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

Original Research Article

Genetic Polymorphisms of DNA Repair Gene (XPD) and Breast Cancer Risk in a Hospital Based Case-Control Study from Maharashtra

Datkhile KD[1], Bhosale SJ[2], Patil MN[1], Durgawale PP[1], Kakade SV[3]

[1]Molecular & Genetic Laboratory, [2]Department of Surgery, [3]Department of Preventive and Social Medicine, Krishna Institute of Medical Sciences University, Taluka-Karad, Dist-Satara, Pin-415 110, Maharashtra, India

Corresponding Author: Datkhile KD

ABSTRACT

Breast cancer is a major concern of women health in developing countries, including India. This study was aimed to determine the polymorphisms in DNA repair gene, Xeroderma pigmentosum complementation group D (*XPD*) at codon (cd) 156, cd199, cd320, cd751 in patients of breast cancer from Maharashtra and to evaluate their association with breast cancer development.

Methods: We conducted a case control study including 170 breast cancer cases and 200 hospital based age and sex matched healthy controls to estimate the role of genetic polymorphisms of XPD gene in the context of breast cancer risk for the Maharashtrian population. We used PCR-RFLP to analyze *XPD* gene polymorphisms.

Results: The result from our study showed that allele frequencies of selected genes were not statistically different between the groups for XPD Arg156, XPD Met199, XPD Gln751 except XPD312. XPD Asn312 (OR= 5.14; 95% CI= (3.04-8.69); *p*= <0.0001) genotype significantly increased the risk of breast cancer.

Conclusions: This study indicates that polymorphisms in cd320 of XPD gene could play a role in modifying genetic susceptibility of individual to breast cancer in Maharashtra patients.

Keywords: Genetic polymorphisms, XPD, breast cancer, genotyping, PCR-RFLP

INTRODUCTION

Breast cancer (BC) is the second most commonly diagnosed cancers and one of the common causes of death among women worldwide. [1-2] India has 17% of the world's population suffering from BC replaced cervical cancer as the leading cause of cancer deaths among women in rural areas. [3] The etiologic factors for BC involve the reproductive events that manipulate the levels of hormones, exposure to heterocyclic aromatic compounds and environmental pollutants. [4-5] For a country like India with huge population and geographical variations, risk factors are likely to be literacy, diet, age at menarche and menopause, age at first delivery, family history of BC. [6-7] It is assumed that along with the environmental factors, a combination of individual's routine 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 remains unrevealed. There are several DNA repair mechanisms which play a crucial role in maintenance of genomic integrity with various repair pathways, but it is not yet clear which DNA repair pathways are most important for protection against breast cancer in case of DNA injury. The nucleotide excision repair

(NER) pathway is an important mechanism involved in DNA repair by the removal of variety of structurally unrelated DNA lesions such as ultra violet light induced pyrimidine dimmer, photoproducts, bulky chemical adducts and oxidative damage. Human xeroderma pigmentosum complementation group D (XPD) is one of the major gene involved in NER pathway through recognition and repair of thymidine dimers. XPD encodes DNA helicase which participates in NER pathway and basal transcription through stabilizing the transcription factor complex TFIIH. [8] Hundreds of polymorphisms in DNA repair genes associated with cancer risk have been identified, [9] but their effects on repair function have not been well characterized. Some of them are XPD, XPC, XPG and XRCC which have been frequently studied and there is growing evidence that polymorphisms of these genes may have some phenotypic significance. [9-10] Also, several polymorphisms in XPD gene have been identified but their impact on DNA repair is not well-known. Previous studies suggested that XPD polymorphisms were associated with risk of skin cancer, head & neck, gliomas, and bladder cancer, [11-15] but in some of the studies the results are contradictory rather than convincing. Very few studies from India have reported the polymorphisms in the XPD gene with respect to a variety of cancer risks including gallbladder, [16] head and neck, [17] prostate cancers. [18] These previous observations suggest that XPD polymorphisms may or may not influence different cancers susceptibility in diverse populations with varied incidence of cancer, whereas few other studies failed to find positive evidence for XPD polymorphisms in breast carcinoma risk [19-20] and therefore the influence of the polymorphisms of XPD genes on DNA repair capacity is still unclear. It is also uncertain which variants of XPD genes may be more important for protection against BC; therefore their contribution to cancer susceptibility needs to be carefully evaluated. In earlier studies we have shown that polymorphisms in BER pathway genes especially XRCC1 cd280 of exon 9 plays an important role in susceptibility of breast cancer in rural population of Maharashtra. In continuation with this, we also hypothesized that the inherited polymorphisms of NER pathway genes especially XPD may contribute to genetic susceptibility to BC. To test this hypothesis we focused on polymorphisms of XPD gene to evaluate its role in BC if any. We performed a hospital based case-control study using a PCR-RFLP assay to genotype the polymorphisms of selected XPD gene in relation to BC in a rural women of western Maharashtra from India. We determined the genotypic frequency of polymorphisms of the (A) XPD C22541A at codon 156 of the exon 6 (B) XPD C23047G at codon199 of the exon 8 (C) XPD G23592A at codon 312 in the exon 10 and (D) XPD A35931C at codon 751 in the exon 23 and evaluated their association with BC development in rural Maharashtrian population.

