RESEARCH ARTICLE

**Editorial Process:** Submission:01/16/2025 Acceptance:07/06/2025

# Association of Tumor Suppressor *TP53* and *TP21* Gene Polymoprhisms with Radiotherapy Induced Adverse Reactions in Head and Neck Cancer Patients

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## Abstract

**Background:** The current study was intended to analyze the genotype distribution of the tumor suppressor genes *TP53* and *TP21*, and to investigate their potential association with acute radiotherapy –induced toxicities, such as skin reactions and mucositis, in normal tissues of head and neck cancer (HNC) patients receiving radiotherapy. **Materials & Methods:** Two hundred and fifty HNC patients undergoing radiotherapy were enrolled in this study and the acute toxicity reactions and radiotherapy response were recorded. The potential association of two single nucleotide polymorphisms (SNPs) (rs1042522, rs28934571) of *TP53* gene and (rs1801270, rs1059234) SNPs of *TP21* gene, with the risk of acute skin toxicity reactions was analyzed using polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) and direct DNA sequencing methods. **Results:** The findings revealed that the homozygous recessive (C/C) genotype of the 72G>C polymorphism in the *TP53* gene exhibited a significantly increased association with acute skin toxicity in (HNC) patients undergoing radiotherapy (OR = 4.32, 95% CI: 1.87–10.01; p = 0.0006, and the heterozygous (G/C) genotype of the same polymorphism demonstrated a robust correlation with acute skin reactions (OR = 9.23, 95% CI: 4.40–19.23; p < 0.0001). The heterozygous variant (G/C) genotype of *TP53* was identified as a significant risk factor for oral mucositis exceeding grade 2 severity in HNC patients receiving radiotherapy, with an odds ratio of 4.15 (95% CI: 2.21–7.76; p < 0.0001). **Conclusion:** The results of genetic polymorphisms of *TP53* and *TP21* genes and their association with radiotherapy induced acute toxicities demonstrated significant association of *TP53*, G72C polymorphism of rs1042522 SNP with both acute radiotherapy induced dermatitis and mucositis.

**Keywords:** Head and neck cancer- Radiotherapy- *TP53*- *TP21*- Genetic polymorphism- acute toxicity

*Asian Pac J Cancer Prev,* **26 (7)**, 2499-2509

## Introduction

Head and Neck cancer (HNC) is the most prevalent cancer in many countries of the world, accounting for approximately 900, 000 cases and over 450, 000 deaths annually [1]. The incidence of HNC has increased significantly worldwide in past few decades and represents a leading cause of cancer-related deaths among both men as well as women in Asian countries like China, Pakistan, Thailand and India [2]. In the Indian subcontinent, HNC has emerged as a major public health burden, representing approximately 30% of all cancers and contributing to 245,811 new cases and 130,139 deaths in 2022 [3-4]. Established risk factors for HNC include the use of tobacco in various forms, excessive alcohol consumption, poor oral hygiene, and exposure to environmental carcinogens [5-6]. Along with, , genetic susceptibility plays a critical role in HNC pathogenesis, involving complex, multistage processes mediated by alterations in DNA repair genes, tumor suppressor genes, oxidative stress-related genes, and epigenetic modifications [7-9]. Radiotherapy (RT) remains a cornerstone of HNC treatment, either as monotherapy or in combination with chemotherapy; however, radiation-induced toxicities in surrounding normal tissues significantly impact patients' quality of life [10-11]. The most common acute radio-toxicities occurred in the HNC patients are oral mucositis, dermatitis and dysphagia, while late toxicities include fibrosis, osteoradionecrosis or xerostomia [12-13]. Emerging evidence highlights the pivotal role of genetic factors in determining individual clinical radiosensitivity and susceptibility to radiation-induced adverse effects [14-15].

Several studies have investigated the role of genetic polymorphisms in tumor suppressor genes in predicting radiosensitivity and the development of normal tissue toxicity following radiotherapy. Polymorphisms such

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as Arg72Pro and Arg249Ser in the *TP53* gene have been extensively associated with an increased risk of various cancers; however, their specific contribution to radiotherapy-induced normal tissue reactions has not been thoroughly elucidated. Nevertheless, emerging evidence suggests that the *TP53* Pro72Arg polymorphism, in conjunction with the *p21* Ser31Ser genotype, may significantly influence the susceptibility to acute radiotherapy-induced toxicities in cancer patients [16-18]. Previous studies have also demonstrated that Arg72Pro SNP (rs1042522) in the *TP53* gene influences radiosensitivity in different cancer patients and contributes to develop adverse effects following radiotherapy in patients with prostate [19], breast [20] gastric [21], lung [22] and head and neck cancers [23-25]. However, the relationship between radiotherapy induced toxicities and genetic polymorphisms in tumor suppressor genes remains controversial which showed no independent effect of *TP53* polymorphisms with the risk of normal tissue toxicity following radiotherapy in breast [26-27] and prostate cancer patients [28-29]. Nevertheless, the combined genotypic effects of *TP53* and *TP21* polymorphisms have been implicated in increased susceptibility to acute radiotherapy induced toxicities in breast cancer patients. In contrast, no significant association of *p21* polymorphisms and radiation-induced toxicity was observed in patients with non-small cell lung carcinoma [30]. Although numerous studies have investigated the role of genetic polymorphisms in radiation-responsive genes, there remains significant scope to further elucidate the association between specific gene variants and radiotherapy-induced normal tissue toxicity, particularly in HNC patients. Therefore, in the present study, we examined the potential association between two single nucleotide polymorphisms (SNPs) of the *TP53* gene Pro72Arg (G>C; rs1042522) and Arg249Ser (G>T; rs28934571) and two SNPs of the *TP21* gene C98A (rs1801270) and C70T (rs1059234) with the risk of developing acute skin toxicity following therapeutic radiotherapy in HNC patients.

