

Germline Mutation of *RAD51* Single Nucleotide Polymorphisms as Susceptibility Factor for Breast and Ovarian Cancer

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ABSTRACT

Background: *RAD51* from the cluster genes which have a vital role in the pathogenesis of squamous cell carcinoma. Present study aimed to analysed polymorphisms of *RAD51* single nucleotide 1(rs2619679, rs2928140 and rs1801320) and their relationship to breast cancer (BC) and ovarian cancer (OC) in Iraqi population. **Methods:** This study included, 35 females with BC, 35 females with OC, who were diagnostic histopathologically, and 30 healthy females as control. Three SNPs (rs2619679, rs2928140 and rs1801320) of *RAD51* were selected for genotyping by using the polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP). **Results:** Statistically differences were found in the distribution of AA and TT genotypes and A/T alleles for rs2619679 in BC ($p < 0.05$) so GG and CC genotypes and G/C alleles for rs2928140. Whereas distribution of genotypes and alleles for rs1801320 not reflect significant differences ($p > 0.05$) between BC and control but mutant genotype GG and mutant allele G appeared in highest frequency (42%, 57%) in BC. Statistically changes of *RAD51* SNPs for OC were found in the incidence of AA and T alleles for rs2619679 ($p < 0.05$) so, GG and CC genotypes and G/C alleles for rs2928140 but distribution of genotypes and alleles for rs1801320 not reproduce significant differences ($p > 0.05$) between OC and control but mutant genotype GG and mutant allele G appeared in OC with highest rate (45%, 59%). Mutant genotype/allele of rs2619679 (TT/T), rs2928140(CC/C) and rs1801320 (GG/G) appeared as effective factors for cancer with acceptable rate for diagnosis of BC and OC. **Conclusion:** mutation in *RAD51* SNPs (rs2619679, rs2928140, rs1801320) associated with increases the possibility of BC and OC.

Keywords: *RAD51*, SNPs, rs2619679, rs2928140, rs1801320, Ovarian Cancer, Breast Cancer

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INTRODUCTION

Among the most common malignant diseases that affect women around the world after puberty are breast and ovarian malignancy. BC is the 2nd cancer-related mortality cause of female in Europe & the U.S. [1]. In 2012, there were an estimated 464 000 women with breast cancer and 131 000 deaths in Europe in a recent report. [2]. Epithelial ovarian cancer is the leading cause of death for gynecological cancer in the U.S and fourth largest cancer mortality cause for women [3,4]. OC is one-tenth as common as breast cancer but three times as lethal. As women with a family history of OC and/or BC possibly inherit genetic changes that alter their risk of OC and/or BC, several studies dealt with the main role of heredity in the development of BC and OC, and most studies focused on germline mutations in the BRCA1/2 genes and that these genes became as indicators to recognize breast cancer [5,6,7]. Human Genesis is continuously exposed to a number of endogenous and exogenous stimuli, including ultraviolet light, ionizing radiation and genotoxic chemicals, which cause DNA damage. Luckily, a repair of DNA has several distinct linear pathways that can maintain genome stability and effectively prevent or restore damage to various types of DNA [8]. Homologous recombination (HR) is part of a DNA repair process and DNAs such as DNA dual-stroke breaks and DNA cross-links need to be assisted and repaired [9,10]. Different risk factors related to cancer have been shown to encourage damage to DNA. DNA damage and related cancer repair mechanisms, for instance stalled DNA replication forks by HR *RAD51*, are associated with many DNA forms. [8,11].

In many cancers including breast, ovary and also prostate, germline and somatic mutations of genes which support homologously guided repairs, notably BRCA1 & BRCA2 are observed frequently. In homology-directed repair the essential biochemical function of BRCA2 is to facilitate the *RAD51* filament assembly on ssDNA from the final resection. [6,12]. To promote the assembly of *RAD51* movies, BRCA2 communicates directly with *RAD51* at various locations. Both *RAD51*'s intracellular and DNA-binding ability have been demonstrated to be regulated by BRCA2. Losses of these controls may be a key event leading to genomic instability and tumorigenesis [13,14]. The human 15q15.1 chromosome *RAD51* plays a key role in restoring double strand DNA. The protein encoded in this gene is part of the *RAD51* protein family. *RAD51* family members, known to be involved in homologous DNA recombinations and reparation, are highly linked to RecA and *Saccharomyces Cerevisiae* Bacteria Rad51. *RAD51* binds to single and double-stranded DNA and demonstrates DNA dependent ATPase behaviour [15]. *RAD51* catalyzes homology recognition and strand exchange forms a joining molecule between the engineered break and repair template between homologous DNA partners. *RAD51* binds the nucleoprotein filaments used in pursuit of homology and in the exchange of strands to one-stranded DNA in ATP dependence [10,16]. In the presence of *RAD51* and XRCC3 in the case of oxidative stress, *RAD51* is responsible for regulating the mitochondrial DNA copy number and for repair of cross-link interstrand. At the DNA damage site with other proteins engaged in homologous recombination, BRCA1 and BRCA2 nuclear focuses

