2R/2R	
Progesterone receptor	-	1 (3.3%)	3 (10%)	13 (43.3%)	0 (0%)	0.068
	+	1 (3.3%)	4 (13.3%)	4 (13.3%)	2 (6.6%)	
HER2	-	1 (3.3%)	2 (6.6%)	9 (30%)	1 (3.3%)	
	+	1 (3.3%)	2 (6.6%)	2 (6.6%)	0 (0%)	
	+2	0 (0%)	2 (6.6%)	1 (3.3%)	1 (3.3%)	
	+3	0 (0%)	1 (3.3%)	3 (10%)	0 (0%)	0.61
P53	-	2 (6.6%)	4 (13.3%)	12 (40%)	1 (3.3%)	0.598
	+	0 (0%)	2 (6.6%)	3 (10%)	1 (3.3%)	
Stress level	None	0 (0%)	3 (10%)	6 (20%)	0 (0%)	0.378
	Low	0 (0%)	3 (10%)	5 (16.6%)	0 (0%)	
	High	2 (6.6%)	1 (3.3%)	8 (26.6%)	2 (6.6%)	
Metastasis	Lymph nodes	0 (0%)	0 (0%)	2 (6.6%)	0 (0%)	0.571
	Lung	1 (3.3%)	2 (6.6%)	2 (6.6%)	1 (3.3%)	
	Liver	0 (0%)	1 (3.3%)	0 (0%)	0 (0%)	
	Breast	0 (0%)	1 (3.3%)	0 (0%)	0 (0%)	
	Bone	0 (0%)	1 (3.3%)	3 (10%)	0 (0%)	
	Brain	0 (0%)	0 (0%)	1 (3.3%)	0 (0%)	
	More than 1 metastasis	1 (3.3%)	2 (6.6%)	11 (36.6%)	1 (3.3%)	
Tumor degree	I	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0.906
	II	0 (0%)	1 (3.3%)	4 (13.3%)	0 (0%)	
	III	2 (6.6%)	4 (13.3%)	12 (40%)	1 (3.3%)	
	IV	0 (0%)	2 (6.6%)	3 (10%)	1 (3.3%)	
Diet	Normal	0 (0%)	4 (13.3%)	11 (36.6%)	0 (0%)	0.148
	Protein-rich	0 (0%)	1 (3.3%)	3 (10%)	0 (0%)	
	Fat-rich	1 (3.3%)	1 (3.3%)	4 (13.3%)	2 (6.6%)	
	Protein- and fat-rich	0 (0%)	1 (3.3%)	0 (0%)	0 (0%)	
	Caffeine-rich	1 (3.3%)	0 (0%)	1 (3.3%)	0 (0%)	
Activity level	Inactive	2 (6.6%)	4 (13.3%)	8 (26.6%)	1 (3.3%)	0.851
	Normal	0 (0%)	2 (6.6%)	9 (30%)	1 (3.3%)	
	Active	0 (0%)	1 (3.3%)	2 (6.6%)	0 (0%)	
Time interval between initial chemotherapy and disease recurrence (year)	<1	0 (0%)	4 (13.3%)	5 (16.6%)	1 (3.3%)	0.069
	1-2	1 (3.3%)	0 (0%)	0 (0%)	0 (0%)	
	2-3	1 (3.3%)	0 (0%)	7 (23.3%)	0 (0%)	
	3-4	0 (0%)	0 (0%)	3 (10%)	0 (0%)	
	<4	0 (0%)	3 (10%)	3 (10%)	1 (3.3%)	

Correlation of the Clinical Information With *XRCC6* Genotypes

According to the calculated *P* values, there was a significant relationship between *XRCC6* genotypes and the number of radiotherapy sessions ($P = 0.007$), as well as a significant relationship between *XRCC6* genotypes and HER2 in patients with mild neglect ($P = 0.087$; Table 4).

