Journal Homepage: -www.journalijar.com

INTERNATIONAL JOURNAL OF ADVANCED RESEARCH (IJAR)

Article DOI:10.21474/IJAR01/18921
DOI URL: http://dx.doi.org/10.21474/IJAR01/18921

RESEARCH ARTICLE

GENETIC POLYMORPHISMS IN RAD51, RAD52 AND RAD54 GENES OF HOMOLOGOUS RECOMBINATION REPAIR PATHWAY IN BREAST CANCER: A CASE-CONTROL STUDY FROM PUNJAB

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Manuscript Info

Manuscript History
Received: 18 April 2024
Final Accepted: 23 May 2024
Published: June 2024

Key words:-
Breast Cancer, Genetic Polymorphism, Homologous Recombination, RAD51, RAD52, RAD54

Abstract

A case-control study was conducted on 600 subjects (300 cases and 300 controls) to understand the association of genetic polymorphisms of RAD51 (rs1801320, rs1801321, rs121917739, rs2619681), RAD52 (rs4987207, rs4987208) and RAD54 (rs2295466) genes in breast cancer patients and controls in population of Punjab. Genotyping of the selected SNPs of RAD51 and RAD52 gene was done by PCR-RFLP, whereas ARMS-PCR was used for RAD54 gene polymorphism. Our study identified significant associations between specific SNPs in the RAD51 and RAD52 genes and breast cancer risk. Notably, three RAD51 SNPs (rs1801320, rs1801321, and rs121917739) and one RAD52 SNP (rs4987208) showed significant genotype frequency differences between cases and controls. Genetic model analysis revealed that minor alleles of four RAD51 SNPs (rs1801320, rs1801321, rs121917739, and rs2619681) and two RAD52 SNPs (rs4987207 and rs4987208) were linked to increased breast cancer risk. Haplotype analysis further supported these findings, with 10 RAD51 haplotypes and two RAD52 haplotypes (G-G and T-G) significantly associated with higher breast cancer risk. Additionally, the GT + TT genotype of RAD52 rs4987207 was associated with lower odds of metastasis, while the TG + GG genotype of rs4987208 was linked to lymph node involvement and higher tumor grade.

Introduction:-

Breast cancer is a malignant tumor, in which cells can invade surrounding tissue or metastasize to distant areas of the body. It occurs almost entirely in women, but men can also get breast cancer (American Cancer Society, 2017). Breast cancer has ranked number one cancer among Indian females with age adjusted rate as high as 25.8 per 100,000 women and mortality 12.7 per 100,000 women (Malviaet al., 2017). According to Globocan (WHO), for the year 2012, India recorded 70,218 deaths due to breast cancer, more than any other country in the world (second: China – 47,984 deaths and third: US – 43,909 deaths). Incidence of breast cancer is predicted to increase to 85 per 100,000 women by 2021(Akramet al., 2017).There are different set of genes that are involved in pathogenesis of breast cancer via different pathways (Brethertonet al., 2001; Rajkumaret al., 2007, Kiranet al., 2010, Anita et al., 2013, Mohammad et al., 2014,Keleman et al., 2002, Zhang et al., 2012).Impaired DNA repair has been proposed to play an important role in genetic instability and cancer development particularly breast tumorigenesis (Ralhanet al., 2007). Double strand breaks (DSBs) are the most dangerous and threatening genotoxic damages with high potential

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in producing chromosomal rearrangements and cell death. DSBs are predominantly repaired by either non-homologous end joining (NHEJ) or homologous recombination (HR). NHEJ is an error-prone repair pathway that is mediated by direct joining of the two broken ends. HR is the major and high fidelity repair mechanism for reparation of DSB lesions. HR eliminates DSB lesions by using the sister chromatids as an undamaged homologous template and repairing damage in an error-free way (Majidinia M. et al., 2017). The chief factors involved in HR include MRN complex, CtlP, replication protein-A (RPA), BRCA1, PALB2, BRCA2 and RAD family (Hosoya N. et al., 2014). Most of the factors involved in homologous recombination (HR) come under RAD52 epistasis group (RAD50, RAD51, RAD52, RAD54, RAD55, RAD57, RAD59, RDH54 (TID1), MRE11 (RAD58) and NBS1). The RAD51 subgroup functions only in homologous recombination (HR) repair pathway (Symington S.L., 2002). RAD51, RAD52 and RAD54 genes play a vital role in homologous recombination repair pathway, the impaired HR repair pathway can lead to tumorigenesis due to accumulation of DSBs (Symington S.L., 2002). Rad51 is a structural and functional eukaryotic homologue of Escherichia coli RecArecombinase. Rad51 is known to function as a part of larger recombination complex that includes Rad52 and Rad54 (Raderschallet al., 2002). RAD51 gene is located at chromosome position 15q15.1, a region that exhibits loss of heterozygosity in a large number of cancers, including those of lung, colorectum and breast. The RAD51 gene consists of 10 exons that span about 30 kb. RAD52 maps to chromosome locus 12p12.2–p13, a frequent site for allelic losses in breast and ovarian cancer. RAD52 encodes a protein of 421 amino acids. Human RAD54 is mapped to chromosome locus 1p32. It encodes a protein, composed of 747 amino acids, that is 52% identical to its yeast counterpart. The RAD54 encoded product is a member of the Swi2/Snf2 protein family of ATPases. Loss of heterozygosity at human chromosome locus 1p32 is observed in breast cancer (Matsuda et al., 1999).Several studies have reported the connection between altered RAD51, RAD52 and RAD54 and breast cancer risk (Matsuda et al., 1999, Bell et al., 1999, Sassiet al., 2013). This could be due to two main reasons: A) the involvement of RAD51, RAD52 and RAD54 genes in the maintenance of genetic stability. B) The potential of these genes to modify penetrance of BRCA1/BRCA2.However, till date no such study has been reported from Punjab. Therefore, the proposed study was designed to find the association between RAD51 (rs1801320, rs1801321, rs121917739, rs2619681), RAD52 (rs4987207, rs4987208) and RAD54 (rs2295466) polymorphisms and breast cancer risk.

