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Original Research Article

Assessment of role of genetic polymorphisms in XRCC1, XRCC2 and XRCC3 genes in cervical cancer susceptibility from a rural population: a hospital based case-control study from Maharashtra, India

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ABSTRACT

Background: Cervical cancer is a major concern of health risk, moreover the leading cause of cancer causing deaths in women of rural parts of India. This study was aimed to assess the risk of cervical cancer development in association with polymorphisms in X-Ray Cross Complementing Group (XRCC1, XRCC2 and XRCC3) genes in the rural population of south-western Maharashtra. We focused to determine the frequency of polymorphisms in DNA repair genes including XRCC1 at codon (cd) 194, cd 280, cd 399, XRCC2 at cd 188 and XRCC3 at cd 241 and their plausible role in cervical cancer risk from rural parts of India.
Methods: This study included 350 proven cases with cervical cancer and 400 age and sex matched controls. We used polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) to analyze the association XRCC1, XRCC2 and XRCC3 gene polymorphisms with cervical cancer development in women of South-Western Maharashtra.
Results: The result from our study showed that allele frequencies of selected genes were not statistically different between the groups for XRCC1 Trp194, XRCC2 His188 and XRCC3 Met241. XRCC1 His280 (OR= 4.36; 95% CI= (3.20-5.95); p= <0.0001) and XRCC1 Gln399 (OR= 2.99; 95% CI= (1.60-5.56); p= <0.0001) genotypes significantly increased the risk of cervical cancer.
Conclusions: This study indicates that polymorphisms in cd 280 of exon 9 and cd 399 of exon 10 of XRCC1 gene could play a role in modifying genetic susceptibility of individuals towards cervical cancer among women from rural Maharashtra. This case-control study suggest that selected DNA repair genes represent genetic determinants in cervical carcinogenesis along with other risk factors in the rural Indian population.

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

INTRODUCTION

Cervical cancer (CC) is one of the most common genital tract carcinomas which has become an exigent health concern and the fourth leading cause of cancer related death among women world over.[1,2] In developing countries, the highest incidence rates of CC are observed in Africa, Caribbean, South, Central and Southeast Asia. India has the highest burden of CC where approximately 1,34,420 women are diagnosed with the disease every year, and of them 72,825 deaths accounting for nearly one-fifth of the global CC burden alone.[3] The number of CC deaths in women from India by the year 2010 was 79,000 and it is estimated that by the year 2025 there will be almost 20 million new cases. Over the last 20 years, it is universally implicated that along with tobacco and alcohol, persistent infection with high risk Human

Papilomavirus (HPV) and other sexually transmitted agents are necessary etiologic factors for cervical carcinogenesis. In India many findings from rural areas have showed higher incidence of HPV infection in different age groups where the prevalence rate could be probably due to preventive measures for management of CC.[4] Although tobacco, alcohol and HPV are the main etiologic factors for three fourth of these cancer cases, no definite etiologic factor can be identified in one fourth of the cases. In those cases development of cervical cancer could be influenced by genetic factors.[5] Identification of such genetic variants associated with CC may contribute to understand mechanisms underlying behind development of cancer.

The exogenous risk factors including environmental chemical agents and carcinogens in tobacco, such as nicotine and nitrosamines can induce DNA damage. DNA repair system is known to play a decisive role in protection of DNA against damage and essential for maintaining the genomic integrity. Until now, in human cells, more than a hundred molecules implicated in different DNA repair pathways such as base excision repair (BER), nucleotide excision repair (NER), double strand break repair (DSBR) and DNA mismatch repair (DMR).The X-Ray cross-complementing (XRCC) genes were initially discovered as an important component of DNA repair pathway contributing to genetic stability which have been extensively studied in the association with various human cancers.[6] The DNA repair enzyme XRCC1 gene is a major component of BER pathway which plays an important role in repair of single strand breaks XRCC2 and XRCC3 are DSB repair genes, key mediators in homologous recombination repair (HRR) of DNA double strand breaks.[7,8]

