International Journal of Health Sciences and Research
www.ijhsr.org ISSN: 2249-9571

Original Research Article

Genetic Polymorphism of DNA Repair Genes (XRCC1, XRCC2 & XRCC3) in Breast Cancer Patients from Rural Maharashtra

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Received: 17/01/2017 *Revised: 28/01/2017* *Accepted: 30/01/2017*

ABSTRACT

Background & Objectives: Breast cancer is a major concern of health risk, moreover the leading cause of cancer causing deaths in women of rural parts of India. In this study, we focused to determine the frequency of polymorphisms in DNA repair genes, XRCC1 at codon (cd) 194, cd 280, cd 399, XRCC 2 at cd 188 and XRCC3 at cd 241 to evaluate their role in breast cancer risk.

Methods: We used polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) to analyze XRCC genes polymorphisms in 150 breast cancer women and 200 age matched disease-free controls.

Results: The result from our study showed that allele frequencies of selected genes were not statistically different between the groups for XRCC1 Trp194, Gln399, XRCC2 His188 and XRCC3 Met241. XRCC1 His280 (OR= 4.14; 95% CI= (2.63-6.53); *p*= <0.0001) genotype significantly increased the risk of breast cancer.

Interpretation & conclusions: This study indicates that polymorphisms in cd280 of XRCC1 gene could play a role in modifying genetic susceptibility of individuals towards breast cancer among women from rural Maharashtra. It is apparent from our findings that larger part of the female had age at first delivery lower than 20 years which could be the probable risk factor for development of breast cancer. Thus, the case-control study suggest that selected DNA repair genes represent genetic determinants in breast carcinogenesis along with other risk factors in the rural Indian population.

Keywords: Genetic polymorphisms, PCR-RFLP, XRCC1, XRCC2, XRCC3

INTRODUCTION

Breast cancer (BC) is the second most common cancer worldwide and major public health burden increasing sporadically in both developed and developing countries. [1] India has seventeen percent of the world's population suffering from BC which has replaced cervical cancer as the leading cause of cancer deaths among women in rural areas. [2] The etiologic factors for BC comprise the reproductive events that influence the levels of hormones, early age of menarche, delayed menopause, use of contraceptives, exposure to heterocyclic aromatic compounds and environmental pollutants. [3-4] For a country like India with huge population, diverse cultures and geographical variations, probable risk factors are literacy, diet, age at first delivery, family history of BC. [5-6] It is assumed that along with the environmental factors, a combination of individual lifestyle habits and genetic factors may contribute to breast carcinogenesis. Though the genetic factors are considered of great importance to cancer risk through the modulation of DNA

repair but the etiology of BC is complex and largely unidentified in this regard. Variety of DNA repair mechanisms play a central role in maintenance of genomic integrity with different repair pathways such as base excision repair (BER), nucleotide excision repair (NER), double strand break repair (DSBR) and DNA mismatch repair (DMR). But it is not yet clear which DNA repair pathways are most important for protection against breast cancer.

The BER pathway is an important mechanism that repairs DNA damage resulting from chemical alterations of a single base. [7] Number of X-ray repair cross complementing group (XRCC) genes is involved in repair steps have been extensively studied in the association with various human cancers. The XRCC1 gene belonging to BER pathway plays an important role in repair of SSBs. XRCC2 and XRCC3 are DSB repair genes are key mediators in homologous recombination repair (HRR) of DNA double strand breaks to maintain chromosome stability. It is possible that polymorphisms of DNA repair genes may be associated with the repair efficiency of damaged DNA and influence an individual's risk of cancer. [8] Amongst the known genetic polymorphisms of the DNA repair genes, several functional genetic variants have been identified in the XRCC genes particularly XRCC1, XRCC2 and XRCC3 which have shown a relationship with the susceptibility to multiple cancers and have provided meaningful results. [9] Three polymorphisms, Arg194Trp, Arg280His and Arg399Gln, in codons 194, 280 and 399 respectively were reported earlier in the XRCC1 gene. Many authors have analyzed these polymorphisms in human populations and have been found associated with susceptibility to gastric, [10] lung, [11] oral [12] and breast [13] cancers. The Arg188His polymorphism in codon 188 of exon 3 of XRCC2 gene plays a role in carcinogenesis of pancreas [14] and colorectal cancer. [15] Similarly, a polymorphism Thr241Met in codon 241 of exon 7 of the XRCC3 gene has been associated with the increased risk of lung [16] and skin cancer. [17] However, the results of former studies remain controversial rather than convincing in terms of the association between genetic polymorphisms of XRCC genes and risk of different cancer types [18-19] and the influence of the polymorphisms of XRCC genes on DNA repair capacity is still ambiguous. Though these XRCC genes are suspected candidates for cancer susceptibility, several association studies on XRCC1 and XRCC3 polymorphisms with BC risk led to conflicting results. [20] Also, XRCC2, and XRCC3 variants were found not to be associated with BC risk. [21] Thus, the association between genetic polymorphism of XRCC1, XRCC2, XRCC3 and susceptibility to BC is still an open question. Therefore, in this study, we focused on the reported polymorphisms of DNA repair genes, XRCC1 at cd 194, cd 280, cd 399, XRCC 2 at cd 188 and XRCC3 at cd 241 to evaluate their role in BC. We performed a hospital based case-control study with a PCR-RFLP assay to determine genotypic frequency of the polymorphisms of selected i) XRCC1 gene at exon 6, exon 9 and exon 10 (ii) XRCC2 gene at exon3 (iii) XRCC3 gene at exon 7 and their associations in the BC risk in rural population of western Maharashtra from India.