Table 4. Relationship between clinical characteristics and *XRCC6* genotypes

Risk Factor		Genotype frequency			*P* value
		AA	AG	GG	
Age	<40	0(0%)	3 (10%)	3 (10%)	0.884
	40-49	0 (0%)	5 (16.6%)	2 (6.6%)	
	50-59	0 (0%)	8 (26.6%)	3 (10%)	
	60-69	0 (0%)	3 (10%)	2 (6.6%)	
	>70	0 (0%)	1 (3.3%)	0 (0%)	
BMI	<16.5	0 (0%)	0 (0%)	0 (0%)	1
	16.5-18.5	0 (0%)	0 (0%)	0 (0%)	
	18.5-25	0 (0%)	9 (30%)	5 (16.6%)	
	25-30	0 (0%)	9 (30%)	5 (16.6%)	
	30-35	0 (0%)	2 (6.6%)	0 (0%)	
	35-40	0 (0%)	0 (0%)	0 (0%)	
	>40	0 (0%)	0 (0%)	0 (0%)	
Menstrual age	<11	0 (0%)	3 (10%)	0 (0%)	0.204
	12	0 (0%)	3 (10%)	0 (0%)	
	13	0 (0%)	4 (13.3%)	3 (10%)	
	14	0 (0%)	3 (10%)	5 (16.6%)	
	>15	0 (0%)	7 (23.3%)	2 (6.6%)	
Radiotherapy sessions	None	0 (0%)	5 (16.6%)	2 (6.6%)	0.007
	<15	0 (0%)	5 (16.6%)	1 (3.3%)	
	16-25	0 (0%)	0 (0%)	5 (16.6%)	
	>26	0 (0%)	10 (33.3%)	2 (6.6%)	
Contraceptive consumption	None	0 (0%)	11 (36.6%)	6 (20%)	1
	LD	0 (0%)	4 (13.3)	2 (6.6%)	
	HD	0 (0%)	2 (6.6%)	0 (0%)	
	LD-HD	0 (0%)	3 (10%)	2 (6.6%)	
Age at first birth	No birth	0 (0%)	1 (3.3%)	1 (3.3%)	1
	<20	0 (0%)	12 (40%)	6 (20%)	
	20-24	0 (0%)	2 (6.6%)	1 (3.3%)	
	25-29	0 (0%)	4 (13.3%)	1 (3.3%)	
	>30	0 (0%)	1 (3.3%)	0 (0%)	
Age at disease infection	<40	0 (0%)	7 (23.3%)	4 (13.3)	1
	>40	0 (0%)	13 (43.3%)	6 (20%)	
Menopause	+	0 (0%)	13 (43.3%)	6 (20%)	1

Risk Factor		Genotype frequency			*P* value
		AA	AG	GG	
Familial history	-	0 (0%)	7 (23.3%)	4 (13.3%)	0.698
	+	0 (0%)	7 (23.3%)	5 (16.6%)	
Estrogen receptor	-	0 (0%)	11 (36.6%)	5 (16.6%)	1
	+	0 (0%)	14 (46.6%)	5 (16.6%)	
Progesterone receptor	-	0 (0%)	6 (20%)	3 (10%)	1
	+	0 (0%)	8 (26.6%)	3 (10%)	
HER2	-	0 (0%)	12 (40%)	5 (16.6%)	0.087
	+3	0 (0%)	1 (3.3%)	3 (10%)	
	+2	0 (0%)	2 (6.6%)	2 (6.6%)	
	+	0 (0%)	4 (13.3%)	1 (3.3%)	
P53	-	0 (0%)	11 (36.6%)	2 (6.6%)	0.344
	+	0 (0%)	3 (10%)	3 (10%)	
	-	0 (0%)	14 (46.6%)	5 (16.6%)	
Stress level	None	0 (0%)	6 (20%)	3 (10%)	0.888
	Low	0 (0%)	6 (20%)	2 (6.6%)	
	High	0 (0%)	8 (26.6%)	5 (16.6%)	
Metastasis	Lymph nodes	0 (0%)	2 (6.6%)	0 (0%)	0.918
	Lung	0 (0%)	4 (13.3%)	2 (6.6%)	
	Liver	0 (0%)	1 (3.3%)	0 (0%)	
	Breast	0 (0%)	1 (3.3%)	0 (0%)	
	Bone	0 (0%)	3 (10%)	1 (3.3%)	
	Brain	0 (0%)	1 (3.3%)	0 (0%)	
	More than 1 metastasis	0 (0%)	8 (26.6%)	7 (23.3%)	
Tumor degree	I	0 (0%)	0 (0%)	0 (0%)	0.283
	II	0 (0%)	5 (16.6%)	0 (0%)	
	III	0 (0%)	11 (36.6%)	8 (26.6%)	
	IV	0 (0%)	4 (13.3%)	2 (6.6%)	
Diet	Normal	0 (0%)	9 (30%)	6 (20%)	0.539
	Protein-rich	0 (0%)	2 (6.6%)	2 (6.6%)	
	Fat-rich	0 (0%)	7 (23.3%)	1 (3.3%)	
	Protein- and fat-rich	0 (0%)	1 (3.3%)	0 (0%)	
	Caffeine-rich	0 (0%)	1 (3.3%)	1 (3.3%)	
Activity level	Low	0 (0%)	12 (40%)	3 (10%)	0.24
	Normal	0 (0%)	6 (20%)	6 (20%)	
	High	0 (0%)	2 (6.6%)	1 (3.3%)	
	<1	0 (0%)	7 (23.3%)	3 (10%)	0.311