METHODS

Study subjects

This study was a hospital based case-control study. Study participants included 170 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 July 2013-August 2016. Trained interviewers used a structured questionnaire to collect personal interview data from the participants regarding demographic factors and known risk factors.

Place of Study

This study was conducted in Krishna Institute of Medical Sciences University from rural areas of South-Western Maharashtra of India.

Selection of cases and controls

Incidence cases of breast cancer were identified using Mammography, tissue biopsy and Fine Needle Aspiration Cytology (FNAC) at Department of

Surgery, Department of Oncology at the Krishna Hospital & Medical Research Centre (KH&MRC) KH &KMRC and Department of Pathology of Krishna Institute of Medical Sciences. Then patients confirmed to have high grade lesions based on their biopsy were recruited to this study. Randomized recruitment was used to frequency match controls to cases. Controls were randomly selected from a group of population visiting to KH&MRC for blood donation and other purposes. Also age and sex matched controls were selected by conducting blood donation camps in vicinity to the Karad area. Control subjects who were relatives of cases or had a prior history of cancer were excluded from the study.

Inclusion criteria & Exclusion criteria:

Control subjects who were relatives of cases or had a prior history of cancer were excluded from the study. All 100 % of cases and controls agreed to provide a blood sample included in this study.

Genomic DNA isolation from whole blood

Genomic DNA was extracted from five milliliter of peripheral blood using Purelink genomic DNA extraction and purification Kit (Invitrogen, Life technologies) following the manufacturer's instructions.

Genotyping assays.

We focused on polymorphisms that have relatively high frequencies of variant alleles with a restriction site: (A) XPD C22541A codon 156 in the exon 6 (B) XPD C23047G at codon199 of the exon 8 (C) XPD G23592A at codon 312 in the exon 10 and (D) XPD A35931C at codon 751 in the exon 23. The primers selected to amplify the exons of XPD containing the polymorphisms of interest were (Forward 5'- tggagtgctatggcacgatctct -3', Reverse 5'- ccatgggcatcaaattcctggga -3') for XPD codon 156, exon6, (Forward 5'- ctgttggtgggtgcccgtatctgttggtct - 3' Reverse 5'- taa tatcggggctcaccctgcagcacttccc -3') for XPD cd199, exon 8, (Forward 5´- ctgttggtgggtgcccgtatctgt tggtct-3' Reverse 5´-taatatcggggctcaccctgcagcacttcc t- 3' for XPD cd312 exon 10 and (Forward 5'- gcccgctctggattatacg -3', Reverse 5'- ctatcatctcctggccccc -3') for cd 751 of exon 23. 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), 0.2 mM each dNTP, 10 picomole (pmol) of each primer, 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). The PCR conditions for amplification of XPD codon 156 of 644 bp (95^0C- 5 min, 30 cycles of 95^0C- 30 sec, 60^0C- 30 sec, 72^0C- 30 sec, 72^0C- 5 min), XPD codon 199 of 357 bp, (95^0C- 5 min, 35 cycles of 95^0C- 30 sec, 55^0C- 30 sec, 72^0C- 30 sec, 72^0C- 5 min), XPD codon 312 of 751 bp (95^0C- 5 min, 35 cycles of 95^0C- 30 sec, 62^0C- 45 sec, 72^0C- 30 sec, 72^0C- 5 min) and XPD codon 751 of 436 bp (95^0C- 5 min, 30 cycles of 95^0C- 30 sec, 55^0C- 30 sec, 72^0C- 30 sec, 72^0C- 5 min). After performing PCR programme for each of the reactions, the PCR products were analyzed by agarose gel electrophoresis. After confirmation of DNA amplification, each PCR product of XPD exon 6, exon 8, exon 10 and exon 23 were digested at 37°C with 1 unit of TfiI, DpnII, StyI and PstI respectively. After the overnight incubation, digestion products were separated on a 2-3% 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 of XPD genotypes with risk of BC were determined using odds ratio (OR). ORs and 95% confidence intervals (CIs) were calculated by the univariate and multivariate logistic regression analyses with adjustment of risk factors.

