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Original Research Article

Role of Genetic Polymorphisms in XRCC4, XRCC5, XRCC6 and XRCC7 in Breast Cancer Susceptibility in Rural Indian Population: A Hospital Based Case-Control Study from Maharashtra

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ABSTRACT

Background & Objectives: Breast cancer is a major concern of health risk, moreover leading cause of cancer causing deaths in women of rural parts of India. In this study, to identify the role of genetic risk factors in the development of breast cancer, functional polymorphisms of DNA repair genes including XRCC4 at codon 247, XRCC4 G1394T, XRCC4 intron7, XRCC5 2R/1R/0R, XRCC6 61 (C>G) and XRCC7 6721 (G>T) were studied among rural population of Maharashtra.
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 and sex 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 XRCC4 (cd247, G1394T, intron 7) and XRCC6 (-61C>G). XRCC5 1R/0R (OR= 3.21; 95% CI= (2.06-5.02); *p*= <0.0001) and XRCC7 6721 G/T (OR= 2.94; 95% CI= (1.88-4.61); *p*= <0.0001) genotypes significantly increased the risk of breast cancer.
Interpretation & Conclusions: This study indicates that XRCC5 1R/0R polymorphisms and heterozygote allele 6721G/T of XRCC6 could play a role in modifying genetic susceptibility of individuals towards breast cancer among women from rural Maharashtra. It is also 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: Breast cancer, Genetic polymorphisms, PCR-RFLP, XRCC4, XRCC5, XRCC6, XRCC7.

INTRODUCTION

Breast cancer (BC) is the most prevalent cancer worldwide increasing public health burden erratically in both developed and developing countries. India has 1/6th of the world's population suffering from BC as the leading cause of cancer deaths among women in rural areas. [1] 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 compounds and environmental pollutants. For a country like India with enormous population, diverse cultures and geographical variations probable risk factors are literacy, diet and early age of first delivery. [2] It is assumed that along with the environmental factors, combination of individual lifestyle habits

and genetic factors may contribute to breast carcinogenesis. 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 BC.

It is postulated that several functional genetic variations in DNA repair genes may be associated with the repair efficiency of damaged DNA and influence an individual's risk of cancer. [3] Number of X-ray repair cross complementing group (XRCC) genes are involved in repair steps have been extensively studied in the association with various human cancers. The XRCC4, XRCC5, XRCC6 and XRCC7 are few of them involved in DNA double strand break repair by two important repair subpathways; homologous recombination repair (HRR) and nonhomologus end joining (NHEJ) to maintain chromosome stability. [4] Amongst the polymorphisms of the DNA repair genes, several functional genetic variants have been identified in the XRCC genes particularly XRCC4, XRCC5 and XRCC6 which have shown a relationship with the susceptibility to multiple cancers. Genetic polymorphisms in those HR & NHEJ genes were reported to influence DNA repair capacity and confer predisposition to several cancer types including skin, [5] lung, [6] bladder, [7] breast, [8] oral [9] and prostate cancers. [10]

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 and the influence of the polymorphisms of XRCC genes on DNA repair capacity are still ambiguous. Several association studies on XRCC4 and XRCC5 polymorphisms with BC risk led to conflicting results. [11,12] Also, XRCC6, and XRCC7 variants were found not to be associated with BC risk. [13] Moreover, the former observations were not consistent in terms of their roles in cancer susceptibility and the influence of polymorphisms of those genes on DNA repair capacity is still unclear. Thus, the association between genetic polymorphism of XRCC genes and susceptibility to BC is still an open question. Therefore, in this study we focused on the reported polymorphisms with the greater allele frequencies of XRCC genes belonging to DSB repair genes to evaluate their role in BC. We performed a hospital based case control study using a PCR-RFLP assay to genotype the polymorphisms of selected DNA repair genes in relation to BC susceptibility in a rural population of south-western Maharashtra from India. We determined the genotypic frequency of polymorphisms of the (i) XRCC4 gene at codon 247 (rs3734091), G-1394T (rs6869366) and Intron 7 (rs1805377). Also the present study intended to investigate the associations between the XRCC5 (2R/1R/0R), XRCC6 promoter 61(61C>G) and XRCC7 (6721 (G>T)) gene polymorphisms and the development of BC in Maharashtrian population.