appear along with *RAD51*. *RAD51* is *XRCC3* binding protein. This combination promotes the production of nucleoprotein filament, the central vector of homologous and heterologous recombination [17,18]. Present study aimed to investigate further SNPs in *RAD51*(rs2619679, rs2928140 and rs1801320) that complicated in double-stranded DNA repair and its link to BC and OC in Iraqi population.

PATIENTS AND METHODS

Study Design: this case-control study included 35 females with BC, 35 females with OC who were diagnostic clinically and histopathologically, and 30 healthy females as control group. Age of participant females ranged from 31- 79 years. In additional, present study was in agreement with ethics of Al-Diwaniyah Teaching Hospital and verbal informed consent was obtained from all participants.

Blood and Tissues Samples: For the analysis of cancer, we used freshly frozen tumor tissues and blood in ovarian epithelial and breast cancer who were operated at the Al-Diwaniyah Obstetrics and Gynecology Hospital / Iraq. A pathologist assessed the proportion of tumour and non-tumour tissue in all newly frozen samples. Blood samples from all patients and controls were collected via venipuncture. Two millilitres of blood were collected directly into the sterile tube for DNA extraction with EDTA.

Molecular Study: The extracted blood genomic DNA was tested with the use of Nanodrop spectrophotometers (THERMO.USA), which measure DNA concentration (ng / μL), and by reading the absorption at (260/280 nm) DNA was calculated. This was the resulting blood genomic DNA was extracted from the blood samples. Three *RAD51* SNPs were included in PCR-RFLP reaction as described in **Table (1)**. PCR temperature time was Pre-PCR: 95 C for 12 minutes' duration; PCR (30 cycles): 95 C for 0.5-minute term, 64 C (rs2619679 and rs2928140) or 65 C (rs1801320) for 0.5-minute term, 72 C for 1-minute term & 5 minutes post-PCR. The amplification components were digested for 16 hours at 37 C using restriction enzymes (**Table 2**). The enzymes originated in New England Bio-Lab Inc. DNA fragments were isolated for UV analysis in a 2 per cent agarose gel of ethidium bromide. The buffers 1x TBE (10x TBE, 89 mM Tris, 89 mM boric acid, 2 M EDTA pH 8.0) and 100V were used.

Statistical analysis: The data is translated into a computerized database system. The database has been tested for errors with selection approaches, logical data purification and incongruities have been corrected. In conjunction with Microsoft Excel 2010 and social science statistics, statistical analysis is conducted using the SPSS version 20 computer software (Statistical Package for Social Sciences). A statistically relevant result was considered significant if the P value < 0.05.

Table 1: Polymorphic sites of *RAD51* according to NCBI [19,20,30]

Gene	SNPs	Others	SNP position	Chromosome	alleles
<i>RAD51</i>	rs2619679	g.3879T > A c.-1285T > A	Promoter	15: 40694039	T/A
	rs2928140	g.7995G > C, c.-2-602G > C	Intron 1	15: 40698155	G/T
	rs1801320	c. -98G > C G135C	UTR-5, Exon	15:40695330	G/C

Table 2: PCR-RFLP reaction and products [19,20,30]