RESULTS

Characteristics of the study subjects:

During the study period we analyzed 170 patients with breast cancer and 200 controls. The characteristics of age and sex matched cases and controls are presented in Table-1. No significant differences were observed between the cases and controls with reference to sex and ethnicity.

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

Variable	Cases N=170		Controls N=200		*P*-Value based on $\chi 2$
Age (Mean ± SD) years	50.04 ±12.06		40.60 ± 13.73		<0.001
	No.	(%)	No.	(%)	
≤ 50	95	55.88	149	74.50	
51-60	38	22.35	32	16.00	
61-70	28	16.47	15	07.50	
>70	9	5.30	4	02.00	
Tobacco smoking Status					0.006
Tobacco users	94	55.30	74	37.00	
Tobacco no users	76	44.70	126	63.00	
Mastectomy status					<0.001
Left MRM	97	57.06	0	0.00	
Right MRM	73	42.94	0	0.00	
Age @ Ist delivery (yrs)					<0.001
15-20	126	74.11	76	38.00	
21-25	37	21.77	111	55.50	
26-30	06	03.53	10	05.00	
31-35	01	00.59	03	01.50	
Hormone Status					0.001
ER/PR+ve	102	60.00	0	0.00	
ER/PR-ve	68	40.00	0	0.00	
Her2 +ve	24	14.11	0	0.00	
Her2 -ve	146	85.89	0	0.00	
ER/PR/Her2+ve	07	04.11	0	0.00	
ER/PR/Her2-ve	46	27.05	0	0.00	
Diet					0.07
Vegeterian	40	23.53	64	32.00	
Mixed	130	76.47	136	68.00	
Education					0.013
High School	71	41.77	75	25.00	
High School graduate (12 y)	08	04.70	26	11.00	
College /Graduate	12	07.05	25	34.00	
No School	79	46.48	74	30.00	
Economic status					0.046
Middle	42	24.70	52	26.00	
Poor	104	61.18	103	51.50	
Rich	24	14.12	45	22.50	
Family history of Cancer					0.002
Yes	42	24.70	0	00.00	
No	128	75.30	200	100.00	

Association of polymorphisms in XPD gene and breast cancer:

We have analyzed the distribution and association between the four previously described polymorphisms of cd156 (silent nucleotide substitution), cd199 (amino acid substitution), cd312 and cd751 (Non-conservative amino acid substitutions) in XPD gene of rural Maharashtrian population. The distribution of XPD genotypes and the concordance of the polymorphisms in patients with BC and controls are presented in Table-2.

(A) Analysis of the XPD C22541A codon 156 exon 6

The association of XPD genotypes and BC risk are shown in table 2. The frequency of wild type *XPD 22541*CC homozygotes was 42.95% in cases and 33.50 % in controls whereas the frequency of variant XPD22541A allele was lower but not significantly in the cases (8.42 %) than in the controls (17.50 %). The frequency of *XPD22541 CA* heterozygotes was 48.23 % in cases and 49.00 % in controls (Table-2). Thus, the C→A polymorphisms in exon 6 at nucleotide does not result in an amino acid

change at codon 156 in cases as compared to controls.