Materials and Methods:-

A case-control molecular genetics study was conducted on 300 breast cancer patients and 300 controls. The blood samples were collected from Govt. Rajindra Hospital, Patiala after obtaining informed consent from all the subjects.**Inclusion criteria: a)** Women who have confirmed breast cancer were taken as cases. b) Age matched healthy women having no family history of breast cancer were taken as controls.**Exclusion criteria**: Males were excluded from the study because of very low incidence. DNA was extracted from each blood sample by salting out method given by Miller et al., 1998 and the quality and quantity of the genomic DNA was determined by absorbance at 260 nm and 280 nm using Spectrophotometer.Genotyping of the selected SNPs of RAD51 and RAD52 gene was done by PCR-RFLP, whereas ARMS-PCR was used for RAD54 gene polymorphism (Table 1).

Table 1:- Primer sequences and genotyping methods used for RAD51, RAD52 and RAD54 SNPs.

SNP& Genotyping method	**Primer sequence 5'…..3'**	**Amplicon size (bp)& Restriction Enzyme**	**Thermocycler conditions**	**Genotypes**
RAD51 rs1801320 135G>C (**PCR-RFLP**)	**Forward Primer**: TGGGAACTGCAA CTCATCTGG **Reverse Primer:** GCGCTCCTCTCTC CAGCAG	**157** **MvaI**	Initial denaturation at 95 °C for 5 min 35 cycles at 95 °C for 30 sec, 30 sec at 58.6 °C annealing temp, and at 72 °C for 1 min Final extension at 72 °C for 5 min	GG - 86,71 GC - 157, 86, 71 CC – 157
RAD51 rs1801321 172G>T (**PCR-RFLP**)	**Forward Primer**: TGGGAACTGCAA CTCATCTGG **Reverse Primer**: GCTCCGACTTCAC CCCGCCGG	**131** **NgoMIV**	Initial denaturation at 95 °C for 5 min 38 cycles at 95 °C for 30 sec, 45 sec at 65 °C annealing temp, and at 72 °C for 50 min Final extension at 72 °C for 10 min	GG –110, 21 GT–131, 110, 21 TT–131

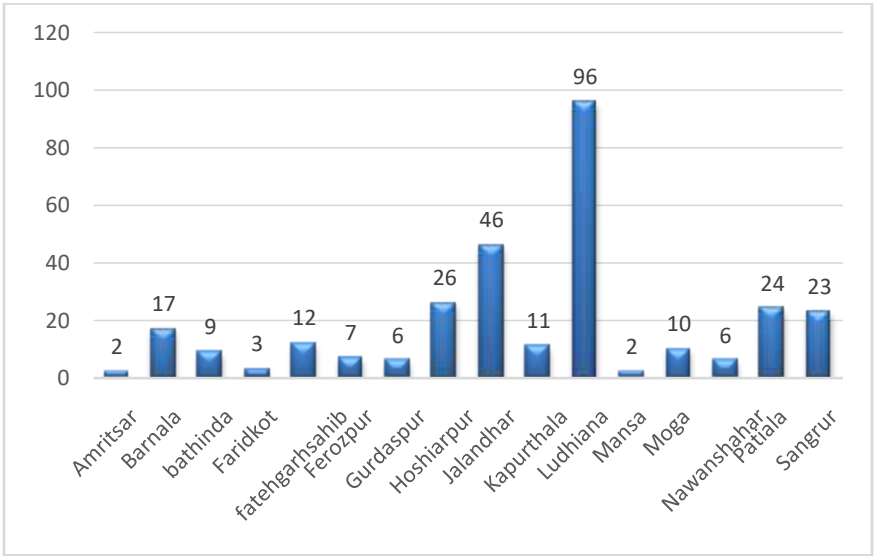
RAD51 rs121917739 449G>A (**PCR-RFLP**)	**Forward Primer:** AAGATGTCATGA GGAGCTTGG **Reverse Primer**: GCCATAGTCTCTC TTATCTAAACCAG	**205** **Msp1**	Initial denaturation at 95 ℃ for 5 min 35 cycles at 95 ℃ for 30 sec, 30 sec at 58.6 ℃ annealing temp, and at 72 ℃ for 1 min Final extension at 72 ℃ for 5 min	GG –89, 116 GC –205, 89. 116 CC –205
RAD51 rs2619681 1640C>T (**PCR-RFLP**)	**Forward Primer:** ACATGCTTGCCA ACACGATA **Reverse Primer:** CATAACTGAGGG CTGATAACCA	**245** **BsmAI**	Initial denaturation at 95 ℃ for 5 min 35 cycles at 95 ℃ for 45 sec, 45 sec at 63 ℃ annealing temp, and at 72 ℃ for 1 min Final extension at 72 ℃ for 5 min	CC–172, 73 GC –245, 172, 73 CC –245
RAD52 rs4987207 806G>T (**PCR-RFLP**)	**Forward Primer:** GTTTTGTTGAGGG GGTTCTGG **Reverse Primer:** TGCCTAAACACCT CTCTGCTAC	**463** **BstNI**	Initial denaturation at 95 ℃ for 5 min 35 cycles at 95 ℃ for 30 sec, 30 sec at 60.5 ℃ annealing temp, and at 72 ℃ for 1 min Final extension at 72 ℃ for 5 min	GG –281, 182 GT–281,182, 463 TT–463
RAD52 rs4987208 1245T>G (**PCR-RFLP**)	**Forward Primer:** ATAGCAGGAAGC GGAAACGA **Reverse Primer:** AAGCCTCACAAG CCGAAGAA	**545** **BamHI**	Initial denaturation at 95 ℃ for 5 min 35 cycles at 95 ℃ for 30 sec, 30 sec at 58.1 ℃ annealing temp, and at 72 ℃ for 1 min Final extension at 72 ℃ for 5 min	TT–545 GT–268,277, 545 GG–268, 277
RAD54 (rs2295466) 451A>G (**ARMS-PCR**)	**Forward inner (A allele):** GACCAATGTTGCT ATTAAAGATGAA **Reverse inner (G allele):** CCATGCCCCGTTG GTTTCTC **Forward outer:** AATCATGCCGGT AGAAAGTAGCA **Reverse outer:** GCCTGTAATACC AGCAACTCAGG	**251** **300** **506** **NA**	Initial denaturation at 95 ℃ for 5 min 35 cycles at 94 ℃ for 40 sec, 35 sec at 54.9 ℃ annealing temp, and at 72 ℃ for 35 sec Final extension at 72 ℃ for 7 min	AA–506, 251 GA–506, 300, 251 GG–506, 300