MATERIALS & METHODS

Study subjects: This study was a hospital based case-control study. Study participants included 150 patients, who were newly diagnosed with BC and 200 healthy, cancer free, age and sex matched individuals as controls. All cases ranged in age from 25-75 years (Mean± SD) 50.04 ±12.06 were recruited immediately after being diagnosed during the year 2013-2015. Trained interviewers used a structured questionnaire to collect personal interview data from the participants regarding demographic factors and known risk factors.

Genomic DNA isolation from whole blood: Five milliliter (mL) of whole blood

from patients and normal controls was collected in sterile vacutainer after receiving informed consent. Genomic DNA extraction was carried out from the peripheral blood sample using Purelink genomic DNA extraction and purification kit (Invitrogen, Life technologies) following the manufacturer's instructions.

Genotyping assays: Genotyping of XRCC1, XRCC2 and XRCC3 genes were performed by PCR-RFLP methods with appropriate primer sets. The primers were designed to amplify the regions of DNA that contain polymorphic sites of interest; *XRCC1* Arg194Trp in the exon 6 (C26304T), *XRCC1* Arg280His in the exon 9 (G27466A), XRCC1 Arg399Gln in the exon 10 (G28152A), XRCC2 Arg188His in the exon 3 (G31479A), *XRCC3* Thr241Met in the exon 7 (C18067T). PCR amplification were carried out separately under specific conditions in 20 micro liter (µL) reaction mixtures containing 1X PCR buffer (10 mili molar (mM) Tris-HCl (pH 9.0), 50 mM KCl 1.5 mM MgCl2), 0.2 mM each dNTP, 10 picomole (pmol) of each primer listed in Table-I, 1U *Taq DNA* polymerase (GeNei, Merck Bioscience) and 100 nanogram (ng) of purified genomic DNA template. The reaction mixtures were subjected to PCR amplification with a Master Cycler Gradient PCR (Eppendorf). After performing PCR programme for each of the reactions, the PCR products were analyzed by agarose gel electrophoresis in Tris-Acetate-EDTA (TAE) buffer. The agarose gels were stained with ethidium bromide (10 mg/mL), visualized under UV Transilluminator and photographed in gel documentation system (BioRad Laboratories). After confirmation of DNA amplification, each PCR product was digested with an appropriate restriction enzyme as shown in table-I for genotyping. Ten µL of the PCR products were digested at 37°C overnight with specific restriction enzymes in 20 µL reaction mixtures containing buffer supplied with each restriction enzyme. After overnight incubation, digestion products were separated on a 2-3% low EEO agarose (GeNei) gel at 100 V for 30 min, stained with ethidium bromide and photographed with gel documentation system.

Statistical analysis: The associations between the *XRCC* genotypes and risk of BC were studied using odds ratio (OR). Both the univariate and multivariate logistic regression analyses were employed to calculate the adjusted ORs and 95% confidence intervals (CIs) to determine the BC risk associated with genotypes.

Table I. Details of PCR and RFLP procedures and expected products.

