RESEARCH ARTICLE

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# Genetic Polymorphisms of *XPC, XPD, XPG* Genes and their Association with Radiotherapy Induced Toxicity among Head and Neck Cancer Patients: A Hospital Based Study from Maharashtra

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## Abstract

**Background:** The present study was planned to investigate possible association of single nucleotide polymorphisms (SNPs) of nucleotide excision repair (NER) genes such as *XPC, XPD, XPG* with acute radiation induced toxicities such as skin reactions and oral mucositis in normal tissue from head and neck cancer (HNC) patients receiving radiotherapy. **Methods:** Two hundred and fifty HNC patients receiving radiotherapy were enrolled in this study and the acute toxicity reactions and radiation response were recorded. Association of SNPs rs2228001 of *XPC*, rs238406, rs13181 of *XPD* and rs17655 of *XPG* gene with normal tissue reactions in the form of dermatitis and mucositis were studied by PCR-RFLP and direct DNA sequencing. **Results:** The results of univariate analysis of SNPs of *XPC*, *XPD* and *XPG* showed that *XPC* polymorphism at codon 939 of exon 15 (A>C) was not associated with dermatitis (OR=0.30, 95% CI: 0.06-1.39; p=0.125), or oral mucositis (OR=1.14, 95% CI: 0.41-3.20; p=0.793). The *XPD* codon 156 of exon 6 (C>A) and codon 751 of exon-23 A>C) polymorphism showed no association with radiosensitivity in HNC patients (OR=1.50, 95% CI: 0.60-3.71; p=0.080) for dermatitis, (OR=1.54, 95% CI: 0.66-3.61; p=0.312) for oral mucositis. The 1104 Asp variant genotype or allele of *XPG* (OR=1.35 95% CI: 0.50-3.64; p=0.541) showed no association with degree of radiotherapy associated dermatitis or mucositis (OR=0.80, 95% CI: 0.32-2.03; p=0.648) in HNC patients. The variant C allele of 2920 A/C genotype of *XPC* gene at codon 939 of exon 15, found protective with developing skin reactions with grade >1 (OR=0.60, 95% CI: 0.36-0.97; p=0.039) in HNC patients treated with radiotherapy. **Conclusion:** The results obtained in this study concluded that the SNPs rs2228001of *XPC*, rs238406, rs13181 SNPs of *XPD* and rs17655 SNP of *XPG* are not associated with normal tissue toxicity in HNC patients treated with radiotherapy. Radiotherapy with high radiation dose was significantly associated with oral mucositis in response to radiotherapy.

**Keywords:** Head and neck cancer- Radiotherapy- *XPC*- *XPD*- *XPG*- Genetic polymorphism- Acute toxicity

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## Introduction

Head and neck cancer (HNC) is a common type of cancer in India constituting 30 % of all cancers and accounting 245, 811 new cases and 130, 139 deaths in year 2022 [1]. The HNC is complex which specifies multiple subsites of lip, oral cavity, larynx, nasopharynx, orapharynx, hypopharynx, thyroid and salivary glands. Tobacco and alcohol habits are dominant risk factors responsible for HNC along with other environmental carcinogens. Genetic susceptibility of an individual towards carcinogenesis depends on genetic variants of genomic determinants such as genes involved in cell cycle regulation, carcinogen detoxification, oxidative stress and DNA repair pathways [2]. The commonly followed treatment of locoregionally advanced HNC is surgery followed by adjuvant radiation therapy or concurrent chemo-radiation [3, 4]. Systemic radiotherapy used in HNC treatment is known to cause significant toxicities to normal healthy cells. When radiation therapy is administered, the normal tissue adjacent to the target cancer area are exposed to radiation and resulted into common acute tissue toxicities such as mucositis, dysphagia, dermatitis [5-7]. Large group of patients suffered with normal tissue reactions and there is no obvious justification for the severity of normal tissue reactions, however, it is usually presumed that genetic components of an individual might be accountable for

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clinical radiosensitivity. Ionizing radiation directly affects DNA structure and induces DNA lesions including single strand breaks (SSBs) and double strand breaks (DSBs) in tumor cells, but also the genomic stability of normal cells is affected by radiotherapy. Nucleotide excision repair (NER) is an important pathway used by individuals for removal bulky DNA lesions formed by radiation or other chemical mutagens [8]. Xeroderma pigmentosum complementation group C (*XPC*), Xeroderma pigmentosum complementation group G (*XPG*) and Human xenoderma pigmentosum complementation group D (*XPD*) are the major components of NER pathway functioning in the repair of ionizing radiation induced oxidative damage [9]

Genetic variations in DNA double strand break repair genes such as single nucleotide polymorphisms (SNPs) could interfere with alteration of gene function, which make them suitable candidate genes for the study of genetic susceptibility of an individual towards acute normal tissue reactions after radiation therapy [10, 11]. Several other reports documented association of genetic variants of DNA repair genes with radiation induced adverse reactions in normal tissue of an individual in response to radiotherapy [12-14], however, some other reports declined their clinical significance in normal tissue toxicity [15, 16]. *XPC* and *XPG* genes are highly polymorphic in nature, where A>C transversion at 2920 position in exon 15 of *XPC* resulted into Lys939 Gln polymorphism (rs2228001) and C>G transversion at position 3507 in exon 15 of *XPG* gene resulted into His1104Asp polymorphism (rs17655). Similarly, *XPD* gene showed Arg156Arg polymorphism at codon156 of exon6, Ile199Met at codon199 of exon 8, Asp312Asn at codon312 of exon 10 and Lys751Gln at codon 751 of exon 23. Both *XPC* and *XPD* gene polymorphism reported their association with radiation induced toxicity in variety of cancers where *XPC* Lys939Gln (rs2228001) and *XPD* Lys751Gln (rs13181) were associated with radiotherapy outcomes in HNC [17, 18], Non-small cell lung cancer [19] however, other studies observed different outcomes with no association of those genes with adverse effects of radiotherapy in breast [20, 21], prostater [22] and lung cancer [23]. However, the association between xenoderma pigmentosum group genes and their role in clinical radiosensitivity is obscure; therefore this postulation brings our interest into research to understand the genetic basis of radiation adverse effects on normal tissue during radiotherapy in HNC patients. In present study, we evaluated possible association of three genes *XPC* (Lys939Gln (A>C) (SNP: rs2228001), *XPD* (Arg156Arg, C>A, SNP: rs238406; Lys751Gln SNP: rs13181) *XPG* (His1104Asp, C>G SNP: rs17655) and risk of acute skin toxicity after therapeutic radiotherapy in HNC patients.

