Iraqi journal of Bioscience and Biomedical
Published Online First: 31/ December /2023.
p-ISSN: 3007-5491 / e-ISSN:3007-5505

Genetic variations of rad51 gene in Iraqi breast cancer patients

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Received 02/10/2023,
Accepted 28/11/2023,
Published 31/12/2023.

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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 polymorphism; repair DNA.

Introduction

Breast cancer, which has a 9% lifetime incidence, is the most frequent form of cancer in women and the most common cause of cancer mortality (1) According to the most recent Iraqi Cancer Registry (2), breast cancer is the most prevalent form of malignancy in women 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 concurrently or sequentially to begin or increase the carcinogenesis of breast cancer (4). Additionally,

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around 10-15% of the risk of getting breast cancer is attributable to mutations in genes that function as repair DNA, such as the RAD51 genes (Khadka et al., 2016).

RAD51 Homologous strand exchange is a critical step in DNA repair through homologous recombination, and this gene, which has 13 exons and is located on chromosome 15p15.1, generates the protein rad51 (HR). binds to both single-stranded and double-stranded DNA and exhibits DNA-dependent ATPase activity. creates a joint molecule between a processed DNA break and the repair template by facilitating strand exchange between homologous DNA partners and the recognition of homology (5).

Materials and Methods

The FFPE tissues samples for this investigation were obtained from women at the Oncology Teaching Hospital in the Medical City and Al-Amal 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 women (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.

DNA Extraction

Using the FlexiGene DNA kit, DNA was extracted (Qiagen). Genomic DNA was extracted from normal 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 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 µl 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 PreMix tubes. A total of 20 µl 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 table (1).

Table (1): PCR Profile program

Step	Temperature	Time	Cycle
Initial denaturation	94ºC	5 minutes	1
Denaturation	94ºC	30second	35
Annealing	55ºC	30 seconds	
Extension	72ºC	30 minutes	
Final extension	72ºC	5 minutes	1

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Statistical analysis

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 25, all statistical computations were carried out (SPSS Inc., Chicago, Illinois, USA).

Results and Discussion

For RAD51 gene exon 6, PCR was carried out using the temperature program. The RAD51 exon 6 product, which was 312 bp 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).

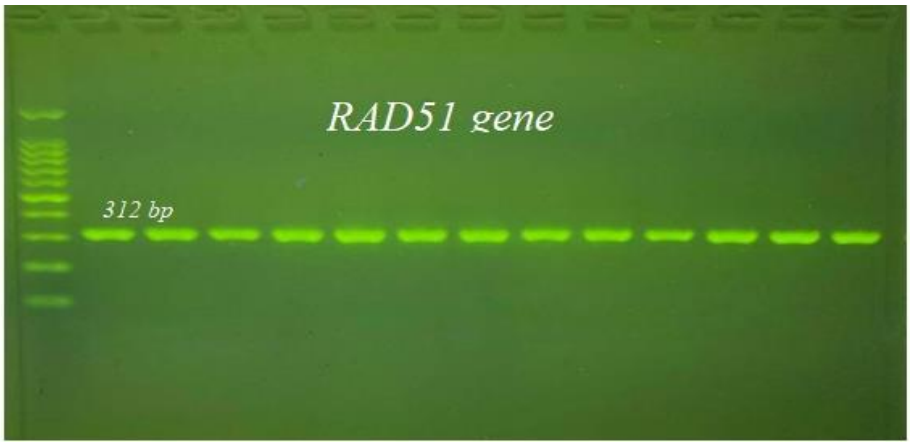

Figure (1): PCR products for RAD51 gene for DNA samples of breast cancer on 2 % agarose gel at 100 V for 75 mints.

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.

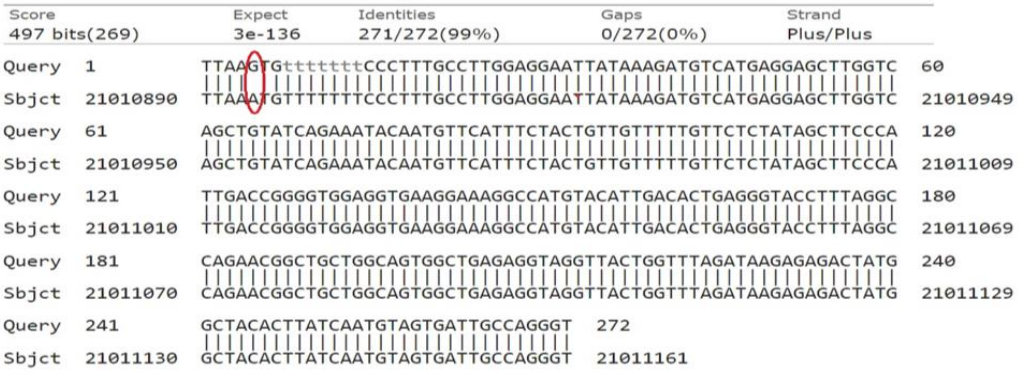

Figure (2): Alignment of exon 6 in *RAD51* gene sequence of women with breast cancer