The PCR reaction mixture included 10µl of hot start Taq2X master-mix, 1 µl of each primer in case of PCR-RFLP and 0.8 µl of each primer in case of ARMS-PCR, 2µl of DNA sample and TDW to make the final reaction volume of 20µl. The thermocycler conditions of all the SNPs are mentioned in the table 1. The restriction digestions conditions for used enzymes were followed as given by the manufacturer. Statistical analysis was performed using online available software Medcalcand SNPStatsoftware for windows. Strength of association between an exposure (risk variables) and an outcome (breast cancer) was analyzed by generating odds ratio given by Andrade, 2015. The 95% confidence interval (CI) was used to see the effect or precision of the odds ratio (OR). P-value of <0.05 (level of significance) was considered significant. Haplotype frequencies were estimated with the help of SNPStatssoftware (https:// www.snpstats.net/start.html).

Results and Discussion:-

Distribution of Study subjects:

The study enrolled 300 clinically confirmed breast cancer cases and equal number of age and gender matched (females) healthy controls. The district wise distribution of breast cancer patients is presented in **figure 1.**



District	Percentage
Amritsar	2(0.7)
Barnala	17(5.7)
Bathinda	9(3)
Faridkot	3(1)
Fatehgarhsahib	12(4)
Ferozpur	7(2.3)
Gurdaspur	6(2)
Hoshiarpur	26(8.7)
Jalandhar	46(15.3)
Kapurthala	11(3.7)
Ludhiana	96(32)
Mansa	2(0.7)
Moga	10(3.3)
Nawanshahar	6(2)
Patiala	24(8)
Sangrur	23(7.7)

Figure 1:- District-wise distribution of breast cancer cases.

The breast cancer distribution across districts revealed significant concentration in Ludhiana (32%) and Jalandhar (15.33%), showcasing the highest percentages of patients among these areas. Additionally, Patiala (8%), Hoshiarpur (8.67%), Sangrur (7.67%), and Barnala (5.67%) also exhibit notable proportions, while the remaining districts indicate lower prevalence, ranging from 2% to 4.67% of reported cases.

Genetic Analysis:

The distribution of the genotypic and allelic frequencies of the studied RAD51 gene polymorphisms (rs1801320, rs1801321, rs121917739, and rs2619681), RAD52 gene polymorphisms (rs4987207, rs4987208), RAD54 gene polymorphism rs2295466 and their comparison among breast cancer patients and controls are presented in **table 2, 3 and 4**.

Table 2:- Frequency distribution of the genotype and allele frequencies of rs1801320, rs1801321, rs121917739 and rs2619681 of RAD51 gene polymorphisms.