Genetic polymorphisms within the genes involved in DNA damage response may contribute to augmented risk of cancer development. In past few years, there has been extensive concern in identifying genetic variability in DNA repair genes and their impact on altering an individual's susceptibility to cancer but the former observations were not consistent in terms of their role in cancer susceptibility.[9-11] Some recent studies proposed the association of the polymorphisms in XRCC1 gene with multiple cancer risk such as lung, gastric, breast prostate and head and neck cancer.[12-14] The polymorphism in XRCC2 gene is studied for its role in carcinogenesis of breast cancer.[15] Likewise, XRCC3 gene polymorphism has been associated with the increased risk of lung and breast cancer.[16,17] 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 and the influence of the polymorphisms of XRCC genes on DNA repair capacity still remains unanswered episode.[17]

Also, significance of those DNA repair genes for cervical cancer is being investigated in recent years. Number of studies were conducted to investigate the association between XRCC1 (Arg194Trp, Arg280His, Arg399Gln) and XRCC3 Thr241Met polymorphisms and risk of cervical cancer in women.[18,19] Some reports stated that XRCC2 (Arg188His) polymorphisms may be involved in repair of HPV related DNA damage in cervical cancer.[20] But the results were debatable where few observations were not consistent in terms to show association between polymorphisms of XRCC1 Arg194Trp and development of cervical cancer, while the influence of the Arg280His polymorphisms acted as protective factor for squamous cervical carcinoma and Arg399Gln polymorphism increased cervical carcinogenesis among women whose age at first delivery was 20 years.[21]

Also, few studies form India have been carried out to investigate association of XRCC1 gene with CC risk especially from Northern India and Southern India but they did not lead to a broad strapping conclusions.[22,23] Consequently, the association between genetic polymorphism of XRCC1, XRCC2, XRCC3 and susceptibility to CC is still an open platform for illustration. Therefore, in this study we focused to evaluate role of genetic polymorphisms of XRCC1 at cd 194, cd 280, cd 399, XRCC 2 at cd 188 and XRCC3 at cd 241 in the development of CC if any. We performed a hospital-based case-control study with a polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) assay to determine genotypic frequency of the selected i) XRCC1 gene at exon 6, exon 9 and exon 10 (ii) XRCC2 gene at exon 3 (iii) XRCC3 gene at exon 7 and their associations in the CC risk from rural population of Western Maharashtra from India.