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 cases 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 XRCC4, XRCC6 and XRCC7 genes were performed by PCR-RFLP methods with appropriate primer sets (Table-1). The primers were designed to amplify the regions of DNA that contain polymorphic sites of interest: XRCC4 codon 247, XRCC4 G1394T, XRCC4 intron7, XRCC6 61 (C>G) and XRCC7 6721 (G>T). The *XRCC5* 2R/1R/0R polymorphisms were identified by PCR. The PCR amplification were carried out separately under different 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, 0.01% gelatin), 0.2 mM each dNTP, 10 picomole (pmol) of each primer listed in Table-1, 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 reaction, 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) and 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-1 for genotyping. Ten micro liters 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 the overnight incubation, digestion products were then 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 (BioRad).

Statistical analysis

The associations between *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 1: Details of PCR and RFLP procedures and expected products

Gene	Primers Forward/ Reverse	PCR conditions	PCR Product	Restriction enzyme	Restriction products
XRCC4-1 cd247	5'-gct aat gag ttg ctg cat ttt a-3' 5'-ttt cta ggg aaa ctg caa tct gt-3'	95^0C- 5 min, 30 cycles of 95^0C- 30 sec, 55^0C- 30 sec, 72^0C- 30 sec, 72^0C- 5 min	308 bp	1U BbsI	WT: C/C: 308 bp VT: A/A:204bp, 104bp
XRCC4-2 G1394T	5'-gat gcg aac tca aag ata ctg a-3' 5'-tgt aaa gcc agt act caa act 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	300 bp	1U HincII	WT : T/T: 300 bp VT: G/G:200bp, 100bp
XRCC4-3 Intron-7	5'-ttc act tat gtg tct ctt ca-3' 5'-aac ata gtc tag tga aca tc-3'	95^0C- 5 min, 30 cycles of 95^0C- 30 sec, 48^0C- 30 sec, 72^0C- 30 sec, 72^0C- 5 min	237 bp	1U Tsp509I	WT: G/G: 237 bp VT: A/A:158bp, 79bp
XRCC5 2R/1R/0R	5'-agg cgg ctc aaa cac cac ac-3' 5'-caa gcg gca gat agc gga aag-3'	95^0C- 5 min, 30 cycles of 95^0C- 30 sec, 62^0C- 30 sec, 72^0C- 30 sec, 72^0C- 5 min	2R/2R:266 bp 1R/1R:245 bp0R/0R: 224bp		
XRCC6- 61C>G	5'-tct cca ctc ggc ttt tct tcc a -3' 5'- tct ccc tcc gct tcg cac tc-3'	95^0C- 5 min, 35 cycles of 95^0C- 30 sec, 56^0C- 30 sec, 72^0C- 30 sec, 72^0C- 5 min	320 bp	1U BanI	WT C/C:262bp, 58bp VT G/G:182bp,80bp,58bp
XRCC7 6721 G > T	5'-cgg ctg cca acg ttc ttt cc -3' 5'-tgc cct tag tgg ttc cct gg -3'	95^0C- 5 min, 30 cycles of 95^0C- 30 sec, 58^0C- 30 sec, 72^0C- 30 sec, 72^0C- 5 min	368 bp	1U PvuII	WT G/G: 368bp VT T/T: 274bp,94bp

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-2. 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.

Association of polymorphisms in XRCC genes and breast cancer

The distribution of *XRCC4, XRCC5, XRCC6 and XRCC7* genotypes and concordance of the polymorphisms are presented in table-3.

Analysis of the polymorphism in the XRCC4 gene

The frequency of the genotypes of XRCC4-1, XRCC4-2 and XRCC4-3 between BC and control groups are shown in table-3. Among the XRCC4 polymorphisms investigated, codon 27, codon 1394 and intron 7 seems not to contribute to increased BC risk. Our findings suggest that the genotype distribution of the A allele at *XRCC4* codon 247 (4.67%) was similar in the BC group to that in the control group (3.00%) (table 3). Also T allele at codon 1394 (33.33%) and A allele at intron 7 (22.00%) in breast cancer cases were not much higher than in controls (40.00% and 27.50% respectively). Also the joint effects of XRCC4 genotype and risk factors, namely hormone receptor status (table-4) and age at first delivery on estimates of BC risk are shown in table-5.

