RESEARCH ARTICLE

Editorial Process: Submission:03/26/2019 Acceptance:08/02/2019

Risk Modulation of Oral Pre Cancer and Cancer with Polymorphisms in *XPD* and *XPG* Genes in North Indian Population

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Abstract

Background: Environmental carcinogens cause DNA damages which if not repaired properly, may increase the risk of cancer. The Xerodermapigmentosum group D (*XPD*) and group G (*XPG*) genes are essential genes for DNA repair and alteration in DNA repair causes cancer. The present study aimed to evaluate the relationship between *XPD* and XPG polymorphisms and risk of oral pre cancer and cancer. **Methods:** Present study genotyped 302 samples of oral diseases and 300 controls for *XPD* (A/C) and *XPG* (G/C) polymorphisms with PCR-RFLP method. **Results:** Our result showed that compared to AA genotype frequency of AC and CC genotype for *XPD*(A/C) polymorphism were significantly lower among cases than in control and are associated with decreased risk of oral diseases (OR= 0.621 and 0.603 respectively). In contrast with reference to GG genotype the frequency of CC genotype of XPG (G/C) was significantly higher in case than in control population (p value=0.004) and found to increase the risk of oral diseases (OR= 2.077). Particularly C allele for *XPD* A/C polymorphism was found to be associated with decreased risk of Lichen planus and increased risk of (OR = 0.470 and 1.541 respectively) oral cancer. While C allele of XPG G/C polymorphism significantly increased the risk of Oral Submucous Fibrosis and Leukoplakia (OR= 1.879 and 1.837 respectively) but not of Lichen planus and oral cancer. In combined genotype analysis from the aforesaid polymorphisms presence of C allele for *XPD* (A/C) polymorphisms were found to decrease the risk of oral diseases. However, the same C allele was observed to increase the chance of having high stage disease (OR= 5.71) with nodal involvement (OR= 6.78) once the cancer been initiated. **Conclusion:** This work shows association of *XPD* (A/C), *XPG* (G/C) polymorphisms with the development of pre oral cancer as well as oral cancer and its clinical courses.

Keywords: Oral pre cancer and cancer- *XPD*- *XPG*- PCR-RFLP- gene polymorphism

Asian Pac J Cancer Prev, **20 (8)**, 2397-2403

Introduction

Oral cancer, a form of head and neck cancer is present in the oral cavity. It is very frequent with 30,000 cases diagnose worldwide per year (Feraly et al., 2015). Around 75 percent of oral cancers are caused due to use of tobacco and excessive alcohol consumption. Poor oral hygiene, nutrition, and some chronic infections by fungus, bacteria and virus are among the other causes of oral cancer. In most of the cases oral cancer is preceded by pre malignant lesions such as Oral Submucous Fibrosis (OSMF), Leukoplakia and Lichen planus. OSMF is characterized by progressive development of fibrosis in oral submucosa with 5-10% of them develop to malignancy (Gupta PC et al., 1992). Leukoplakia appears as white patches while Lichen planus is chronic inflammation to mucous membrane of oral cavity.

Cancer is a complex disease related to environmental factors, genetic susceptibility and gene environment interactions. DNA damage caused by environmental factors, such as consumption of tobacco and alcohol, exposure to UV radiation are important risk factors for oral cancer (Wood et al., 2001). This exposures may results in DNA damages such as formation of DNA adducts and cross-links. Damaged DNA if not restored properly with DNA repair system (Cheng et al., 1998) can causes dis-regulation of cell growth and apoptosis which may lead to development of cancer including oral cancer (Kawakami et al., 2015).

DNA repair mechanism is a complex biological system, including five different pathways namely; nucleotide excision repair (NER), Double –strand break repair,

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Base excision repair, Mismatch repair and Homologus recombination repair (Yang, 2012). Gene mutation in NER pathway can develop to several human diseases, including Xerodermapigmentosum (XP) (Spivak et al., 2015). The XP patients can be classified as 8 complementation groups, XPA through XPG. The XPG gene is located on chromosome 13 and synthesizes a DNA endonuclease of 1,186 amino acids. This enzyme is specific for single strand and cleaves the damaged DNA strand at the 3'end (O'Donovan et al., 1994). Any alteration in XPG gene can impair its DNA repair efficiency which can further cause genomic instability and carcinogenesis (Cheng et al., 2002). XPG gene harbored SNPs and many of those SNPs have been reported to alter the risk of different cancer including colorectal (Moreno et al., 2006), lung (Shen et al., 2005; Sakoda et al., 2012), gastric (He et al., 2012; Yang et al., 2012) and laryngeal (Abbasi et al., 2009).