METHODS

This study was a hospital-based case-control study. Study participants included 350 newly diagnosed CC patients and 400 healthy, cancer free, age and sex matched individuals as controls. All cases ranged in age from 20-80 years (Mean±SD) 48.67±13.78 were recruited immediately after being diagnosed during the year 2014-2017. Trained interviewers used a structured questionnaire to collect personal interview data from the participants regarding demographic factors and known risk factors. The study was approved by the Institutional Ethics Committee of the Krishna Institute of Medical Sciences 'Deemed to be University'. Five milliliter (mL) of whole blood from patients and normal controls was collected in sterile vacutainer after receiving written 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 of XRCC1, XRCC2 and XRCC3 genes was 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 100 nanogram (ng) of purified genomic DNA template, 1X PCR buffer (10 mili molar (mM) Tris-HCl (pH 9.0), 50 mM KCl 1.5 mM MgCl2), 0.2 mM each dNTP, 1U Taq DNA polymerase (GeNei, Merck Bioscience) and 10 picomole (pmol) of each primes. The primers selected to amplify the exons of XRCC1 containing the polymorphisms of interest were*;* XRCC1 (exon 6) sense primer: 5'-GCCAGGGCCCCTCCTTCAA-3' antisence primer: 5'-TACCCTCAGACCCACGAGT-3'; XRCC1 (exon 9) sense primer: 5'-CCAGCTCCAACTCGTACC-3' antisence primer: 5'-ATGAGGTGCGTGCTGTCC-3'; XRCC1 (exon 10) sense primer: 5'-CAGTGGTGCTAACCTAATC-3' antisence primer: 5'-AGTAGT CTGCTGGCTCTGG-3';XRCC2(exon 3)sense primer:5'-GTTGCTGCCATGCCTTACA-3'antisence primer: 5'-TGTAGTCACCCATCTCTCTGC-3' and XRCC3 (exon 7) sense primer: 5'-GGTCGAGTGACAGTCCAAAC-3' antisence primer: 5'-TGCAACGGCTGAGGGTCTT-3'. The PCR conditions for amplification of XRCC1 codon 194 in the exon 6 of 485 bp and XRCC1 codon 280 in the exon 9 of 257 bp were initial denaturation at 95^0C for 10 minutes (min) followed by 30 cycles of 95^0C- 30 seconds (sec), 61^0C- 30 sec, 72^0C- 30 sec and final extension at 72^0C for 10 min. The conditions for XRCC1 codon 399 in the exon 10 of 871 bp were initial denaturation at 95^0C for 10 min followed by 30 cycles of 95^0C-20 sec, 56^0C- 30 sec, 72^0C-20 sec and final extension at 72^0C-10 min. The PCR conditions for XRCC2 codon 188 in the exon 3 of 290 bp initial denaturation at 95^0C for 10 min, followed by 30 cycles of 95^0C-30 sec, 58^0C-30 sec, 72^0C- 30 sec and72^0C-10 min and XRCC3 codon 241 in the exon 7 of 455 bp (95^0C-10 min, 30 cycles of 95^0C- 30 sec, 53^0C-30 sec, 72^0C-30 sec, 72^0C-10 min).

After performing PCR programme for each of the reactions with a Master Cycler Gradient PCR (Eppendorf), the PCR products were analyzed by agarose gel electrophoresis in Tris-Acetate-EDTA (TAE) buffer after staining with ethidium bromide (10mg/mL) and visualized under UV-transilluminator and photographed in gel documentation system (BioRad Laboratories).

After confirmation of DNA amplification, each PCR product of XRCC1 exon 6, exon 9 and exon 10 were digested overnight at 37°C with 1 unit (U) of restriction enzymes PvuII, RsaI and NciI respectively. Similarly, PCR product of XRCC2 exon 3 and XRCC3 exon 7 were digested overnight at 37^0C with 1U of restriction enzyme HphI and 1U NlaII respectively. After the overnight incubation at 37^0C, restriction digestion products were separated on a 2-3% low EEO agarose (GeNei, Merck Biosciences) gel at 100 V for 30 min, stained with ethidium bromide and photographed with gel documentation system.

Statistical analysis

The associations between the XRCC1, XRCC2 and XRCC3 genotypes and risk of CC development 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 CC risk associated with genotypes. For each polymorphism the χ^2 test was used to evaluate differences in the frequency distribution of selected demographic variables and the frequencies of allele and genotype of the polymorphisms between CC cases and the controls.

RESULTS

Characteristics of the study subjects

During the study period 350 patients with CC met the eligibility criteria for this study and 400 controls were selected to match these cases. The characteristics of age and sex matched cases and controls are presented in Table 1.

The mean age in years was 48.67 (median: 47, range 20-80) for the cases and 42.37 (median: 40 range 20-80) for the controls. There were no significant differences between the cases and controls with respect to sex and ethnicity. Stratification of other risk factors was performed by considering factors such as age, tobacco chewing status, diet and age at first pregnancy. Among women having their first intercourse and child birth after the age of 20, there was a significant association for cervical carcinogenesis determined using multivariate logistic regression analysis (Table 3).