Table 2: 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	57.33	74	37.00	
Tobacco no users	64	42.67	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
Vegetarian	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	

Table 3: 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
XRCC4-1 cd247	C/C	143 (95.33)	194 (97.00)	1		1	
	A/A	7 (4.67)	6 (3.00)	1.58 (0.52-4.81)	0.41	0.335 (0.09- 1.20)	0.094
XRCC4-2 G1394T	G/G	100 (66.67)	120 (60.00)	1		1	
	T/T	50 (33.33)	80 (40.00)	0.75(0.48-1.16)	0.20	1.33 (0.81-2.20)	0.25
XRCC4-3 Intron-7	G/G	117 (78)	145 (72.50)	1		1	
	A/A	33 (22)	55 (27.50)	0.74 (0.45-1.22)	0.24	1.13 (0.65-1.98)	0.65
XRCC5 2R/1R/0R	2R/2R	47 (31.33)	119 (59.50)	1		1	
	1R/1R	86 (57.33)	53 (26.50)	4.10 (2.54-6.64)	<0.0001	0.24 (0.14-0.40)	0.0001
	0R/0R	17 (11.34)	28 (14.00)	1.53 (0.77-3.06)	0.22	0.56 (0.26-1.18)	10.12
	1R/1R+0R/0R	103 (68.67)	81 (40.50)	3.21 (2.06-5.02)	<0.0001	0.54 (0.16-1.84)	0.33
XRCC6-61C>G	C/C	93 (62.00)	94 (47.00)	1		1	
	C/G	41 (27.33)	85 (42.50)	0.48 (0.30-0.78)	0.002	2.27 (1.35-3.81)	0.002
	G/G	16 (10.67)	21 (10.50)	0.77 (0.37-1.56)	0.47	1.20 (0.54-2.65)	0.65
	C/G+G/G	57 (38.00)	106 (53.00)	0.54 (0.35-0.83)	0.005	1.87 (1.19-2.93)	0.006
XRCC7 6721 G > T	G/G	44 (29.33)	110 (55.00)	1		1	
	G/T	65 (43.33)	46 (23.00)	3.53 (2.11-5.91)	<0.0001	0.31 (0.17-0.54)	0.0001
	T/T	41 (27.34)	44 (22.00)	2.32 (1.34-4.04)	0.002	0.39 (0.21-0.70)	0.002
	G/T+T/T	106 (77.67)	90 (45.00)	2.94 (1.88-4.61)	<0.0001	0.33 (0.21-0.53)	0.0001

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

Analysis of polymorphism in the XRCC5 gene

In our study, *XRCC5* 2R/1R/ 0R selected to investigate the associations between the polymorphisms and risk of BC in a hospital-based case-control study in a Maharashtrian population. As shown in table-3, the genotypes of 1R/1R, 1R/0R was more common (57.33%, 68.67%) and that of 2R/2R was less common (31.33%) among the cases than among the controls (25.50%, 40.50%, and 59.50% respectively). The 1R/1R variant conferred an increased risk of BC development compared to the 2R/2R wild-type genotype in the age below fifty years OR 3.30 (95% CI=1.87- 1.87, p<0.0001), smoking stratification (OR 2.86 (95% CI=1.49- 5.48, p<0.0001) and age for the first delivery OR 3.90 (95% CI=4.71- 9.05, p<0.0001). We found a statistically significant increased risk of BC in group of patients with 1R/1R and the combined 1R/1R and 0R/0R genotype.

Table 4: Genotype Frequencies of XRCC genes Polymorphism in Breast Cancer Cases with ER/PR status

Gene	Genotype	ER/PR +ve (%)	ER/PR -ve (%)	OR	95% CI	P-value
XRCC4-1 cd247	C/C	86 (57.33)	57 (38.00)	1		
	A/A	3 (2.00)	4 (2.67)	0.49	(0.10-2.30)	0.37
XRCC4-2 G1394T	G/G	54 (36.00)	46 (30.67)	1		
	T/T	32 (21.33)	18 (12.00)	1.51	(0.75-3.04)	0.24
XRCC4-3 Intron-7	G/G	63 (42.00)	54 (36.00)	1		
	A/A	24 (16.00)	9 (6.00)	1.28	(0.97-5.33)	0.05
XRCC5 2R/1R/0R	2R/2R	22 (14.67)	25 (16.67)	1		
	1R/1R	53 (35.33)	33(22.00)	1.82	(0.88-3.74)	0.10
	0R/0R	10 (6.67)	7 (4.66)	1.62	(0.52-4.99)	0.39
	1R/1R+0R/0R	63 (42.00)	40 (26.66)	1.78	(0.89-3.59)	0.10
XRCC6-61C>G	C/C	54 (36.00)	39 (26.00)	1		
	C/G	29 (19.34)	12 (8.00)	1.74	(0.79-3.84)	0.16
	G/G	8 (5.33)	8 (5.33)	0.72	(0.24-2.09)	0.54
	C/G+G/G	37 (24.67)	20 (13.33)	1.33	(0.67-2.64)	0.40
XRCC7 6721 G > T	G/G	26 (17.33)	18 (12.00)	1		
	G/T	40 (26.67)	25 (16.67)	1.10	(0.50-2.42)	0.79
	T/T	22 (14.67)	19 (12.66)	0.80	(0.33-1.89)	0.61
	G/T+T/T	62 (41.34)	44 (29.33)	0.97	(0.47-1.99)	0.94