## Materials and Methods

*Study Subjects and Clinical Information*

Two hundred and fifty patients receving radiotherapy treatment at Department of Oncology of Krishna Hospital & Medical Research Center, Karad were enrolled in this study.

Inclusion critera: Patients with 25 to 85 years age, histopathologically confirmed for HNC; Patients with clinically localised or locally advanced stage with normal skin and oral mucosa before the first radiotherapy fraction.

Exclusion criteria: No pathological diagnosis; relapsed disease or metastasis; severe co-morbidities; patients with auto immune disease; incomplete treatment taken; incomplete follow-up; missing or incomplete data.

Written informed consent was obtained and patients were explained about their participation in the study. The detailed demographic, pathology, clinical examination findings and three months post radiotherapy follow up information were recorded for all patients as described in Table 1.

Response Evaluation Criteria in Solid Tumors (RECIST) criteria was followed for the documentation of clinical and radiological responses. For determining acute normal skin toxicity effects, Patients were followed up for three months post radiotherapy treatment to review the clinical response (Partial/complete/no response), stable, progressive disease, early death from disease, toxicity or any other cause.

*Treatment of patients with radiotherapy & chemo-radiotherapy*

All patients were treated using Linear accelerator (Model: Unique Performance, Make: Varian Medical System, USA) 6-Mega Volt (MV) (X-ray) with total radiotherapy dose of 60- 66 Gy (2 Gy per fractions for 5 days a week) with volumetric modulated arc therapy (VMAT) technique. Patients after surgical resection having positive margins were given a dose of 66 Gy in 33 fractions. Patients with no positive margins were given 60 Gy in 30 fractions. Chemotherapy was added if clinically indicated and the drug used was Cisplatin at doses of 40 mg/m$^2$ every week given for 5-6 doses along with RT.

*Follow up and Toxicity Assessment*

Patients were followed up for three months after completion of radiotherapy for assessment of toxicity and response. Acute adverse effects (oral mucositis and skin reaction) were recorded during and after completion of radiatiotherapy according to Radiation Therapy Oncology Group (RTOG) criteria. Acute radiation toxicity effects were defined as injuries appearing from the initial day of radiotherapy treatment until 3 months after the end of radiotherapy. The post-radiotherapy toxicity effects on normal skin and oral mucosa were recorded weekly. The severity of acute radiation injury was determined by radiation oncologist and severity of acute radiation dermatitis and oral mucositis was graded according the RTOG grading system. For comparing the severity of radiation toxicity, the skin reactions were graded (>1 grade) severe skin reactions versus ≤ 1 grade skin reactions. The patients with oral mucositis grade >2 are radiosensitive group (cases) compared with ≤2 grade (controls).

*DNA extraction and single nucleotide polymorphism genotyping assays*

Five milliliter (mL) of whole blood from patients was collected before initiation of radiotherapy treatment.

Genomic DNA extraction from peripheral blood sample was carried out using HipurA®Blood genomic DNA miniprep purification kit following the manufacturer's instructions. This purified DNA was used for genotyping assays. Genotyping of *XPC*, *XPD* and *XPG* gene SNPs was performed by PCR-RFLP and direct DNA sequencing methods with appropriate primer sets presented in Table 2. The polymerase chain reaction amplification was performed in a total 20 micro liter (µL) reaction mixtures containing1X PCR buffer (10 mili molar (mM) Tris-HCl (pH 9.0), 50 mM KCl 1.5 mM MgCl2, 0.01% gelatin), 200 µM 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. Each PCR reaction was performed with DNA denaturation at 95 °C for 10 minutes followed by 35 cycles of 95 °C for 30 seconds, and primer annealing for 30 seconds and extension at 72°C for 30 seconds lastly final extension at 72°C for 10 minutes for all gene amplification mixtures. After confirmation of DNA amplification, each PCR product (10 µl) was digested with an appropriate restriction enzyme at 37°C for 16 hours in 20 µL reaction mixtures containing buffer supplied with each restriction enzyme (Table 2). After the overnight incubation, digestion products were separated on 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 genotypic frequencies for NER genes (*XPC*, *XPD* and *XPG*) in the patient's were determined. The Odds Ratio (OR) and corresponding 95% confidence intervals (CI) were determined through unconditional multiple logistic regression. The OR estimated to test whether any association exists between the grade of acute toxicity and selected SNPs. The correlation between confounding factors and radiation toxicities were evaluated by the $\chi 2$ test. The p values <0.05 were considered as statistically significant. The event of occurrence of clinical severity of post-radiotherapy adverse effects are defined as skin reactions with >1grade and oral mucositis scored as grade >2. All statistical analyses were carried out using SPSS 11 Software.