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

The distribution of XRCC genotypes and concordance of the three polymorphisms are presented in Table 2. The frequency of XRCC1 26304CC homozygotes was 79.71% in cases and 79.00% in controls whereas XRCC1 26304 TT homozygotes was 0.00% in cases and 1.50% in controls. The frequency of XRCC1 26304 CT heterozygotes was 20.29% in cases and 21.0% in controls. In codon 280, genotype frequencies for wild and variant genotypes were 39.14 and 60.86 per cent respectively in cases and 73.75 and 26.25 percent respectively in controls which showed significant difference in cases than controls. The frequency of XRCC1 28152GG homozygous wild type alleles at codon 399 of exon 10 was 39.14%, 28152GA heterozygote alleles was 52.29% and for 28152AA homozygous alleles was 8.57% in the cases where that of the frequencies for the controls were 61.50, 34.00 and 4.50 % respectively (Table 3).

Table 1: Distribution comparisons of selected demographic characteristics of cervical cancer cases and healthy controls from rural areas of Maharashtra in India.

Variable	Cases N=350		Controls N=400		*P*-Value based on $\chi 2$
Age (Mean ± SD) years	48.67±13.78		42.37±13.90		
	No.	**(%)**	**No.**	**(%)**	
≤ 50	216	61.71	286	71.50	0.003
51-60	58	16.58	68	17.00	
61-70	56	16.00	33	08.25	
>70	20	5.71	13	03.25	
Tobacco smoking Status					
Tobacco users	189	54.00	112	28.00	<0.001
Tobacco no users	161	46.00	288	72.00	
Age @ Ist Pregnancy (yrs)					
15-20	277	79.14	183	45.75	<0.001
21-25	72	20.58	178	44.50	
26-30	00	0.00	34	08.50	
31-35	01	0.28	05	01.25	
Diet					
Vegeterian	96	27.42	118	29.50	0.59
Mixed	254	72.58	282	70.50	
Education					
High School	139	39.71	108	27.00	<0.001
High School graduate (12 y)	24	06.86	49	12.25	
College /Graduate	43	12.29	129	32.25	
No School	144	41.14	114	28.50	
Economic status					
Poor	198	56.58	132	33.00	<0.001
Middle	97	27.71	161	40.25	
Rich	55	15.71	107	26.75	
Family history of Cancer					
Yes	62	17.71	10	02.50	<0.001
No	288	82.29	390	97.50	

Analysis of the Arg188His polymorphism in the XRCC2 gene

Table 2 displays the distribution of genotypes and frequency of alleles of the G31479A polymorphisms in patients with CC and controls. We did not find any significant difference in genotype or allele frequencies between patients and controls. The frequency of *XRCC2* 31479*GG* wild type alleles at codon 188 of exon 3 was 78.86%, 31479*GA* heterozygote alleles was 19.43% and for 31479AA homozygous alleles was 1.71% in the cases where that of the frequencies for the controls were 79.25, 18.00 and 2.75% respectively (Table 2). Thus, the haplotype analysis according to variant type of G31479A showed a lack of association with CC.

Analysis of the Thr241Met polymorphism in the XRCC3 gene

Allele frequencies and distribution of genotypes of *XRCC3* codon 241 are shown in Table 2. In frequency distribution of codon 241 of XRCC3 gene at C18067T, genotype frequencies for wild, heterozygote and variant genotypes were 68.00, 26.57 and 5.43% respectively in cases where as the frequencies in controls were 71.00, 24.25 and 4.75% respectively. XRCC3 241 Met/Met, Thr/Met and Met/Met genotypes did not show significant association with development of CC (OR=1.19, 95% CI=0.61-2.30; *p*= 0.59 for 241 Met/Met) (Table 2).

Association of age of cancer occurrence, tobacco status and age at Ist pregnancy with cervical cancer risk

In the studied population, the median age of commencement of CC is 47 years, substantially lower than observed in other reports. To evaluate the association of the polymorphisms with the young age at diagnosis of cervical cancer, we stratified the patients as ≤50 (n=216) or >50 (n=134) years of age and compared with age matched sample of controls which interestingly showed that the XRCC1 cd 280 (OR=4.19; CI=2.87-6.12; p<0.0001) showed significant risk of CC at the age

bellow median. Also, when we studied plausible association of demographic factors and polymorphism in XRCC1, XRCC2, XRCC3 genes, our results indicated non-vegetarian diet and tobacco chewing habits also showed connection with CC development (OR=6.53; CI=4.43-9.61; p<0.0001) for mixed diet and (OR=5.14; CI=3.03-8.70; p<0.0001) for tobacco chewing status (Table 3).