## Materials and Methods

*Patient enrollment and Clinical Information*

A total of 250 patients, histopathologically confirmed with HNC and visiting to Medical Oncology Out Patient Department (OPD) for the treatment at the Krishna Institute of Medical Sciences were enrolled in accordance based on predefined inclusion and exclusion criteria. Eligible participants included individuals aged between 25 to 85 years diagnosed with HNC on histopathology and no evidence of metastatic disease at the time of enrollment.

*Treatment of HNC patients with radiotherapy & chemo-radiotherapy*

All HNC patients were treated using three-dimensional conformal radiation therapy (3DCRT) or Intensity modulated radiation therapy (IMRT), based on computed tomography (CT)-guided planning, simulation, verification, and rigorous quality assurance protocols. 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 ranging from 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. Concurrent chemotherapy was added where clinically indicated, using cisplatin at a dose of 40 mg/m2 , administered weekly for up to 6 cycles in combination with radiotherapy.

*Follow up and Toxicity Assessment*

To assess acute normal tissue toxicity, patients were prospectively followed for three months post-radiotherapy to evaluate clinical outcomes, including partial, complete, or no response, disease stability or progression, early mortality, and treatment-related toxicity. The facial and cervical skin regions were designated as target areas for monitoring acute radiation-induced skin reactions. Acute adverse effects, specifically oral mucositis and skin reactions, were documented weekly during radiotherapy at 1 and 3 months post-treatment, according to the Radiation Therapy Oncology Group (RTOG) criteria. Acute radiation toxicity was defined as any injury manifesting from the initiation of radiotherapy up to three months post-treatment. Severity grading of radiation-induced dermatitis and oral mucositis was performed by radiation oncologists using the RTOG scale. Patients presenting with grade >2 skin reactions or mucositis were classified as radiosensitive and compared against patients with grade ≤2 toxicity to investigate associations with polymorphisms in tumor suppressor genes. All patient information was systematically recorded, and participants were monitored for a minimum of three months following therapy. Written informed consent was obtained from all participants prior to enrollment. The study protocol was reviewed and approved by the Institutional Ethics Committee of Krishna Institute of Medical Sciences.

*Blood sample collection and genomic DNA isolation*

Five milliliter (mL) of whole blood from patients was collected in sterile EDTA containing vacutainer after receiving informed consent. The blood sample from patients was collected before initiation of radiotherapy treatment. The genomic DNA extraction was carried out by salting out method where the whole blood was processed with lysis buffer-1 containing 10mM Tris-HCl pH-7.6, 320mM sucrose, 5mM $MgCl_2$, 1% triton X-100, pH 7.6 to lyse RBCs, thereafter the sample was treated with the lysis buffer 2 to lyse out WBCs (10mM Tris- HCl, 11.4mM sodium citrate, 1mM EDTA, 1% SDS, pH-8.0). The sample was further treated with Proteinase K (200µg/µl) to digest the proteins and subsequently RNase A (200µg/µl). The genomic DNA was precipitated by addition of twice the volume of ice cold ethanol and 1/10th volume of 3M Sodium acetate (pH-5.2). The precipitated DNA was aggregated together by centrifugation. The obtained pellet of DNA was then resuspended in T10E1 buffer and was checked on 1% agarose gel for its quality and quantity. This purified DNA was used for further genotyping assays after quantitative and qualitative analysis.

*Genotyping assays of Tumor suppressor (TP53 & TP21) genes*

The genotyping assay of tumor suppressor genes (*p53, p21*), was performed by polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) using appropriate primer set as shown in Table 1. The PCR conditions for amplification of *TP53* (309 bp) codon -72, exon-4, Pro72Arg, G>C) and codon249 of exon-7 with Arg249Ser (G>T) polymorphisms and *TP21* with codon 31 of exon-2, C98A (272bp) and *TP21* at exon 3 (C70T) polymorphisms with 300 bp are depicted in Table 1. 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).

*Restriction Fragment Length Polymorphism*

After confirmation of DNA amplification, each PCR product was digested with an appropriate restriction enzyme for genotyping. Ten 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 (Table1). 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.

*Statistical Analysis*

The genotypic frequencies for tumor suppressor genes in the patient's were determined. The relative risk and odds ratios (OR) with corresponding 95% confidence intervals (CI) were calculated using unconditional multiple logistic regression analysis to assess the association between acute toxicity grades and SNPs. Statistical significance was defined as a p-value less than 0.05. Acute post-radiotherapy adverse events were categorized based on the severity of skin reactions and oral mucositis, with events classified as grade >2 considered clinically significant. The patients with oral mucositis grade >2 are radiosensitive groups (cases) compared with ≤2 grade (controls) for determining their association with polymorphism of different tumor suppressor genes. All statistical analyses were performed using SPSS version 11.0 software.

