RESEARCH ARTICLE

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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

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Abstract

Background: The genetic polymorphisms in DNA repair genes and their correlation with normal tissue toxicity in response to radiation therapy has not been consistently proven in many of the studies done in head and neck cancers (HNC). This study was intended to investigate the association of most common single nucleotide polymorphisms of DNA repair genes with acute radiation induced toxicities such as skin reactions and oral mucositis in normal tissue from HNC patients receiving radiotherapy from South-Western Maharashtra. **Methods:** Two hundred HNC patients receiving radiotherapy were enrolled in this study and the radiation injuries in the form of skin reactions and oral mucositis were recorded. Three single nucleotide polymorphisms (SNPs) rs1799782, rs25489) rs25487 of *XRCC1* gene, rs3218536in *XRCC2* gene and rs861539 SNP of *XRCC3* gene were studied by PCR-RFLP and direct DNA sequencing. **Results:** The univariate analysis of SNPs of *XRCC1*, *XRCC2* and *XRCC3*, the obtained results verified that *XRCC1* polymorphism at 194Trp of exon 6 (OR=0.69, 95% CI: 0.28-1.71; p=0.433), codon 280 at exon 9 ((OR=1.05, 95% CI: 0.42-2.63; p=0.911) and codon 399 of at exon 10(OR=1.06, 95% CI: 0.52-2.15; p=0.867) and *XRCC2* polymorphism at codon 188 at exon 3 (OR=1.07, 95% CI: 0.46-2.47; p=0.866) and 241Met variant genotype of *XRCC3* (OR=2.63 95% CI: 0.42-16.30; p=0.298) showed no association with degree of radiotherapy associated dermatitis or mucositis in HNC patients. **Conclusion:** The findings from this study postulated that none of rs1799782, rs25489, rs25487 SNPs of *XRCC1*, rs3218536 SNP of *XRCC2* nor rs861539 SNP of *XRCC3* were associated with increased toxicity of radiotherapy in HNC patients of south-western Maharashtra.

Keywords: Head and Neck cancer- *XRCC1- XRCC2- XRCC3* SNP- genetic polymorphism PCR-RFLP

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Introduction

Head and neck cancer (HNC) is a leading cancer in many countries of the world and accounting for approximately 900, 000 cases and over 400, 000 deaths annually (Global Cancer Observatory 2021). HNC increased significantly worldwide in past few decades and represents the leading cause of cancer related deaths in men as well as women in Asian countries like China, Pakistan, Thailand and India (Chaturvedi et al., 2013). HNC includes the most common neoplasms of the oral cavity, oropharynx, larynx, hypopharynx, paranasal sinuses, salivary glands and constitute a major public health problem (Mehanna et al., 2010). HNC is observed as a multifactorial disease which affects people from the middle or low income group especially in developing countries like India and found to be frequently associated with environmental, lifestyle and genetic factors. It is generally assumed that among the major documented risk factors associated with HNC aretobacco in various forms, heavy alcohol consumption followed by poor oral health or exposure to environmental carcinogens (Marcu et al., 2009; HariRam et al., 2011). Meanwhile, genetic factors are found to be responsible for determining host susceptibility towards developing HNC along with combination of lifestyle and environmental factors. The individual's genetic susceptibility plays an important role in HNC carcinogenesis by regulating the genes of cell cycle or genes involved in DNA repair mechanisms (Sabir et al., 2013). The mainstay treatment of HNC is either surgery followed by adjuvant radiation therapy (aRT) or concurrent chemoradiation (CRT) (Anderson et al., 2021).

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Radiation therapy and systemic chemotherapy are known to cause significant acute or late toxicity to normal cells in the vicinity (McDonald et al., 2016). Although radiation therapy is a commonly used modality in treatment of HNC, but the normal tissues around the target area are certainly exposed to radiation and result into a spectrum of normal tissue toxicity. These toxicities may be acute (mucositis, dysphagia and dermatitis) due to damage to cells with rapid cell division rate and late radiotoxicity (subcutaneous skin fibrosis, osteoradionecrosis) due to toxicity to slow dividing cells (Bentzen et al., 2003;Ghazali et al., 2012). This became a hotspot area of the research to understand the factors and mechanisms associated with radiation induced toxicity caused by radiotherapy.