## Results

*Demographic and Clinical characteristics of study population*

Two hundred and fifty patients of age range 25-85 years with median age 55 years were enrolled in the study and distribution of patients based on clinical characteristics, demographic information, histopathological grading and toxicity grades are presented in Table 1. 187 patients were males and 63 were females. Majority of the patients were tobacco smokers (74.80%) as compare to non-smokers (25.20%). When we grouped the HNC patients based on primary site of cancer we observed oropharynx (42.0%), hypopharynx (23.0%), oral cavity (20.0%), nasopharynx (7.0 %), larynx (4.0%) and remaining 4% were other sites. A total of 72.40 % patients underwent radiotherapy alone whereas 66.00 % patients receive combination of radiation therapy and chemotherapy. A total of 65.60 % patient's tumor was more than 2 cm size where as 34.40 % tumor was ≤ 2cm in size. Out of total 250 patients, 132 (52.80%) patients showed oral mucositis reactions (grade >2) (grade 3 and 4) and 47.20 % patients experienced grade ≤2 mucositis. Preliminary objective of the study was to investigate acute radiation induced toxicities such as dermatitis and mucositis in HNC patients after exposure to radiotherapy. When acute skin reactions were observed in response to radiation adverse effects, out of 250 patients

Table 1. Details of Baseline Demographic and Clinico-pathological Characteristics of Head and Neck Cancer Patients Enrolled in the Study

| Variables | Number/Percentage (%) |
|---|---|
| Total Number of Patients | 250 |
| Age (Mean ± SD) years | 54.92 ±13.55 (Range:25-85) Median:55 |
| ≤ 50 | 90 (36.00) |
| >50 | 160 (64.00) |
| Sex | |
| Male | 187 (74.80) |
| Female | 63 (25.20) |
| BMI Kg/m$^2$ | |
| ≤20 | 111 (44.40) |
| >20 | 139 (55.60) |
| Smoking/Tobacco chewing | |
| Smokers | 219 (87.60) |
| Non-Smokers | 31 (12.40) |
| Alcohol Consumption | |
| Drinkers | 105 (42.00) |
| Non-Drinkers | 145 (58.00) |
| Tumor size in cm | |
| ≤ 2 | 86 (34.40) |
| > 2 | 164 (65.60) |
| Radiation Response | |
| Tumor Response | |
| Complete Response | 209 (83.060) |
| Partial Response | 41 (16.40) |
| Node Response | |
| Complete Response | 217 (86.80) |
| Partial Response | 33 (13.20) |
| Skin reaction (RTOG Grading) | |
| Grade 0 | 19 (7.60) |
| Grade 1 | 168 (67.20) |
| Grade 2 | 61 (24.40) |
| Grade 3 | 2 (0.80) |
| Mucositis (RTOG Grading) | |
| Grade 1 | 8 (3.20) |
| Grade 2 | 110 (44.00) |
| Grade 3 | 128 (51.20) |
| Grade 4 | 4 (1.60) |

Table 2. The List of Candidate *XPC, XPD, XPG* Genes with Details of PCR and RFLP Procedures Including Primers and Restriction Enzymes and Expected Products of Selected Genes.

| Gene Genotype | rs number | Amino acid/ nucleotide change | Primer Sequence Forward/Reverse | PCR product size | Enzyme / Digestion conditions | Dominant (Wild type) | Heterozygous | Recessive (Mutant) |
|---|---|---|---|---|---|---|---|---|
| XPC A2920C Lys939Gln cd-939 Ex-15 | rs2228001 | Lys939Gln (A>C) | FP:5'-5'-GGA GGT GGA CTC TCT TCT GAT G-3' RP: 5'-TAG ATC CCA GCA GAT GAC C-3' | 765 bp | PvuII 37°C for 16h | 765 bp | 765 bp, 585 bp, 180 bp | 585 bp , 180 bp |
| XPD C22541A Arg156Arg cd-156 Ex-6 | rs238406 | Arg156Arg (C>A) | FP:5'-TGG AGT GCT ATG GCA CGA TCT CT-3' RP:5'-CCA TGG GCA TCA AAT TCC TGG GA-3' | 644 bp | TfiI 37°C for 16h | 587 bp, 57 bp | 587 bp, 474 bp, 113 bp, 57 bp | 474 bp, 113 bp, 57 bp |
| XPD A35931C Lys751Gln cd-751 Ex-23 | rs13181 | Lys751Gln (A>C) | FP: 5'- GCC CGC TCT GGA TTA TAC G -3' RP: 5'-CTA TCA TCT CCT GGC CCC C- 3' | 436 bp | PstI 37°C for 16h | 290 bp, 146 bp, | 290 bp, 227 bp, 146 bp, 63 bp | 227 bp, 146 bp, 63 bp |
| XPG C3507G His1104Asp cd-1104 Ex-15 | rs17655 | His1104Asp (C>G) | FP: 5'-GAC CTG CCT CTC AGA ATC ATC-3' RP: 5'-CCT CGC ACG TCT TAG TTT CC-3' | 271 bp | NlaIII 37°C for 16h | 271 bp | 271 bp, 227 bp, 44 bp | 227 bp, 44 bp |

we observed 187 (74.80%) patients with grade ≤1 skin reactions as compared to 25.20% patients with >1 (grade 2 and 3) severe skin reactions. There was no signal of grade 4 toxicity of skin reaction and more than grade 4 of oral mucositis observed during follow up of patients treated with radiotherapy.

*Genotype distribution of XPC, XPD and XPG gene SNPs and radiotherapy toxicity in HNC patients*

The univariate analysis of genotype polymorphism of *XPC*, *XPD* and *XPG* genes and their association with radiation induced acute normal tissue toxicity such as skin dermatitis and oral mucositis are presented in Table 3. Based on severity of radiation toxicity we grouped patient normal tissue toxicity as grade ≤1 or >1 for skin reactions and grade ≤2 or >2 for oral mucositis as prescribed by RTOG criteria. Out of 250 patients treated with radiotherapy 63 patients showed grade >1 normal skin reactions such as bright erythema, moderate edema, patchy moist desquamation, increased pain, secretion of yellow exudates, itching and tightening of skin, and 187 patients reported grade ≤1 skin reactions including faint erythema, itching and tightening of skin. A total of 132 patients showed grade >2 oral mucositis reactions including severe pain, fibrinous mucositis, ulceration, hemorrhage and necrosis whereas 118 patients with grade ≤ 2 mucositis reactions with irritation, patchy mucositis, serosanguinitis discharge with moderate pain in response to radiation exposure. When we studied rs2228001 SNP of *XPC*, rs238406, rs13181 SNPs of *XPD* and rs17655 SNP of *XPG*, the results of univariate analysis showed that none of the variant genotypes of SNPs showed association with radiotherapy induced toxicity effects on normal tissue reactions. When we analysed polymorphism of *XPC*