## Results

*Demographic and Clinical characteristics of study population*

A total of 250 patients, aged 25 to 85 years (median age: 55 years), were enrolled in the study. Of these, 187 were male (74.80%) and 63 were female (25.20%). The majority of patients were tobacco smokers (87.60%) compared to non-smokers (12.40%). When stratified by the primary site of cancer, the distribution was as follows: oropharynx (36.0%), hypopharynx (22.0%), oral cavity (23.60%), nasopharynx (2.80%), larynx (8.0%), and other sites (4.80%). Of the total cohort, 26% underwent radiotherapy alone, while 74% received a combination of radiation therapy and chemotherapy. Regarding tumor size, 65.60% of patients presented with tumors larger than 2 cm, while 34.40% had tumors ≤ 2 cm. Among the 250 patients treated with radiotherapy, 63 (25.20%) exhibited grade >2 (grade 3) severe skin reactions, including intense erythema, moderate edema, patchy moist desquamation, increased pain, yellow exudate secretion, itching, and skin tightening. The remaining 187 (74.80%) patients reported grade ≤2 skin reactions, such as faint erythema, itching, and skin tightening. Additionally, 132 (52.80%) patients exhibited grade >2 (grade 3 or 4) oral mucositis, characterized by severe pain, fibrinous mucositis, ulceration, hemorrhage, and necrosis. In contrast, 118 (47.20%) patients experienced grade ≤2 mucositis, with symptoms including irritation, patchy mucositis, serosanguineous discharge, and moderate pain in response to radiation exposure.

*Genotype Distribution of TP53 and TP21 genes and radiotherapy toxicity in HNC patients*

The tumor suppressor *TP53* and *TP21* were analyzed to assess the association of polymorphisms with genes radiotherapy-induced normal tissue toxicity. Univariate logistic regression analysis of the polymorphisms in *TP53* and *TP21* revealed a significant association between the *TP53* G72C polymorphism (rs1042522 SNP) and both acute skin reactions and mucositis (Table 2). The analysis indicated that the homozygous recessive (C/C) genotype of the *TP53* 72G>C polymorphism was associated with a 4.32-fold increased risk of acute skin reactions (OR=4.32, 95% CI: 1.87–10.01; p=0.0006), while the heterozygous (G/C) genotype was linked to a 9.23-fold increased risk (OR=9.23, 95% CI: 4.40–19.23; p<0.0001) in HNC patients following radiotherapy. Additionally, the univariate analysis indicated that the odds ratio for severe oral mucositis (grade >2) in patients with the recessive allele of the *TP53* gene was 3.54 times higher (95% CI: 1.74–7.14, p=0.0005) (Table 2). The heterozygous (G/C) genotype of *TP53* also showed a 4.15-fold increased risk of severe oral mucositis (OR=4.15, 95% CI: 2.21–7.76; p<0.0001) in HNC patients exposed to radiotherapy. However, no significant association was observed between the rs28934571 SNP of *TP53* and the rs1801270 and rs1059234 SNPs of *TP21* with normal tissue toxicity, specifically regarding skin reactions or mucositis, as recessive and heterozygous genotypes did not show significantly higher frequencies in affected patients.

*Association of TP53 and TP21 gene polymorphisms with risk of toxicity effects of radiotherapy in HNC patients*

The logistic regression analysis in the current study revealed no significant association between the 249G>T polymorphism of *TP53* and the 98C>A and 70C>T polymorphisms of *TP21* with skin reactions in HNC patients following radiotherapy (Table 3). The multivariate analysis produced odds ratios (ORs) with 95% confidence intervals (CIs) for patients experiencing acute dermatitis with the recessive allele of *TP53* (rs28934571), which showed an OR of 1.10 (95% CI: 0.54–2.23; p=0.783), and

Table 2. Univariate Analysis of Polymorphisms of Tumor Suppressor *TP53*, and *TP21* Genes and Radiation Induced Skin Reactions and Mucositis in Head and Neck Cancer Patients