Xerodermapigmentosum complementation group D (*XPD*) is involved in NER pathway through recognition and repair of thymidine dimers. The *XPD* gene contains 23 exons, encodes the 761-amino acid protein and is present in chromosome 19q13.3. The *XPD* protein is a part of transcription factor TFIIH and is involved in different functions including nucleotide excision repair (NER), transcription coupled repair, transcription, cell cycle regulation and apoptosis (Sturgis et al., 2000) and therefore, any sequence variation in *XPD* gene may lead to repair and transcription defects. Several SNPs been identified in *XPD* gene, and it is thought that certain *XPD* polymorphisms may be associated with the development of cancer (Goode et al., 2002).

In this study, we investigated the association between *XPD* (A/C, *rs13181*) and *XPG* (G/C, *rs17655*) polymorphisms with the risk of oral cancer and pre cancer.

Materials and Methods

Study Subjects

The study was evaluated on 602 subjects including 302 patients with previously treated and pathologically confirmed oral pre cancer and cancer who were registered at department of Oral Pathology and Microbiology, King George's Medical University and 300 healthy controls. This study was approved by the Institutional Ethics Committee of the King George's Medical University, Lucknow. An informed written consent was obtained from all subjects. Venous blood samples were collected in EDTA tubes and stored at −80°C, till DNA extraction. Genomic DNA extraction from blood samples was carried out by salting out method (Suguna et al., 2014).

Genotyping by RFLP

PCR-RFLP method was employed to determine the genotypes of *XPD* A/C and *XPG* G/C polymorphisms. PCR primers used for the amplification of *XPD* A/C and XPG G/C polymorphisms were same as used by Sanyal et al., (2004). PCR reactions were carried out with 10 ng of genomic DNA in 10 ul volume reactions containing 0.3 mM each primer, 0.11 mM each dNTP, 20 mMTris--HCl, 50 mMKCl, 2.0 mM $MgCl_2$, and 0.5 U TaqDNApolymerase (Sigma Aldrich,USA). PCR products were digested with Pst I and Nla III respectively to obtain genotypes for *XPD* and *XPG* polymorphisms. The digested PCR products were visualize on a 2% agarose gel stained with ethidium bromide. The genotype results were regularly checked and compared with known genotypes as controls.

Statistical analysis

The distributions of genotype were checked for the Hardy–Weinberg equilibrium (HWE) and any deviation from HWE was measured with Pearson chi-square test. Comparison between the genotype and allele frequencies among case and control were assessed by the chi-square test. Odds ratio (OR) was calculated to measure the chances of having diseases with the relative frequency of different genotypes and allele among the cases and controls. Odds ratio and p values were calculated with Epi-Info programme (http://wwwn.cdc.gov/epiinfo/). P value <0.05 was considered to be significant.

Results

Demographics of the study population

The demographic profile including gender, age, habitual risk which may responsible for the development of oral cancer and pre oral cancer along with the tumor's clinical parameters, are shown in Table 1. This study involved 302 oral cancer patient, including 203 (67%) male and 99 (33%) female. Calculated mean age of subject was 46.67. The mean age of controls [219 (73%) males and 81 (27%) females] was 38.02. Tobacco, smoking chewing and alcohol consumption have been observed

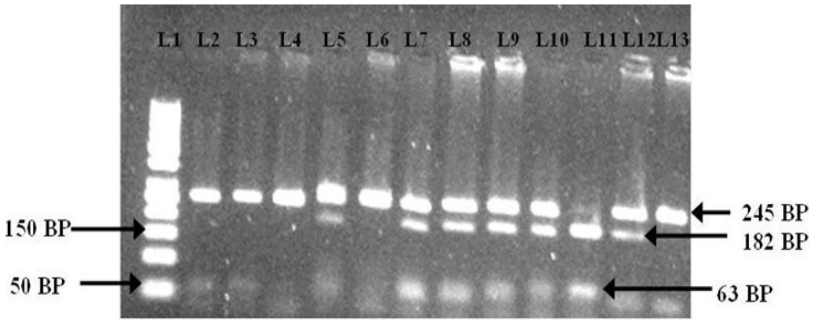

Figure 1. 2.5% Agarose Gel Analysis of XPD (A/C) Polymorphism. Lane 1 50 bp Ladder. Lane 2,3,4,5,6,13 AA genotype 245bp.Lane 7,8,9,10,12 AC genotype 245,182, 63bp. Lane 11 CC genotype 182, 63bp.