Gene	SNPs	Primer	PCR product (bp)	Restriction enzymes	genot ypes	Fragment sizes (bp)
<i>RAD51</i>	rs2619679	(F) 5'-CCGTGCAGGCCTTATATGAT-3' (R)5'-AGATAAACCTGGCCAACGTG3'	286	Hinfl	AA TA TT	286, 114 286, 172,114 172, 114
	rs2928140	(F) 5'-GCTTCTGGCTATTTTCAAGT-3' (R) 5'-TGAGGCAGGTAATGGCTTC-3'	332	EaiI	GG GC CC	332 332,185, 147 185,147
	rs1801320	(F)5'-TGGGAAGTCAACTCATCTGG-3' (R) 5'-GCGTCCTC TCTCCAGCAG-3'	157	MvaI	CC GC GG	157 71, 86, 157 71, 86

RESULTS

In the present case –control study, samples are collected from 35 females with BC their ages range from 31 to 79 years (Mean ± SD = 45.63±11.64), 35 females with OC their ages range from 32 to 78 years (Mean ± SD = 43.22±10.14) and 30 healthy females as control groups

with the age range from 32 to 79 (Mean ± SD = 45.36±11.88) as shown in **Table (3)**. Furthermore, present result revealed there are no significant differences between patients and healthy individuals in the mean of age (P= 0.327).

Table 3: The case-control difference in mean of age

Age /years	Females with BC	Females with OC	Healthy control females	P value
range	31- 79	32- 78	32-79	
Mean ± SD	45.63±11.64	43.22±10.14	45.36±11.88	0.327[NS]
SE	3.71	2.89	2.55	
Total number	35	35	30	

NS= No Significant ($p > 0.05$); SD= Standard Deviation; SE= Standard Error

The current study, **Figure (1)**, showed that 46% and 37% of patients with BC and OC respectively have a positive family history for developing the intended types of cancer

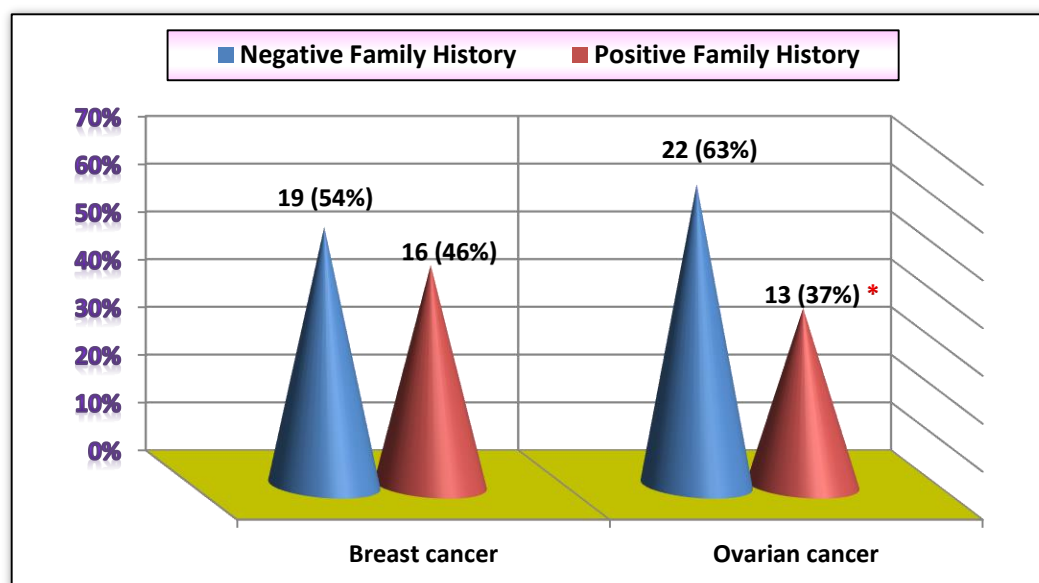


Figure 1: Frequency of family history among cases. * mean significant difference in compared with control group ($P=0.021$)

Histopathological examination of tumor samples in **Table (4)** showed that most patients with BC and OC were in the stages II&III (71.4 % and 60% respectively) of cancer development when the cancer spread into nearby tissues or lymph nodes. Moreover, 11.4%, 8.6% and 8.6% of BC patients undergo from 'in-situ' (stage 0), localized (stage I) and metastatic cancer (stage IV) respectively as in

table (4). On other hand, 17.1%, 14.3% and 8.6% of OC patients undergo from localized (stage I), metastatic cancer (stage IV) and in 'in-situ' (stage 0). Also, **Table (4)** presented significant differences in distribution of cancer stages among patients with BC ($X^2=11.60$, $P= 0.019$) and OC ($X^2= 9.88$, $P= 0.023$).

