Genetic Variation of Rad51 Gene in Breast Cancer Patient

Mahmoud H. Khalaf AL-Fahdawi[1], Akeel H. Ali ALAssie[2], Ismail H. Aziz[3]
[1] Al-Farahidi University/College of Medical Technology, Iraq*
[2] Al Kitab University/Medical Technical College, Iraq
[3] University of Baghdad/Institute of Genetic Engineering and Biotechnology for Postgraduate Studies, Iraq
Email: Mahmoud.hamid30@gmail.com

Abstract
In a group of women with early-stage breast cancer in Baghdad/Iraq, this study has been done to assess the impact of certain SNPs situated on RAD51 genes on its repair effects on DNA damage. Between November 2020 and March 2021, (100) FFPE samples from breast cancer patients who were diagnosed in the early stages of the disease were obtained at the Tumor Teaching Hospital in the Medical City and the Al-Amal National Hospital in Baghdad. As a control group, (50) samples from healthy women were also obtained. All of the study samples' DNA was taken in order to find SNPs, and their ages varied from (40 to) 60 years. Additionally, DNA sequence analysis was used to identify single nucleotide polymorphisms (SNPs) in exon 6 of the RAD51 gene. Then, these exons' nucleotide sequences were matched with NCBI and the control group (healthy women). In exon 6 of the RAD51 gene, one polymorphism, rs121917739 GA, was discovered.
Keywords: RAD51, Breast Cancer, RAD51 Variation

1. Introduction

Breast cancer, which has a 9% lifetime incidence, ranked number 1 among other cancer types in women and number 1 cause of cancer mortality [1]. According to the most recent Iraqi Cancer Registry [2]. It is the most prevalent form of malignancy and makes up one-third of all cancer cases in women who have been registered [3]. In the previous 20 years, breast cancer incidence rates have increased, making it one of the biggest hazards to the health of Iraqi women. several forms of breast cancer the illness is identified in its early stages by a number of techniques, including biopsy, magnetic resonance imaging (MRI), ultrasound, mammography, and clinical cancer examination (CCE). In certain situations, the tumor may be a mix of many types. Numerous risk factors for breast cancer, including sex, age, smoking, radiation, and genetic history, have been estimated by epidemiological research to either work at the same time or in sequence to begin or increase the breast cancer carcinogenesis [4]. Additionally, around 10-15% of breast cancer vulnerability is attributable to mutations in genes that function as repair DNA, such as the RAD51 genes [5]. The exchange if RAD51 Homologous strands is critical in DNA repairing by recombining homologies, and this gene, which has 13 exons and is located on chromosome 15p15.1, generates the protein rad51 (HR) binding to both single- and double-stranded DNA showing DNA-dependent ATPase activities. So, joint molecule is created by this between a processed DNA breaks and the repair templates through the facilitation of strand exchanges between DNA partner homologies and the homology recognition [6].

2. Methods

The FFPE tissues samples for this investigation were obtained from women at the Oncology Teaching Hospital in the Medical City and AlAmal Al-Watanii Hospital in Baghdad between November 20 (2020) and March 30. Of these women, (100) had just been diagnosed with breast cancer, and (50) were healthy (2021). They received an early diagnosis. based on clinical data, a mammogram, and the hospital's medical advisory panel. The study's female subjects, including the patients and control group, varied in age from (40 to) 60 years.

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DNA Extraction

Using the FlexiGene DNA kit, DNA was extracted (Qiagen). Genomic DNA was extracted from normal and tumor breast tissue at TJU following surgical excision. The DNeasy Tissue Kit was used to extract gDNA (Qiagen). 39 macroscopically dissected breast cancer tissue specimens provided the sequencing information. The tissue samples were kept at -80°C in a solution of a protease inhibitor (Roche Applied Science, Indianapolis, IN, USA). 19,20 After thoroughly washing the preserved tissue in phosphate-buffered saline (PBS) to eliminate any remaining traces of the stabilizing solution, the DNA was extracted from the tissue using the QIAamp DNA Mini kit (Qiagen).

DNA amplification

5 l of Template DNA in total were put to PreMix tubes. Two milliliters of a specific primer—F-AAGGGAATGCCTCCTTCCTA and two milliliters of a reverse primer—R-CCAAACTAACCCTGGCAATC—were added to the

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PreMix tubes. A total of 20 l of distilled water was poured into PreMix tubes. Pipetting was used to dissolve the blue lyophilized pellet, followed by a quick spindown. The tubes were moved to a thermal cycler using the PCR process shown in the following table (1)

Table 1. PCR Profile program			
Step	Temperature	Time	Cycle
Initial denaturation	94°C	5 minutes	1
Denaturation	94°C	30 second	35
Annealing	55°C	30 seconds	
Extension	72°C	30 minutes	
Final extension	72°C	5 minutes	1

3. Statistical Methods

Kaplan-Meier survival estimates were used, and pairwise log-rank tests were used to compare survival rates across groups. Using Pearson's c2 test, differences in the distribution of variables across groups were computed. A Cox regression model was used to determine each variable's relative influence on survival after univariate analysis revealed any potential confounders. Using SPSS version 12.0.1, all statistical computations were carried out (SPSS Inc, Chicago, Illinois, USA).

4. Results And Discussion

For RAD51 gene exon 6, PCR was carried out using the temperature program. The RAD51 exon 6 products, which was 312bp as indicated in the figures, was electrophoresed after the PCR product was loaded on an agarose gel without the use of a loading-dye combination (1),

Figure 1. PCR products for RAD51 gene for DNA samples of breast cancer on 2 % agarose gel at 100 V for 75 mints.

polymorphism of RAD51 gene

As shown in figure (2), the sequencing findings and alignment of 100 samples with breast cancer revealed that 84% of the samples had a mutation from G A whereas the control samples are G G.