SNP	Genotype	Genotype frequency		P-value	Allele frequencies		P-value
		Cases (N=300) n (%)	Controls (N=300) n (%)		Cases n (%)	Controls n (%)	
RAD51 (rs1801320)	GG	197(65.67)	208 (69.33)	**<0.0001*** (19.67)	G-449 (74.83)	G-492 (82)	**0.0032*** (8.69)
	GC	55 (18.33)	76 (25.33)		C-151 (25.17)	C-108 (18)	
	CC	48(16)	16 (5.34)				
RAD51 (rs1801321)	GG	168 (56)	133 (44.33)	**0.0123*** (8.79)	G-414 (69)	G-357 (59.5)	**0.0007*** (11.38)
	GT	78 (26)	91 (30.33)		T-186 (31)	T-243 (40.5)	
	TT	54 (18)	76 (25.34)				
51 (rs12 1917	GG	236 (78.7)	228 (76)	**0.0111***	G-511 (85.2)	G-511 (85.2)	1.000
	GA	39 (13)	55 (18.3)		A-89 (14.8)	A-89 (14.8)	

				(4.39)			(0.00)
	AA	25 (8.3)	17 (5.7)				
RAD51 (rs2619681)	CC	116 (38.7)	135 (45)		C-363 (60.5)	C-399 (66.5)	
	CT	131 (43.7)	129 (43)	0.095 (4.70)	T-237 (39.5)	T-201 (33.5)	**0.031*** (4.65)
	TT	53 (17.6)	36 (12)				

The frequency distribution of genotype and allele frequencies of studies SNPs of RAD51 gene showed statistically significant differences among cases and controls in case of rs1801320(genotypic p-value <0.0001, allelic p-value= 0.0032), rs1801321 (genotypic p-value <0.0123, allelic p-value= 0.0007); rs121917739 showed significant differences at genotypic level only (p-value= 0.111), whereas in case of rs2619681 significant differences were shown only at allelic level only (p-value=0.031).

Table 3:- Frequency distribution of the genotype and allele frequencies of rs4987207 and rs4987208 of RAD52 gene polymorphisms:

SNP	**Genotype**	**Genotype frequency**		**P-value**	**Allele frequencies**		**P-value**
		Cases (N=300) n (%)	**Controls (N=300) n (%)**		**Cases n (%)**	**Controls n (%)**	
RAD52 (rs4987207)	GG	173 (57.7)	196 (65.5)		G-454 (75.7)	G-486 (81)	
	GT	108 (36)	94 (31.5)	0.07 (5.19)			0.02* (5.02)
	TT	19 (6.3)	10 (3)		T-146 (24.3)	T-114 (19)	
RAD52 (rs4987208)	TT	101 (33.7)	142 (47.3)		T-350 (58.3)	T-408 (68)	
	TG	148 (49.3)	124 (41.3)	0.002* (12.42)			0.0005* (12.04)
	GG	51 (17)	34 (11.3)		G-250 (41.7)	G-192 (32)	

***P<0.05 is considered statistically significant.**

Frequency distribution of the allele frequencies of rs4987207 showed significant difference with p-value =0.02 and rs4987208 of RAD52 gene showed significant differences at both genotypic (p-value = 0.002) as well as allelic levels (p-value = 0.0005) (Table=3). The rs2295466 of RAD54 gene did not show any significant differences among cases and controls at either genotypic or allelic levels.

Table 4:- Frequency distribution of the genotypes and alleles frequencies of rs2295466 of RAD54 gene polymorphisms:

SNP	**Genotype**	**Genotype frequency**		**P-value**	**Allele frequencies**		**P-value**
		Cases (N=300) n (%)	**Controls (N=300) n (%)**		**Cases n (%)**	**Controls n (%)**	
RAD54 (rs2295466)	AA	126 (42)	109 (36.3)		A-384 (64)	A-365 (60.8)	
	AG	132 (44)	147 (49)	0.35 (2.08)			0.25 (1.28)
	GG	42 (14)	44 (14.7)		G-216 (36)	G-235 (39.2)	

***p-value <0.05 (statistically significant)**

Further to evaluate the association of selected SNPs with breast cancer genetic model analysis was done (Table 5). In case of rs1801320 genetic model analysis revealed higher risk for breast cancer under the co-dominant model (GG vs. CC) (p-value=0.0002, OR=3.17; 95%CI= 1.74-5.76), recessive model (CC vs. GG+GC) (OR=3.38; 95% CI=1.87-6.10) and allele model (G vs. C) ((p-value=0.003, OR=1.53; 95% CI=1.16-2.02). These results were in concordance with the results reported by various previously conducted studies which showed positive association between rs1801320 and breast cancer risk (Kadouri et al., 2004, Krupa et al., 2009, Gao et al., 2011, Zhou et al., 2011, Hosseini et al., 2013, Romanowicz et al., 2017). Genetic model analysis revealed lower risk for breast cancer under both the dominant (OR=0.63; 95% CI= 0.45-0.86, p-value = 0.004) and the recessive models (OR=0.65; 95% CI=0.44-2.96, p-value = 0.037) for rs1801321 but the previously published studies showed conflicting results in this regard (Kushchel et al., 2002, Loizidou et al., 2009, Silva et al., 2010, Michlaska et al., 2015). For rs121917739, genetic model analysis revealed higher risk for breast cancer only under the co-dominant model (GG vs. AA) (OR=3.16; 95% CI=1.74-5.76, p-value = 0.0002) which supports the results provided by Kato et al., 2000 whereas contradicting to those of Lose et al., 2006. Further, genetic model analysis for rs2619681 of RAD51 revealed significant association of rs2619681 with breast cancer under co-dominant model (CC vs. TT) (p- value = 0.03, OR=1.71; 95% CI= 1.04-2.79), recessive model (TT vs. CC+CT) (p- value = 0.05, OR=1.57; 95% CI= 0.99-2.48) and allele model (C vs. T) (p- value = 0.03, OR=1.29; 95% CI= 1.02-1.64) . Similar results were shown in a study done by Sehl et al., 2009.