Table 1. Demographic Parameters of Patient and Controls and Their Association with Risk of Oral Pre Cancer and Cancer

Demographic Character	Cases (n=302) (%)	Control (n=300) (%)	P- value
Sex			
Male	203 (67)	219 (73)	Ref
Female	99 (33)	81 (27)	0.144
Age distribution			
10 – 40	89 (29)	160 (53)	Ref
40- 90	213 (71)	140 (47)	<0.0001*
Mean age	46.67	38.02	
Median age	47	37.5	
Habitual risk			
Alcohol consumption			
Yes	41 (14)	11 (04)	Ref
No	261 (86)	289 (96)	<0.0001*
Smoking			
Yes	141 (46)	78 (26)	Ref
No	161 (54)	222 (74)	<0.0001*
Tobacco chewing			
Yes	134 (44)	73 (25)	Ref
No	168 (56)	227 (75)	<0.0001*
Type of oral cancer			
Leukoplakia	70 (23.33)	-	-
O.S.M.F	90 (30.00)	-	-
Lichen planus	70 (23.33)	-	-
Malignancy	72 (23.33)	-	-
TNM staging of oral cancer (Malignancy)			-
Tumor Stage		-	-
I	8 (11)		
II	11 (15)		
III	18 (25)		
IV	35 (49)		
Tumor T Status		-	-
T1+T2	10(13)		
T3+T4	62 (87)		
Lymph Node		-	-
N0	25 (34)		
N1+N2	47 (66)		
Metastasis		-	-
M0	54 (75)		
M1	18 (25)		
Cell differentiated grade		-	-
Grade 1	31 (45)		
>Grade 1	39 (55)		

*, significant value

to be significantly associated with the development of oral diseases (p value <0.0001). Types of oral diseases included in this study are also mentioned in Table 1, which includes 23.33% leukoplakia, 30% OSMF, 23.33% lichen planus, and 23.33% malignancy.

Genotypes of XPD and XPG polymorphisms and risk of oral diseases

The different genotype distribution and allele frequency for *XPD* A/C, *XPG* G/C polymorphisms both in control and cases are represented in Table 2. Both the case and control population were at Hardy-Weinberg equilibrium. With reference to the AA genotype frequency of AC and CC genotype for *XPD* (A/C) polymorphism was significantly low among cases of oral diseases and found to decrease the risk of oral diseases (OR= 0.621 and 0.603 respectively). Compared to A allele a significant risk reduction was also observed with C allele (OR=0.758). In contrast with reference to GG genotype the frequency of CC genotype of XPG (G/C) was significantly higher in case than in control population (p value=0.004) and found to increase the risk of oral diseases (OR= 2.077). Increased risk of oral disease was also documented with C allele of XPG (G/C) polymorphism (OR= 1.425, p value= 0.003).

Since both *XPD* and *XPG* are proteins of NER pathway and functions in collaboration we hypothesized that combined genotypes from *XPD* A/C and *XPG* G/C polymorphisms might synergistically altered the risk of oral disease. Distribution of different combined genotypes from *XPD* (A/C) and *XPG* (G/C) polymorphisms are shown in Table 3. Compared to combined common allele genotype AA/GG the risk of oral disease were found to be significantly reduced with AA/CC, AC/GC, AC/CC and CC/CC genotype (OR=0.393, 0.377, 0.237 and 0.147 respectively).