In this regard, numerous DNA repair pathways like the homologous recombination (HR), non-homologous end-joining (NHEJ), nucleotide excision repair (NER) and base excision repair (BER) are involved in maintaining the genomic stability and integrity (Hoeijmakers 2001; Jorgensen 2009). Single nucleotide polymorphisms (SNPs) in DNA repair genes like X-ray repair cross complementing (XRCC) genes are found to influence the radiation induced adverse effects which may interfere with an individual's DNA repair capacity (Ghazali et al., 2012; Liu et al., 2015) and associated with intrinsic radiosensitivity. X-ray repair cross-complementing group 1 (*XRCC1*) was the first human gene identified in the BER pathway, and cells lacking this gene product can be overly sensitive to ionizing radiation (Churchill et al., 1991; Popanda et al., 2009). X-ray repair cross-complementing group 2 (*XRCC2*) and *XRCC3*, participate in homologous recombination (HR) repair for DNA double strand breaks (DSBs) (Araujo 2002; Thacker and Zdzienicka 2004) and play an important role in DNA damage repair and maintaining chromosome stability. In recent years, evidences support the hypothesis that the risk of radiotoxicity correlates with genetic susceptibility where SNPs account for most of the known genetic variations (Liu et al., 2015; Song et al., 2015; Zhao et al., 2018; Gupta et al., 2019;Yang et al., 2020;Gupta et al., 2021). However, studies linking association of DNA repair gene polymorphisms and clinically relevant radiation induced toxicity are rare. The polymorphisms in DNA repair genes including *XRCC1* and *XRCC3* genes are correlated with either adverse effects (Alsbeih et al., 2010; Langsenlehner et al., 2011) or protective effects (Chang-Claudeet al., 2005;Song et al., 2015) resulted from radiation therapy in several cancers, but limited information is accessible on head and neck cancer. It was evidenced that the SNPs of *XRCC1* and *XRCC2* has a potential in predicting radiation responses for HNC, gastrointestinal and Non Small Cell Lung cancer patients (Liu et al., 2015; Zhao et al., 2018; Gupta et al., 2019; Yang and Liu 2020) but the association between other SNPs of *XRCC1* and *XRCC2* and the risk of radiation-induced adverse effects on normal tissue remained controversial in other reports (Langsenlehner et al., 2011;Xie et al., 2012;Li et al., 2013; Wang et al., 2017). It is further predicted that polymorphisms of *XRCC2* and *XRCC3* may be positively associated with radiation hypersensitivity in variety of cancers (Zou et al., 2014; Oliva et al., 2018; Gupta et al., 2021; Goricar et al., 2022) but other studies reported opposite results where SNPs of *XRCC2* and *XRCC3* showed no association with side effects of radiotherapy in other cancers (Popanda et al., 2006). Studies conducted on different ethnic population stated contradictory opinion on the correlation of genetic variations and post radiotherapy complications; however some of the common genetic variants were overlooked. Therefore, this study was intended to investigate the association of three most common single nucleotide polymorphisms of *XRCC1* Arg194Trp (exon 6), Arg280His (Exon 9) and Arg 399 Gln (exon 10) *XRCC2*, *XRCC3* and its predictive potential with normal tissue adverse reactions in HNC patients of South-Western Maharashtra undergoing chemo-radiotherapy or radiotherapy alone. In addition we explored the correlation between SNPs of *XRCC1*, *XRCC2 XRCC3* genes and their association with acute radiation induced toxicities such as skin reactions and oral mucositis from HNC patients receiving radiotherapy.

Materials and Methods

Patients

Two hundred patients dignosed with HNC and initially treated at the Department of Oncology of Krishna Hospital & Medical Research Center, Karad were enrolled based on predefined inclusion and exclusion criteria. The inclusion critera were; patients with 18 to 85 years age diagnosed with HNC on histopathology , no metastasis at diagnosis, clinically localised or locally advancedaccording tostandard staging system, and normal skin and oral mucosa before the first radiotherapy fraction. The exclusion criteria were no pathological diagnosis; relapsed disease or metastasis; severe co-morbidities; dermatoses or auto immune disease; incomplete treatment taken; incomplete follow-up; or missing or incomplete data. The study enrolled patients were conveyed about their participation in the protocol. After obtaining written informed consent from them , the clinical details with examination findings and relevant reports were noted down in the proforma.The study protocol was approved by Institutional Ethics Committee. The information of all the patients was recorded and followed up for 3 months after radiotherapy.

Clinical data

Detailed information of HNC patient characteristics including clinicopathological record and data on demographic factors, carcinogen exposure were collected and details of are described in table 2. The clinical and radiological responses are documented as per Response Evaluation Criteria in Solid Tumors (RECIST) criteria at planned initial and end of treatment assessment.After giving radiation therapy patients are followed up at regular prespecified intervals for three months to assess for the clinical response such as complete response, partial response, stable disease, progressive disease, early death from disease or toxicity or any other cause.