| Gene Name | Genotype | Skin reaction ≤2 n=187 | Skin reaction >2 n=63 | OR 95% CI | p value | Oral mucositis ≤2 n=118 | Oral mucositis >2 n=132 | OR 95% CI | p value |
|---|---|---|---|---|---|---|---|---|---|
| TP53 | G/G | 120 | 13 | 1 (Reference) | | 83 | 50 | 1(Reference) | |
| G72C | 35 | 35 | 35 | 9.23 (4.40-19.33) | <0.0001* | 20 | 50 | 4.15 (2.21-7.76) | <0.0001* |
| (rs1042522) | C/C | 32 | 15 | 4.32 (1.87-10.01) | 0.0006* | 15 | 32 | 3.54 (1.74-7.14) | 0.0005* |
| | G/C +C/C | 67 | 50 | 6.88 (3.49-13.59) | <0.0001* | 35 | 82 | 3.88 (2.29-6.60) | <0.0001* |
| TP53 | G/G | 114 | 43 | 1 (Reference) | | 74 | 83 | 1(Reference) | |
| G249T | G/T | 43 | 7 | 0.43 (0.18-1.03) | 0.059 | 27 | 23 | 0.75 (0.40-1.43) | 0.398 |
| (rs28934571) | T/T | 30 | 13 | 1.14 (0.54-2.40) | 0.713 | 17 | 26 | 0.83 (0.39-1.77) | 0.647 |
| | G/T +T/T | 73 | 20 | 0.72 (0.39-1.33) | 0.301 | 44 | 49 | 0.99 (0.59-1.65) | 0.978 |
| TP21 | C/C | 146 | 50 | 1 (Reference) | | 94 | 102 | 1(Reference) | |
| C98A | C/A | 40 | 12 | 0.87 (0.42-1.80) | 0.718 | 24 | 28 | 1.07 (0.58-1.98) | 0.816 |
| (rs1801270) | A/A | 1 | 1 | 2.92 (0.17-47.55) | 0.451 | 1 | 1 | 0.92 (0.05-14.94) | 0.954 |
| | C/A +A/A | 41 | 13 | 0.92 (0.45-1.86) | 0.829 | 25 | 29 | 1.06 (0.58-1.95) | 0.828 |
| TP21 | C/C | 134 | 48 | 1 (Reference) | | 88 | 94 | 1 (Reference) | |
| C70T | C/T | 47 | 11 | 0.65 (0.31-1.36) | 0.256 | 25 | 33 | 1.23 (0.68-2.24) | 0.486 |
| (rs1059234) | T/T | 6 | 4 | 1.86 (0.50-6.87) | 0.351 | 5 | 5 | 0.93 (0.26-3.34) | 0.919 |
| | C/T +T/T | 53 | 15 | 0.79 (0.40-1.53) | 0.485 | 30 | 38 | 1.18 (0.67-2.07) | 0.55 |

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 1. The List of Candidate Tumor Suppressor Genes (*TP53* and *TP21*) Genes with Details of PCR and RFLP Procedures Including Primers and Restriction Enzymes

| Gene Genotype | rs number | Amino acid/ nucleotide change | Primer Sequence Forward/Reverse | PCR Conditions | PCR product size | Enzyme / Digestion conditions | Dominant (Wild type) | Hetero-zygous | Recessive (Mutant) |
|---|---|---|---|---|---|---|---|---|---|
| *TP53 Codon-72 Exon-4 Pro72Arg* | rs1042522 | (G>C) | FP: 5'-TTC ACC CAT CTA CAG TCC -3' RP: 5'- CTC AGG GCA ACT GAC CGT-3' | 950C- 5 min, 30 cycles of 950C- 30 sec, 520C-30 sec, 720C-30 sec, 720C- 10 min | 309 bp | 1 U of BstuI (Bsh12361) 370C Incubation for 16 hrs | 175bp, 134bp | 309bp, 175bp, 134bp | 309bp |
| *TP53 Codon-249 Exon-7 Arg249Ser* | rs28934571 | (G>T) | FP: 5'-GGC GAC AGA GCG AGATTC CA -3' RP: 5'-GGG TCA GCG GCA AGCAGA GG -3' | 950C- 5 min, 30 cycles of 950C- 20 sec, 600C-20 sec, 720C-20 sec, 720C- 10 min | 286 bp | 1 U of HaeIII (BsuRI) 370C Incubation for 16 hrs | 92bp, 66bp, Small fragments | | 158bp, 92bp, 66bp, Small fragments |
| *TP21 codon-31 exon-2 C98A* | rs1801270 | (C>A) | FP: 5'-GTC AGA ACC GGC TGG GGA TG-3' RP: 5'-CTC CTC CCA ACT CAT CCC GG-3' | 950C- 5 min, 30 cycles of 950C- 30 sec, 580C- 30 sec, 720C-30 sec, 720C- 10 min | 272 bp | 1 U of BpuI (BlpI) 370C Incubation for 16 hrs | 183 bp, 89 bp | 272 bp, 183 bp, 89 bp | 272bp |
| *TP21 exon-3 C70T* | rs1059234 | (C>T) | FP: 5'-CCC AGG GAA GGG TGT CCT-3' RP: 5'-GGG CGG CCA GGG TAT GTA-3' | 950C- 5 min, 30 cycles of 950C- 20 sec, 580C- 20 sec, 720C-20 sec, 720C- 10 min | 300 bp | 1 U of PstI 370C Incubation for 16 hrs | 126bp, 174bp | 300 bp, 174 bp, 126 bp | 300 bp |