Genotypes of XPD and XPG polymorphisms and risk of pre oral cancer and oral cancer

The study showed impact of *XPD* and *XPG* polymorphisms on the risk of oral disease. In order to investigate the association of them separately with pre oral cancer and oral cancer we further divided the case population into four groups: (i) patients with Oral Submucous Fibrosis (ii) patients with Lichen planus, (iii) patients with Leukoplakia and (ii) patients with oral cancer. The frequency of different genotypes and alleles for *XPD* A/C and *XPG* G/C polymorphisms among patients of different pre oral cancer diseases and oral cancer are listed in Table 4. Compared to the AA genotype of *XPD* A/C polymorphism the risk of developing Lichen planus was lower with AC as well as CC genotypes (OR= 0.495 and 0.222 respectively) (Table 4). In contrast the CC genotype and C allele for the same *XPD* A/C polymorphism was found to be associated with high risk for oral cancer (OR= 2.708 and 1.541 respectively) (Table 4). However no such association was observed with other pre oral cancer lesions namely OSMF and Leukoplakia. For XPG G/C polymorphism the CC genotype as well as the C allele significantly increased the risk of OSMF and Leukoplakia but not of Lichen planus and oral cancer (Table 4).

XPD and XPG gene polymorphisms in relation to different clinical parameters of patients with oral cancer

The distribution of different genotypes of *XPD* A/C and *XPG* G/C polymorphisms among different disease

Table 2. Distribution of Different Genotypes and Alleles from XPD (A/C) and (G/C) Polymorphisms among Cases of Oral Diseases and Controls

	Cases N (%)	Controls N (%)	p- value	Odds Ratio	95% CI
XPD(A/C) Genotypes					
AA	98 (33.00)	70 (23.33)	Ref	1	1
AC	127 (43.00)	146 (48.66)	0.020*	0.621	0.421-0.916
CC	71 (24.00)	84 (28.00)	0.032*	0.603	0.388-0.937
A	323 (54.50)	286 (47.66)	Ref	1	1
C	269 (45.50)	314 (52.34)	0.020*	0.758	0.604-0.952
XPG (G/C) Genotypes					
GG	100 (34.12)	122 (42.36)	Ref	1	1
GC	130 (44.36)	129 (44.79)	0.3	1.229	0.858-1.761
CC	63 (21.50)	37 (12.84)	0.004*	2.077	1.28-3.37
G	330 (56.32)	373 (64.75)	Ref	1	1
C	256 (43.68)	203 (35.25)	0.003*	1.425	1.12-1.80

*, significant value

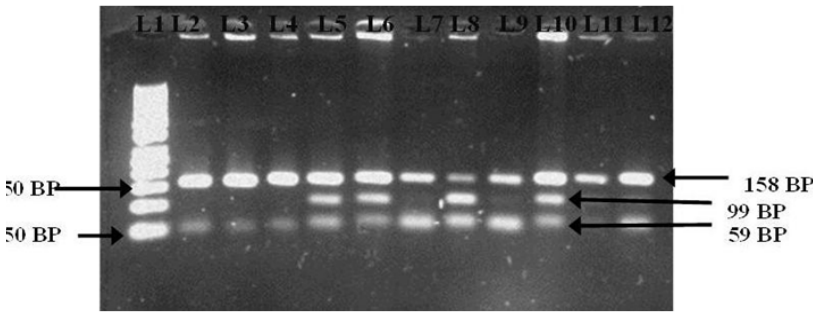

Figure 2. 2.5% Agarose Gel Analysis of XPG (G/C) Polymorphism. Lane 1 50 bp Ladder. Lane 2,3,4,7,911,12 GG genotype 158bp. Lane 5,6,10 GC genotype 158,99,59bp. Lane 8 CC genotype 99,59bp.

categories are shown in Table 5. Prevalence of patients with variant allele genotypes (A/C + C/C) for *XPD* (A/C) polymorphism were more (96%) among patients who had cancer with lymph node involvement (number of lymph node involved N1+N2+N3) compared to those who did not (76%). Similarly compared to patients with AA genotype, patients with variant allele genotypes (A/C + C/C) for *XPD* (A/C) polymorphism had developed high stage tumor at diagnosis (OR= 5.71, p value= 0.049; Table 5). Rest of the clinical parameters of oral cancer did not show any association with *XPD*, *XPG* genotypes Table 5.

Discussion

Oral cancer is the most commonly found cancer and has been prime health issue in the growing countries including India. Pre oral cancer develops into oral cancer and early treatment of oral lesion could therefore decrease prevalence of oral cancer. This could be achieved by identifying the SNP markers for pre and malignant oral lesion. In this study we investigated the relationship between genotypes of *XPD* (A/C) and *XPG* (G/C) polymorphism and risk of oral pre cancer and oral cancer.