Table 3. Univariate Analysis of Candidate SNPs of *XPC, XPD, XPG* Genes and Radiation Induced Skin Reactions and Mucositis in Head and Neck Cancer Patients

| Gene Name (SNP) | Genotype/ Allele | Skin reaction ≤1 n=187 | Skin reaction >1 n=63 | OR 95% CI | p value | Oral mucositis ≤2 n=118 | Oral mucositis >2 n=132 | Odds Ratio (OR) | p value |
|---|---|---|---|---|---|---|---|---|---|
| *XPC* | A/A | 89 | 39 | 1 (Reference) | | 57 | 71 | 1(Reference) | |
| A2920C | A/C | 83 | 22 | 0.60 (0.33-1.10) | 0.101 | 54 | 51 | 0.75 (0.45-1.27) | 0.294 |
| rs2228001 | C/C | 15 | 2 | 0.30 (0.06-1.39) | 0.125 | 7 | 10 | 1.14 (0.41-3.20) | 0.793 |
| | A allele | 261 | 100 | 1 (Reference) | | 168 | 193 | 1 (Reference) | |
| | C Allele | 113 | 26 | 0.60 (0.36-0.97) | 0.039* | 68 | 71 | 0.90 (0.61-1.34) | 0.632 |
| *XPD* C22541A | C/C | 45 | 15 | 1 (Reference) | | 28 | 32 | 1(Reference) | |
| rs238406 | C/A | 118 | 36 | 0.91 (0.45-1.83) | 0.802 | 77 | 77 | 0.87 (0.48-1.59) | 0.661 |
| | A/A | 24 | 12 | 1.50 (0.60-3.71) | 0.08 | 13 | 23 | 1.54 (0.66-3.61) | 0.312 |
| | C Allele | 208 | 66 | 1 (Reference) | | 133 | 141 | 1 (Reference) | |
| | A Allele | 166 | 61 | 1.15 (0.77-1.73) | 0.475 | 103 | 123 | 1.12 (0.79-1.60) | 0.508 |
| *XPD* | A/A | 85 | 25 | 1 (Reference) | | 55 | 55 | 1(Reference) | |
| A35931C | A/C | 72 | 30 | 1.41 (0.76-2.62) | 0.268 | 46 | 56 | 1.21 (0.70-2.08) | 0.475 |
| rs13181 | C/C | 30 | 8 | 0.90 (0.36-2.22) | 0.83 | 17 | 21 | 1.23 (0.58-2.59) | 0.576 |
| | A allele | 242 | 80 | 1 (Reference) | | 156 | 166 | 1 (Reference) | |
| | C Allele | 132 | 46 | 1.05 (0.69-1.60) | 0.805 | 80 | 98 | 1.15 (0.79-1.66) | 0.452 |
| *XPG* | C/C | 95 | 35 | 1 (Reference) | | 55 | 75 | 1 (Reference) | |
| C3507G | C/G | 78 | 21 | 1.07 (0.61-1.90) | 0.793 | 53 | 46 | 0.63 (0.37-1.07) | 0.092 |
| rs17655 | G/G | 14 | 7 | 1.35 (0.50-3.64) | 0.541 | 10 | 11 | 0.80 (0.32-2.03) | 0.648 |
| | C allele | 268 | 91 | 1 (Reference) | | 163 | 196 | 1 (Reference) | |
| | G Allele | 106 | 35 | 0.97 (0.62-1.52) | 0.903 | 73 | 68 | 0.77 (0.52-1.14) | 0.199 |

SNP, Single nucleotide polymorphism; OR, Odds ratio, CI, Confidential interval; Significance p< 0.05; *, Indicates significant Odds Ratio (p<0.05), p value determined based on $\chi 2$

gene at 939 Lys/Gln of exon 15, we observed significant decrease of variant 'C' allele in patients with skin reactions with grade >1 (OR=0.60, 95% CI: 0.36-0.97; p=0.039) which indicated negative association with protective effects of C allele in developing severe toxicity reactions in normal skin of the patients exposed to radiotherapy. When the polymorphism of *XPD* codon 156 (exon-6) and codon 751 (exon-23) were analyzed, there was no statistically significant difference between homozygous recessive or heterozygous genotypes with either of skin reactions or oral mucositis. Similarly, *XPG* 1104 His/Asp showed no association with any of the toxicity effects in HNC patients in response to radiotherapy treatment.

*Correlation between of clinical factors and effects of radiotherapy*

The relationship between acute normal skin reactions, mucositis and the confounding factors of age, gender, alcohol, tobacco consumption, chemotherapy, radiotherapy, radiation dose were evaluated and represented in Table 4. Out of 250 HNC patients investigated in this study, 63 patients showed acute radiation dermatitis (>1 grade) and 132 patients with oral mucositis (>2 grade). The tobacco smoking habit in HNC patients found to be associated with grade >2 oral mucositis (p=0.002). The radiotherapy with combination of chemotherapy also showed significant association with oral mucositis in HNC patients treated with radiotherapy (p<0.0001). We also observed that 45.45 % patients treated with 66 Gy radiation showed significant correlation with development of oral mucositis (p=0.006). None of the confounding factors showed significant correlation with development of severe skin reaction in HNC patients.

*Association of XPC, XPD, XPG gene polymorphisms with histological tumor grade, tumor and node response towards radiotherapy in HNC patients*

The distribution of SNPs of *XPC* (A2920), *XPD* (C22541A, A35931C) and *XPG* (C3507G) genotypes in 250 HNC patients was studied by logistic regression analysis. The results of univariate analysis carried out to study the association of *XPC*, *XPD* and *XPG* genotypes with histological tumor stage along with histopathological grade were represented in Table 5. The univariate analysis by logistic regression analysis showed that neither of rs2228001 SNP of *XPC* nor rs17655 SNP of *XPG* was associated with advanced tumor stages (T3, T4). Regarding *XPD* (C22541A, A35931) SNPs, no significant difference was found between early or advanced tumor stages and variant genotype distribution (p=0.207; 0.396), where C/C, C/A, A/A genotype of (C22541) and A/A, A/C, C/C genotypes of (A35931C) polymorphisms were found with same frequency in early and advanced tumor stages. When rs238406 and rs13181 SNPs of *XPD* studied for their association with histological tumor grade, the variant C/C genotype of (A35931C) polymorphisms of *XPD* showed significant negative association with the advanced tumor grades (grade III and IV) (OR=0.26, 95% CI: 0.12-0.56; p=0.0007). The association between genotypes of *XPC*, *XPD*, and *XPG* genes and the response to radiotherapy in HNC patients are shown in Table 6. None of the SNPs of *XPC* (rs2228001), *XPD* (rs238406, rs13181) and *XPG*