Table 2: The genotype frequencies of XRCC gene polymorphisms in untreated cervical cancer patients and controls.

Gene	Genotype	Cases (n= 350) (%)	Control (n = 400) (%)	Odds' ratio (95% CI)	P value	Adjusted odds ratio (95% CI)	*P* value
XRCC1 cd194 ex-6	Arg/Arg	279 (79.71)	316(79.00)	1		1	
	Arg/Trp	71 (20.29)	78(19.50)	1.03 (0.72-1.48)	0.86	0.85 (0.58-1.27)	0.44
	Trp/Trp	0 (0.00)	6 (1.50)	0.08 (0.04-1.55)	0.09	NA	NA
	Arg/Trp+Trp/Trp	71 (20.29)	84 (21.00)	1.06 (0.75-1.50)	0.72	0.84 (0.54-1.19)	0.27
XRCC1 cd280 ex-9	Arg/Arg	137 (39.14)	295 (73.75)	1		1	
	Arg/His	0 (0.00)	0(0.00)	NA	-	-	-
	His/His	213 (60.86)	105 (26.25)	4.36 (3.20-5.95)	<0.0001*	4.27 (3.096-5.882)	<0.0001
	Arg/His+His/His	213 (60.86)	105 (26.25)	4.36 (3.20-5.95)	<0.0001*	4.27 (3.096-5.882)	<0.0001
XRCC1 cd399 ex-10	Arg/Arg	137 (39.14)	246 (61.50)	1		1	
	Arg/Gln	183 (52.29)	136 (34.00)	2.41 (1.78-3.27)	0.0001	2.38 (1.72- 3.31)	<0.001
	Gln/Gln	30 (08.57)	18 (04.50)	2.99 (1.60-5.56)	0.0001*	2.91 (1.49- 5.66)	<0.001
	Arg/Gln+ Gln/Gln	213 (60.96)	154 (38.50)	2.48 (1.85-3.33)	0.0001*	2.43 (1.77-3.33)	<0.001
XRCC2 cd188 ex-3	Arg/Arg	276 (78.86)	317(79.25)	1		1	
	Arg/His	68 (19.43)	72 (18.00)	1.08 (0.75-1.56)	0.66	0.94 (0.62-1.42)	0.77
	His/His	6 (01.71)	11 (02.75)	0.62 (0.22-1.71)	0.36	0.57 (0.19-1.72)	0.32
	Arg/His+His/His	74 (21.14)	83 (20.75)	1.02 (0.71-1.45)	0.89	0.89 (0.60-1.32)	0.56
XRCC3 Cd241 Ex-7	Thr/Thr	238 (68.00)	284 (71.00)	1		1	
	Thr/Met	93 (26.57)	97 (24.25)	1.14 (0.82-1.56)	0.42	1.04 (0.72-1.51)	0.82
	Met/Met	19 (05.43)	19 (04.75)	1.19 (0.61-2.30)	0.59	1.23 (0.61-2.49)	0.56
	Thr/Met+Met/Met	112 (32.00)	116 (29.00)	1.15 (0.84-1.57)	0.37	1.07 (0.77-1.51)	0.67

*Indicates significant Odds Ratio (p<0.001), p value determined based on χ^2

Table 3: Stratification of age of cancer occurrence, tobacco smoking and distribution of XRCC1, XRCC2 and XRCC3 genotypes in the patients with CC and control group from rural population of Maharashtra.