Table 3. Distribution of Combined Genotype from XPD (A/C) and XPG (G/C) Polymorphism among the Subject of Oral Diseases and Controls

Combined genotype	Cases N (%)	Controls N (%)	p- value	Odds Ratio	95% CI
AA/GG	29 (10.03)	14 (04.86)	Ref		
AA/GC	40 (13.84)	28 (09.72)	0.477	-	-
AA/CC	22 (07.61)	27 (09.37)	0.050*	0.393	0.168-0.921
AC/GG	49 (16.95)	17 (05.90)	0.581	-	-
AC/GC	50 (17.30)	64 (22.22)	0.014*	0.377	0.180-0.788
AC/CC	29 (10.03)	59 (20.48)	0.0004*	0.237	0.109-0.516
CC/GG	20 (06.92)	06 (02.08)	0.57		
CC/GC	39 (13.49)	37 (12.84)	0.129		
CC/CC	11 (03.80)	36 (12.50)	0.0001*	0.147	0.058-0.373

*, significant value

XPG protein serves as structure-specific endonuclease which cleaves different kinds of substrates with ss/ds DNA junction in NER (Evans et al., 1997). The NER complex including XPG removes the lesion from DNA strand. Some study showed that XPG is related to carcinogenesis and prognosis of different types of Cancer (Cheng et al., 2002; Guo et at., 2005; Cheng et al., 2000). Genetic polymorphisms in the XPG gene show significant association with the risk of development of different cancers (Closas et al., 2006; Zienolddiny et al., 2006; Rouissi et al., 2011). In the present study we have observed that the C allele for XPG G/C polymorphism increased the risk of pre oral cancer namely OSMF, Leukoplakia. To the best of our knowledge our study is first to evaluate the relation of XPG G/C polymorphism with the risk of pre oral cancer. However, we did not find any impact of this XPG polymorphism on oral cancer; neither on its susceptibility nor on its clinical parameters.

XPD contribute to NER by eliminating bulky chemical adducts, certain cross-links, ultraviolet (UV) photo lesions. *XPD* protein posses a 59-39 DNA helicase activities and it is a single-strand DNA-dependant ATPase which plays an important role in transcription and NER pathway (Lunn, 2000). In the present study the C allele from *XPD* A/C polymorphism were associated with reduced risk of Lichen planus. Similar protective association of this allele with the development of oral cancer has also been reported previously (Pereiraa et al., 2016). They have also reported that the C allele of *XPD* A/C polymorphism was associated with high grade oral squamous cell carcinoma. Similarly, we have also observed that once oral cancer is established the C allele enhanced the risk of having high stage malignant disease with nodal involvement. Several other cases have reported the link of *XPD* A/C polymorphism with the development of different cancer in different population indicates its universal role in carcinogenesis (Du et al., 2016; Smolarz et al., 2019; Avci et al., 2018; Zhu et al., 2018).

Since a single polymorphism shows low penetrance we combined the genotypes from studied polymorphism to find any combined effect of them on disease susceptibility. Our results from the present study indicate polymorphisms from *XPD* and *XPG* exert synergistic effect on the development of oral diseases. The protective effect of C allele from *XPD* A/C polymorphism suppressed the risk enhancing effect of C allele of XPG G/C polymorphism.

In conclusion, this study shows association of *XPD* (A/C), XPG (G/C) polymorphisms with the risk for development of pre oral cancer as well as oral cancer. However, due to small sample size in different disease group our finding are rather suggestive than conclusive and warn larger studies.

Table 4. Distribution of Different Genotypes and Allele from XPD (A/C), XPG (G/C) Polymorphisms Among the Subjects of Oral Submucous Fibrosis, Lichenplanus, Leukoplakia, Oral Cancer and Controls