Table 4: Distribution stages of BC and OC among patient (staging according to Canadian cancer society)

Type of cancer	Cancer Stages				X^2	P value
	0	I	II & III	IV		
	N (%)	N (%)	N (%)	N (%)		
Breast cancer (N=35)	4 (11.4)	3 (8.6)	25 (71.4)	3 (8.6)	11.60	0.019*
Ovarian cancer (N=35)	3 (8.6)	6 (17.1)	21 (60)	5 (14.3)	9.88	0.023*

N= Number of Cases; X^2 = chi square; *= Statically Significant ($P<0.05$)

The distribution of *RAD51* genotypes and alleles in the tested and control group of BC or OC corresponding to Hardy-Weinberg equilibrium (**Tables 5 & 6**). Statistically Significant distribution variations have been found of AA and TT genotypes and A/T alleles for rs2619679 in BC ($p<0.05$). So, statistically significant variation was appeared in the dissemination of GG and CC genotypes

and G/C alleles for rs2928140. Whereas genotypes and alleles distribution for rs1801320 not reflect significant differences ($p>0.05$) between BC and control but mutant genotype GG and mutant allele G appeared in highest frequency (42%, 57%) in BC cases. On the other hand, significant differences of *RAD51* polymorphisms for OC were found in the frequency of AA and T alleles for

rs2619679 ($p < 0.05$). So, statistically differences ($p < 0.05$) were appeared in distribution of GG and CC genotypes and G/C alleles for rs2928140. Whereas distribution of genotypes and alleles for rs1801320 not reflect significant differences ($p > 0.05$) between OC and control but mutant genotype GG and mutant allele G appeared in highest frequency (45%, 59%) in cases with OC.

Etiology fractions or effective factors of *RAD51* for BC and OC are mutant homozygous genotype TT (EF=0.390, 0.085 respectively), heterozygous genotype TA (EF=0.09, 0.183 respectively) and mutant T alleles (EF=0.547, 0.466 respectively) for rs2619679 so mutant homozygous genotypes CC (EF=0.332, 0.331 respectively), heterozygous genotype GC (EF=0.131, 0.111 respectively) and mutant C alleles (EF=0.481, 0.496 respectively) for rs2928140 while mutant homozygous genotype GG (EF=0.154, 0.292 respectively) and mutant G alleles (EF=0.095, 0.262 respectively) for rs1801320 whereas protective fractions of *RAD51* against BC and OC are wild homozygous genotype AA (PF= 0.088, 0.395 respectively) and wild allele A (PF= 0.031, 0.390 respectively) for

rs2619679 as well wild homozygous genotype GG (PF= 0.391, 0.482 respectively) and wild allele G (PF= 0.153, 0.311 respectively) for rs2928140 in addition wild homozygous genotype CC (PF= 0.285, 0.363 respectively), heterozygous genotype (PF= 0.402, 0.379 respectively) and wild allele C (PF= 0.322, 0.451 respectively) for rs1801320.

In the figure below, **Figure (2)**, we compared the percentage of the effective genotypes/ alleles of rs2619679, rs1801320 and rs2928140 in cancer cases, we find that the mutant genotype TT and mutant allele T is higher in BC (43%, 59% respectively) than OC (31%, 51% respectively) while the highest percentage of mutant genotype GG and allele G of rs1801320 are appeared in OC (45%, 59% respectively) in matched with BC (42%, 57% respectively). Moreover, an equal proportion (37%) of mutant genotype CC of rs2928140 appeared in patients with breast cancer and ovarian cancer, while the percentage of mutant allele C was higher in OC (56%) when compared with BC (54%).

Table 5: Comparison SNPs of *RAD51* in BC and healthy control.