Radiotherapy & Chemoradiotherapy Regimen

All patients treated using 3DCRT or Intensity

modulated radiation therapy (IMRT). Gross tumor volume (GTV), Clinical Target Volume (CTV) and Planning target volume (PTV) were defined by using computed tomography (CT) positioning. Gross tumor volume included all known gross disease as defined by clinical, physical examination and imaging findings. Patients were treated using Linear accelerator (Model: Unique Performance, Make: Varian Medical System, USA) 6-Mega Volt (MV) (X-ray) with the total radiotherapy dose of 60- 66 Gy (2 Gy per fractions for 5 days a week) with 3D-CRT, IMRT or VMAT techniques. Patients after surgical resection having positive margins were given a dose of 66 Gy in 33 fractions. Patients with no positive margins were given 60Gy 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.

Evaluation of adverse radiation effects

Acute adverse effects (oral mucositis and skin reaction) were recorded during and after completion of therapy according to Radiation Therapy Oncology Group (RTOG) criteria and the association between single nucleotide polymorphisms in *XRCC1*, *XRCC2 XRCC3* genes and adverse radiotoxicity reactions were evaluated for the increased risk of developing these normal tissue adverse reactions. Acute radiation injury is defined as injury appearing from the initial day of radiotherapy until 3 months after the end of radiotherapy. In the process of patients' radiotherapy, the radiation injuries to the skin and oral mucosa were observed and recorded weekly. At the 1st and 3rd months after radiotherapy, the evaluation was performed again. Skin over face and neck was selected as the observing area in patients with acute radiation dermatitis. The severity of acute radiation injury was determined by radiation oncologist and severity of oral mucositis was graded according the RTOG grading system.

Genomic DNA isolation from blood

Five milliliter (mL) of whole blood from patients was collected in sterile purple top vacutainer after receiving informed consent. Genomic DNA extraction was carried out from the peripheral blood sample using HipurA®Blood genomic DNA miniprep purification kit. (Cat no. MB504-250PR) (HiMedia Laboratories) following the manufacturer's instructions. After the quantitative and qualitative analysis of genomic DNA was used for genotyping analysis.

Genotyping assays

Genotyping of *XRCC1*, *XRCC2*, *XRCC3* genes was performed by PCR-RFLP and direct DNA sequencing methods with appropriate primer sets presented in table 1. The PCR amplification were carried out separately under different conditions in 20 micro liter (µL) reaction mixtures containing 1X PCR buffer (10 mili molar (mM) Tris-HCl (pH 9.0), 50 mM KCl 1.5 mM MgCl2, 0.01% gelatin), 0.2 mM each dNTP, 10 picomole (pmol) of each primer listed in Table-1, 1U Taq DNA polymerase (GeNei, Merck Bioscience) and 100 nanogram (ng) of purified genomic DNA template. The reaction mixtures subjected to PCR amplification with a Master Cycler Gradient PCR (Eppendorf). After performing PCR programme for each of the reaction, the PCR products were analyzed by agarose gel electrophoresis in Tris-Acetate-EDTA (TAE) buffer. The agarose gels stained with ethidium bromide (10 mg/mL) and visualized under UV Transilluminator and photographed in gel documentation system (BioRad Laboratories). After confirmation of DNA amplification, each PCR product was digested with an appropriate restriction enzyme for genotypingTen micro liters of the PCR products digested at 37°C overnight with specific restriction enzymes in 20 µL reaction mixtures containing buffer supplied with each restriction enzyme (Table 1). After the overnight incubation, digestion products were separated on a 2-3% low EEO agarose (GeNei) gel at 100 V for 30 min stained with ethidium bromide and photographed with Gel Documentation System (BioRad).

Statistical Analysis

The genotypic frequencies for the DNA repair genes (*XRCC1*, *XRCC2*and *XRCC3*) in the patient's were determined. The Odds Ratio (OR) and corresponding 95% confidence intervals (CI) were determined through unconditional multiple logistic regression. OR estimated to test whether any association exists between the grade of acute toxicity and selected SNPs. The event of the occurrence of clinical radiosensitivity defined as skin reactions and oral mucositis scored as grade ≥1.Statistical analysis was carried out using SPSS 11 Software.