Table 3. Association of Polymorphisms ofTumor Suppressor *TP53* and *TP21* Genes 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 |
|---|---|---|---|---|---|
| *TP53* (rs1042522) | G/G | 133 | 13 | 1 (Reference) | |
| | G/C | 70 | 35 | 5.11 (2.54-10.29) | <0.0001* |
| | C/C | 47 | 15 | 3.26 (1.44-7.36) | 0.004* |
| | G/C +C/C | 117 | 50 | 4.37 (2.26-8.44) | <0.0001* |
| *TP53* (rs28934571) | G/G | 157 | 43 | 1 (Reference) | |
| | G/T | 50 | 7 | 0.51 (0.21-1.20) | 0.126 |
| | T/T | 43 | 13 | 1.10 (0.54-2.23) | 0.783 |
| | G/T +T/T | 93 | 20 | 0.78 (0.43-1.41) | 0.421 |
| *TP21* (rs1801270) | C/C | 196 | 50 | 1 (Reference) | |
| | C/A | 52 | 12 | 0.90 (0.44-1.82) | 0.779 |
| | A/A | 2 | 1 | 1.96 (0.17-22.05) | 0.585 |
| | C/A +A/A | 54 | 13 | 0.84 (0.47-1.86) | 0.867 |
| *TP21* (rs1059234) | C/C | 182 | 48 | 1 (Reference) | |
| | C/T | 58 | 11 | 0.71 (0.35-1.47) | 0.368 |
| | T/T | 10 | 4 | 1.51 (0.45-5.04) | 0.497 |
| | C/T +T/T | 68 | 15 | 0.83 (0.43-1.59) | 0.586 |

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 $\chi^2$.

for the heterozygous G/T genotype, which showed an OR of 0.51 (95% CI: 0.21–1.20; p=0.126). Both associations were non-significant with respect to acute skin reactions in HNC patients. The ORs for the heterozygous variant alleles of *TP21* rs1801270 (OR=1.96, 95% CI: 0.17–22.05; p=0.585) and rs1059234 (OR=1.51, 95% CI: 0.45–5.04; p=0.497) also indicated no significant association with skin toxicity following radiotherapy in HNC patients. However, the odds ratio for patients with grade >2 oral mucositis showed a significant increase associated with the recessive (C/C) genotype of *TP53* (rs1042522) with an OR of 3.26 (95% CI: 1.44–7.36; p=0.004) and the heterozygous (G/C) genotype with an OR of 5.11 (95% CI: 2.54–10.29; p<0.0001). The multivariate logistic regression analysis also evaluated the association of tumor suppressor genes, particularly radiosensitive genes, with oral mucositis in HNC patients treated with radiotherapy. The OR for patients experiencing grade >2 oral mucositis with the recessive (C/C) genotype of *TP53* (rs1042522) was 1.81 (95% CI: 1.04–3.15; p=0.035), and

Table 4. Association of Polymorphisms of Tumor Suppressor *TP53* and *P21* Genes with Risk of Mucositis after Radiotherapy in Head and Neck Cancer Patients

| Gene /SNP | Genotypes | All Patients | Radiosensitive patients | OR 95% CI | p value |
|---|---|---|---|---|---|
| *TP53* (rs1042522) | G/G | 133 | 50 | 1 (Reference) | |
| | G/C | 70 | 50 | 1.90 (1.16-3.09) | 0.009* |
| | C/C | 47 | 32 | 1.81 (1.04-3.15) | 0.035* |
| | G/C+ C/C | 117 | 82 | 1.86 (1.21-2.86) | 0.004* |
| *TP53* (rs28934571) | G/G | 157 | 83 | 1 (Reference) | |
| | G/T | 50 | 23 | 0.87 (0.49-1.52) | 0.626 |
| | T/T | 43 | 26 | 1.14 (0.65-1.99) | 0.635 |
| | G/T +T/T | 93 | 49 | 0.99 (0.64-1.54) | 0.987 |
| *TP21* (rs1801270) | C/C | 196 | 102 | 1 (Reference) | |
| | C/A | 52 | 28 | 1.03 (00.61-1.73) | 0.897 |
| | A/A | 2 | 1 | 0.96 (0.08-10.72) | 0.974 |
| | C/A+ A/A | 54 | 29 | 1.03 (0.61-1.71) | 0.903 |
| *TP21* (rs1059234) | C/C | 182 | 94 | 1 (Reference) | |
| | C/T | 58 | 33 | 1.10 (0.67-1.80) | 0.701 |
| | T/T | 10 | 5 | 0.96 (0.32-2.91) | 0.954 |
| | C/T+ T/T | 68 | 38 | 1.08 (0.67-1.72) | 0.741 |

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 $\chi^2$.

for the heterozygous (G/C) genotype, it was 1.90 (95% CI: 1.16–3.09; p=0.009), both indicating significant associations with oral mucositis toxicity following radiotherapy in HNC patients (Table 4). Therefore, no significant association was observed between genetic variants of *TP53* (rs28934571) and *TP21* (rs1801270, rs1059234) and the development of increased acute skin toxicity or oral mucositis reactions after radiotherapy.