Gene	Primers Forward/ Reverse	PCR conditions	PCR Product	Restriction enzyme	Restriction products
XRCC1 **Arg194Trp cd 194 exon 6 (C26304T)**	5'-gcc agg gcc cct cct tca a-3' 5'-tac cct cag acc cac gag t-3'	95^0C- 5 min, 30 cycles of 95^0C- 30 sec, 61^0C- 20 sec, 72^0C- 30 sec, 72^0C- 5 min	485 bp	1U of PvuII	Arg/Arg: 485bp Arg/Trp: 485 bp, 396bp, 89 bp Trp/Trp: 396 bp,89 bp
XRCC1 Arg280His cd280 exon 9 (G27466A)	5' cca gct cca act cgt acc 3' 5' atg agg tgc gtg ctg tcc 3'	95^0C- 5 min, 30 cycles of 95^0C- 30 sec, 61^0C- 30 sec, 72^0C- 30 sec, 72^0C- 5 min	257 bp	2U of RsaI	Arg/Arg: 241 bp His/His: 257 bp
XRCC1 **Arg399Gln cd399 exon 10 (G28152A)**	5'-cag tgg tgc taa cct aat c -3' 5'-agt agt ctg ctg gct ctg g- 3'	95^0C- 5 min, 30 cycles of 95^0C- 20 sec, 56^0C- 30 sec, 72^0C- 20 sec, 72^0C- 5 min	871 bp	2U of NciI	Arg/Arg:593bp, 461bp, 278 bp, 132bp Arg/Gln: 461bp, 278,bp,132bp, Gln/Gln:593bp,278bp
XRCC2 Arg188His cd188 Exon 3 (G31479A)	5'- agt tgc tgc cat gcc tta ca-3' 5'-tgt agt cac cca tct ctc tgc-3'	95^0C- 5 min, 30 cycles of 95^0C- 30 sec, 58^0C- 30 sec, 72^0C- 30 sec, 72^0C- 5 min	290 bp	1U HphI	Arg/Arg :290bp Arg/His: 290bp,148bp 142bp His/His: 148bp, 142bp
XRCC3 **Thr241Met cd241 exon7 (C18067T).**	5'-ggt cga gtg aca gtc caa ac-3' 5'-tgc aac ggc tga ggg tct t- 3'	95^0C- 5 min, 30 cycles of 95^0C- 30 sec, 53^0C- 30 sec, 72^0C- 30 sec, 72^0C- 5 min	455 bp	2U NlaIII	Thr/Thr : 315bp, 140bp Thr/Met: 315bp/210bp, 140bp, 105bp Met/Met : 210bp,140bp, 105bp

RESULTS

Characteristics of the study subjects:

During the study period 150 patients with BC met the eligibility criteria for this study and 200 controls were selected to match these cases. The characteristics of age and sex matched cases and controls are presented in table-II. The mean age in years was 50.04 (median: 50, range 25-75) for the cases and 40.60 (median: 37.5 range 24-75) for the controls. There were no significant differences between the cases and controls with respect to sex and ethnicity.

Table: II Distribution comparisons of selected demographic characteristics of breast cancer cases and healthy controls from rural areas of Maharashtra in India.

Variable	Cases N=150		Controls N=200		*P*-Value based on $\chi 2$
Age (Mean ± SD) years	50.04 ±12.06		40.60 ± 13.73		<0.05
	No.	(%)	No.	(%)	
≤ 50	84	56.00	149	74.50	
51-60	33	22.00	32	16.00	
61-70	27	18.00	15	07.50	
>70	6	4.00	4	02.00	
Tobacco smoking Status					<0.001
Tobacco users	86	90.00	74	37.00	
Tobacco no users	64	40.00	126	63.00	
Mastectomy status					<0.001
Left MRM	90	60.00	0	0.00	
Right MRM	60	40.00	0	0.00	
Age @ Ist delivery (yrs)					<0.0001
15-20	108	72.00	72	36.00	
21-25	33	22.00	109	54.50	
26-30	07	04.67	11	05.50	
31-35	02	01.33	08	04.00	
Hormone Status					0.00
ER/PR+ve	91	49.33	0	0.00	
ER/PR-ve	59	34.67	0	0.00	
Her2 +ve	20	11.33	0	0.00	
Her2 -ve	130	66.00	0	0.00	
ER/PR/Her2+ve	04	02.67	0	0.00	
ER/PR/Her2-ve	41	27.33	0	0.00	
Diet					0.03
Vegeterian	33	24.67	64	32.00	
Mixed	117	75.33	136	68.00	
Education					<0.001
High School	72	48.00	50	25.00	
HighSchoolgraduate (12 y)	08	5.33	22	11.00	
College /Graduate	10	6.67	68	34.00	
No School	60	40.00	60	30.00	
Economic status					<0.001
Middle	35	22.67	73	36.50	
Poor	99	65.33	32	16.00	
Rich	16	12.00	95	47.50	
Family history of Cancer					0.04
Yes	07	4.67	0	00.00	
No	143	95.33	200	100.00	

Association of polymorphisms in XRCC genes and breast cancer:

The distribution of *XRCC* genotypes and concordance of the three polymorphisms are presented in table-III.