Results

Demographic and Clinical characteristics of study population

Two hundred patients were enrolled in the study. There were 146 males and 54 females. The age range was 22 to 85 years, with a median age of 55 years. The distribution of patients based on clinical characteristics, demographic information, histopathological grading and toxicity grades are presented in Table 2.Radiation doses planned were 60 Gy given in 30 fractions in the adjuvant setting and 66 Gy in 33 fractions in the curative setting. Median number of weekly chemotherapy cycles was 5. A total of 148 patients underwent chemo-radiotherapy, while the remaining 52 were given radiotherapy alone. Primary site of disease was oropharynx (41.0%), hypopharynx (23.0%), oral cavity (20.0%), hypopharynx (7.0 %), larynx (8.0%) and metastasis of unknown origin to neck (6.0%). 40.5% had stage IV (locally advanced), 17% had stage III, 16.0 % were stage II and 20.5 % stage I disease (Table 2). Out of 200 patients, 97 (48.5%) patients experienced mucositis (grade ≤1) and 51.5% patients experienced grade >1mucositis (grade 2 and 3) and 79.5% experienced skin reactions (grade ≤1) where as only 20.5 % patients showed >1 severe skin reactions.

Correlation between of confounding factors and effects of radiotherapy

The association of effects of confounding factors including age of cancer occurrence, alcohol drinking

and tobacco smoking habits as well as chemotherapy in relation with toxicity effects of radiotherapy with different grades of skin reactions and mucositis were represented in Table 3. When we analyzed the presence of confounding factors like tobacco and alcohol we found that patients with tobacco smoking habit (92.68%) was associated with skin reactions >1 grade than alcohol consumption (34.14). We also observed that 93.20 % patients with tobacco habit was associated with grade >1 mucositis (p = 0.04)

Table 2. Demographic and Clinical Details of Head and Neck Cancer Patients

Patient Clinical details		Number/Percentage (%)
Total number of patients		200
Mean age		55.43 (22-85) Median;55
Sex	Males	146 (73.0)
	Females	54 (27.0)
Smoking/Tobacco chewing	Smokers	161 (80.5)
	Non-Smokers	39 (19.50)
Alcohol Consumption	Drinkers	87 (43.5)
	Non-Drinkers	113 (56.5)
Diet	Mixed	154 (77.0)
	Vegeterian	46 (23.0)
Education	High School	130 (65.0)
	Graduate	70 (35.0)
Economic status	Middle	60 (30.0)
	Poor	140 (70.0)
Family history	Yes	24 (12.0)
	No	176 (88.0)
	Hypopharynx	46 (23.0)
	Oropharynx	82 (41.0)
	Nasopharynx	2 (1.0)
Subsites	Larynx	16 (8.0)
	Oral Cavity	40 (20.0)
	Thyroid	2 (1.0)
	Unknown origin of region	12 (6.0)
Treatment	Chemo-radiotherapy	148 (74.0)
	Radiotherapy alone	52 (26.0)
Radiation Response	CR	161(80.5)
	PR	31 (15.5)
	No Response	8 (4.0)
Tumor Staging	T1	41(20.5)
	T2	32 (16.0)
	T3	34 (17.0)
	T4	81 (40.5)
	Tx	12 (6.0)
Skin reaction (RTOG Grading)	Grade 0	17 (8.5)
	Grade 1	142 (71.0)
	Grade 2	39 (19.5)
	Grade 3	2 (1.0)
	Grade 4	0
Mucositis (RTOG Grading)	Grade 0	8 (4.0)
	Grade 1	89 (44.5)
	Grade 2	102 (51.0)
	Grade 3	1 (0.5)

Table 1. The List of Candidate Genes Selected in the Present Study 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)
XRCC1 codon-194 exon-6 (C26304T)	rs1799782	Arg194Trp (C>T)	FP:5'-GCCAGGCCCCTCCTTCAA-3' RP:5'-TACCCTCAGACCCACGAGT-3'	485 bp	PvuII 37°C for 16h	485 bp	485bp, 396bp, 89bp	396bp, 89 bp
XRCC1 codon-280 exon-9 (G27466A)	rs25489	Arg280His (G>A)	FP:5'-CCA GCT CCA ACT CGT ACC-3'; RP: 5' ATG AGG TGC GTG CTG TCC-3'	257bp	RsaI 37°C for 16h	241bp	NIL	257bp
XRCC1 codon-399 exon-10 (G28152A)	rs25487	Arg399Gln (G>A)	FP:5'-CAGTGGTGCTAACCTAATC-3' RP:5'-AGTAGT CTGCTGGCTC TGG-3	871 bp	NciI 37°C for 16h	461bp, 278bp, 132bp	593bp, 461bp, 278bp, 132bp	593bp, 278bp
XRCC2 codon-188 exon-3 (G31479A)	rs3218536	Arg188His (G>A)	FP: 5'- AGT TGC TGC CAT GCC TTA CA -3' RP:5'-TGTAGTCACCCATCTCTCTGC-3'	290 bp	HphI 37°C for 16h	290bp	290bp, 148bp, 142bp	148bp, 142bp
XRCC3 codon-241 exon-7 (C18067T)	rs861539	Thr241Met (C>T)	FP: 5'-GGTCGAGTGACAGTCCAAAC-3' RP:5'-TGCAACGGC TGAGGGTCTT- 3'	455 bp	NlaII 37°C for 16h	315bp, 140bp	315bp, 210bp, 140bp, 105bp	210bp, 140bp, 105bp