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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		

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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 (Antoniou et al., 2007). In their investigation of the relationship between the RAD51 G/C variant and breast cancer risk, Rajagopal et al. (2022) found that, in contrast to the current study, which found that the (G/A) genotype was associated with an elevated risk of breast cancer in Iraqi women, homozygous mutant (C/C) genotype was associated with an elevated risk of breast cancer in South Indian women.

Previous results suggest that the G/C polymorphism of the RAD51 can be independent markers of breast cancer risk in Pakistan. (6). Authors showed that RAD51 G > C substitution may serve as a useful marker for screening of breast cancer risk; Nevertheless, its use may be restricted to the Caucasian populations. Since about 65% of the studies included in this meta-analysis were undertaken on Caucasian subjects, (7). Previous data presented here may suggest that the RAD51 G>C polymorphism is not associated with breast cancer risk in Iranian Azeri population (8). In the Saudi females, G>C polymorphism did not show an association with risk of breast cancer (9). In the Bangladesh females, study results indicate an association of G>C and C>C polymorphisms with breast cancer patients older than 60 years (10).

Previous study showed G/A and G/G polymorphism of Rad51 increase breast cancer risk, but A/A polymorphism decrease the risk of European women with breast cancer (11). (12) found the G>T polymorphism in RAD51 increase the risk of neck cancer. (13) investigated whether RAD51 SNPs polymorphisms may increase risk of breast cancer in Iraqi population with and non-family history, this study agreed with the current study.

Previous studies showed that RAD51mutations mainly associated with high risk of ovarian cancer primarily more often in women with breast cancer in the context of family history of ovarian cancer than in without family history, so that the risk of breast cancer is kept unincreased if no family cases of ovarian cancer are reported (14). The mutation of RAD51 was thought to be associated with some dysfunction in the human body, RAD51 mutation has been found to be associated with the disorder in breast cancer (15). A study mentioned the analysis towards the correlation of RAD51 gene mutation with malignant transformation of breast cells showed the significance of the G allele in odds ratio (OR = 2.04), and these results contrast to present study that showed A allele scored highest risk factor (odd ratio = 2.219) in patients' breast cancer. These differences may be due to genetic background and environmental factors among the population, as each community shares its pattern of disequilibrium. Accordingly, the functional SNP may be in disequilibrium with distinct markers in different ethnic groups (16).

Conclusion

The results of the present study revealed that Rad51 gene had three SNPs namely (GA, AA and GG). The GA polymorphism of RAD51 gene was associated with a higher risk of breast cancer. The AA polymorphism was not statistically associated with the risk of breast cancer, while the GG polymorphism might show protective role against breast cancer in Iraqi female populations.

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Acknowledgment

We express our profound gratitude to all those who contributed to the successful completion of this study. Our appreciation also extends to the College of Biotechnology / Al-Nahrain University represented by the Dean of the college, his assistants and to the heads of Plant and Medical Biotechnology Departments for their support and help.

Authors' Declaration

- We hereby confirm that all the Figures and Tables in the manuscript are original and have been created by us.
- We have obtained ethical clearance for our study, which involved human participants, from the local ethical committee at [Al-Nahrain University/College of Biotechnology]. This approval underscores our commitment to ethical research practices and the well-being of our participants.
- Ethical Clearance: The project was approved by the local ethical committee at [Al-Nahrain University/College of Biotechnology], ensuring adherence to ethical standards and the protection of participants' rights and welfare.

Authors' Contribution Statement

[Mahmoud Hamid Khalaf]: Contributed to the conception and design of the study, conducted the experiments, and drafted the initial manuscript.

[Mustafa K. Al-Bayaty]: Performed the statistical analysis, assisted in conducting the experiments and aided in preparation and construction of the manuscript.

[Akeel H. Ali]: Played a role in samples collection and interpretation of the results.

[Ismail H. Aziz]: Played a role in samples collection, editing the manuscript and interpretation of the results.

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