SNPs of <i>RAD51</i>	Genotype/ allele	BC	Control	OR	χ^2	P value	EF	PF
		N (%)	N (%)					
rs2619679	Genotype							
	AA	9 (26)	12 (40)	0.93	11.40	0.013*	-----	0.088
	TA	11 (31)	10 (33)	1.41	1.95	0.722	0.09	----
	TT	15(43)	8 (27)	10.73	16.25	0.009*	0.390	----
	allele							
	A	29 (41)	34 (57)	0.93	5.41	0.047*	----	0.031
	T	41 (59)	26 (33)	13.82	22.44	0.0019*	0.547	----
rs2928140	Genotype							
	GG	10 (29)	12 (40)	0.52	9.63	0.014*	----	0.391
	GC	12 (34)	13 (43)	1.63	2.01	0.052	0.131	----
	CC	13 (37)	5 (17)	9.64	10.57	0.010*	0.332	----
	allele							
	G	32 (46)	37 (62)	0.83	0.04	0.012*	----	0.153
	C	38 (54)	23 (38)	9.10	11.99	0.011*	0.481	----
rs1801320	Genotype							
	CC	10 (29)	10 (33)	0.65	0.773	0.511	----	0.285
	GC	10 (29)	9 (30)	0.08	0.111	0.934	----	0.402
	GG	15 (42)	11 (37)	1.14	1.58	0.496	0.154	----
	allele							
	C	30 (43)	29 (48)	0.15	0.61	0.433	----	0.322
	G	40 (57)	31 (52)	1.20	1.95	0.472	0.095	----

OR=Odd ratio, EF= Etiology fraction, PF=Preventive Fraction, *= Statistically Significant ($P < 0.05$).

Table 6: Comparison SNPs of *RAD51* in OC and healthy control.

SNPs of <i>RAD51</i>	Genotype/allele	OC	Control	OR	χ^2	P value	EF	PF
		N (%)	N (%)					
rs2619679	Genotype							
	AA	10 (29)	12 (40)	0.50	9.61	0.014*	----	0.395
	TA	14 (40)	10 (33)	1.84	2.11	0.362	0.183	----
	TT	11 (31)	8 (27)	1.38	0.66	0.411	0.085	----
	allele							
	A	34 (49)	34 (57)	0.51	2.82	0.084	----	0.390
T	36 (51)	26 (33)	11.7	14.83	0.003*	0.466	----	
rs2928140	Genotype							
	GG	9 (26)	12 (40)	0.24	5.38	0.022*	----	0.482
	GC	13 (37)	13 (43)	1.06	1.91	0.226	0.111	----
	CC	13 (37)	5 (17)	9.47	11.95	0.019*	0.331	----
	allele							
	G	31 (44)	37 (62)	0.87	10.91	0.023*	---	0.311
C	39 (56)	23 (38)	8.72	9.94	0.0229*	0.496	----	
rs1801320	Genotype							
	CC	10 (29)	10 (33)	0.83	1.05	0.293	----	0.363
	GC	9 (26)	9 (30)	0.57	0.81	0.420	----	0.379
	GG	16 (45)	11 (37)	2.85	3.91	0.053	0.292	----
	allele							
	C	29 (41)	29 (48)	0.74	1.22	0.136	----	0.451
G	41 (59)	31 (52)	1.80	1.07	0.140	0.262	----	