Analysis of the Arg194Trp, Arg280His and Arg399Gln polymorphism in the XRCC1 *gene*

The amplification of XRCC1 codon 194 resulted in the product of 485 bp. The PvuII enzyme was used to detect the *XRCC1* C26304T at codon 194 of exon 6. The PCR amplified products upon treatment with PvuII yielded wild-type (26304C) allele of single 485 bp fragment containing exons 5 and 6, and the polymorphic (T) allele produces 2 fragments of 396 and 89 bp (Figure 1A). The PCR amplified product of 290 bp for XRCC1 (G27466A) at codon 280, showed the presence of 148 and 142 bp bands after RsaI digestion depicting homozygous wild and variant genotypes respectively (Figure 1B). The amplification of XRCC1 codon 399 resulted in the

product of 871 bp. The *Nci*I enzyme was used to detect the *XRCC1* G28152A at codon 399 of exon 10. The 28152A variant allele has lost an NciI site. The wild-type (28152G) allele, which has 2 NciI sites, produces 3 bands (461, 278 and 132 bp), and the variant A allele produces only 2 (593 and 278 bp) (Figure 1C). The frequency of *XRCC1* 26304CC homozygotes was 74.0% in cases and 78.50% in controls whereas *XRCC1* 26304 TT homozygotes was 4.0 % in cases and 18.50 % in controls. The frequency of *XRCC1* 26304 CT heterozygotes was 22.0% in cases and 3.0% in controls. In codon 280, genotype frequencies for wild and variant genotypes were 41.33 and 58.67 per cent respectively in cases and 74.50 and 25.50 percent respectively in controls. The frequency of *XRCC1* 28152GG homozygous wild type alleles at codon 399 of exon 10 was 68.67%, 28152GA heterozygote alleles was 27.33% and for 28152AA homozygous alleles was 4.00% in the cases where that of the frequencies for the controls were 67.50, 30.50 and 2.00 % respectively (Table-III)

Analysis of the Arg188His polymorphism in the* XRCC2 *gene

Table-III displays the distribution of genotypes and frequency of alleles of the G31479A polymorphisms in patients with BC and controls. We did not find any significant difference in genotype or allele frequencies between patients and controls. We investigated the association between the polymorphisms and hormone receptor status (Table-IV). The frequency of *XRCC2* 31479*GG* wild type alleles at codon 188 of exon 3 was 70.67%, 31479*GA* heterozygote alleles was 26.00% and for 31479AA homozygous alleles was 3.33% in the cases where that of the frequencies for the controls were 78.0, 19.50 and 2.50 % respectively(Table-III). Thus, the haplotype analysis according to variant type of G31479A showed a lack of association with BC.

The pattern of genotype polymorphism by PCR-RFLP of XRCC2 is represented as shown in agarose gel (Figure 1D)