*Association of TP53 and TP21 gene polymorphisms with tumor and node response towards radiotherapy in HNC patients*

Univariate logistic regression analysis was performed to evaluate the association between *TP53* (rs1042522, rs28934571) and *TP21* (rs1801270, rs1059234) gene polymorphisms with clinically and histopathologically confirmed tumor grades and tumor response to radiotherapy, as presented in Tables 5 and 6. The univariate analysis revealed that the rs1042522 SNP of the *TP53* gene was not associated with histological tumor grades >III, and the rs28934571 SNP showed no association with either clinical or histopathological tumor grades. However, the recessive (C/C) genotype of *TP53* (rs1042522) was significantly associated with high tumor stage (stage >3), with an odds ratio (OR) of 2.22 (95% CI: 1.12–4.38; p=0.02). Similarly, the heterozygous C/A genotype of *TP53* (rs1801270) was associated with histological tumor grade >3 (OR=2.16, 95% CI: 1.10–4.25; p=0.024), while the rs1059234 SNP of *TP21* did not show any significant association with clinical stage >3 or histopathological grade >III (Table 5). Regarding the relationship between *TP53* and *TP21* polymorphisms and tumor response to radiotherapy, no association was found between the rs1042522 and rs28934571 SNPs of *TP53* and tumor or nodal response to radiotherapy, as shown in Table 6. Furthermore, the C98A polymorphism of *TP21* (rs1801270) showed that neither the recessive nor the heterozygous genotypes were associated with tumor or nodal response in HNC patients following radiotherapy, evaluated three months post-treatment. A significant positive association was observed for the heterozygous (C/T) genotype of the *TP21* (rs1059234) SNP with complete tumor response to radiotherapy (OR=2.19, 95% CI: 1.06–4.51; p=0.033), whereas the recessive T/T genotype was not associated with tumor or nodal response. Additionally, the C70T polymorphism of *TP21*, with the recessive T/T genotype (OR=3.47, 95% CI: 0.83–14.50; p=0.088) and the heterozygous C/T genotype (OR=1.68, 95% CI: 0.73–3.84; p=0.213), was not associated with nodal response in HNC patients to radiotherapy. When HNC patients were stratified based on body mass index (BMI), no significant association was found between any *TP53* or *TP21* gene polymorphisms and the risk of acute radiotherapy toxicity (Table 7).

## Discussion

Radiotherapy is a crucial treatment modality for HNC, typically administered in fractions to enhance treatment efficacy. However, radiotherapy can induce toxic reactions in normal tissues. Prominent toxicities

Table 5. Association between Genotypes of Tumor Suppressor *TP53* and *TP21* 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=134 | T3, T4 n=116 | | | I, II n=103 | III, IV n=147 | | |
| TP53 | G/G | 80 | 53 | 1 (Reference) | | 58 | 75 | 1(Reference) | |
| G72C | G/C | 35 | 35 | 1.50 (0.84-2.70) | 0.166 | 30 | 40 | 1.03 (0.57-1.84) | 0.918 |
| (rs1042522) | C/C | 19 | 28 | 2.22 (1.12-4.38) | 0.020* | 15 | 32 | 1.64 (0.817-3.33) | |
| | G/C+ C/C | 54 | 63 | 0.76 (1.06-2.91) | 0.027* | 45 | 72 | 1.23 (0.74-2.05) | 0.409 |
| TP53 | G/G | 85 | 72 | 1 (Reference) | | 66 | 91 | 1(Reference) | |
| G249T | G/T | 28 | 22 | 0.92 (0.48-1.76) | 0.818 | 20 | 30 | 1.08(0.56-2.08) | 0.799 |
| (rs28934571) | T/T | 21 | 22 | 1.23 (0.62-2.42) | 0.537 | 17 | 26 | 1.10 (0.55-2.20) | 0.767 |
| | G/T+ T/T | 49 | 44 | 1.06 (0.63-1.77) | 0.823 | 37 | 56 | 1.09 (0.65-1.85) | 0.726 |
| TP21 | C/C | 106 | 90 | 1 (Reference) | | 87 | 109 | 1(Reference) | |
| C98A | C/A | 28 | 24 | 1.00 (0.54-1.86) | 0.975 | 14 | 38 | 2.16 (1.10-4.25) | 0.024* |
| (rs1801270) | A/A | 0 | 2 | 5.88 (0.27-124.15) | 0.254 | 2 | 0 | 0.15 (0.007-3.37) | 0.238 |
| | C/A+ A/A | 28 | 26 | 1.09 (0.59-1.99) | 0.771 | 16 | 38 | 1.89 (0.99-3.62) | 0.053 |
| TP21 | C/C | 95 | 87 | 1 (Reference) | | 73 | 109 | 1 (Reference) | |
| C70T | C/T | 33 | 25 | 0.82 (0.45-1.50) | 0.532 | 24 | 34 | 0.94 (0.52-1.73) | 0.863 |
| (rs1059234) | T/T | 6 | 4 | 0.72 (0.19-2.66) | 0.631 | 6 | 4 | 0.44 (0.12-1.63) | 0.223 |
| | C/T+ T/T | 39 | 29 | 0.81 (0.46-1.42) | 0.467 | 30 | 38 | 0.84 (0.48-1.48) | 0.566 |