Risk Factor		Genotype frequency			*P* value
		AA	AG	GG	
Time interval between initial chemotherapy and disease recurrence (year)	1-2	0 (0%)	0 (0%)	1 (3.3%)	
	2-3	0 (0%)	6 (20%)	2 (6.6%)	
	3-4	0 (0%)	1 (3.3%)	2 (6.6%)	
	>4	0 (0%)	6 (20%)	1 (3.3%)	

Discussion

Breast cancer is a highly heterogeneous disease caused by the interplay of hereditary and environmental risk factors, leading to the progressive accumulation of genetic and epigenetic changes in breast cells (25). The DNA-dependent protein kinase (DNA-PK) consists of 2 subunits, including DNA-PKcs as a catalytic subunit and a Ku heterodimer. The role of DNA-PK has been appointed in DSBs through the NHEJ pathway (27). Ku70 and Ku80 form the Ku complex and are expressed by the *XRCC5* and *XRCC6* genes, respectively (28-30). In this study, we evaluated the frequency of rs132793 and rs6147172 polymorphisms in *XRCC6* and *XRCC5* in patients with metastatic breast cancer and reported their association with clinical-pathological characteristics. Our results revealed that women in the 50-59 age group had the highest percentage (36.7%) of breast cancer. Moreover, no significant difference was found between breast cancer in women and parameters such as age at first pregnancy, contraceptive consumption, and age at first menstruation. However, other factors (including a family history of cancer and age) show a significant relationship between breast cancer and incidence index.