(B) Analysis of the XPD C23047G codon199 exon 8

The amplification of XPD codon 199 resulted in the product of 357 bp. The DpnII enzyme was used to detect the *XPDC23047G* at codon 199 of exon 8. The PCR amplified products upon treatment with DpnII yielded wild-type (23047C) alleles of 176, 73bp fragments, and the polymorphic (G) allele produces 1 fragment of 243 bp. The frequency of *XPD 23047 CC* wild type homozygotes was 63.52 % in cases and 67.00 % in controls whereas *XPD23047GG* variant homozygotes was 15.30 % in cases and 17.00 % in controls. The frequency of *XPD23047CG* heterozygotes was 21.18% in cases and 16.00% in controls (Table-2). This allele frequency in the controls was similar to previously reported studies. We did not find any significant difference in genotype or allele frequencies in patients with cancer and controls.

Table: 2. The genotype frequencies of *XPD* gene variants in untreated breast cancer patients and controls.

GENE	Genotype	CASES (n= 170) (%)	CONTROL (n = 200) (%)	Odds' Ratio (OR) (95% CI)	*P* value	Adjusted Odds Ratio (95% CI)	*P* value
XPD Arg156Arg codon 156 Ex-6 C22541A	CC/CC	73 (42.95)	67 (33.50)	1		1	
	CC/AA	82 (48.23	98 (49.00)	0.76 (0.49-1.19)	0.24	1.13 (0.68-1.89)	0.62
	AA/AA	15 (8.82)	35 (17.50)	0.39 (0.19-0.78)	0.008	2.32 (1.008-5.38)	0.04
	CC/AA+ AA/AA	97(57.05)	133 (66.50)	0.66 (0.43-1.02)	0.06	1.73 (0.73-1.88)	0.51
XPD Ile199Met codon199 Ex-8 C23047G	CC/CC	108 (63.52)	134 (67.00)	1		1	
	CC/GG	36 (21.18)	32 (16.00)	1.39 (0.81-2.39)	0.22	0.84 (0.44-1.57)	0.589
	GG/GG	26 (15.30)	34 (17.00)	0.94 (0.53-1.67)	0.85	1.04 (0.54-2.02)	0.88
	CC/GG+ GG/GG	62 (36.48)	66 (33.00)	1.16 (0.75-1.79)	0.48	0.91 (0.56-1.48)	0.73
XPD Asp312Asn codon312 Ex-10 G23591A	GG/GG	38 (22.35)	112 (56.00)	1		1	
	GG/AA	57 (33.53)	45 (22.50)	3.73 (2.18-6.38)	<0.0001	0.25 (0.13-0.45)	<0.0001
	AA/AA	75 (44.12)	43 (21.50)	5.14 (3.04-8.69)	<0.0001	0.15 (0.08-0.28)	<0.0001
	GG/AA+ AA/AA	132 (77.65)	88 (44.00)	4.42 (2.80-6.97)	<0.0001	0.21 (0.12-0.34)	<0.0001
XPD Lys751Gln codon751 Ex-23 A35931C	AA/AA	64 (37.65)	106 (53.00)	1		1	
	AA/CC	91 (53.53)	77 (31.50)	1.95 (1.26-3.02)	0.002	0.50 (0.30-0.83)	0.007
	CC/CC	15 (08.82)	17 (8.50)	1.46 (0.68-3.12)	0.32	0.66 (0.27-1.57)	0.35
	AA/CC+ CC/CC	106 (62.35)	94 (47.00)	1.86 (1.23-2.83)	0.003	0.49 (0.31-0.79)	0.003

*: Indicates significant Odds Ratio (p<0.05)
p value determined based on $\chi 2$

(C)Analysis of the XPD G23592A codon 312 exon 10

Table-2 displays the distribution of genotypes and frequency of alleles of the G23592A polymorphisms in patients with breast cancer and controls. The G>A polymorphism in codon 312 of the exon 10 results in Asp>Asn substitution in an evolutionary conserved region. The frequency of *XPD 23592GG* homozygotes was 22.35 % in cases and 56.00 % in controls whereas *23592AA* homozygote was 44.12 % in cases and 21.50 % in controls. The frequency of *23592GA* heterozygotes was 33.53% in cases and 22.50% in controls (Table-2). The frequency of the XPD *23592A* allele was significantly higher in cases (0.44) than in controls (0.21). Compared to *23592GG* genotype, the variant genotype *23592AA* genotype was associated with breast cancer risk (0R=5.14; 95% CI= (3.04-8.69); *p*=<0.0001) of BC. The 23592 variants of the DNA repair gene XPD are extremely high and contribute significantly to the risk of BC in the rural population of south western Maharashtra.