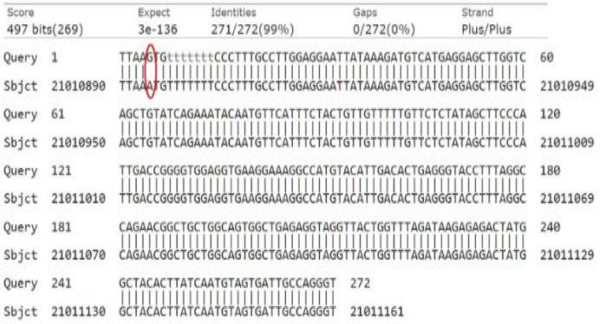

Figure 2. Alignment of exon 6 in RAD51 gene sequence of women with breast cancer

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The current findings revealed that exon 6 of this gene has one mutation, designated as rs121917739. The following describes the RAD51 gene genotype frequency in breast cancer patients: GA (84.0%), AA (14.0%), and GG (2.0%). While GG (76.0%), GA (0.0%), and AA (24.0%) were present in healthy individuals. In patients with breast cancer (84.0%), the GA genotype had the highest risk factor (OR=84.92), while the GG genotype had the lowest risk factor (OR=0.021), which is protective for patients. The differences between the research groups and the RAD51 gene genotype (GG and GA) were significant (p< 0.05).

In comparison to G allele (46.73%), which represents protective factor (OR=0.6150), A allele is more frequent in breast cancer patients (53.27%), and it represents risk factor (OR=2.219), with no statistically significant difference (p>0.05). In contrast, the G allele scored well in healthy groups (52.9%) compared to the A allele (47.01%) (Table 2).

Table 2. Comparative genotypes and allele frequency of SNP rs121917739 RAD51 gene in breast cancer patient

Genotypes			Groups		Total	P value	OR (C.I.)
			Patients	Healthy			
SNP rs121917739 RAD51 G/A gene	GG	N	2	38	40	P<0.001 ***	0.021 (0.003-1.21)
		%	2.0%	76.0%	26.7%		
	GA	N	84	0	84	P<0.001 ***	84.92 (3.22-111.21)
		%	84.0%	0.0%	56.0%		
	AA	N	14	12	26	P>0.05	0.5833 (0.09-2.10)
		%	14.0%	24.0%	17.3%		
	Total	N	100	50	150	OR= Odd Ratio C.I.= Confidence intervals	
		%	100.0%	100.0%	100.0%		
	P value		P<0.001***	P<0.001***	P<0.001***		
	Allele frequency						
	G	N	86	38	124	*p>0.05*	0.6150 (0.21-4.21)
		%	46.73%	76.0%	52.99%		
	A	N	98	12	110	*P<0.05**	2.219(1.21-4.22)
		%	53.27%	24.0%	47.01%		
	Total	N	184	50	234	OR= Odd Ratio C.I.= Confidence intervals	
		%	100.0%	100.0%	100.0%		

	P value	p>0.05	P<0.001***	p>0.05	

The GA genotype of the RAD51 gene was identified in the current study as a risk factor for BC, whereas the GG genotype was identified as a protective factor. Interesting studies have found that the RAD51 G/C variation raises the chance of developing breast cancer [7]. They investigated how the RAD51 G/C variant is related to breast cancer risk. [8]. (2022) found that, in contrast to the current study stating that the (G/A) genotype was linked to a higher breast cancer vulnerability in Iraqi women, homozygous mutant (C/C) genotype was associated with an elevated risk of breast cancer in South Indian women.

In past results, the RAD51 G/C polymorphism is an independent breast cancer vulnerability marker of in Pakistan [9].

Authors showed that RAD51 G > C substitution could be a suitable marker to screen breast cancer vulnerability. Yet it is used with restriction limited to the Caucasian populations. As 65% of the investigations in this meta-analysis were conducted on Caucasian subjects [10].

According to the previous data, the RAD51 G>C polymorphism is not linked with breast cancer vulnerability in Iranian Azeri population [11]. The Saudi females showed no G>C polymorphism link with breast cancer vulnerability [12] while the Bangladesh females showed that G>C and C>C polymorphisms are linked with breast cancer patients older than 60 years [13].

Past investigations indicated that G/A and G/G polymorphism of Rad51 could raise the breast cancer vulnerability. Yet, A/A polymorphism reduced European women vulnerability to breast cancer [14]. According to [15], the G>T polymorphism in RAD51 increase the risk of neck cancer.

[16] investigated whether RAD51 SNPs polymorphisms could enhance breast cancer vulnerability in Iraqi population with non-family history. This study agreed with the current study.

. Previous studies showed that RAD51mutations has a key association to with a high vulnerability ovarian cancer usually in breast cancer women in the ovarian cancer family history than with those with no family history. Thus, breast cancer vulnerability is stable if there are no family cases of ovarian cancer [17].

It seems the mutation of RAD51 is linked with some that dysfunction in the human bodies, RAD51 mutation is related to the disorder in breast cancer [18].

[19] analyzed how RAD51 gene mutation is correlated to malignant breast cell transformation showing the G allele importance in odds ratio (OR = 2.04). The results contracted the current study when A allele scored highest risk factors (odd ratio = 2.219) in patients' breast cancer. These contrasts could be linked to genetic background and environmental factors in the population, as all community have some disequilibrium patterns. So, the functional SNP is in a possible disequilibrium with discrete markers in various ethnic groups.

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