OR=Odd ratio, EF= Etiology fraction, PF=Preventive

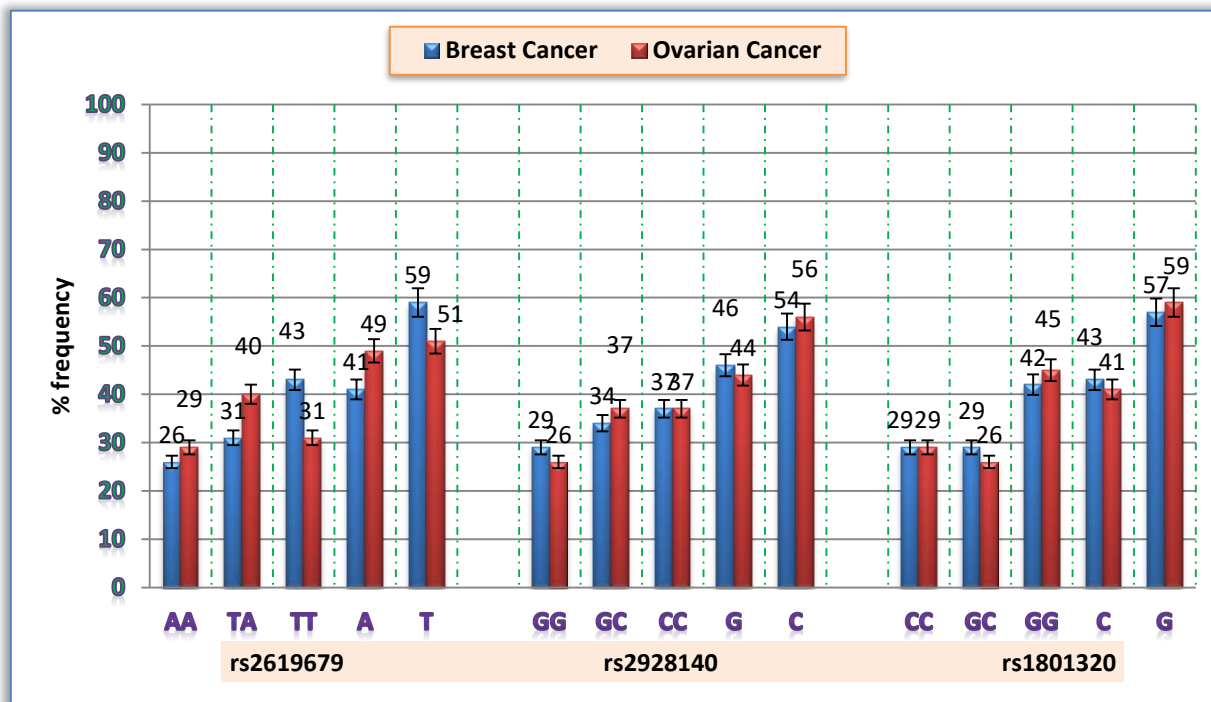


Figure 2: Frequency *RAD51* genotype/ allele among BC and OC

The findings of this analysis have also been shown a clear impact on the family history in transferring mutant genes to the offspring which leading to an increased incidence of cancer as in **Table (7)**. Significant variations have been found in both genotype and alleles of rs2619679 according to the family history of patient with BC and OC ($P < 0.05$), the most important of which was shown when high levels of mutant genotype TT and mutant allele T appeared in BC patients who had a positive family history of BC (67%, 59% respectively) so in mutant genotype TT of OC (55%). Statistically differences ($p < 0.05$) also seen in all rs2928140 genotypes and alleles distribution according to the family history of patient with OC and for GC, CC and

C in BC however the highest frequency of mutant genotype CC and mutant allele C detected in patient with positive family history for BC (69%, 53% respectively) and in patients with negative family history for OC (64% for each one).

Table 7: Distribution SNPs of *RAD51* according to family history of cancer.

SNPs of <i>RAD51</i>	Genotype/ allele	Family history of BC				Family history of OC			
		Total number	Positive (N=16)	Negative (N=19)	P value	Total number	Positive (N=13)	Negative (N=22)	P value
			N (%)	N (%)			N (%)	N (%)	
rs2619679	Genotype								
	AA	9	2 (22)	7 (78)	0.0015*	10	3 (30)	7 (70)	0.0013
	TA	11	4 (36)	7 (64)	0.0022*	14	4 (29)	10 (71)	0.0179
	TT	15	10 (67)	5 (33)	0.002*	11	6 (55)	5 (45)	0.049*
	allele								
	A	29	8 (28)	21 (72)	0.0016*	34	10 (29)	24 (71)	0.0017*
T	41	24 (59)	17 (41)	0.0392*	36	16 (44)	20 (56)	0.045*	
rs2928140	Genotype								
	GG	10	5 (50)	5 (50)	1.00	9	3 (33)	6 (67)	0.002*
	GC	12	2 (17)	10 (83)	0.0004*	13	5 (38)	8 (62)	0.0025*
	CC	13	9 (69)	4 (31)	0.0018*	13	5 (38)	8 (62)	0.0024*
	allele								
	G	32	12(37.5)	20(62.5)	0.0023*	31	11 (35)	20 (65)	0.0021*
C	38	20 (53)	18 (47)	0.384	39	15 (38)	24 (62)	0.0024*	
rs1801320	Genotype								
	CC	10	3 (30)	7 (70)	0.0019*	10	1 (10)	9 (90)	0.0001*
	GC	10	5 (50)	5 (50)	1.00	9	2 (22)	7 (78)	0.0015*
	GG	15	8 (53)	7 (47)	0.381	16	10(62.5)	6(37.5)	0.0023*
	allele								
	C	30	11 (37)	19 (63)	0.00224*	29	4 (14)	25 (86)	0.0003*
G	40	21(52.5)	19(47.5)	0.428	41	22 (54)	19 (46)	0.0778	

Statistically variations ($p < 0.05$) as well showed in the distribution of all rs1801320 genotypes and alleles according to the family history of patient with OC and only for homozygous genotype CC and wild allele C in BC but the highest frequency of mutant genotype GG and mutant allele G detected in patient with positive family history for BC (53%, 52.5% respectively) and OC (62.5%, 54% respectively).