(D) Analysis of the XPD A35931C codon 751 exon23

The amplification of XPD codon 751 resulted in the product of 436 bp. The PstI enzyme was used to detect the *XPDA35931C* at codon 751 of exon 23. The

PCR amplified products upon treatment with PstI yielded wild-type (*35931A*) alleles of 290, 146bp fragments, and the polymorphic (C) allele produces 3 fragments of 227, 146 and 63 bp. The A→C polymorphism in exon 23 at nucleotide position 35931 gives rise to the amino acid substitution Lys→Gln in the codon 751. Table-2 displays the distribution of genotypes and frequency of alleles of the A35931C polymorphisms in patients with breast cancer and controls. The frequency of the XPD 35931C allele was not significantly higher in the cases (8.82%) than in the controls (8.50%) which is at the lower end of the previously reported allele frequencies.

Table 3: Stratification analysis of the demographic factors including age, tobacco smoking status, age at first delivery and distribution of genotypes with odds ratio of the XPD genes in the patients with breast cancer and the control group from rural population of south-western Maharashtra.

		Demographic Factors							
Gene	**Genotype**	**Age (Cases/Control)**		**Tobacco status (Cases/Control)**		**Age @ 1st Delivery (Cases/Control)**			
		≤ 50 N=95/149	> 50 N=75/51	Tobacco Users N=94/82	Tobacco nonusers N=76/118	15-20 N=126/76	21-25 N=37/111	26-30 N=6/10	31-35 N=1/3
XPD *Arg156Arg codon 156 Ex-6 C22541A*	CC/CC	31/49	42/18	45/30	28/37	54/21	15/42	4/3	0/1
	CC/AA+ AA/AA	64/100	33/33	49/52	48/81	72/55	22/69	2/7	1/2
	OR (95% CI)	1.01 0.58-1.75	0.42 0.20-0.89	0.62 0.34-1.15	0.78 0.42-1.43	0.50 0.27-0.94	0.89 0.41-1.90	0.21 0.02-1.87	1.80 0.04-79-42
	P value	0.96	0.02	0.13	0.42	0.03	0.76	0.16	0.76
XPD Ile199Met codon199 Ex-8 C23047G	CC/CC	56/99	52/35	62/53	46/81	84/52	21/73	3/8	0/1
	CC/GG+ GG/GG	39/50	23/16	32/29	30/37	42/24	16/38	3/2	1/2
	OR (95% CI)	1.37 0.82-2.34	0.54 0.25-1.16	1.06 0.59-1.97	1.42 0.78-2.60	1.08 0.58-1.99	1.46 0.68-3.12	4.00 0.43-37.10	1.80 0.04-79-42
	P value	0.23	0.11	0.85	0.24	0.79	0.32	0.22	0.76
XPD Asp312Asn codon312 Ex-10 G23591A	GG/GG	19/88	19/24	21/45	17/67	29/27	9/82	0/3	0/0
	GG/AA+ AA/AA	76/61	56/27	73/37	59/51	97/49	28/29	6/7	1/3
	OR (95% CI)	5.77 3.16-10.50	2.61 1.22-5.58	4.22 2.20-8.11	4.55 2.37-8.74	1.84 0.98-3.44	0.11 0.04-0.26	6.06 0.26-140.2	0.42 0.005-33.5
	P value	<0.0001	0.01	<0.0001	<0.0001	0.05	0.0001	0.26	0.70
XPD Lys751Gln codon751 Ex-23 A35931C	AA/AA	35/79	29/27	33/42	31/64	49/23	12/73	3/9	0/1
	AA/CC+ CC/CC	60/70	46/24	61/40	45/54	77/53	25/38	3/1	1/2
	OR (95% CI)	1.93 1.14-3.24	1.78 0.86-3.66	1.94 1.05-3.55	1.72 0.95-3.08	0.68 0.37-1.25	0.24 0.11-0.55	9.0 0.65-122.8	1.80 0.04-79-42
	P value	0.01	0.11	0.03	0.06	0.21	0.006	0.09	0.76