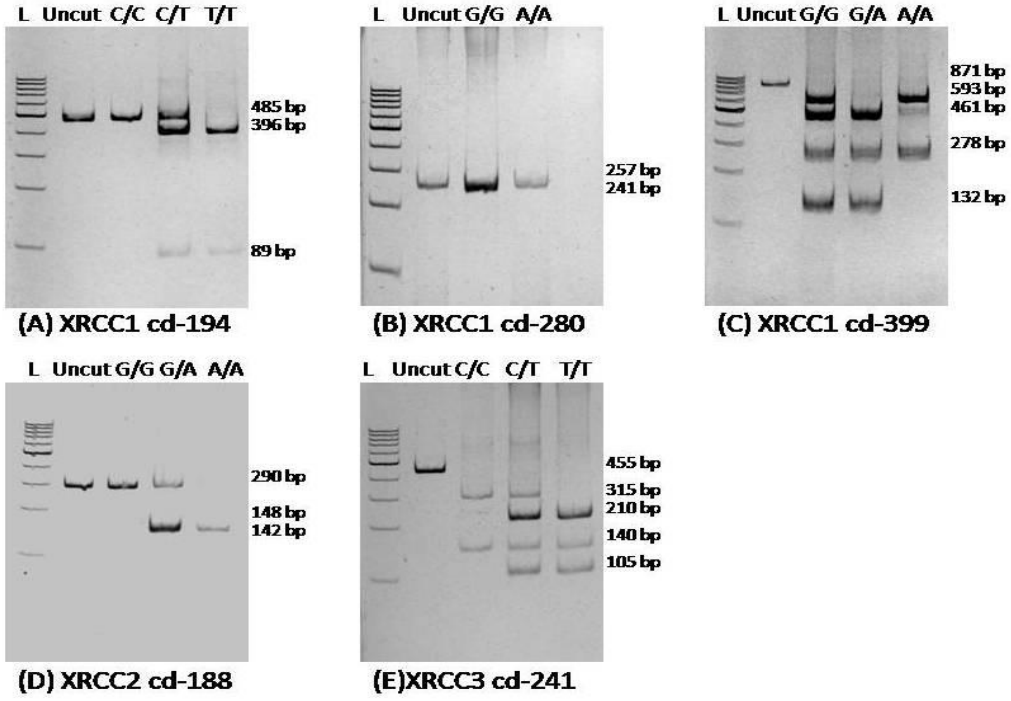

Figure I: Representative agarose gel image showing nucleotide polymorphism by PCR-RFLP of (A) XRCC1 Codon 194 Ex-6 by PvuII (Lane 1: 100 bp ladder, lane 2: Uncut PCR product, lane 3: WT genotype, lane 4: HT genotype, lane 5: VT genotype (B) XRCC1 codon280 Ex-9 by RsaI, (C) XRCC1 cd399 Ex-10 by NciI (D) XRCC2 cd188 Ex-3 by HphI (E) XRCC3 cd241 Ex-7 by NlaIII.

Analysis of the Thr241Met polymorphism in the XRCC3 *gene*

Allele frequencies and distribution of genotypes of *XRCC3* codon 241 are shown in Table-III. In frequency distribution of codon 241 of XRCC3 gene at C18067T, genotype frequencies for wild, heterozygote and variant genotypes were 70.00, 21.33 and 8.67 % respectively in cases where as the frequencies in controls were 82.0, 12.0 and 6.0 % respectively. XRCC3 241 Met/Met, Thr/Met and Met/Met genotypes did not show significant association with development of BC (OR=1.69, 95% CI=0.74-3.84; *p*= 0.20 for 241 Met/Met) (Table-III). The RFLP pattern of XRCC3 genotypes after digestion with NlaIII is as shown in figure1E.

Table: III. The genotype frequencies of *XRCC* gene polymorphisms in untreated breast cancer patients and controls.

GENE	Genotype	CASES (n= 150) (%)	CONTROL (n = 200) (%)	Odds' Ratio (95% CI)	*P* value	Adjusted Odds Ratio (95% CI)	*P* value
XRCC1 cd194 ex-6	Arg/Arg	111(74.00)	157 (78.50)	1		1	
	Arg/Trp	33(22.00)	37 (18.50)	1.26 (0.74-2.14)	0.38	1.48 (0.41-5.28)	0.54
	Trp/Trp	6 (4.00)	6 (03.00)	1.41 (0.44-4.50)	0.55	1.20 (0.31- 4.63)	0.78
	Arg/Trp+Trp/ Trp	39 (26.00)	43 (21.50)	1.28 (0.78-2.10)	0.32	1.23 (0.71-2.12)	0.21
XRCC1 cd280 ex-9	Arg/Arg	62 (41.33)	149 (74.50)	1		1	
	Arg/His	0(0.00)	0 (0.00)	-----	-----	-----	-----
	His/His	88 (58.67)	51 (25.50)	**4.14 (2.63-6.53)**	**<0.0001***	**4.79 (2.99-7.68)**	**<0.0001**
	Arg/His+His/ His	88 (58.67)	51 (25.50)	**4.14 (2.63-6.53)**	**<0.0001***	**4.79 (2.99-7.68)**	**<0.0001**
XRCC1 cd399 ex-10	Arg/Arg	103 (68.67)	135 (67.50)	1		1	
	Arg/Gln	41 (27.33)	61 (30.50)	0.88 (0.41-1.41)	0.59	2.45 (0.62-9.68)	0.20
	Gln/Gln	6 (04.00)	4 (02.00)	1.96 (0.54-7.14)	0.30	2.88 (0.69-11.87)	0.14
	Arg/Gln+ Gln/Gln	47 (31.33)	69 (32.50)	0.89 (0.56-1.40)	0.62	0.93 (0.56-1.54)	0.80
XRCC2 cd188 ex-3	Arg/Arg	106 (70.67)	156 (78.00)	1		1	
	Arg/His	39 (26.00)	39 (19.50)	1.47 (0.88-2.44)	0.13	1.49 (0.38-5.83)	0.56
	His/His	5 (03.33)	5 (02.50)	1.47 (0.41-5.20)	0.54	1.09 (0.26-4.55)	0.9
	Arg/His+His/ His	44 (29.33)	44 (22.00)	1.47 (0.90-2.39)	0.11	1.44 (0.84-2.45)	0.17
XRCC3 Cd241 Ex-7	Thr/Thr	105 (70.00)	164 (82.00)	1		1	
	Thr/Met	32 (21.33)	24 (12.00)	2.08 (1.16-3.73)	0.01	2.13 (0.86-5025)	0.09
	Met/Met	13 (08.67)	12 (06.00)	1.69 (0.74-3.84)	0.20	0.91 (0.32-2.57)	0.86
	Thr/Met+Met/ Met	45 (30.00)	36 (18.00)	1.95 (1.18-3.22)	0.009	2.22 (1.28-3.84)	0.004