N= number, *= Statistically Significant ($P < 0.05$).

As shown in **Tables (8) and (9)**, a positive mutant TT genotype test of rs2619679 in patient with BC is 42.86% sensitive and 73.33% specific in diagnosing BC. The overall test accuracy is 56.92% testing positive for TT genotype can establish diagnosis of BC with high confidence whereas a positive TT genotype test of

rs2619679 in patient with OC is 31.43% sensitive and 73.33% specific in diagnosing BC. The complete test accuracy is 50.77% testing positive for TT genotype can establish diagnosis of OC with confidence. The positive GC genotype test of rs2928140 in patient with BC or OC showed 37.14% sensitivity and the highest specificity 83.33% in diagnosing BC or OC with accuracy 58.46% which can establish diagnosis of cancer with high confidence. Furthermore, The positive GC genotype test of rs1801320 in patient with BC or OC showed 28.57% sensitivity and specificity 70.00% in diagnosing BC or 25.71% sensitivity and specificity 70.00% in diagnosing OC with accuracy 47.69% for BC and 46.15% for OC those can establish diagnosis of cancer with good confidence.

Table 8: Validity parameters for positive *RAD51* SNPs genotypes or alleles when used as a test to diagnosis BC differentiating it from healthy control

SNPs of <i>RAD51</i>	Genotype/ allele	Sensitivity%	Specificity%	PPV%	NPV%	Accuracy%
rs2619679	Genotype					
	AA	25.71	60.00	42.86	40.91	41.54
	TA	31.43	66.67%	52.38	45.45	47.69
	TT	42.86%	73.33	65.22	52.38	56.92

	allele					
	A	41.43	43.33	46.03	38.81	42.31
	T	58.57	56.67	61.19	53.97	57.69
rs2928140	Genotype					
	GG	28.57	60.00	45.45	41.86	43.08
	GC	34.29	56.67	48.00	42.50	44.62
	CC	37.14	83.33	72.22	53.19	58.46
	allele					
	G	45.71	38.33	46.38	37.70	42.31
rs1801320	C	54.29	61.67	62.30	53.62	57.69
	Genotype					
	CC	28.57	66.67	50.00	44.44	46.15
	GC	28.57	70.00	52.63	45.65	47.69
	GG	42.86	63.33	57.69	48.72	52.31
	allele					
C	42.86	51.67	50.85	43.66	46.92	
G	57.14	48.33	56.34	49.15	53.08	

*PPV =Positive Predictive Value; NPV =Negative Predictive Value

Table 9: Validity parameters for positive *RAD51* SNPs genotypes or alleles when used as a test to diagnosis OC differentiating it from healthy control

SNPs of <i>RAD51</i>	Genotype/ allele	Sensitivity%	Specificity%	PPV%	NPV%	Accuracy%
rs2619679	Genotype					
	AA	28.57	60.00	45.45	41.86	43.08
	TA	40.00	66.67	58.33	48.78	52.31
	TT	31.43	73.33	57.89	47.83	50.77
	allele					
	A	48.57	43.33	50.00	41.94	46.15
rs2928140	T	51.43	56.67	58.06	50.00	53.85
	Genotype					
	GG	25.72	60.00	42.86	40.91	41.54
	GC	37.14	56.67	50.00	43.59	46.15
	CC	37.14	83.33	72.22	53.19	58.46
	allele					
G	44.29	38.33	45.59	37.10	41.54	
rs1801320	C	55.71	61.67	62.90	54.41	58.46
	Genotype					
	CC	28.57	66.67	50.00	44.44	46.15
	GC	25.71	70.00	50.00	44.68	46.15
	GG	45.71	63.33	59.26	50.00	53.85
	allele					
C	41.43	51.67	50.00	43.06	46.15	
G	58.57	48.33	56.94	50.00	53.85	