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

Gene	**Genotype**	**ER/PR +ve (frequency)**	**ER/PR-ve (frequency)**	**OR**	**95% CI**	**χ^2**	**P-value**
XPD *Arg156Arg codon 156 Ex-6 C22541A*	CC/CC	41 (0.41)	32 (0.47)	1			
	CC/AA	53 (0.52)	29 (0.43)	1.42	0.74-2.72	1.07	0.28
	AA/AA	8 (0.07)	7 (0.10)	0.89	0.29-2.71	0.20	0.84
	CC/AA+ AA/AA	61(0.59)	36 (0.53)	1.32	0.71-2.45	0.88	0.37
XPD Ile199Met codon199 Ex-8 C23047G	CC/CC	65 (0.64)	43 (0.64)	1			
	CC/GG	22 (0.21)	14 (0.20)	1.03	0.47-2.25	0.09	0.92
	GG/GG	15 (0.15)	11 (0.16)	0.90	0.37-2.14	0.23	0.81
	CC/GG+ GG/GG	37 (0.36)	25 (0.36)	0.97	0.51-1.85	0.06	0.94
XPD Asp312Asn codon312 Ex-10 G23591A	GG/GG	24 (0.23)	14 (0.20)	1			
	GG/AA	29 (0.29)	28 (0.41)	0.60	0.26-1.39	1.17	0.23
	AA/AA	49 (0.48)	26 (0.39)	1.09	0.48-2.47	0.22	0.81
	GG/AA+ AA/AA	78 (0.77)	54 (0.80)	0.84	0.40-1.77	0.45	0.65
XPD Lys751Gln codon751 Ex-23 A35931C	AA/AA	43 (0.42)	21 (0.31)	1			
	AA/CC	47 (0.46)	44 (0.65)	0.52	0.26-1.01	1.92	0.05
	CC/CC	12 (0.12)	3 (0.04)	1.95	0.49-7.67	0.95	0.33
	AA/CC+ CC/CC	59 (0.58)	47 (0.69)	0.61	0.32-1.17	1.48	0.13

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

In Maharashtrian patients, the age of beginning of BC is 50 years, considerably lesser than reported in other reports. To assess the relationship of analyzed polymorphisms with the age at diagnosis of BC, we grouped the patients as ≤50 (n=95) or >50 (n=75) years of age and compared with age matched controls which surprisingly showed that the XPD cd 312 (OR=5.77; CI=3.16-10.50); p<0.0001) displayed significant risk of BC at the age bellow 50. Also, the association of BC with first delivery age was reviewed in this study which showed that15-20 yrs age of first delivery, considerably associated with increased BC risk. The distribution of genotype polymorphisms along with the statistical analysis are shown in table-3. Also when we carried out the relationship of BC risk with the hormone receptor status, we found that (ER/PR +ve (n= 102) and ER/PR-ve (n= 68) (table-4), showed higher BC risk.

DISCUSSION

The relationship between recently studied genotype polymorphisms of NER pathway genes and the risk for BC was investigated in the rural areas of western Maharashtra. The genotypic frequencies of (A) XPD at codon 156 of the exon 6 (B) XPD at codon199 of the exon 8 (C) XPD at codon 312 in the exon 10 and (D) XPD at codon 751 in the exon23. The crude and adjusted ORs and their 95% CIs were calculated to determine the association XPD variants and risk of BC. Wild type genotype frequencies of XPD codon 156, codon 199, codon 751 showed broad distribution in the Maharashtrian population in both controls as well as BC cases. The occurrence of XPD polymorphisms at 22541(C-A), 23047 (C-G), 35931 (A-C) did not show much variation with the BC risk in western Maharashtrian population. Also, when we carried out investigation to study the relationship between the XPD polymorphisms and the BC risk in a western Maharashtrian population, we found positive association between the XPD codon 312 at Asp 312 Asn polymorphism and BC. Earlier, Xing et al (2002) [21] reported that the frequencies of Asn allele of XPD 312 and the Gln allele of XPD 751 are 0.06 and 0.07 in hospital based case control study of lung cancer. In our study the frequency of Asn allele XPD 312 was 0.44 that of XPD 156 allele Arg was 0.08 and that of XPD 199 Asn allele was 0.15 and frequency of XPD 751 allele Gln was 0.08 which were to some extent different from other reports. The investigations into the connection between polymorphisms NER genes and cancer susceptibility have not yet produced constant results and the results were disagreeing in different types of cancer in different populations [8,22] Several molecular epidemiological studies have investigated the association between genetic polymorphisms in XPD and susceptibility to several cancers including head & neck, bladder and breast cancer among different ethnic groups. [13,23-24] Few other studies were unsuccessful to discover positive proof for XPD polymorphisms in of the oral and breast cancer. [8,14,24] Exceptionally only some studies from Northern and Southern parts of India observed the genetic polymorphisms in the DNA repair genes with respect to a variety of cancer risks including prostate, breast and head and neck cancers. [17,25-27] However, no information available on the association of polymorphisms of NER pathway genes and their susceptibility to BC from rural population of Maharashtra where the prevalence of breast carcinogenesis is very high. Therefore, we aimed to examine the connection between the BC development and polymorphisms in XPD genes from a pool of unexplored rural Maharashtrian population.