Table 4. Association of Clinicopathological Factors and the Risk of Radiation Induced Acute Skin Reactions in Head and Neck Cancer Patients

| Confounding factors | Grade ≤1 Skin reaction (n =187) | Grade >1 Skin reaction (n=63) | p-value | Grade ≤2 Mucositis (n =118) | Grade >2 Mucositis (132) | p-value |
|---|---|---|---|---|---|---|
| Age (Years) | | | | | | |
| Mean ± SD) | 55.00 ±13.50 | 55.42 ± 12.28 | | 54.70 ± 13.18 | 55.20 ± 13.90 | |
| Gender | | | | | | |
| Male | 135 | 52 | 0.105 | 86 | 101 | 0.508 |
| Female | 52 | 11 | | 32 | 31 | |
| Alcohol | | | | | | |
| Yes | 82 | 23 | 0.308 | 58 | 47 | 0.030* |
| No | 105 | 40 | | 60 | 85 | |
| Smoking | | | | | | |
| Yes | 160 | 59 | 0.101 | 95 | 124 | 0.002* |
| No | 27 | 4 | | 23 | 8 | |
| Chemotherapy | | | | | | |
| Yes | 134 | 47 | 0.651 | 65 | 116 | <0.0001* |
| No | 53 | 16 | | 53 | 16 | |
| RT + Chemotherapy | | | | | | |
| Yes | 123 | 42 | 0.917 | 60 | 105 | <0.0001* |
| No | 64 | 21 | | 58 | 27 | |
| Irradiation Dose (Gy) | | | | | | |
| 60 Gy | 119 | 37 | 0.486 | 84 | 72 | 0.006* |
| 66 Gy | 68 | 26 | | 34 | 60 | |

Abbreviations, SD, Standard deviation; Gy, Gray; Significance p< 0.05; p value determined based on $\chi 2$

(rs17655) showed significant difference for partial or no response of tumor or nodes. The genotypes of *XPC*, *XPG* or *XPD* showed no association with radiotherapy response in tumor or nodes after three months of evaluation.

## Discussion

Head and neck cancer is a second most common cancer and leading cause of cancer-causing deaths in India. The commonly pursued treatment modality for HNC is surgery followed by chomoradiation or radiotherapy alone. Radiotherapy is an important treatment of HNC given in fractions for improving treatment outcomes. However, radiotherapy causes radiation induced toxic reactions to the normal tissue of patients. It is important to identify genetic determinants of the patients which may be associated with decreasing efficacy of radiotherapy in patients. Numerous studies have been carried out on the genetic variants of various pathway genes including DNA repair genes and their role in cancer development; however limited literature is available on their association with radiotherapy treatment outcomes. In this regard, we tried to explore the role of SNPs of *BER* genes *XRCC1*, *XRCC2, XRCC3* and found no association of either of the gene with the susceptibility towards radiotherapy toxicity in HNC patients of south-western Maharashtra [24]. In continuation with our earlier study on base excision repair (BER) genes, this study was intended to investigate possible association of genetic variants of NER pathway genes including *XPC*, *XPD*, *XPG* genes with risk of developing radiotherapy induced severe normal tissue toxicity in HNC patients. The SNPs of three NER pathway genes; *XPC* (2920 A>C, rs2228001), *XPD* (22541 C>A rs238406; 35931 A>C, rs13181), *XPG* (3507 C>G, rs17655) were studied, which showed no significant association with radiation induced severe skin reactions or mucositis in studied HNC patients.

To the best of our knowledge no published information available about the association of xenoderma pigmentosum group genes (*XPC*, *XPD*, *XPG*) polymorphisms and the increased acute tocxicity caused by radiotherapy in HNC patients. The only studies are available on association of base excision repair genes including *XRCC1* and *XRCC3* SNPs and their significant correlation with increased risk of developing acute normal skin reactions in response to radiotherapy in HNC patients [25-28]. The significant association of polymorphism in NER genes including *XPC*, *XPD* and *XPG* genes with acute toxicity of radiotherapy was reported earlier for bladder [29], lung cancer [19]. Few studies derived negative outcomes with no association of any of the polymorphisms in *XPC* or *XPD* genes with radiosensitivity in breast [20, 21], prostate [22] and lung cancer [23]. The polymorphism of *XPC*, *XPD*, *XPG* gene and their association with radiosensitivity is reported earlier in HNC for *XPC* Lys939Gln (rs2228001) and *XPD* Lys751Gln (rs13181) SNPs [17, 18].

In contrast, our results depicted no association of SNPs of three *XPC* (rs2228001), *XPD* (rs238406; rs13181), and *XPG* (rs17655) with radosensitivity producing adverse