Table 3. Effects of Confounding Factors on Set of Samples Analyzed

Confounding factors	Grade ≤1 Skin reaction (n =159	Grade >1 Skin reaction (n=41	p-value	Grade ≤1 Mucositis (n =97	Grade >1 Mucositis n=103	p-value
Age	55.35 ±13.95	55.73 ± 12.34	0.873	55.15 ± 12.75	55.68 ± 14.42	0.782
Gender	113 M	33 M	<0.0001	69 M	77 M	0.366
	46 F	8 F		28 F	26 F	
Alcohol	74	14	<0.0001	48	40	0.401
Smoking	136	38	<0.0001	77	96	0.043
Chemotherapy	111	27	<0.0001	52	88	0.0001
Radiotherapy & Chemotherapy	111	27	<0.0001	54	88	0.0003
Dose in Gray	60 Gy=99 66Gy=60	60 Gy=27 66Gy=22	0.293	60 Gy=70 66Gy=27	60 Gy=52 66Gy=50	0.435
Radiation Response	CR=129 PR=23	CR=32 PR=8	<0.0001	CR= 76 PR=18	CR=87 PR=13	0.549

CR: Complete Response; PR, Partial Response; Gy, Gray

(Table 3). Patients tolerate Grade 1 toxicities without interventions, whereas toxicities >1 require therapeutic interventions. Based on this, and considering reports from earlier studies, we grouped the patient normal tissue toxicity data as grade ≤1 or >1.Out of 200 patients with HNC included in the study, 41 patients showed acute radiation dermatitis (>1 grade) and103 patients with oral mucositis (>1 grade).

Distribution of XRCC1, XRCC2, XRCC3SNPs and radiotherapy toxicity in HNC patients

The univariate analysis showed that none of the polymorphisms presented any significant association to skin reaction as well as mucositis (Table 4).All the genotype distributions for *XRCC1*, *XRCC2* and *XRCC3* were found to deviate from Hardy- Weinberg equilibrium. Out of 200 patients, 41 patients represented grade >1 skin reactions and 103 patients reported grade >1 oral mucositis in response to radiotherapy. Univariate analysis for depicting association of *XRCC1*, *XRCC2*, XRCC3, polymorphisms showed that none of the SNPs studied in the current study presented any significant association tothe degree of any dermatitis or mucositis after radiotherapy. When we studied, *XRCC1* polymorphism at 194Trp of exon 6, we noted wild type (Arg) genotype in 76.73 % of patients who demonstrated dermatitis of grade≤1 dermatitis and 20.75%of patients with>1 dermatitis whereas 23.27% of patients had polymorphic heterozygous Arg194Trp genotype and showed≤1 skin reactions. Statistically this was not significant and hence any association of these polymorphisms with degree of radiotherapy induced dermatitis could not be proven.(OR=0.69, 95% CI: 0.28-1.71; p=0.433). When polymorphism of *XRCC1* codon 280 at exon 9 was investigated, we observed 83.64% 280Arg and 16.36 280His genotypes in the patients with ≤1 skin reactions

Table 4. Univariate Analysis of Candidate SNPs and Radiation Induced Skin Reactions and Mucositis in Head and Neck Cancer Patients