Table 5:- Comparison of frequency distribution of RAD51, RAD52 and RAD54 gene polymorphisms between breast cancer patients and controls under different genetic models:

Models	**Genotype**	**Cases N=300 n (%)**	**Controls N=300 n (%)**	**OR (95%CI)**	**P-value**
rs1801320					
Co-dominant	GG	197 (65.67)	208 (69.33)	Referent	
	GC	55 (18.33)	76 (25.33)	0.76 (0.51-1.14)	0.22
	CC	48 (16)	16 (5.33)	3.17 (1.74-5.76)	0.0002*
Allele	G	449 (74.83)	492 (82)	1.53 (1.16-2.02)	0.003*
	C	151 (25.17)	108 (18)		
Dominant	GG	197 (65.67)	208 (69.33)	1.18 (0.84-1.66)	0.38
	GC+CC	103(34.33)	92 (30.67)		
Recessive	CC	48 (16)	16 (5.33)		<0.0001*
	GG+GC	252 (84)	284 (94.67)	3.38 (1.87-6.10)	
rs1801321					
Co-dominant	GG	168 (56)	133 (44.33)	Referent	
	GT	78 (26)	91 (30.33)	0.68 (0.46-0.99)	0.06
	TT	54 (18)	76 (25.33)	0.56 (0.37-0.85)	0.009*
Allele	G	414 (69)	357 (59.5)	0.66 (0.52-0.84)	0.0006*
	T	186 (31)	243 (40.5)		
Dominant	GG	168 (56)	133 (44.33)	0.63 (0.45-0.86)	0.004*
	GT+TT	132 (44)	167 (55.67)		
Recessive	TT	54 (18)	76 (25.33)	0.65 (0.44-2.96)	0.037*
	GT+GG	246 (82)	224 (74.67)		
rs121917739					
Co-dominant	GG	236 (78.7)	228 (76)	Referent	
	GA	39 (13)	55 (18.3)	0.68 (0.43-1.07)	0.098
	AA	25 (8.3)	17 (5.7)	3.16 (1.74-5.76)	0.0002*
Allele	G	511 (85.2)	511 (85.2)		
	A	89 (14.8)	89 (14.8)	1.00 (0.72-1.37)	1.000
Dominant	GG	236 (78.7)	228 (76)		
	GA+AA	64 (21.3)	72 (24)	0.85 (0.58-1.25)	0.435
Recessive	AA	25 (8.3)	17 (5.7)		
	GG+GA	275 (91.7)	283 (94.3)	1.51 (0.79-2.86)	0.203
rs2619681					

Co-dominant	CC	116 (38.7)	135 (45)	Referent	
	CT	131 (43.7)	129 (43)	1.18 (0.83-1.67)	0.34
	TT	53 (17.6)	36 (12)	1.71 (1.04-2.79)	0.03*
Allele	C	363 (60.5)	399 (66.5)		
	T	237 (39.5)	201 (33.5)	1.29 (1.02-1.64)	0.03*
Dominant	CC	116 (38.7)	135 (45)		
	CT+TT	184 (61.3)	165 (55)	1.29 (0.93-1.79)	0.11
Recessive	TT	53 (17.6)	36 (12)		
	CC+CT	247 (82.3)	264 (88)	1.57 (0.99-2.48)	0.05*
rs4987207					
Co-dominant	GG	173 (57.7)	196 (65.5)	Referent	
	GT	108 (36)	94 (31.5)	1.30 (0.92-1.83)	0.13
	TT	19 (6.3)	10 (3)	2.15 (0.97-4.75)	0.05*
Allele	G	454 (75.7)	486 (81)		
	T	146 (24.3)	114 (19)	1.37 (1.03-1.80)	0.02*
Dominant	GG	173 (57.7)	196 (65.3)		
	GT+TT	127 (42.3)	104 (34.7)	1.38 (0.99-1.92)	0.05*
Recessive	TT	19 (6.3)	10 (3)		
	GG+GT	281 (93.7)	390 (97)	2.63 (1.20-5.75)	0.014*
rs4987208					
Co-dominant	TT	101 (33.7)	142 (47.3)	Referent	
	TG	148 (49.3)	124 (41.3)	1.67 (1.18-2.37)	0.003*
	GG	51 (17)	34 (11.3)	2.10 (1.27-3.48)	0.003*
Allele	T	350 (58.3)	408 (68)		
	G	250 (41.7)	192 (32)	1.51 (1.19-1.92)	0.0005*
Dominant	TT	101 (33.7)	142 (47.3)		
	TG+GG	199 (66.3)	158 (52.7)	1.77 (1.27-2.46)	0.0007*
Recessive	GG	51 (17)	34 (11.3)		
	TT+TG	249 (83)	266 (88.7)	1.60 (1.00-2.55)	0.047*
rs2295466					
Co-dominant	AA	126 (42)	109 (36.3)	Referent	
	AG	132 (44)	147 (49)	0.77 (0.54-1.09)	0.15
	GG	42 (14)	44 (14.7)	0.82 (0.50-1.35)	0.44
Allele	A	384 (64)	365 (60.8)		
	G	216 (36)	235 (39.2)	0.87 (0.69-1.10)	0.25
Dominant	AA	126 (42)	109 (36.3)		
	AG+GG	174 (58)	191 (63.7)	0.78 (0.56-1.09)	0.15
Recessive	GG	42 (14)	44 (14.7)		
	AA+AG	258 (86)	256 (85.3)	0.94 (0.59-1.49)	0.81