***: Indicates significant Odds Ratio (p<0.005)**
***p* value determined based on $\chi 2$**

Association of age at Ist delivery and hormone receptor status with breast cancer risk

In Maharashtrian patients, the median age of onset of breast cancer is 50 years, substantially lower than observed in other reports. To evaluate the association of the polymorphisms with the young age at diagnosis of BC, we stratified the patients as ≤50 (n=84) or >50 (n=66) years of age and compared with age matched sample of controls which interestingly showed that the XRCC1 cd 280 (OR=5.35; CI=3.01-9.52; *p*<0.0001) showed significant risk of BC at the age bellow median. Also, the association of BC with age at first delivery was considered in this study which showed that early age of first delivery i.e.15-20 yrs, significantly associated with increased BC risk. The genotype distributions for the individual polymorphisms along with the statistical analysis are shown in table-V. When we conducted the association of BC risk with the hormone receptor (ER/PR) status of the tumors we found that (ER/PR +ve (n= 91) and ER/PR-ve (n= 59) (table-II and IV), showed higher risk of BC.

Table IV: Genotype Frequencies of XRCC1, XRCC2 & XRCC3 gene Polymorphism in Breast Cancer Cases with ER/PR status

Gene	Genotype	ER/PR +ve (%)	ER/PR -ve (%)	OR	95% CI	χ^2	P-value
***XRCC1* Arg194Trp cd 194 exon 6 *(*C26304T)**	Arg/Arg	71 (0.48)	40 (0.27)	1			
	Arg/Trp	16 (0.11)	17 (0.12)	0.53	0.24-1.16	1.58	0.11
	Trp/Trp	5 (0.03)	1 (0.006)	2.81	0.31-24.96	0.93	0.35
	Arg/Trp+ Trp/Trp	21 (0.14)	18 (0.12)	0.65	0.31-1.37	1.13	0.26
XRCC1 Arg280His cd280 exon 9 (G27466A)	Arg/Arg	36 (0.24)	24 (0.16)	1			
	Arg/His	0	0	-----	-----	-----	-----
	His/His	55 (0.37)	35 (0.23)	1.04	0.53-2.04	1.13	0.089
	Arg/His+ His/His	55 (0.37)	35 (0.23)	1.04	0.53-2.04	1.13	0.089
***XRCC1* Arg399Gln cd399 exon 10 *(*G28152A)**	Arg/Arg	60 (0.40)	43 (0.29)	1			
	Arg/Gln	25 (0.17)	16 (0.11)	1.11	0.53-2.34	0.30	0.76
	Gln/Gln	4 (0.026)	2 (0.013)	1.43	0.25-8.18	0.40	0.68
	Arg/Gln+ Gln/Gln	29 (0.19)	18 (0.12)	1.15	0.56-2.34	0.39	0.69
XRCC2 Arg188His cd188 Exon 3 (G31479A	Arg/Arg	65 (0.44)	41 (0.27)	1			
	Arg/His	25 (0.17)	14 (0.1)	1.12	0.52-2.41	0.36	0.75
	His/His	1(0.006)	4 (0.02)	0.15	0.01-1.46	1.46	0.16
	Arg/His+ His/His	26 (0.17)	18 (0.12)	0.91	0.44-1.86	0.25	0.79
***XRCC3* Thr241Met cd241 exon7 (C18067T)**	Thr/Thr	62 (0.41)	43 (0.29)	1			
	Thr/Met	20 (0.13)	13 (0.08)	1.06	0.47-2.37	0.15	0.87
	Met/Met	5 (0.03)	7 (0.04)	0.49	0.14-1.66	1.13	0.25
	Thr/Met+ Met/Met	25 (0.17)	20 (0.14)	0.86	0.42-1.75	0.39	0.69