Gene Name	Genotype	Skin reaction ≤1 n=159	Skin reaction >1 n=41	OR 95% CI	p value	Oral mucositis ≤1 n=97	Oral mucositis >1 n=103	OR 95% CI	p value
XRCC1 (rs1799782)	CC	122	33	1 (Reference)		77	78	1(Reference)	
	CT	37	7	0.69 (0.28-1.71)	0.433	20	24	1.18 (0.60- 2.31)	0.621
	TT	0	1	10.97 (0.43-27.49)	0.145	0	1	2.96 (0.11-73.83)	0.508
XRCC1 (rs25489)	GG	133	34	1 (Reference)		85	82	(Reference)	
	AA	26	7	1.05 (0.42-2.63)	0.911	12	21	1.81 (0.83-3.92)	0.13
XRCC1 rs25487)	GG	71	18	1 (Reference)		47	42	1(Reference)	
	GA	78	21	1.06 (0.52-2.15)	0.867	46	53	1.28 (0.72-2.28)	0.385
	AA	10	2	0.78 (0.15-3.92)	0.772	4	8	2.23 (0.62-7.97)	0.213
XRCC2 rs3218536	GG	126	32	1 (Reference)		80	78	1(Reference)	
	GA	33	9	1.07 (0.46-2.47)	0.866	17	25	1.50 (0.75-3.00)	0.243
	AA	0	0	NC		0	0	NC	
XRCC3 rs861539	CC	154	39	1 (Reference)		92	101	1 (Reference)	
	CT	2	0	0.78 (0.03-16.62)	0.874	2	0	0.18 (0.008-3.84)	0.273
	TT	3	2	2.63 (0.42-16.30)	0.298	3	2	0.60 (0.09-3.71)	0.589

SNP, Single nucleotide polymorphism; OR, Odds ratio, CI, Confidence interval; p value, 0.05; NC, Not calculated

Table 5. Association of *XRCC1, XRCC2, XRCC3*, Polymorphisms with Risk of Skin Reaction after Radiotherapy in Head and Neck Cancer Patients

Gene /SNP	Genotypes	All Patients	Radiosensitive patients	OR 95% CI	p value
	CC	155	33	1 (Reference)	
*XRCC1*rs1799782	CT	44	7	0.74 (0.30-1.80)	0.517
	TT	1	1	4.69 (0.28-77.02)	0.278
*XRCC1*rs25489	GG	167	34	1 (Reference)	
	AA	33	7	1.04 (0.42-2.55)	0.928
	GG	89	18	1 (Reference)	
*XRCC1*rs25487	GA	99	21	1.04 (0.52-2.09)	0.892
	AA	12	2	0.82 (0.16-4.00)	0.81
	GG	158	32	1 (Reference)	
XRCC2 rs3218536	GA	42	9	1.05 (0.46-2.38)	0.892
	AA	0	0	NC	
	CC	193	39	1 (Reference)	
XRCC3 rs861539	CT	2	0	0.97 (0.04-20.80)	0.989
	TT	5	2	1.97 (0.37-10.57)	0.424

SNP, Single nucleotide polymorphism; OR, Odds ratio, CI, Confidence interval; p value: 0.05; NC, Not calculated

and 82.92% 280Arg and 17.08% 280Arg genotypes in >1 grade dermatitis respectively with (OR=1.05, 95% CI: 0.42-2.63; p=0.911) in current population. For codon 399 of *XRCC1* at exon 10 with Arg>Gln polymorphism, 44.65% were 399Arg genotype, 49.60% (Arg399Gln) genotype for ≤1 skin reactions and 43.90% (99Arg), 51.22% (Arg399Gln) genotypes for >1 grade dermatitis with (OR=1.06, 95% CI: 0.52-2.15; p=0.867) and 6.29% and 4.88% for homozygous 399Gln genotype in grade ≤1 and >1skin reactions respectively with (OR=0.78, 95% CI: 0.15-3.92; p=0.772). Similarly, for codon 188 of *XRCC2* at exon 3 the wild (Arg) genotype frequency was 0.79 and heterozygous (Arg188His) genotype frequency was 0.21 in response to skin reaction with grade ≤1 and the frequency of Arg188 genotype with 0.78 and Arg188His genotype with 0.22 in grade >1skin reactions (OR=1.07, 95% CI: 0.46-2.47; p=0.866). The Odds ratios with 95% confidence intervals of the patients experiencing skin reactions with 241Met variant genotype of *XRCC3* (Thr241Met) was OR=2.63 95% CI: 0.42-16.30; p=0.298, and the heterozygous (Thr241Met) genotype (OR=0.78 95% CI: 0.03-16.62; p=0.874).