***p-value <0.05 (statistically significant)**

The genetic model and disease association analysis of rs4987207 showed strong association of this SNP with breast cancer under all the models i.e. co-dominant model (GG vs. TT) (p-value = 0.05, OR = 2.15; 95% CI = 0.97-4.75), dominant model (GG vs. GT+TT) (p-value = 0.05, OR = 1.38; 95% CI = 0.99-1.92), recessive model (TT vs. GT+GG) (p-value = 0.014, OR = 2.63; 95% CI = 1.20-5.75) and allele model (G vs. T) (p-value = 0.02, OR = 1.37; 95% CI = 1.03-1.80), whereas in case of rs4987208, comparison of frequency distribution between breast cancer patients and controls under different genetic models revealed statistically significant association under all the models i.e. co-dominant model (TT vs. TG) (p-value = 0.003, OR = 1.67; 95% CI = 1.18-2.37), (TT vs. GG) (p-value = 0.003, OR = 2.10; 95% CI = 1.27-3.48), dominant model (TT vs. TG+GG) (p-value = 0.0007, OR = 1.77; 95% CI = 1.27-2.46), recessive model (GG vs. TT+TG) (p-value = 0.047, OR = 1.60; 95% CI = 1.00-2.55) and allele model (T vs. G) (p-value = 0.0005, OR = 1.51; 95% CI = 1.19-1.92). The study conducted by Han et al., 2002 on ovarian cancer did not show anyAssociation between the disease and rs4987208 of RAD52 gene, similarly no association was reported by Keleman et al., 2005 between breast cancer and rs4987207 of RAD52 gene. None of the genetic

model analysis revealed any significant risk of breast cancer associated with rs2295466 of RAD54 gene in the present study, agreeing with the results of Matsuda et al., 1999.

The association analysis of the studied SNPs was done to understand its potential relationship with various clinical variables which included cancer stage, tumor status, lymph node involvement, metastasis, tumor grade, and CA 15.3 levels (Table 6).The findings revealed no significant association between the rs1801320, rs1801321, rs121917739, rs2619681 and rs2295466 genotypes and the clinical variables examined. In case of rs4987207 of RAD52 gene, a notable exception was observed for metastasis, where the GT + TT genotype exhibited significantly lower odds of metastasis compared to the GG genotype (OR: 0.263; 95% CI = 0.087-0.795, p = 0.017). Statistically significant associations were observed between the TG+GG genotypeod rs4987208 and lymph node status (p = 0.02, OR = 1.72, 95% CI: 1.064-2.810) as well as grade (p = 0.03, OR = 2.54, 95% CI: 1.061-6.120).

Table 6:- Association analysis of SNPsof RAD51, RAD52and RAD54 gene with clinico-pathological variables.

Clinical variable / Genotypes	Stage		Tumor status		Lymph node status		Metastasis		Grade		CA 15.3 U/ml	
	I,IIA, IIB	IIIA, IIIB, IIIC,IV	T_1	T_2 – T_4	N_O	$N_1 – N_3$	M_O	M_1	Low (1)	High (2,3,4)	<30	≥30
rs1801320												
GC + CC	70	33	12	91	42	61	92	11	5	98	82	21
GG	136	61	28	169	80	117	185	12	17	180	163	34
OR (95% CI)	1.05 (0.630-1.755)		1.25 (0.609-2.588)		0.99 (0.611-1.613)		1.843 (0.783-4.336)		1.85 (0.663-5.170)		1.227 (0.670-2.249)	
P-value	0.85		0.54		0.98		0.16		0.24		0.506	
rs1801321												
GT + TT	93	39	13	119	56	76	123	9	9	123	107	25
GG	113	55	27	141	66	102	154	14	13	155	138	30
OR (95% CI)	0.862 (0.526-1.411)		1.752 (0.866-3.548)		0.878 (0.552-1.396)		0.805 (0.337-1.921)		1.146 (0.474-2.770)		1.075 (0.597-1.953)	
P-value	0.55		0.12		0.58		0.62		0.76		0.81	
rs121917739												
GA + AA	45	19	6	58	25	39	59	5	7	57	52	12
GG	161	75	34	202	97	139	218	18	15	221	193	43
OR (95% CI)	0.906 (0.496-1.655)		1.627 (0.651-4.065)		1.088 (0.618-1.916)		1.026 (0.365-2.879)		0.553 (0.215-1.419)		1.035 (0.509-2.105)	
P-value	0.75		0.29		0.77		0.96		0.22		0.92	
rs2619681												
CT + TT	125	59	26	158	74	110	171	13	13	171	155	29
CC	81	35	14	102	48	68	106	10	9	107	90	26
OR (95% CI)	1.092 (0.660-1.806)		0.834 (0.416-1.673)		1.049 (0.654-1.683)		0.806 (0.341-1.903)		1.106 (0.457-2.677)		0.647 (0.359-1.168)	
P-value	0.73		0.60		0.84		0.62		0.82		0.15	
rs4987207												