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 Tumor Suppressor *TP53* and *TP21* Genes with Tumor and Node Response in Head and Neck Cancer Patients towards Radiotherapy

| Gene Name (SNP) | Genotype | Tumor Response | | Risk Ratio (RR) 95% CI | p value | Node Response | | Risk Ratio (RR) 95% CI | p value |
|---|---|---|---|---|---|---|---|---|---|
| | | CR | PR/NR | | | CR | PR/NR | | |
| | | n=209 | n=41 | | | n=217 | n=33 | | |
| *TP53* G72C (rs1042522) | G/G | 108 | 25 | 1 (Reference) | | 113 | 20 | 1(Reference) | |
| | G/C | 61 | 9 | 0.63 (0.27-1.45) | 0.284 | 61 | 9 | 0.83 (0.35-1.94) | 0.673 |
| | C/C | 40 | 7 | 0.75 (0.30-1.88) | 0.548 | 43 | 4 | 0.52 (0.16-1.62) | 0.264 |
| | G/C+ C/C | 101 | 16 | 0.68 (0.34-1.35) | 0.276 | 104 | 13 | 0.70 (0.33-1.49) | 0.361 |
| *TP53* G249T (rs28934571) | G/G | 130 | 27 | 1 (Reference) | | 134 | 23 | 1(Reference) | |
| | G/T | 41 | 9 | 1.05 (0.45-2.42) | 0.896 | 43 | 7 | 0.94 (0.38-2.36) | 0.909 |
| | T/T | 38 | 5 | 0.59 (0.18-1.94) | 0.394 | 40 | 3 | 0.43 (0.12-1.53) | 0.195 |
| | G/T+ T/T | 79 | 14 | 0.80 (0.32-2.02) | 0.647 | 83 | 10 | 0.70 (0.31-1.54) | 0.38 |
| *TP21* C98A (rs1801270) | C/C | 166 | 30 | 1 (Reference) | | 174 | 22 | 1(Reference) | |
| | C/A | 41 | 11 | 1.48 (0.68-3.20) | 0.315 | 42 | 10 | 1.88 (0.82-4.27) | 0.13 |
| | A/A | 2 | 0 | 1.09 (0.05-23.30) | 0.955 | 1 | 1 | 7.90 (0.47-130.99) | 0.148 |
| | C/A+ A/A | 43 | 11 | 1.41 (0.65-3.05) | 0.375 | 43 | 11 | 2.02 (0.91-4.48) | 0.083 |
| *TP21* C70T (rs1059234) | C/C | 157 | 25 | 1 (Reference) | | 162 | 20 | 1 (Reference) | |
| | C/T | 43 | 15 | 2.19 (1.06-4.51) | 0.033* | 48 | 10 | 1.68 (0.732-3.84) | 0.213 |
| | T/T | 9 | 1 | 0.69 (0.08-5.74) | 0.738 | 7 | 3 | 3.47 (0.83-14.50) | 0.088 |
| | C/T+ T/T | 52 | 16 | 1.93 (0.95-3.89) | 0.065 | 55 | 13 | 1.91 (0.89-4.10) | 0.094 |

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 $\chi^2$

Table 7. Association of Tumor Suppressor *TP53* and *TP21* Gene Polymorphisms with Risk of Acute Toxicity Effects of Radiotherapy in Head and Neck Cancer Patients Stratified by BMI

| Gene Name (SNP) | Genotype | Normal Weight (BMI ≤20) | | | | Overweight (BMI ≤20) | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | All Patients | RS patients | OR 95% CI | p value | All Patients | RS patients | OR 95% CI | p value |
| *TP53* G72C (rs1042522) | G/G | 55 | 22 | 1 (Reference) | | 78 | 36 | 1 (Reference) | |
| | G/C | 33 | 10 | 0.75 (0.31-1.79) | 0.528 | 47 | 17 | 0.78 (0.39-1.54) | 0.482 |
| | C/C | 23 | 16 | 0.71 (0.33-1.50) | 0.375 | 33 | 14 | 0.91 (0.43-1.92) | 0.823 |
| | G/C+ C/C | 56 | 26 | 1.16 (0.58-2.28) | 0.667 | 80 | 31 | 0.83 (0.47-1.48) | 0.549 |
| *TP53* G249T (rs28934571) | G/G | 76 | 32 | 1 (Reference) | | 81 | 36 | 1 (Reference) | |
| | G/T | 17 | 9 | 1.25 (0.50-3.11) | 0.62 | 33 | 13 | 0.88 (0.41-1.88) | 0.753 |
| | T/T | 18 | 9 | 1.18 (0.48-2.92) | 0.708 | 25 | 18 | 1.62 (0.78-3.33) | 0.19 |
| | G/T+ T/T | 35 | 18 | 1.22 (0.60-2.46) | 0.576 | 58 | 31 | 1.20 (0.66-2.16) | 0.537 |
| *TP21* C98A (rs1801270) | C/C | 83 | 36 | 1 (Reference) | | 113 | 54 | 1 (Reference) | |
| | C/A | 27 | 12 | 1.02 (0.46-2.24) | 0.951 | 25 | 22 | 1.84 (0.95-3.55) | 0.069 |
| | A/A | 1 | 0 | 0.76 (0.03-19.16) | 0.869 | 1 | 1 | 2.09 (0.12-34.09) | 0.604 |
| | C/A+ A/A | 28 | 12 | 0.98 (0.45-2.15) | 0.976 | 26 | 23 | 1.85 (0.96-3.53) | 0.062 |
| *TP21* C70T (rs1059234) | C/C | 80 | 43 | 1 (Reference) | | 102 | 48 | 1 (Reference) | |
| | C/T | 28 | 18 | 1.19 (0.59-2.40) | 0.615 | 30 | 15 | 1.06 (0.52-2.15) | 0.866 |
| | T/T | 3 | 2 | 1.24 (0.19-7.71) | 0.817 | 7 | 3 | 0.91 (0.22-3.67) | 0.895 |
| | C/T+ T/T | 31 | 20 | 1.20 (0.61-2.35) | 0.595 | 37 | 18 | 1.03 (0.53-1.99) | 0.921 |