Association of XRCC1, XRCC2, XRCC3, polymorphisms with risk of toxic effects of radiotherapy in HNC patients

Univariate analysis showed that none of the polymorphisms studied in the current study presented any significant association to skin reaction in patients of HNC after giving radiotherapy (Table 5). The Odds ratios with 95% confidence intervals of the patients experiencing acute dermatitis with recessive allele of *XRCC1* (rs1799782) OR=4.69 95% CI: 0.28-77.2; p=0.278, *XRCC1* (rs25489) OR=1.04 95% CI: 0.42-2.55; p=0.278, *XRCC1* (rs25487) OR=0.82 95% CI: 0.16-4.00; p=0.810; *XRCC3* (rs861539)

Table 6. Association of *XRCC1, XRCC2, XRCC3*, Polymorphisms with Risk of Mucositis after Radiotherapy in Head and Neck Cancer Patients

Gene /SNP	Genotypes	All Patients	Radiosensitive patients	OR 95% CI	p value
	CC	155	79	1 (Reference)	
*XRCC1*rs1799782	CT	44	24	1.07 (0.60-1.88)	0.814
	TT	1	1	1.96 (0.12-31.78)	0.635
*XRCC1*rs25489	GG	167	82	1 (Reference)	
	AA	33	21	1.29 (0.70-2.37)	0.402
	GG	89	42	1 (Reference)	
*XRCC1*rs25487	GA	99	53	1.13 (0.69-1.86)	0.618
	AA	12	8	1.41(0.53-3.71)	0.483
	GG	158	78	1 (Reference)	
XRCC2 rs3218536	GA	42	25	1.20 (0.68-2.12)	0.516
	AA	0	0	NC	
	CC	193	101	1 (Reference)	
*XRCC3*rs861539	CT	2	0	0.38 (0.01-8.01)	0.535
	TT	5	2	0.76 (0.14-4.00)	0.75

SNP, Single nucleotide polymorphism; OR, Odds ratio, CI, Confidence interval; p value: 0.05; NC, Not calculated

OR=1.97 95% CI: 0.37-10.57; p=0.424. The ORs with 95% CI of heterozygous variant alleles of *XRCC1* (rs1799782) OR=0.74 95% CI: 0.30-1.80; p=0.517, *XRCC1* (rs25487) OR=1.04 95% CI: 0.52-2.09; p=0.892, *XRCC2* (rs3218536) OR=1.05 95% CI: 0.46-2.38; p=0.989, *XRCC3* (rs861539) OR=0.97 95% CI: 0.37-10.57; p=0.424. Univariate analysis for depicting association of *XRCC1*, *XRCC2*, *XRCC3*, polymorphisms showed that none of the polymorphisms studied in the current study presented any significant association to mucositis in HNC patients after giving radiotherapy (Table 6). We found no significant association between the genetic variants of *XRCC1*, *XRCC2*, *XRCC3* and the development of increased toxicity in either univariate or multivariate analysis. It was indicated that OR of patients experiencing oral mucositis (>1) with recessive allele of *XRCC1* (rs1799782) was OR=1.96 95% CI: 0.12-31.78; p=0.635); rs 25489 (OR=1.29 95% CI: 0.70-2.37; p=0.402; rs25487) OR=1.41 95% CI: 0.53-3.71; p=0.483); *XRCC2* (rs3218536) with no recessive variant allele in single patient and *XRCC3* (rs861539) OR=0.76 95% CI: 0.14-4.00; p=0.750).