GT + TT	93	34	13	114	54	73	123	4	5	122	104	23
GG	113	60	27	146	68	105	154	19	17	156	141	32
OR (95% CI)	0.688 (0.416-1.138)		1.621 (0.801-3.284)		0.875 (0.549-1.394)		0.263 (0.087-0.795)		2.659 (0.954-7.410)		0.974 (0.538-1.762)	
P-value	0.15		0.18		0.57		**0.017***		0.061		0.93	
rs4987208												
TG + GG	134	65	25	174	72	127	180	19	10	189	157	42
TT	72	29	15	86	50	51	97	4	12	89	88	13
OR (95% CI)	1.20 (0.713-2.031)		1.21 (0.608-2.420)		1.72 (1.064-2.810)		2.55 (0.846-7.737)		2.54 (1.061-6.120)		1.81 (0.922-3.55)	
P-value	0.48		0.58		**0.02***		0.095		**0.03***		0.08	
rs2295466												
AG + GG	124	50	25	149	77	97	162	12	14	160	148	26
AA	82	44	15	111	45	81	115	11	8	118	97	29
OR (95% CI)	0.751 (0.459-1.229)		0.805 (0.405-1.598)		0.699 (0.436-1.121)		0.774 (0.330-1.816)		0.774 (0.314-1.907)		0.587 (0.326-1.058)	
P-value	0.25		0.54		0.13		0.56		0.58		0.07	

***p-value <0.05 (statistically significant)**

Linkage disequilibrium estimation and haplotype analysis of RAD51 SNPs (rs1801320, rs1801321, rs121917739, rs2619681) and RAD52 SNPs (rs4987207, rs4987208).

To elucidate the combined effect of rs1801320, rs1801321, rs121917739 and rs2619681 of RAD51 gene on the risk of breast cancer, haplotype analysis was performed. The pair-wise linkage disequilibrium (LD) between the polymorphic sites of RAD51 gene showed a strong LD between them (**Figure 2**). Frequency distributions of haplotypes of RAD51 SNPs (rs1801320, rs1801321, rs121917739 and rs2619681) among cases and controls are summarized in **Table 7.** Haplotype G-G-A-C showed highest frequency in the control group and therefore was taken as a reference for the haplotype association estimation with breast cancer. The frequencies of the 10 haplotypes out of total 16 haplotype constructs showed statistically significant differences among cases and controls. Comparison of these 10 haplotypes with the reference haplotype showed significant results **GGGC** (p-value < 0.0001), **GGGT** (p-value < 0.0001), **GTAC** (p-value = 0.025), **GTGC** (p-value < 0.0001), **CGGC** (p-value < 0.0001)**, CGAC** (p-value = 0.021)**, GTGT** (p-value < 0.0001)**, CGGT** (p-value = 0.0036)**, CTGC** (p-value = 0.013)**, CTAT** (p-value < 0.0001). Out of these 10 haplotypes, the odds ratio values of two haplotypes (GTGC and CTAT) showed increased risk of breast cancer and the other 8 showed reduced risk of breast cancer (**Table 6**).