Genotypes	Oral submucous fibrosis N (%)	P- value	Lichenplanus N (%)	P- value	Leukoplakia N (%)	P- value	Malignancy N (%)	P- value	Controls N (%)
XPD (A/C) Genotypes									
AA	30 (34.09)	Reference	30 (43.47)	Reference	24 (34.78)	Reference	8 (11.42)	Reference	70 (23.34)
AC	36 (40.01)	0.073	31 (44.94)	0.024* 0.495(0.278-0.882)	30 (43.47)	0.133	36 (51.44)	0.089 2.158(0.952-4.887)	146 (48.66)
CC	22 (25.00)	0.171	8 (11.59)	0.0004* 0.222(0.095-0.515)	15 (21.75)	0.106	26 (37.14)	0.031* 2.708(1.153-6.360)	84 (28.00)
A	96 (54.55)	-	91 (65.95)	-	78 (56.52)	-	52 (37014)	-	286 (47.66)
C	80 (46.45)	0.128	47 (34.05)	0.0002* 0.470(0.319-0.692)	60 (43.47)	0.074	88 (62.86)	0.031* 1.541(1.056-2.251)	314 (52.34)
XPG (G/C) Genotypes									
GG	24 (26.96)	Reference	23 (34.32)	Reference	20 (28.57)	Reference	33 (49.25)	Reference	122 (42.36)
GC	40 (44.94)	0.147	33 (49.25)	0.382	30 (42.85)	0.338	27 (40.29)	0.455	129 (44.79)
CC	25 (28.08)	0.0004* 3.435(1.757-6.714)	11 (16.41)	0.373	20 (28.57)	0.001* 3.297 (1.604-6.780)	7 (10.44)	0.566	37 (12.84)
G	88 (49.43)	-	79 (58.95)	-	70 (50.00)	-	93 (69.40)	-	373 (64.75)
C	90 (50.56)	0.0003* 1.879(1.338-2.640)	55 (41.04)	0.246	70 (50.00)	0.001* 1.837 (1.266-2.668)	41 (30.59)	0.358	203 (35.24)

*, significant value

Acknowledgments

We are thankful to Prof J.K Srivastava, Director, Amity institute of Biotechnology, Amity University Uttar Pradesh Lucknow Campus, Lucknow, India. We are also grateful to Dr Sabyasachi Sanyal for helpful comments.

Table 5. Association of Different Genotypes for XPD (A/C) and XPG (G/C) Polymorphism with Tumor Stage, Tumor T Status, Lymph Node, Metastasis and Grade in Oral Cancer Patients

	XPD (A/C), XPG (G/C) Genotypes/Alleles				
	T3+T4 N (%)	T1+T2 N (%)	P-value	Odds Ratio	95% CI
Tumor T Status					
XPD (A/C)					
AA	03 (08)	05 (15)	Reference	Reference	Reference
AC+CC	34 (92)	28 (85)	0.583	2.024	0.444-9.222
XPG (G/C)					
GG	21 (58)	12 (38)	Reference	Reference	Reference
GC+CC	15 (42)	19 (62)	0.174	0.451	0.169-1.203
Lymph Node	N1+N2+N3 N (%)	N0 N (%)			
XPD (A/C)					
AA	02 (04)	06 (24)	Reference	Reference	Reference
AC+CC	43 (96)	19 (76)	0.038*	6.789	1.254-36.77
XPG (G/C)					
GG	23 (53)	10 (42)	Reference	Reference	Reference
GC+CC	20 (47)	14 (58)	0.5	0.621	0.226-1.704
Metastasis	M1 N (%)	M0 N (%)			
XPD (A/C)					
AA	01 (06)	07 (13)	Reference	Reference	Reference
AC+CC	16 (94)	46 (87)	0.698	2.435	0.277-21.36
XPG (G/C)					
GG	11 (65)	22 (44)	Reference	Reference	Reference
GC+CC	06 (35)	28 (56)	0.232	0.428	0.136-1.341
Tumor stage	III+1V N (%)	I+II N (%)			
XPD (A/C)					
AA	03 (06)	05 (26)	Reference	Reference	Reference
AC+CC	48 (94)	14 (74)	0.049*	5.714	1.212-26.93
XPG (G/C)					
GG	26 (53)	07 (39)	Reference	Reference	Reference
GC+CC	23 (47)	11 (61)	0.451	0.562	0.187-1.694
Cell differentiated grade	>Grade 1 N (%)	Grade 1 N (%)			
XPD (A/C)					
AA	02 (05)	06 (20)	Reference	Reference	Reference
AC+CC	38 (95)	24 (80)	0.115	4.75	0.885-25.49
XPG (G/C)					
GG	20 (55)	13 (48)	Reference	Reference	Reference
GC+CC	16 (45)	14 (52)	0.743	0.742	0.272-2.022

*, significant value

Funding

The work was supported by Science and Engineering Board (SERB), India.

Ethical Approval

All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

Conflict of Interest

None of the author had any conflict of interest

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