CONCLUSION

Our results showed that the XPD gene at position 23592 of codon 312 of exon 10 polymorphism which could be associated with the risk of BC. 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. Such genotyping analysis will also enhance our ability to identify those individuals most susceptible breast carcinogenesis in the rural population of India.

Conflict of Interest: None declared

Ethical approval: The study protocol was approved by the Institutional Ethics Committee of Krishna Institute of Medical Sciences University for the Use of human subjects in research

Funding: Intramural funding by Krishna Institute of Medical Sciences.

ACKNOWLEDGEMENTS

We greatly acknowledge Dr. Neelima Malik, Vice Chancellor, Krishna Institute of Medical Sciences for guidance & support to carry out this research. The experimental assistance of Mr. Santosh Jadhav is duly acknowledged.

REFERENCES

1. Ferlay J, Shin HR, Bray F, Forman D, Mathers C, Parkin DM. Estimates of worldwide burden of cancer in 2008: GLOBOCAN 2008. Int. J. Cancer.2010; 127: 2893-2917.
2. Ferlay J, Soerjomataram I, Ervik M, Dikshit R. et al. GLOBOCAN 2012 v1.1, Cancer Incidence and Mortality Worldwide: IARC Cancer Base No. 11 [Internet]. Lyon, France: International Agency for Research on Cancer. 2014.
3. Gupta S, Rao YN, Agarwal SP. Emerging strategies for cancer control for women in India. 50 Years of Cancer control in India. 2003.
4. Hulka BS and Moorman PG. Breast cancer: Hormones and other risk factors. Maturitas. 2001; 38:103-116.
5. Boffetta P, Hashibe M. Alcohol and cancer. Lancet Oncol. 2006; 7:149–156.
6. Mathew A, Gajalakshmi V, Rajan B, Kanimozhi V. et al. Anthropometric factors and breast cancer risk among urban and rural women in south India A multicentric case control study. Br J Cancer. 2008; 99:207-213.
7. Meshram II, Hiwarkar PA, Kulkarni PN. Reproductive risk factors for breast cancer: A case control study. Online J Health Allied Sci. 2009; 83:5.
8. Benhamou S, Sarasin A. *ERCC2/XPD* gene polymorphisms and cancer risk. Mutagenesis. 2002; 17: 463-469.
9. Manuguerra M, Saletta F, Karagas MR, Berwick M, Veglia F, Vineis P, Matullo G. XRCC3 and XPD/ERCC2 single nucleotide polymorphisms and the risk of cancer: a HuGE review. Am J Epidemiol. 2006; 164:297-302.
10. Clarkson SG, Wood RD. Polymorphisms in the human XPD (ERCC2) gene, DNA repair capacity and cancer susceptibility: an appraisal. DNA Repair (Amst). 2005; 4:1068-1074.
11. Cagana M, Kilgallen J, Conroy JM, Wiencke JK, Kelsey KT, Miike R. et al. Associations between ERCC2 polymorphisms and gliomas. Cancer Epidemiol. Biomarkers Prev. 2001; 10: 355-360.
12. An J, Liu Z, Hu Z, Li G, Wang LE, Sturgis EM, El-Naggar AK, Spitz MR, Wei Q. Potentially functional single nucleotide polymorphisms in the core nucleotide excision repair genes and risk of squamous cell carcinoma of the head and neck. Cancer Epidemiol Biomarkers Prev. 2007; 16: 1633-1638
13. Shao J, Gu M, Xu Z, Hu Q, Qian L. Polymorphisms of the DNA gene XPD and risk of bladder cancer in a Southeastern Chinese population. Cancer Genet. Cytogenet. 2007; 177: 30-36.
14. Tomescu D, Kavanagh G, Ha T, Campbell H, Melton D. Nucleotide excision repair gene XPD polymorphisms and genetic predisposition to melanoma. Carcinogenesis. 2001; 22: 403-408.
15. Vogel U, Olsen A, Wallin H, Overvad K, Tjonneland A, Nexo BA. Effect of polymorphisms in XPD, RAI, ASE-1 and ERCC1 on the risk of basal cell carcinoma among Caucasians after age 50. Cancer Detect. Prev. 2005; 29: 209-214.