Discussion

Head and neck cancer is treated either by surgery followed by adjuvant radiation therapy or concurrent chemoradiation. Subcutaneous fibrosis, osteoradionecrosis, oral mucositis and skin reactions including dermatitis are the prominent toxicities induced in normal tissue of HNC patients after exposure to adjuvant radiation therapy or concurrent chemo radiotherapy. Number of factors are involved in increasing radiation induced toxicities such as total radiation dose, dose per fraction, volume irradiated, irradiation site and dose in homogeneity and also additional treatment like concomitant chemotherapy (Straub et al., 2015;Deng et al., 2019). Genetic factors are found to affect the individual's susceptibility towards radiation induced adverse reactions and such an understanding might help in minimizing the aftereffects of radiotherapy. The single nucleotide polymorphisms in genes involved in DNA repair pathway may alter the ability of adjacent cells to repair radiation induced DNA damage ultimately resulting into more severe toxicity. Different ethnic population possesses varied genetic susceptibility which may lead to mixed treatment responses and toxicity profiles in response to various treatment modalities. Therefore, it is necessary to understand the genetic variations which could help to personalize the therapy in order to achieve a safer and improved outcome. Several epidemiological data on single nucleotide polymorphism of different pathway genes depicted the genetic susceptibility towards radiotherapy induced adverse effects. Some studies reported correlation of SNPs in DNA repair genes with radiotherapy response in cancers like rectum (Qin et al., 2015), HNC (Jin et al., 2014) and breast cancer (Lee et al., 2020). However, there remained further opportunity to explore the role of SNPs of DNA repair genes in predicting normal tissue toxicity in response radiotherapy induced adverse effects. Considering the functional influence of radiation responsive genes, we attempted to investigate if there is significant association of any of the important genes involved in DNA repair pathway with normal tissue toxicity such as dermatitis or oral mucositis in radiotherapy treated HNC patients. Our study failed to show any correlation of any of the selected SNPs of DNA repair genes with risk of developing clinically significant skin reactions or oral mucositis in the HNC patients. This is in accordance with the earlier studies which reported that neither of rs1799782, rs25489nor rs25487 of *XRCC1* were associated with adverse side effects of radiotherapy in prostate (Langsenlehner et al., 2011) and breast cancer (Xie et al., 2012) patients. Also other studies reported no association for rs1799782, rs25489,rs25487 SNPs with radiation toxicities in nasopharyngeal carcinoma patients (Li et al., 2013; Wang et al., 2017). Similarly, the polymorphisms in the DNA double strand break repair genes *XRCC2* and *XRCC3* are not associated with side effects of radiotherapy in breast cancer patients (Popanda et al., 2006).There are few studies which have reported the association of genetic polymorphisms in BER pathway genes and DNA double strand break repair genes with normal tissue toxicity in variety of cancers (Alsbeih et al., 2010, Song et al., 2015), including breast (Chang-Claudeet al., 2005) nasopharyneal (Zou et al., 2014)and Non-Small-Cell Lung carcinoma (Yang and Liu 2020). The polymorphism of *XRCC3* noted to have important predictive role in acute skin reactions in breast cancer patients undergoing adjuvant radiotherapy (Oliva et al., 2018' Goricar et al., 2022) Similarly very recently, the rs25487 SNP of *XRCC1*and rs861539 SNP of *XRCC3* presented to play an important predictive role in increased acute and late radio toxicity in oropharyngeal cancer patients (Gupta et al., 2019; 2021). Conversely, our report did not show any such association of SNPs of *XRCC1*, *XRCC2* or *XRCC3* for the risk of developing normal tissue toxicity in HNC patients exposed to radiotherapy treatment. Our results were consistent in accordance with previously reported research in 180 HNC patients from Karnataka state (Venkatesh et al., 2014) indicating no association of any of the *XRCC1* or *XRCC3* SNPs with radiotherapy induced toxicity effects in HNC patients. Moreover, Wang et al reported similar findings with no association of rs1799782, rs25489, rs25487 SNPs of *XRCC1* with radiotherapy induced toxicities in nasopharyngeal carcinoma patients in Chinese population (Wang et al., 2017). Furthermore, no statistically significant correlations were identified between any of the reported SNPs (rs1799782, rs25489, rs25487) and early or late radiotherapy induced toxicity when studied in meta-analysis of forty studies (Zhao et al., 2018).

In conclusion, the results obtained in this study postulated that the SNPs rs1799782, rs25489, rs25487of *XRCC1*, rs3218536 SNP of *XRCC2* and rs861539 SNP of *XRCC3* which theoretically increase the susceptibility towards radiotoxicity are not associated with the increased normal tissue toxicity of radiotherapy in HNC patients of south-western Maharashtra. This is the first study of the kind in this geographic and ethnic background and hence may serve as a benchmark for further evaluations to know clinical correlations of various treatment modalities.

Abbreviations

HNC: Head and Neck Cancer
XRCC:X-ray repair cross complementing
PCR-RFLP: Polymerase Chain Reaction-Restriction Fragment Length Polymorphism
SNP: Single Nucleotide Polymorphism
OR: Odds Ratio
CI: Confidence Interval
DNA: Deoxyribose Nucleic Acid
aRT: Adjuvant Radiation Therapy
CRT: Concurrent Chemoradiation
HR: Homologous Recombination
NHEJ: Non-homologous end-joining
NER: Nucleotide excision repair
BER: Base excision repair
DSBs: Double strand breaks
IMRT: Intensity modulated radiation therapy
GTV: Gross tumor volume
CTV: Clinical Target Volume
PTV: Planning target volume
CT: Computed Tomography
RTOG: Radiation Therapy Oncology Group (RTOG
VRS: Verbal Rating Scales
Gy: Gray

Author Contribution Statement

Concept: AKG, SJB, KDD, RAG, 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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Declaration of competing interest

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

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