Analysis of the polymorphism in XRCC6 gene

Our study revealed that the *XRCC6* promoter T-61C genotype (Table 3) was not associated with the risk of breast cancer. For the *XRCC6 (*61C > G) polymorphism, the frequencies of the CC, CG, and GG genotypes were 62.00%, 27.33%, and

10.67%, respectively, among BC cases, and 47.00%, 42.50%, and 10.50%, respectively, among controls (Table 3). XRCC6 gene polymorphisms did not show association with increase in the risk of BC.

Analysis of the polymorphism in XRCC7 gene

For the *XRCC7 (*6721G>T) polymorphism, the frequencies of the GG and G/T genotypes were 29.33% and 77.67 % respectively, among BC cases, and 55.00% and 45.00%, respectively, among controls. However, the difference was statistically significant (*P*=0.0001) in cases than in controls for the heterozygote GT alleles in cases than controls. In this study, we found that *XRCC7 (*6721G >T) polymorphism was associated with the risk of BC (Table 3 & 5)

Table 5: 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 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**
XRCC4-1 cd247	C/C	79/146	64/48	82/71	61/123	102/69	32/106	7/11	2/8
	A/A	3/3	4/3	4/3	3/3	6/3	1/3	0/0	0/0
	OR (95% CI)	1.84 0.36-9.37)	1.00 (0.21-4.67)	1.15 0.24-5.33)	2.01 (0.39-10.28)	1.35 (0.32-5.59)	1.10 (0.11-10.98)	1.53 (0.02-85.96)	0.34 (0.05-2.19)
	P value	0.45	1.00	0.85	0.39	0.67	0.93	0.83	0.56
XRCC4-2 G1394T	G/G	55/91	45/29	58/38	42/82	71/41	22/69	5/6	2/4
	T/T	30/58	20/22	29/36	21/44	37/31	11/40	2/5	0/4
	OR (95% CI)	0.85 (0.49-1.48)	0.58 (0.27-1.25)	0.52 (0.27-0.99)	0.93 (0.49-1.76)	0.68 (0.37-1.27)	0.86 (0.37-1.96)	0.48 (0.06-3.63)	0.20 (0.007-5.45)
	P value	0.58)	0.17	0.04	0.82	0.23	0.72	0.47	0.33
XRCC4-3 Intron-7	G/G	64/115	53/30	63/50	54/95	79/52	30/84	6/4	2/5
	A/A	21/34	12/21	27/24	6/31	29/20	3/25	1/7	0/3
	OR (95% CI)	1.10 (0.59-2.07)	0.32 (0.13-0.74	0.89 (0.45-1.73)	0.34 (0.13-0.86)	0.95 (0.48-1.86)	0.33 (0.09-1.19)	0.09 (0.008-1.10)	0.31 (0.01-8.68)
	P value	0.74	0.008	0.73	0.02	0.89	0.09	0.05	0.49
XRCC5 2R/1R/0R	2R/2R	26/89	21/30	26/41	21/78	31/44	16/59	2/9	0/7
	1R/1R+0R/0R	58/60	46/21	60/33	43/48	77/28	17/50	5/2	2/1
	OR (95% CI)	3.30 (1.87-5.83)	2.11 (1.03-4.35)	2.86 (1.49-5.48)	3.32 (1.76-6.27)	3.90 (2.07-7.33)	1.25 (0.57-2.73)	1.25 (0.19-10.61)	0.25 (0.075-0.18)
	P value	0.0001	0.04	0.001	0.0002	0.0001	0.56	0.03	0.07
XRCC6-61C>G	C/C	59/72	34/22	53/32	40/62	64/34	23/54	5/3	1/3
	C/G+G/G	26/77	31/29	33/42	24/64	44/38	10/55	2/8	1/5
	OR (95% CI)	0.41 (0.23-0.72)	0.69 (0.33-1.44)	0.47 (0.25-0.89)	0.58 (0.31-1.07)	0.61 (0.33-1.12)	0.42 (0.18-0.98)	0.15 (0.01-1.23)	0.60 0.02-13.58)
	P value	0.002	0.32	0.02	0.08	0.11	0.04	0.07	0.74
XRCC7 6721 G > T	G/G	26/80	18/30	64/41	42/69	33/39	7/60	2/6	1/5
	G/T+T/T	58/69	48/21	24/33	20/57	75/33	26/49	5/5	1/3
	OR (95% CI)	2.58 (1.47-4.54)	3.80 (1.75-8.28)	0.46 (0.24-0.89)	0.57 (0.30-1.09)	2.68 (1.44-4.98)	4.54 (1.81-11.36)	0.08 (0.01-0.37)	1.66 (0.07-37.72)
	P value	0.001	0.0007	0.02	0.09	0.001	0.001	0.001	0.74