16. Srivastava K, Srivastava A and Mittal B. Polymorphisms in ERCC2, MSH2, and OGG1 DNA repair genes and gallbladder cancer risk in a population of Northern India. Cancer. 2010; 116: 3160-3169.
17. Kumar A, Pant MC, Singh HS, Khandewal S. Associated risk of XRCC1 and XPD cross talk and life style factors in progression of head and neck cancer in north Indian population. Mutat Res. 2012; 729: 24-34
18. Sobti RC, Berhane N, Melese S et al. Impact of XPD gene polymorphism on risk of prostate cancer on north Indian population. Mol Cell Biochem. 2012; 362:263-268.
19. Smith TR, Levine EA, Freimanis RI, Akman SA, Allen GO, Hoang KN, et al. Polygenic model of DNA repair genetic polymorphisms in human breast cancer risk. Carcinogenesis. 2008; 29:2132–2138.
20. Yin J, Wang C, Liang D, Vogel U, Yue L, Liu J, Qi R, Sun X. No evidence of association between the synonymous polymorphisms in XRCC1 and ERCC2 and breast cancer susceptibility among nonsmoking Chinese. Gene. 2012; 503: 118–122.
21. Xing D, Tan W, Wei Q and Lin D. Polymorphisms of the DNA repair gene XPD and the risk of Lung cancer in Chinese population. Lung Cancer. 2002; 38: 123-129.
22. Mohrenweiser HW, Wilson DM, Jones IM. Challenges and complexities in estimating both the functional impact and the disease risk associated with the extensive genetic variation in human DNA repair genes. Mutat Res.2003; 526: 93-125.
23. Sturgis EM, Zheng R, Li L, Castillo EJ, Eicher SA, Chen M, Strom SS, Spitz MR, Wei Q. XPD/ERCC2 polymorphisms and risk of head and neck cancer: a case-control analysis. Carcinogenesis. 2000; 21: 2219-2223.
24. Kipikasova L, Wolaschka T, Bohus, P, Baumohlová H, Bober J, Blazejov, J, et al. Polymorphisms of the XRCC1 and XPD genes and breast cancer risk: a case–control study. Pathol. Oncol. Res. 2008; 14: 131–135.
25. Ramachandran S, Ramadas K, Hariharan R, Rejnish Kumar R, Radhakrishna Pillai M. Single nucleotide polymorphisms of DNA repair genes XRCC1 and XPD and its molecular mapping in Indian oral cancer. Oral Oncol. 2006; 42: 350-362.
26. Sobti RC, Singh J, Kaur P, Pachouri S, Siddiqui EA and Bindra HS. XRCC1codon 399 and ERCC2 codon 751 polymorphism, smoking, and drinking and risk of esophageal squamous cell carcinoma in a North Indian population. Cancer Genet Cytogenet. 2007; 175: 91-97.
27. Mandal R, Gangwar R, Kapoor R and Mittal R. Polymorphisms in base-excision & nucleotide-excision repair genes & prostate cancer risk in north Indian population. Indian J Med Res. 2012; 135: 64-71.

How to cite this article: Datkhile KD, Bhosale SJ, Patil MN et al. Genetic polymorphisms of DNA repair gene (XPD) and breast cancer risk in a hospital based case-control study from Maharashtra. Int J Health Sci Res. 2017; 7(7):290-298.

****