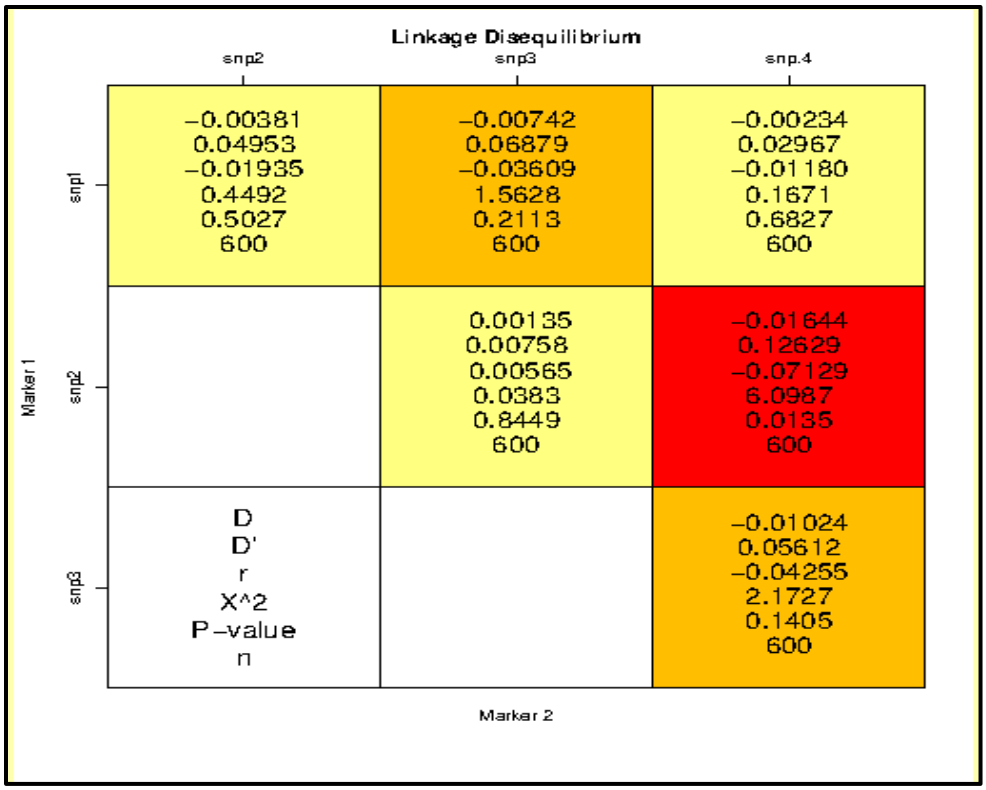


Figure 2:- LD plot showing haplotype block for SNPs (rs1801320, rs1801321, rs121917739, rs2619681) of RAD51 gene.

Table 7:- Frequency distributions of haplotypes of RAD51 SNPs (rs1801320, rs1801321, rs121917739 and rs2619681) among cases and controls.

Haplotypes	**Cases (%)**	**Controls (%)**	**OR (95% CI)**	**p- value**
G-G-A-C	0.0426	0.2888	1.00	-
G-G-G-C	0.2387	0.0377	0.09 (0.04-0.19)	**<0.0001***
G-G-G-T	0.1803	0.0339	0.07 (0.03-0.15)	**<0.0001***
G-T-G-C	0.1546	0.0451	4.67 (1.22-17.89)	**0.025***
G-T-A-C	0.0073	0.179	0.12 (0.06-0.23)	**<0.0001***
G-G-A-T	0.0466	0.1335	0.64 (0.29-1.39)	0.26
G-T-A-T	0.0073	0.0874	0.07 (0.02-0.21)	**<0.0001***
C-G-G-C	0.0836	0.0112	1.44 (0.37-5.60)	0.6
C-G-A-C	0.031	0.0539	0.30 (0.11-0.83)	**0.021***
G-T-G-T	0.071	0.0145	0.09 (0.03-0.24)	**<0.0001***
C-G-G-T	0.0576	NA	0.02 (0.00-0.28)	**0.0036***
C-T-G-C	0.0449	0	3.18	0.34

			(0.30-34.12)	
C-T-A-C	0.0023	0.0493	0.04 (0.00-0.50)	**0.013***
C-G-A-T	0.0113	0.0361	0.48 (0.15-1.61)	0.24
C-T-G-T	0.021	0.006	0.15 (0.02-1.09)	0.062
C-T-A-T	NA	0.0236	100862400979.78 (100862400978.52 - 100862400981.05)	**<0.0001***

***p-value <0.05 (statistically significant)**

Table 8:- Frequency distributions of haplotypes of RAD52 SNPs (rs4987207 and rs4987208) among cases and controls.

Haplotypes	**Cases %**	**Controls %**	**OR (95% CI)**	**p- value**
G-T	0.4554	0.555	1.00	-
G-G	0.3013	0.255	0.69 (0.50-0.94)	0.019
T-T	0.128	0.125	0.78 (0.51-1.20)	0.26
T-G	0.1154	0.065	0.49 (0.31-0.77)	0.0024

***p-value <0.05 (statistically significant)**

The pair-wise LD analysis showed a strong LD between SNPs located at two different loci **rs4987207 and rs4987208**within RAD52 gene (D'=0.081 and r^2=0.0558). Frequency distributions of haplotypes of RAD52 SNPs (rs4987207 and rs4987208) among cases and controls are summarized in **Table 8.** On performing the haplotype analysis, the frequency of the G-T haplotype out of four haplotypeswas found to be significantly higher in controls and it was considered as reference for association analysis. Comparison of other haplotypes with reference haplotypes indicated 0.69 fold (95% CI= 0.50-0.94, p-value = 0.019) and 0.78 fold (95% CI= 0.31-0.77, p-value = 0.0024) reduced risk of breast cancer in GG and TG haplotypes respectively**.**

Conclusion:-

Our study concludes that certain single nucleotide polymorphisms (SNPs) in the RAD51 and RAD52 genes are significantly linked to breast cancer risk. Specifically, three RAD51 SNPs (rs1801320, rs1801321, and rs121917739) and one RAD52 SNP (rs4987208) demonstrated significant genotype frequency differences between cases and controls. Genetic model analysis indicated that minor alleles of four RAD51 SNPs (rs1801320, rs1801321, rs121917739, and rs2619681) and two RAD52 SNPs (rs4987207 and rs4987208) were associated with an elevated risk of breast cancer. Haplotype analysis reinforced these associations, revealing 10 of 16 RAD51 haplotypes and two RAD52 haplotypes (G-G and T-G) as significantly increasing breast cancer risk. While most SNPs showed no significant correlation with clinical variables, notable exceptions in the RAD52 gene included the GT + TT genotype of rs4987207, which was linked to lower metastasis odds, and the TG + GG genotype of rs4987208, which was associated with lymph node involvement and higher tumor grade. These results suggest that RAD51 and RAD52 polymorphisms play a significant role in breast cancer susceptibility and progression, highlighting their potential as genetic markers for risk assessment and therapeutic targets. Further research is needed to elucidate the mechanisms behind these associations and to confirm these SNPs as reliable biomarkers for breast cancer.

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