Gene	Genotype	Demographic Factors			
		Age, Cases/Controls		**Tobacco Status, Cases/Controls**	
		≤50, N=216/286	>50, N=134/114	Users, N=189/112	Non-Users, N=161/288
XRCC1 Arg194Trp cd 194 exon 6*(*C26304T)	Arg/Arg	174/225	105/91	153/89	126/227
	Arg/Trp+ Trp/Trp	42/61	29/23	36/23	35/61
	OR (95% CI)	0.89 (0.57-1.38)	1.09 (0.59-2.02)	0.91 (0.50-1.63)	1.03 (0.64-1.65)
	p value	0.60	0.77	0.7	0.88
XRCC1 Arg280His cd280 exon 9 (G27466A)	Arg/Arg	83/207	54/88	74/86	63/209
	Arg/His+ His/His	133/79	80/26	115/26	98/79
	OR (95% CI)	4.19 (2.87-6.12)	0.19 (0.11-0.34)	5.14(3.03-8.70)	4.11 (2.73-6.19)
	p value	<0.0001	<0.0001	<0.0001	<0.0001
XRCC1 Arg399Gln cd399 exon 10*(*G28152A)	Arg/Arg	78/190	59/56	73/68	64/178
	Arg/Gln+ Gln/Gln	138/96	75/58	116/44	97/110
	OR (95% CI)	3.50 (2.41-5.07)	1.11 (0.74-2.02)	2.45 (1.52-3.96)	2.45 (1.65-3.64)
	p value	<0.0001	0.42	0.002	<0.0001
XRCC2 Arg188His cd188 exon 3 (G31479A	Arg/Arg	173/234	103/103	151/85	125/232
	Arg/His+ His/His	43/52	31/31	38/27	36/56
	OR (95% CI)	0.89 (0.57-1.40)	1.00 (0.56-1.76)	0.79 (0.45-1.38)	1.19 (0.74-1.91)
	p value	0.62	1.00	0.41	0.46
XRCC3 Thr241Met cd241 exon7 (C18067T	Thr/Thr	152/212	86/72	130/86	108/198
	Thr/Met+ Met/Met	64/74	48/42	59/26	53/90
	OR (95% CI)	0.80 (0.55-1.22)	1.04 (0.62-1.75)	1.50 (0.87-2.56)	1.07 (0.71-1.63)
	p value	0.35	0.86	0.13	0.71

Table 4: Stratification of age at first pregnancy and distribution of XRCC1, XRCC2 and XRCC3 genotypes in the patients with CC and control group from rural population of Maharashtra.

Gene	Genotype	Demographic Factors			
		Age@1st Pregnancy, Cases/Controls			
		15-20 N=277/183	21-25 N=72/178	26-30 N=0/34	31-35 N=1/5
XRCC1 Arg194Trp cd 194 exon 6(C26304T)	Arg/Arg	220/138	58/143	0/31	1/4
	Arg/Trp+ Trp/Trp	57/45	14/35	0/3	0/1
	OR (95% CI)	0.79 (0.50-1.23)	0.98 (0.49-1.96)	9.00 (0.15-528)	1.00 (0.02-40.27)
	p value	0.31	0.96	0.29	1.0
XRCC1 Arg280His cd280 exon 9 (G27466A)	Arg/Arg	110/126	27/145	0/19	0/5
	Arg/His+ His/His	167/57	45/33	0/15	1/0
	OR (95% CI)	3.35 (2.26-4.98)	7.32 (3.98-13.46)	1.25 (0.02-67.0)	33.00 (0.44-2470.7)
	p value	<0.0001	<0.0001	0.90	0.11
XRCC1 Arg399Gln cd399 exon 10(G28152A)	Arg/Arg	113/96	24/123	0/14	0/3
	Arg/Gln+ Gln/Gln	164/87	48/55	0/10	1/2
	OR (95% CI)	1.60 (1.09-2.33)	4.47 (2.49-8.02)	1.38(0.02-75.3)	4.20 (0.11-151.97)
	p value	0.01	<0.0001	0.87	0.43
XRCC2 Arg188His cd188 exon 3	Arg/Arg	219/136	56/149	0/27	1/5
	Arg/His+ His/His	58/47	16/29	0/7	0/0
	OR (95% CI)	0.76 (0.49-1.19)	1.46 (0.74-2.90)	3.66 (0.06-200.)	3.66 (0.04-274.53)
(G31479A	p value	0.23	0.27	0.52	0.55
XRCC3 Thr241Met cd241 exon7 (C18067T	Thr/Thr	190/121	47/135	0/24	1/4
	Thr/Met+ Met/Met	87/62	25/43	0/10	0/1
	OR (95% CI)	0.89 (0.60-1.32)	1.67 (0.92-3.02)	2.33 (0.04-125)	1.00 (0.02-40.27)
	p value	0.57	0.09	0.67	1.00