Table 5. Association between Genotypes of *XPC, XPD, XPG* Genes with Tumor Stage and Tumor Grade in Head and Neck Cancer Patients

| Gene Name (SNP) | Genotype | Tumor stage | | OR 95% CI | p value | Histological Grade | | OR 95% CI | p value |
|---|---|---|---|---|---|---|---|---|---|
| | | T1, T2 n=121 | T3, T4 n=129 | | | I, II n=90 | III, IV n=160 | | |
| *XPC* | A/A | 60 | 68 | 1 (Reference) | | 47 | 81 | 1 (Reference) | |
| A2920C | A/C | 56 | 49 | 0.77 (0.46-1.29) | 0.326 | 39 | 66 | 0.98 (0.57-1.67) | 0.946 |
| rs2228001 | C/C | 5 | 12 | 2.11 (0.70-6.35) | 0.181 | 4 | 13 | 1.88 (0.58-6.11) | 0.29 |
| | A/C+C/C | 61 | 61 | 0.88 (0.53-1.44) | 0.621 | 43 | 79 | 1.06 (0.63-1.78) | 0.808 |
| *XPD* C22541A | C/C | 33 | 27 | 1 (Reference) | | 24 | 36 | 1(Reference) | |
| rs238406 | C/A | 73 | 81 | 1.35 (0.74-2.46) | 0.318 | 56 | 98 | 1.16 (0.63-2.15) | 0.621 |
| | A/A | 15 | 21 | 1.71 (0.74-3.94) | 0.207 | 10 | 26 | 1.73 (0.70-4.23) | 0.227 |
| | C/A+A/A | 88 | 102 | 1.41 (0.79-2.53) | 0.241 | 66 | 124 | 1.25 (0.68-2.27) | 0.459 |
| *XPD* | A/A | 52 | 58 | 1 (Reference) | | 34 | 76 | 1(Reference) | |
| A35931C | A/C | 48 | 54 | 1.00 (0.58-1.73) | 0.975 | 32 | 70 | 0.97 (0.54-1.75) | 0.942 |
| rs13181 | C/C | 21 | 17 | 0.72 (0.34-1.52) | 0.396 | 24 | 14 | 0.26 (0.12-0.56) | 0.0007* |
| | A/C+C/C | 69 | 71 | 0.92 (0.55-1.52) | 0.751 | 56 | 84 | 0.67 (0.39-1.13) | 0.138 |
| *XPG* | C/C | 68 | 62 | 1 (Reference) | | 54 | 76 | 1 (Reference) | |
| C3507G | C/G | 46 | 53 | 1.26 (0.74-2.13) | 0.381 | 31 | 68 | 1.55 (0.89-2.70) | 0.113 |
| rs17655 | G/G | 7 | 14 | 2.19 (0.83-5.78) | 0.112 | 5 | 16 | 2.27 (0.78-6.58) | 0.129 |
| | C/G+G/G | 53 | 67 | 1.38 (0.84-2.28 | 0.198 | 36 | 84 | 1.65 (0.98-2.79) | 0.058 |

SNP, Single nucleotide polymorphism; OR, Odds ratio; CI, confidence interval; Significance p< 0.05; *, Indicates significant Odds Ratio (p<0.05); p value determined based on $\chi 2$

Table 6. Association between Genotypes of *XPC, XPD, XPG* Genes with Tumor and Node Response in Head and Neck Cancer Patients Towards Radiotherapy

| Gene Name (SNP) | Genotype | Tumor Response CR n=209 | PR/NR n=41 | Risk Ratio 95% CI | p value | Node Response CR n=217 | PR/NR n=33 | Risk Ratio 95% CI | p value |
|---|---|---|---|---|---|---|---|---|---|
| *XPC* | A/A | 107 | 21 | 1 (Reference) | | 111 | 17 | 1(Reference) | |
| A2920C | A/C | 86 | 19 | 0.12 (0.56-2.22) | 0.733 | 91 | 14 | 1.00 (0.46-2.14) | 0.99 |
| rs2228001 | C/C | 16 | 1 | 0.31 (0.04-2.53) | 0.279 | 15 | 2 | 0.87 (0.18-4.14) | 0.861 |
| | A/C+C/C | 102 | 20 | 0.99 (0.51-1.95) | 0.997 | 106 | 16 | 0.98 (0.47-2.05) | 0.969 |
| *XPD* C22541A | C/C | 47 | 13 | 1 (Reference) | | 54 | 6 | 1(Reference) | |
| rs238406 | C/A | 132 | 22 | 0.60 (0.28-1.29) | 0.192 | 134 | 20 | 1.34 (0.51-3.52) | 0.549 |
| | A/A | 30 | 6 | 0.72 (0.24-2.10) | 0.552 | 29 | 7 | 2.17 (0.66-7.07) | 0.197 |
| | C/A+A/A | 162 | 28 | 0.62 (0.30-1.30) | 0.209 | 163 | 27 | 1.49 (0.58-3.80) | 0.403 |
| *XPD* | A/A | 97 | 13 | 1 (Reference) | | 98 | 12 | 1(Reference) | |
| A35931C | A/C | 80 | 22 | 2.05 (0.97-4.33) | 0.058 | 83 | 19 | 1.86 (0.85-4.07) | 0.115 |
| rs13181 | C/C | 32 | 6 | 1.39 (0.49-3.98) | 0.529 | 36 | 2 | 0.45 (0.09-2.12) | 0.316 |
| | A/C+C/C | 112 | 28 | 1.86 (0.91-3.80) | 0.086 | 119 | 21 | 1.44 (0.67-3.07) | 0.344 |
| *XPG* | C/C | 110 | 20 | 1 (Reference) | | 117 | 13 | 1 (Reference) | |
| C3507G | C/G | 84 | 15 | 0.98 (0.47-2.03) | 0.961 | 84 | 15 | 1.60 (0.72-3.55) | 0.241 |
| rs17655 | G/G | 15 | 6 | 2.20 (0.76-6.34) | 0.144 | 16 | 5 | 2.81 (0.88-8.93) | 0.079 |
| | C/G+G/G | 99 | 21 | 1.16 (0.59-2.27) | 0.652 | 100 | 20 | 1.80 (0.85-3.80) | 0.123 |

SNP, Single nucleotide polymorphism; CR, Complete Response; PR, Partial Response; NR, No Response; RR, Risk ratio, CI, Confidence interval; Significance p< 0.05; *, Indicates significant Odds Ratio (p<0.05), p value determined based on $\chi 2$

toxicity reactions in normal tissue of HNC patients. Only in *XPC* exon-15 (2920 A>C, rs2228001) polymorphism, the C allele had highly significant protective effect (p=0.039) on the risk of developing severe toxicity reactions in normal skin of HNC patients in response to radiotherapy. The detailed analysis of clinical factors including chemotherapy, radiotherapy and radiation dose showed significant association with adverse acute toxicity reactions such as oral mucositis in response to radiotherapy treatment. When we studied polymorphism and their association with tumor stage and histological grade by multivariate analysis, we observed that variant genotype of 35931A>C polymorphism of rs13181 SNP of *XPD* showed significant association with advanced tumor grades (grade III and IV) (p=0.0007). Despite some positive findings, there are limitations in this study

where we are unable to prove association of *XPC*, *XPD* or *XPG* gene polymorphisms with risk of developing clinically significant skin reactions or oral mucositis in the HNC patients treated with radiotherapy from Indian population. This could be because of small sample size and few numbers of SNPs for genotyping.