Table V: Stratification analysis of the demographic factors including age, tobacco smoking and age at first delivery and distribution of genotypes with odds ratio of the XRCC genes in the patients with breast cancer and the control group from rural population of western Maharashtra.

		Demographic Factors							
Gene	**Genotype**	**Age (Cases/Control)**		**Tobacco status (Cases/Control)**		**Age @ 1st Delivery (Cases/Control)**			
		≤ 50 N= 86/149	> 50 N= 64/51	Tobacco Users N=86/74	Tobacco nonusers N=64/126	15-20 N= 108/72	21-25 N= 33/109	26-30 N= 7/11	31-35 N= 2/8
***XRCC1* Arg194Trp cd 194 exon 6 *(*C26304T)**	Arg/Arg	67/117	44/41	59/61	52/96	82/58	21/84	5/10	2/7
	Arg/Trp+ Trp/Trp	19/32	20/10	27/14	12/29	26/14	12/25	2/1	0/1
	OR (95% CI)	1.03 0.54-1.97	1.86 0.78-4.44	1.99 0.95-4.17	0.76 0.35-1.62	1.31 0.63-2.73	1.92 0.83-4.43	4.00 0.28-55.47	1.00 0.03-33.31
	P value	0.91	0.16	0.06	0.48	0.46	0.12	0.30	1.00
XRCC1 Arg280His cd280 exon 9 (G27466A)	Arg/Arg	29/109	30/40	34/89	25/59	39/60	13/82	6/8	2/6
	Arg/His+ His/His	57/40	34/11	52/34	39/18	69/12	20/27	1/3	0/2
	OR (95% CI)	5.35 3.01-9.52	4.12 1.79-9.43	4.00 2.22-7.19	5.11 2.46-10.59	8.84 4.24-18.42	0.21 0.09-0.48	0.44 0.03-5.40	0.52 0.01-15.09
	P value	0.0001	0.0008	0.0001	0.0001	**0.0001***	0.0002	0.52	0.70
***XRCC1* Arg399Gln cd399 exon 10 *(*G28152A)**	Arg/Arg	58/108	44/39	65/88	39/47	76/46	21/79	4/8	2/7
	Arg/Gln+ Gln/Gln	28/41	20/12	21/38	25/27	32/26	12/30	3/3	0/1
	OR (95% CI)	1.27 0.71-2.26	1.47 0.64-3.40	0.74 0.40-1.39	1.11 0.55-2.22	0.74 0.39-1.40	1.50 0.65-3.43	2.00 0.27-14.78	1.00 0.03-33.31
	P value	0.41	0.36	0.36	0.75	0.36	0.33	0.49	1.00
XRCC2 Arg188His cd188 Exon 3 (G31479A	Arg/Arg	62/116	44/40	58/102	53/55	78/60	25/87	5/7	2/6
	Arg/His+ His/His	20/33	24/11	28/23	11/20	30/12	8/22	2/4	0/2
	OR (95% CI)	1.13 0.60-2.14	1.98 0.86-4.55	2.14 1.13-4.05	0.57 0.24-1.30	1.92 0.90-4.06	1.26 0.50-3.18	0.70 0.09-5.43	0.52 0.01-15.09
	P value	0.69	0.10	0.01	0.18	0.08	0.61	0.73	0.70
***XRCC3* Thr241Met cd241 exon7 (C18067T)**	Thr/Thr	60/123	45/38	58/100	47/64	75/61	24/89	5/11	2/8
	Thr/Met+ Met/Met	25/26	20/13	28/25	17/11	33/11	9/20	2/0	0/0
	OR (95% CI)	1.97 1.05-3.70	1.29 0.57-2.95	1.93 10.2-3.62	2.10 0.90-4.90	2.44 1.13-5.22	1.66 0.67-4.13	10.45 0.42-256	0.29 0.004-18.9
	P value	0.03	0.53	0.04	0.04	0.02	0.26	0.15	0.56