PPV =Positive Predictive Value; NPV =Negative Predictive Value

DISCUSSION

Previous studies have found that the repair of the DNA system is essential to genomic integrity, as threats from DNA lesions are countered. A lack of the DNA repair pathways may result in these lesions being unrepaired or improperly repaired and finally in genome instability or mutations that can result in an increased cancer susceptibility. [21]. *RAD51*, a kind of ubiquitous strand exchange protein, is known to be a core component involved in DNA double-strand break repair in HR repair pathway [22]. In this study, we investigated whether

RAD51 SNPs rs2619679, rs2928140 and rs1801320 polymorphisms are increase risk of BC and OC in Iraqi population. Current results showed that mutant genotype/ allele of rs2619679 (TT/T), rs2928140 (CC/C) and rs1801320(GG/G) play a clear role in pathological development of BC and OC. Current research is in agreement to meta-analytical A by Zeng et al. suggests that polymorphism of *RAD51* rs1801320 is a risk factor for three different gynecological tumors, i.e. breast, ovarian and endometrial cancers in particular [15]. But the current study contradicts with study of who found BC

no associated with rs2619679, rs2928140 rs1801320 under any of genetic models [23].

Wang *et al.* observed that the *RAD51* gene rs1801320 polymorphism reduces the risk of developing ovarian cancer in *BRCA2* mutation transporters [26], in comparison to current research. Ribeiro Junior *et al.* further proposed a link between the *RAD51* gene rs1801320 and the decreased risk of developing myelodysplastic syndrome [27]. Polymorphism *RAD51* rs1801320 is also has arule in other cancer types. A significant association between *RAD51* rsl801320 polymorphism and an increased risk for prostate cancer has been identified in the previous analysis of Nowacka-Zawisza *et al.* [20]. The possibility of glioblastoma has been shown in subjects with genotype *RAD51* rs1801320 GC (GC vs GG, $x(2) = 10.75$; OR 3.0087; $p = 0.0010$). In addition, the probability of developing glioblastoma was increased by *RAD51* rs1801320 C allele in combination with *XRCC1* rs25487 G allele and *XRCC3* rs861539 C allele ($x(2) = 6.558$; $p = 0.0053$) [24]. The combination of the host *Helicobacter pylori* infection and *RAD51* rs1801320 genotype has shown leads to higher bowel metaplasia in Trang *et al.*, this suggests that *RAD51* rs18001320 may be an effective predictor for patients with gastric cancer with *Helicobacter pylori*-infected [25]. Although, the *RAD51* SNPs rs2619679 and rs2928140 polymorphism have role in HR and DNA repair there are very limited study about their role in cancer pathology. However, Inconsistent results might be due to a different role of *RAD51* gene polymorphisms in different cell types or tissues. So, another explanation for the different findings may be result from gene-gene and gene-environment interactions. Additionally, large and well - designed studies are needed to confirm this conclusion [19].

In the current study, there was a clear effect of family history on the susceptibility of women to cancer, when most mutant *RAD51* SNPs (especially rs2619679 and rs1801320) appeared in patients who had a positive family history of BC or OC. Previous studies showed that *RAD51* mutations 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 [28,29]. The apparent excess of *RAD51* mutation carrier cases among breast cancer cases in ovarian / breast cancer pedigrees is possibly attributable exclusively to the determination of distortion [28]. Osher *et al* also found that *RAD51D* testing was likely to be used in 1–5% of cases in women with ovarian carcinoma, who have at least one relative with ovarian carcinoma. [30]. In current study, diagnostic test for measurement of *RAD51* SNPs sensitivity, specificity, positive and negative predictive value as well as accuracy are expressed as percentages by using MedCalc statistical software and results of this test showed that mutant CC genotype of rs2928140 It is considered the best indicator for the genetic predisposition to BC and OC when it showed the highest specificity (83.33%).

CONCLUSION

In present study, mutant genotype/allele of rs2619679 (TT/T), rs2928140 (CC/C) rs1801320(GG/G) mainly appeared in BC and OC so these mutant genotypes/alleles act as eitological factor for cancer development especially in patients who have positive family history for cancer.

Moreover, mutant CC genotype of rs2928140 It is considered the highest validity parameter to investigate about BC and OC because it showed the highest specificity.

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