In conclusion, the results obtained in this study concluded that the SNPs rs2228001of *XPC*, rs238406, rs13181 SNPs of *XPD* and rs17655 SNP of *XPG* are not associated with increased normal tissue toxicity HNC patients treated with radiotherapy. Further investigation of genetic variants and their association with radiotherapy response and its adverse toxicity effects need to be carried out to confirm our findings by using more number of samples and SNPs of genes for genotyping.

*Abbreviations*

HNC: Head and Neck Cancer; RT: Radiotherapy; Gy: Gray; VMAT: Volumetric Modulated Arc Therapy; RECIST: Response Evaluation Criteria in Solid Tumors; RTOG: Radiation Therapy Oncology Group; DNA: Deoxyribose Nucleic acid; BER: Base excision repair; NER: Nucleotide excision repair; DSBs: Double strand breaks; *XPC*: Xeroderma pigmentosum complementation group C; *XPD*: Xeroderma pigmentosum complementation group D; *XPG*: Xeroderma pigmentosum complementation group G; *XRCC1:* X-ray repair cross complementing 1; XRCC2: X-ray repair cross complementing 2; *XRCC3:* X-ray repair cross complementing 3; PCR-RFLP: Polymerase Chain Reaction-Restriction Fragment Length Polymorphism; SNP: Single Nucleotide Polymorphism; OR: Odds Ratio; CI: Confidence Interval

## Author Contribution Statement

Concept: AKG, SJB Design: KDD, SJB, AKG, Experimental Studies: SRK, KDD Clinical studies: AKG, RAG, Data analysis: KDD, AKG, Statistical analysis: KDD, Manuscript preparation: KDD, SJB, AKG, RAG. All authors read and approved the final manuscript.

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The study protocol was approved by protocol committee of Krishna Vishwa Vidyapeeth (Deemed to be University)

*Declaration of Conflict of interest*

The authors declare that they have no competing financial or any other conflict of interests that could have appeared to influence the work reported in this paper.

*Ethics Committee Approval*

The study protocol was approved by Institutional Ethics Committee of Krishna Vishwa Vidyapeeth 'Deemed to be University', Karad.

## References

1. Sathishkumar K, Chaturvedi M, Das P, Stephen S, Mathur P. Cancer incidence estimates for 2022 & projection for 2025: Result from national cancer registry programme, india. Indian J Med Res. 2022;156(4&5):598-607. https://doi.org/10.4103/ijmr.ijmr_1821_22.
2. Sabir M, Baig RM, Mahjabeen I, Kayani MA. Novel germline cdk4 mutations in patients with head and neck cancer. Hered Cancer Clin Pract. 2012;10(1):11. https://doi.org/10.1186/1897-4287-10-11.
3. Mendenhall WM, Hinerman RW, Amdur RJ, Malyapa RS, Lansford CD, Werning JW, et al. Postoperative radiotherapy for squamous cell carcinoma of the head and neck. Clin Med Res. 2006;4(3):200-8. https://doi.org/10.3121/cmr.4.3.200.
4. Anderson G, Ebadi M, Vo K, Novak J, Govindarajan A, Amini A. An updated review on head and neck cancer treatment with radiation therapy. Cancers (Basel). 2021;13(19). https://doi.org/10.3390/cancers13194912.
5. Cox JD, Stetz J, Pajak TF. Toxicity criteria of the radiation therapy oncology group (rtog) and the european organization for research and treatment of cancer (eortc). Int J Radiat Oncol Biol Phys. 1995;31(5):1341-6. https://doi.org/10.1016/0360-3016(95)00060-c.
6. Ghazali N, Shaw RJ, Rogers SN, Risk JM. Genomic determinants of normal tissue toxicity after radiotherapy for head and neck malignancy: A systematic review. Oral Oncol. 2012;48(11):1090-100. https://doi.org/10.1016/j.oraloncology.2012.08.002.
7. McDonald MW, Liu Y, Moore MG, Johnstone PA. Acute toxicity in comprehensive head and neck radiation for nasopharynx and paranasal sinus cancers: Cohort comparison of 3d conformal proton therapy and intensity modulated radiation therapy. Radiat Oncol. 2016;11:32. https://doi.org/10.1186/s13014-016-0600-3.
8. Marteijn JA, Lans H, Vermeulen W, Hoeijmakers JH. Understanding nucleotide excision repair and its roles in cancer and ageing. Nat Rev Mol Cell Biol. 2014;15(7):465-81. https://doi.org/10.1038/nrm3822.
9. Satoh MS, Jones CJ, Wood RD, Lindahl T. DNA excision-repair defect of xeroderma pigmentosum prevents removal of a class of oxygen free radical-induced base lesions. Proc Natl Acad Sci USA. 1993;90(13):6335-9. https://doi.org/10.1073/pnas.90.13.6335.
10. Bourguignon MH, Gisone PA, Perez MR, Michelin S, Dubner D, Giorgio MD, et al. Genetic and epigenetic features in radiation sensitivity. Part ii: Implications for clinical practice and radiation protection. Eur J Nucl Med Mol Imaging. 2005;32(3):351-68. https://doi.org/10.1007/s00259-004-1731-6.
11. Ho AY, Atencio DP, Peters S, Stock RG, Formenti SC, Cesaretti JA, et al. Genetic predictors of adverse radiotherapy effects: The gene-pare project. Int J Radiat Oncol Biol Phys. 2006;65(3):646-55. https://doi.org/10.1016/j.ijrobp.2006.03.006.
12. Barnett GC, West CM, Dunning AM, Elliott RM, Coles CE, Pharoah PD, et al. Normal tissue reactions to radiotherapy: Towards tailoring treatment dose by genotype. Nat Rev Cancer. 2009;9(2):134-42. https://doi.org/10.1038/nrc2587.
13. Goutham HV, Mumbrekar KD, Vadhiraja BM, Fernandes DJ, Sharan K, Kanive Parashiva G, et al. DNA double-strand break analysis by γ-h2ax foci: A useful method for determining the overreactors to radiation-induced acute reactions among head-and-neck cancer patients. Int J Radiat Oncol Biol Phys. 2012;84(5):e607-12. https://doi.org/10.1016/j.ijrobp.2012.06.041.
14. West CM, Dunning AM, Rosenstein BS. Genome-wide