***: Indicates significant Odds Ratio (p<0.005)**

***p* value determined based on $\chi 2$**

DISCUSSION

In this hospital based case-control study we assessed the genotypic frequency of polymorphisms of the (i) XRCC1 gene at cd 194, cd 280, cd 399 (ii) XRCC2 gene at cd188 (iii) XRCC3 gene in exon 7 to study the association with BC susceptibility particularly from the rural areas of Maharashtra. Comparable wild type genotype frequencies of XRCC1 cd 194, cd 280, cd 399 showed wide distribution in the Maharashtrian population in controls. We found no evidence for a combined effect of the 194Trp and 399Gln alleles and BC. The analysis of the individual polymorphisms showed that only the 280His allele of XRCC1 was associated with an increased risk of BC. The frequency of allele of the XRCC2 polymorphism at 31479 (G-A) did not show association with the BC risk in Maharashtrian population. Also, when we conducted a case-control study to investigate the relationship between the polymorphisms XRCC3 codon 241 and the risk BC in a western Maharashtrian population, we did not find association between the XRCC3 codon 241 polymorphism and BC.

There is evidence that reduced DNA repair capacity, resulting from genetic polymorphisms of various DNA repair genes, is associated with increased risk and susceptibility to various types of human cancers. [12,16] Very few studies from India have reported the genetic polymorphisms in the DNA repair genes with respect to a variety of cancer risks including prostate, breast and oral cancer. [22-23] Several molecular epidemiological studies have been conducted to evaluate the association between XRCC1, XRCC2, XRCC3 genes and BC risk. Some studies revealed the positive association of Arg194Trp variant of XRCC1 with BC development risk [24] while other demonstrated a strong association of the Arg399Gln polymorphism with an increased risk of BC. [15, 25] On the other hand, in a more recent report [26] showed a weak association of XRCC1 gene in development of BC, however Breast Cancer Association Consortium (BCAC) reported no evidence of association of XRCC genes with BC development. [27] Thus, there is a controversy between association, or no association between XRCC1 gene and BC development risk. Also, XRCC2 variants were found not to be associated with BC risk. [21] Although polymorphisms of XRCC3 gene may results in reduced DNA repair capacity, the direct evidence in terms of the associations with cancer susceptibility have proved conflicting. [28]

However, no information is available on the association of genetic polymorphisms of DNA repair genes and their susceptibility to BC from rural population of Maharashtra. Therefore in this study, we determined the relationship between the development of BC and genetic polymorphisms in XRCC genes from a pool of unexplored rural Maharashtrian population. We found no evidence for a combined effect of the 194Trp and 399Gln alleles of XRCC1 and BC but, XRCC1 cd 280 genotype may be related to BC risk. However the direction and magnitude of associations observed in this study are difficult to interpret on the basis of current knowledge of the functional status of XRCC1 alleles. Overall, we observed that only 280His allele was associated with an increased risk of BC, whereas the 194Trp & 399Gln allele did not show any association with a risk of this cancer. A similar observation was also reported in French population by Moulan N et al. (2003) [29] but in combination with 399Gln and also weak association with 194Trp to increase frequency of BC, but Duell et al., [30] reported no association of 399Gln with BC in white American women. Smith et al., [31] also reported a weak association of the 194 Trp allele with a risk of BC occurrence in American women. However, Kim *et al.* [32] found that the 194 variant allele had no influence on BC risk in Korean women. Thus there is an inconsistency in the previous findings about the role of XRCC1 gene in BC association.

In conclusion, our study implies the possibility of no direct involvement of the Arg194Trp, Arg399Gln polymorphism of XRCC1 gene in the development and/or progression of BC, except Arg280His which showed strong association with BC. The results also showed the increased risk of BC in patients with ER/PR positive category. It is also evident from our findings that larger part (72%) of the female had age at first delivery below 20 years which could be the probable risk factor for development of BC. The mechanistic basis for the present findings remains unclear, therefore confirmation of our results in larger populations is warranted to clarify this point.

Ethical approval: The study protocol was approved by the Institutional Ethics Committee of KIMSDU for the use of human subjects in research.

Funding: Research project was funded be Intramural funding by Krishna Institute of Medical Sciences University.

Acknowledgements

The experimental assistance of Mr. Santosh Jadhav is duly acknowledged.

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How to cite this article: Datkhile KD, Bhosale SJ, Patil MN et. al. Genetic Polymorphism of DNA repair genes (XRCC1, XRCC2 & XRCC3) in breast cancer patients from rural Maharashtra. Int J Health Sci Res. 2017; 7(2):281-290.

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