*: Indicates significant Odds Ratio (p<0.001), p value determined based on $\chi 2$

Also, the association of CC with age at first pregnancy was considered in this study which showed that early age of first pregnancy i.e.15-20 yrs, significantly associated with increased CC risk (OR=3.25; CI=2.26-4.98; p<0.0001) (Table 4).

DISCUSSION

In this hospital based case-control study, we examined 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 at cd 241 of exon 7 to study the associated risk of cervical carcinoma among the rural population of south-western Maharashtra. The results interpreted that the OR associated with His 280 genotype (OR = 4.36; 95% CI = 3.20-5.95) and Gln399 genotype (OR= 2.99; 95% CI= 1.60-5.56) of XRCC1 were significantly increased in relation to the relative risk of cervical cancer in the study. Over the past few years, a number of epidemiological studies have been conducted to demonstrate the association of XRCC1, XRCC2, XRCC3 polymorphism with susceptibility to gastric, lung, breast cancer, and other types of cancers.[24,25] However, little published information is available regarding the association between polymorphism of XRCC1, XRCC2 and XRCC3 gene and susceptibility to cervical carcinoma.[26] Also, few studies from Northern and Southern India have reported the genetic polymorphisms in the DNA repair genes with respect to a variety of cancer risks including prostate, breast, oral and esophageal cancers, but only couple of studies available to reveal the association of polymorphisms in XRCC1, XRCC2 and XRCC3 genes with CC development and the results were with incompatible conclusions.[27,28] Some recent studies discovered positive association of Arg194Trp variant of XRCC1 with CC development risk while other demonstrated a strong association of the Arg399Gln polymorphism with an increased risk of CC, but other failed to find the significant result.[29,30] No apparent association observed between Arg280His polymorphisms of XRCC1 and cervical cancer risk in any of the published study.[29] On the other hand, in a more recent report showed a weak association of XRCC1 gene in development of CC, however two studies by Zeng et al and Cai et al reported no evidence of association of XRCC genes with CC development.[31,32] Thus, there is a debate between association or no association between XRCC1 gene and risk of development of CC. Also, reports on XRCC2 Arg188His polymorphisms stated that XRCC2 variants were found not to be associated with CC risk.[20] Similarly, studies have been conducted to prove that polymorphisms of XRCC3 gene may be involved in reducing DNA repair capacity but the direct evidence in terms of the associations with CC susceptibility have been proved contradictory.[19]

However, no information is available on the association of genetic polymorphisms of DNA repair genes including

XRCC1, XRCC2, XRCC3 and their susceptibility to CC from Indian population. Therefore, in this study, we determined the relationship between the development of CC and genetic polymorphisms of those 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 CC development but, 280His and 399Gln genotype of XRCC1 may be collectively related to cervical cancer risk. A similar observation was also reported by Bajpai et al in cervical cancer risk in combination with 399Gln and also weak association with 194Trp to increase frequency of cervical cancer.[24] It is not obvious to observe the association of 280 His and 399 Gln in cervical carcinogenesis and there is an inconsistency in the previous findings about the role of XRCC1 gene in cervical cancer association.

CONCLUSION

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 CC, except Arg280His which showed strong association with CC. It is also evident from our findings that larger part (72%) of the female had age at first pregnancy below 20 years which could be the probable risk factor for development of cervical cancer. The mechanistic basis for the present findings remains unclear; therefore confirmation of our results in larger populations is warranted to clarify this point.

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