association studies and prediction of normal tissue toxicity. Semin Radiat Oncol. 2012;22(2):91-9. https://doi.org/10.1016/j.semradonc.2011.12.007.
15. Popanda O, Marquardt JU, Chang-Claude J, Schmezer P. Genetic variation in normal tissue toxicity induced by ionizing radiation. Mutat Res. 2009;667(1-2):58-69. https://doi.org/10.1016/j.mrfmmm.2008.10.014.
16. Andreassen CN, Dikomey E, Parliament M, West CM. Will snps be useful predictors of normal tissue radiosensitivity in the future? Radiother Oncol. 2012;105(3):283-8. https://doi.org/10.1016/j.radonc.2012.11.003.
17. Carles J, Monzo M, Amat M, Jansa S, Artells R, Navarro A, et al. Single-nucleotide polymorphisms in base excision repair, nucleotide excision repair, and double strand break genes as markers for response to radiotherapy in patients with stage i to ii head-and-neck cancer. Int J Radiat Oncol Biol Phys. 2006;66(4):1022-30. https://doi.org/10.1016/j.ijrobp.2006.06.029.
18. Lopes-Aguiar L, Costa EF, Nogueira GA, Lima TR, Visacri MB, Pincinato EC, et al. Xpd c.934g>a polymorphism of nucleotide excision repair pathway in outcome of head and neck squamous cell carcinoma patients treated with cisplatin chemoradiation. Oncotarget. 2017;8(10):16190-201. https://doi.org/10.18632/oncotarget.7668.
19. Yang Z, Liu Z. Potential functional variants in DNA repair genes are associated with efficacy and toxicity of radiotherapy in patients with non-small-cell lung cancer. J Oncol. 2020;2020:3132786. https://doi.org/10.1155/2020/3132786.
20. Chang-Claude J, Popanda O, Tan XL, Kropp S, Helmbold I, von Fournier D, et al. Association between polymorphisms in the DNA repair genes, xrcc1, ape1, and xpd and acute side effects of radiotherapy in breast cancer patients. Clin Cancer Res. 2005;11(13):4802-9. https://doi.org/10.1158/1078-0432.Ccr-04-2657.
21. Chang-Claude J, Ambrosone CB, Lilla C, Kropp S, Helmbold I, von Fournier D, et al. Genetic polymorphisms in DNA repair and damage response genes and late normal tissue complications of radiotherapy for breast cancer. Br J Cancer. 2009;100(10):1680-6. https://doi.org/10.1038/sj.bjc.6605036.
22. Gao R, Price DK, Dahut WL, Reed E, Figg WD. Genetic polymorphisms in xrcc1 associated with radiation therapy in prostate cancer. Cancer Biol Ther. 2010;10(1):13-8. https://doi.org/10.4161/cbt.10.1.12172.
23. Du L, Yu W, Dai X, Zhao N, Huang X, Tong F, et al. Association of DNA repair gene polymorphisms with the risk of radiation pneumonitis in lung cancer patients. Oncotarget. 2018;9(1):958-68. https://doi.org/10.18632/oncotarget.22982.
24. Gudur AK, Gudur RA, Bhosale SJ, Kale SR, Datkhile KD. Single nucleotide polymorphisms in DNA repair genes (xrcc1, xrcc2, xrcc3) and their association with radiotherapy toxicity among head and neck cancer patients:A study from south-western maharashtra. Asian Pac J Cancer Prev. 2023;24(9):3049-57. https://doi.org/10.31557/apjcp.2023.24.9.3049.
25. Li H, You Y, Lin C, Zheng M, Hong C, Chen J, et al. Xrcc1 codon 399gln polymorphism is associated with radiotherapy-induced acute dermatitis and mucositis in nasopharyngeal carcinoma patients. Radiat Oncol. 2013;8:31. https://doi.org/10.1186/1748-717x-8-31.
26. Zou Y, Song T, Yu W, Zhao R, Wang Y, Xie R, et al. Xrcc3 polymorphisms are associated with the risk of developing radiation-induced late xerostomia in nasopharyngeal carcinoma patients treated with intensity modulation radiated therapy. Jpn J Clin Oncol. 2014;44(3):241-8. https://doi.org/10.1093/jjco/hyt202.
27. Wang J, Guo C, Gong X, Ao F, Huang Y, Huang L, et al. The impacts of genetic polymorphisms in genes of base excision repair pathway on the efficacy and acute toxicities of (chemo)radiotherapy in patients with nasopharyngeal carcinoma. Oncotarget. 2017;8(45):78633-41. https://doi.org/10.18632/oncotarget.20203.
28. Gupta A, Mathew D, Bhat SA, Ghoshal S, Pal A. Genetic variants of DNA repair genes as predictors of radiation-induced subcutaneous fibrosis in oropharyngeal carcinoma. Front Oncol. 2021;11:652049. https://doi.org/10.3389/fonc.2021.652049.
29. Sakano S, Hinoda Y, Sasaki M, Wada T, Matsumoto H, Eguchi S, et al. Nucleotide excision repair gene polymorphisms may predict acute toxicity in patients treated with chemoradiotherapy for bladder cancer. Pharmacogenomics. 2010;11(10):1377-87. https://doi.org/10.2217/pgs.10.106.