*: 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. When, the association of BC with age at first delivery was considered in this study 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-5. 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-2), showed higher risk of BC. The joint effects of XRCC5 genotype and risk factors, namely

hormone receptor status (Table-4) and age at first delivery on estimates of BC risk are shown in table-5 where early age of delivery showed significant association with development of breast cancer (OR= 3.90 ; 95% CI: 2.07-7.33 *p* <0.0001). Also the results of XRCC7 (6721G>T) for early age of delivery showed association with development of breast cancer (OR= 2.68; 95% CI: 1.44-4.98 *p* <0.001)

DISCUSSION

In this hospital based case-control study we investigated the relationship between newly reported genotype polymorphisms of HRR & NHEJ genes and the elevated risk for BC particularly in the rural areas of Maharashtra. To the best of our knowledge, there are no reports concerning any *XRCC* polymorphism in BC risk, therefore the present study was planned to determine the genotypic frequency of polymorphisms of the DNA repair genes (i) XRCC4 at codon 247 (rs3734091), G-1394T (rs6869366) and Intron 7 (rs1805377). The polymorphisms *XRCC5 (*2R/1R/0R), *XRCC6 (*-61C>G) and *XRCC7 (*6721G>T) were also selected to investigate the associations between the polymorphisms and risk of BC in Maharashtrian population from India. We found a significant association with the polymorphisms of *XRCC5 (2R*/1R/0R), *XRCC7 (*6721G>T) and the risk of BC. However, there was no evidence for an association between the XRCC4 and XRCC6 (-61C>G) variants and BC. To the best of our knowledge, this is the first report that the *XRCC5* 2R/1R/0R and *XRCC7* 6721G>T polymorphisms are associated with the risk of BC.

There are several reports that support our finding. The XRCC4 codon 247 A/C genotype conferred an evidence for association with the development of oral cancer in Taivanese population. [14] Repeats (1R/1R, 1R/0R) of XRCC5 in the promoter region were associated with increased risk of gastric, [15] esophageal [16] and head and neck cancer. [17] Holgersson et al. 2004 [18] reported increased expression of XRCC5 and XRCC7 protein in high-grade lymphoma patients. XRCC6 was significantly associated with a reduction of breast cancer risk in a Taiwanese population, [13] whereas another study in a Belgian population found, a marginally significant protective effect of these polymorphisms on breast cancer. [19] In contrast other studies failed to identify significant associations with risk of oral [20] lung cancer [21] and hepatocellular carcinoma risk. [22] XRCC6 -61C>G and XRCC7 6721G>T were associated with an increased risk of breast cancer, [13] and glioma, [23] respectively, while few studies reported that there were no significant associations between the XRCC7 6721G>T polymorphism and risk of renal carcinoma and differentiated thyroid cancer. [17,24]

Very few studies from northern and southern India have reported polymorphisms in the DNA repair genes with respect to a variety of cancer risks including prostate, breast, oral and esophageal cancer. [10,25] However, no information is available on the association of polymorphisms of those 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. Such genotyping analysis of DNA repair genes will enhance our ability to identify those individuals most susceptible to breast carcinogenesis in the rural Indian population.

CONCLUSION

The study suggests that functional XRCC5 2R/1R/0R and XRCC7 6721G>T polymorphisms could play an important role in the development of BC. However, the polymorphisms of XRCC4 and XRCC had no main effect on risk of BC from rural population of Maharashtra. Thus this analysis of correlation of DNA repair genes and BC may provide a deeper insight into the genetic and environment factors to

cancer risk in the rural unexplored population but larger scale studies, including more detailed environmental exposure status and more detailed patient clinical information, are needed to verify these findings.

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

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