We observed that the genotype frequencies of the *XRCC5* gene in the examined subjects were 6.6%, 63.3%, 6.6%, and 23.3% for 0R/0R, 1R/1R, 2R/2R, and 1R/R, respectively. These results indicated no association between this polymorphism in the *XRCC5* gene and metastatic breast cancer. Ku80 is a heterodimer encoded by *XRCC5*, binding to the broken ends of DNA through the NHEJ pathway. The role of *XRCC5* can be affected by various factors, including the insertion of VNTR in the promoter region of this gene. This phenomenon can alter the expression of *XRCC5*, leading to a subsequent change in the synthesis of Ku80. The mentioned process may modify NHEJ and HR pathways, which can consequently lead to the development of cancers, including breast cancer (25). In line with previous studies, AL-Eitan et al evaluated the correlation between VNTR in *XRCC5* genotypes and the development of breast cancer. They reported a strong significant correlation between the VNTR polymorphism and breast cancer risk. After performing PCR, they stated a remarkable association between 2R/2R, 3R/2R, and 3R/3R genotypes with breast cancer. Also, the allele frequency showed significant differences between the patient and healthy groups (26). Cui et al investigated the association between the VNTR polymorphism and 3 types of familial breast cancer ($BRCA1^+$, $BRCA2^+$, and wild-type BRCAx) at the germline level. Different techniques, including PCR, PAGE, and Sanger sequencing, were used to compare the VNTR polymorphism of *XRCC5* between healthy and breast cancer cases with familial history. The statistical analysis of VNTR genotypes showed significant differences between healthy and 2 mutated groups ($BRCA1^+$ and $BRCA2^+$) but not the BRCAx group. They indicated that in the BRCA1+ group, the increased risk was related to 2R/2R and 2R/1R genotypes, and decreased risk was associated with 1R/1R and 1R/0R genotypes. However, 2R/1R was associated with increased risk factors in the BRCA2+ group. Overall, the different VNTR genotypes of the *XRCC5* promoter were related to the altered risk of breast cancer in the mutated carriers ($BRCA1^+$ and $BRCA2^+$ individuals) (25). Regarding myeloma patients, a study on 27 SNPs in *XRCC3, XRCC4*, and *XRCC5* genes reported that rs1051685 in the *XRCC5* gene located in the 3′-UTR was associated with the susceptibility of myeloma, while GG genotype carriers remained at a lower risk of cancer development (27). In our study, the *XRCC6* gene was found to be associated with breast cancer. The frequencies of AA, GG, and AG genotypes of this gene were 0%, 33.3%, and 66.7%, respectively. Furthermore, *XRCC6*, as a Ku70 protein-coding gene and a component of the NHEJ pathway, has a key role in the inhibiting rearrangements of chromosomes and preservation of the genome integrity, resulting in the stability of the genome and cell survival. It was suggested that polymorphisms of *XRCC6* may have a critical role in tumorigenesis (28). In this regard, Jia et al investigated the association of the *XRCC6* polymorphisms with the risk of cancer development in a meta-analysis study. They proposed that the rs132793 polymorphism reduced the risk of breast cancer, while it could increase the incidence of other neoplasia (28). Li et al in 2011 investigated genetic variants of *XRCC5/XRCC6* genes and their association with hepatocellular carcinoma (HCC). They assessed the genotypes of 13 common SNPs in these genes and reported a significant relationship between the reduced risks of HCC associated with the *XRCC5* rs16855458 polymorphism, as well as a significantly increased risk of HCC associated with the *XRCC5* rs9288516

polymorphism. The effects of rs16855458 and rs9288516 were more considerable in the subjects infected with hepatitis B surface antigen compared with non-infected subjects. The haplotype-based analysis revealed that in *XRCC5*, AA in block 1 and CGGTT in block 2 were associated with decreased risk of HCC. Moreover, *XRCC5* variants could determine the susceptibility of HCC, but the reliability of these results requires further studies and validation on a larger scale **(29)**. Besides, the investigation of SNPs in *XRCC5* and *XRCC6* genes in prostate cancer patients showed that rs2267437 in *XRCC6* could be considered a risk factor in aggressive prostate cancer tumors. Compared with CC/CG genotypes, GG genotype-carrying patients showed increased risks of developing bigger tumors. They suggested that these results could be taken into account for malignant prostate cancer tests along with genetic variants of the major vault protein (MVP) gene **(30)**.

Conclusion

We evaluated the prognosis of *XRCC5* and *XRCC6* polymorphisms in 30 patients with metastatic breast cancer undergoing chemotherapy. Our findings showed no significant relationship between the *XRCC5* rs6147172 polymorphism and metastatic breast cancer, while a significant relationship was detected between the *XRCC6* rs132793 polymorphism and metastatic breast cancer. We speculated that the *XRCC6* rs132793 polymorphism could be used as a diagnostic biomarker in breast cancer. In addition, the efficacy of chemotherapy-based approaches can be improved by tracking the rs132793 polymorphism of the *XRCC6* gene as a prognostic factor in breast cancer.

Acknowledgments

None.

Conflict of Interest

Authors declare that they have no conflict of interest.

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How to Cite This Article:

Asaadi Tehrani G, Karimi Moghaddam Z, Karimkhanilouei S. Association between Polymorphisms of X-ray Repair Cross Complementing 5 and 6 Promoter Genes and the Risk of Metastatic Breast Cancer. J Adv Med Biomed Res. 2023; 31(144): 46-56.