RS, Radiosensitive; 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 $\chi^2$

resulting from radiotherapy in HNC patients include subcutaneous fibrosis, oral mucositis, and skin reactions such as dermatitis, particularly following adjuvant radiation therapy or concurrent chemoradiotherapy. Host genetic factors play a significant role in determining an individual's susceptibility to radiation-induced adverse effects, and a better understanding of these factors could help mitigate the long-term consequences of radiotherapy. Single nucleotide polymorphisms (SNPs) in genes involved in the DNA repair pathway may influence the ability of adjacent cells to repair radiation-induced DNA damage, potentially leading to more severe toxicity. Additionally, polymorphisms in tumor suppressor genes may offer insights into their role in the radiation response during treatment. Although extensive research has been conducted on the genetic variants of tumor suppressor genes and their involvement in cancer development, limited literature exists regarding their association with radiotherapy outcomes. Therefore, identifying genetic variations in these genes is essential for personalizing therapy, improving treatment safety and outcomes, and addressing the potential reduction in radiotherapy efficacy in patients. Tumor suppressor proteins, such as *p53* and *p21*, are key regulators of cellular responses and play essential roles in controlling cell growth and apoptosis in response to radiation-induced damage. Upon exposure to ionizing radiation, *p53* is rapidly activated and facilitates G1 phase cell cycle arrest by transactivating the expression of *p21*, thereby inducing cell cycle checkpoint control [31]. Polymorphisms in the *TP53* and *TP21* genes have been associated with increased susceptibility to both acute and chronic radiation-induced toxicities across various cancers, although findings have been inconsistent. In this study, we aimed to investigate the polymorphisms of the tumor suppressor genes *TP53* and *TP21* and their potential association with radiotherapy-induced acute toxicities in HNC patients. Our results demonstrated that the Arg72Pro polymorphism of *p53* was significantly associated with an increased risk of acute radiation-induced toxicities, including dermatitis (OR=4.32, 95% CI: 1.87–10.01; p=0.0006) and mucositis (OR=3.54, 95% CI: 1.74–7.14; p=0.0005) following radiotherapy. These findings are consistent with previous studies that have linked *p53* polymorphisms to adverse toxicity outcomes in HNC [32] and breast cancer [17] patients undergoing radiotherapy, either alone or in combination with chemoradiotherapy. Several studies have also reported an association between the *p53* Arg72Pro polymorphism and the risk of skin toxicities in patients undergoing radiotherapy for breast [26], lung [22], and prostate cancer [29]. In the present study, no significant correlation was found between the *p21* Ser31Arg polymorphism and the risk of acute toxicities, such as dermatitis or mucositis, which is consistent with other studies on *p21* polymorphisms in breast cancer [17, 33]. When examining the clinicopathological features and tumor and nodal response of head and neck cancer (HNC) patients to radiotherapy, as well as their association with *p53* and *p21* gene polymorphisms, our results confirmed that the recessive (C/C) genotype of *TP53* (rs1042522) was associated with a higher tumor stage (p=0.02). Similarly, the heterozygous (C/T) genotype of *TP21* (rs1059234) was significantly associated with a complete tumor response to radiotherapy (p=0.033).

Conclusion: The results obtained from this study evidenced a significant association between Arg72Pro polymorphism of the *TP53* gene with risk of acute radiation-induced toxicities, such as dermatitis and mucositis in HNC patients treated with radiotherapy. Specifically, the results suggest a significant correlation between *TP53* G72C polymorphism (rs1042522 SNP) with both acute dermatitis and mucositis. Further investigation of genetic variants and their association with radiotherapy response and its adverse toxicity effects is required to be out to confirm our findings by using large number of samples and broad range of SNPs for comprehensive genotyping.

## Author Contribution Statement

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

## Acknowledgements

*Funding statement*

Authors are thankful to Indian Council of Medical Research (ICMR) for financial assistance to the research project (Grant No. NCD/Ad-hoc/120/2021-22 dated 22/11/2021.

*Approval*

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

*Ethics Committee Approval*

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

*Declaration of Conflict of interes*t

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.

*Abbreviations*

HNC: Head and neck cancer
*TP53*: Tumor suppressor 53
*TP21* Tumor suppressor 21
SNP: Single nucleotide polymorphism
PCR-RFLP: Polymerase Chain Reaction-Restriction Fragment Length Polymorphism
DNA: Deoxyribose Nucleic Acid
OR: Odds ratio
CI: Confidence interval
RT: Radiotherapy
OPD:Out Patient Department
MV: Mega Volt

VMAT: volumetric modulated arc therapy
RTOG: Radiation Therapy Oncology Group
mL: milliliter
µl: Microliter
EDTA: Ethylenediamdie Tetra acetate
SDS: Sodium dodecyl Sulphate
TAE: Tris-Acetate-EDTA

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