

Role of Oral Microbiota as Predictive Biomarkers for Radiation-Induced Toxicities.

Dr. Prince Prakash¹, Dr. Surjya Shankar Meher², Dr. Rajlaxmi Paikray³, Dr. Ayesha Sitam⁴, Dr. Suparna Ghosh Ray^{5*}

¹PGT 3, Department of Radiation Oncology, Burdwan Medical College and Hospital, West Bengal, India.

²Senior Resident, Department of Pulmonary Medicine, IMS and SUM Hospital, Bhubaneswar, Odisha, India.

³PGT 3, Department of Forensic Medicine and Toxicology, VIMSAR, Burla, Odisha, India.

⁴Senior Resident, Department of Dermatology, AIIMS, Patna, Bihar, India.

⁵Professor Cum HOD, Department of Radiation Oncology, Burdwan Medical College and Hospital.

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Abstract

Background:

Radiotherapy for head and neck cancers commonly causes oral toxicities that can affect treatment tolerance and quality of life. Alterations in oral microbiota may help identify patients at increased risk of these complications.

Methods:

A retrospective observational study was conducted at Burdwan Medical College and Hospital between June 2024 and July 2025. Medical records of 79 patients with histologically confirmed head and neck cancer who completed definitive radiotherapy, with or without concurrent chemotherapy, were reviewed. Demographic characteristics, clinical parameters, treatment details, oral microbiological findings, and radiation-induced toxicities graded according to the Common Terminology Criteria for Adverse Events (CTCAE) version 5.0 were retrieved. Associations between microbial profiles and treatment-related toxicities were analyzed using chi-square test, Student's t-test, and multivariate logistic regression. Statistical significance was considered at $p < 0.05$.

Results:

Among 79 patients, 53.2% developed Grade II–III oral mucositis, while xerostomia and oral candidiasis occurred in 44.3% and 27.8% of patients, respectively. Patients demonstrating higher baseline colonization with *Candida* species and pathogenic Gram-negative bacteria experienced significantly greater rates of severe mucositis and xerostomia ($p < 0.05$). Reduced abundance of commensal *Streptococcus* species was independently associated with increased radiation-induced toxicity. Multivariate analysis identified baseline dysbiosis as an independent predictor of Grade II–III mucositis (adjusted OR 3.42, 95% CI 1.48–7.92, $p = 0.003$).

Conclusion:

Baseline oral microbial dysbiosis was associated with radiation-induced oral toxicities and may help identify patients at increased risk of severe treatment-related complications.

Recommendation:

Prospective multicenter studies incorporating microbiome sequencing and longitudinal microbial profiling are recommended to validate these findings and support the development of microbiota-based preventive strategies.

Keywords: Oral microbiota, radiotherapy, head and neck cancer, oral mucositis, xerostomia, predictive biomarkers, dysbiosis.

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Corresponding Author: Dr. Suparna Ghosh Ray

Email: suparnasupa@rediffmail.com

Professor Cum HOD, Department of Radiation Oncology, Burdwan Medical College and Hospital.

Introduction

Approximately 900,000 new cases of head and neck cancer are reported each year, making them a major global health burden. These cancers are among the most prevalent in India and are primarily caused by human papillomavirus

infection, tobacco usage, alcohol intake, chewing betel nut, and poor oral hygiene. For locally advanced head and neck malignancies, radiotherapy is still a crucial part of treatment, either by itself or in conjunction with chemotherapy and surgery. Treatment-related oral toxicities continue to

provide significant therapeutic problems that typically impair nutritional status, speech, swallowing, quality of life, and treatment adherence, despite significant advancements in radiation delivery modalities. Oral mucositis, xerostomia, dysgeusia, oral candidiasis, and secondary bacterial infections are some of the most frequent side effects. These issues result from radiation-induced harm to the salivary glands, oral mucosa, and local immune systems. Severe oral toxicities frequently result in treatment discontinuation, dose adjustments, hospital stays, and higher medical expenses, all of which have an impact on patient survival and disease control. As a result, one of the main objectives of contemporary radiation oncology is to identify patients who are more vulnerable before serious damage manifests(1).

The significance of the oral microbiota in preserving mucosal homeostasis and immunological control has been brought to light by recent developments in microbiome research. *Streptococcus*, *Veillonella*, *Actinomyces*, *Neisseria*, and many anaerobic bacteria make up the majority of the complex microbial community seen in a healthy mouth cavity. By decreasing salivary secretion, altering mucosal integrity, and changing the local immunological milieu, radiation therapy upsets this ecological balance and encourages microbial dysbiosis. Increased inflammation and delayed mucosal healing have been linked to an increase in opportunistic organisms such as *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, *Candida* species, and other pathogenic Gram-negative bacteria. There is mounting evidence that an individual's vulnerability to radiation-induced toxicities may be influenced by their baseline oral bacteria composition. The onset and severity of xerostomia, treatment-related infections, and oral mucositis have all been connected to certain bacterial and fungal fingerprints. As a result, oral microbiota profiling has become a promising non-invasive biomarker for anticipating radiation-related complications and identifying patients who might benefit from specific preventive measures like probiotics, antimicrobial therapy, better oral hygiene, and individualised supportive care.(2). Nevertheless, there is still little research assessing the prognostic usefulness of the oral microbiome in Indian patients, and there are especially few data from eastern India. Microbial composition and treatment effects may be impacted by differences in food habits, dental hygiene practices, socioeconomic status, and local microbial ecology. In order to assess the relationship between oral microbiota and radiation-induced toxicities in patients undergoing radiotherapy for head and neck tumours at Burdwan Medical College and Hospital, the current retrospective study was conducted. Finding microbial indicators that could aid in early risk assessment and enhance supportive management techniques in standard clinical practice was another goal of the study.(3).

Materials and Methods

Study Design

Retrospective observational study.

Study Setting

Department of Radiation Oncology in collaboration with the Department of Microbiology, Burdwan Medical College and Hospital, West Bengal.

Study Duration

The study was conducted from **June 1, 2024, to July 31, 2025**.

Study Population

Patients with histopathologically confirmed head and neck malignancies who underwent definitive radiotherapy during the study period.

Sample Size

A total of **79 eligible patients** were included based on the availability of complete clinical, microbiological, and treatment records.

Inclusion Criteria

- Patients aged ≥ 18 years.
- Histologically confirmed head and neck malignancy.
- Completion of external beam radiotherapy with or without concurrent chemotherapy.
- Availability of baseline oral microbiological evaluation before radiotherapy.
- Complete clinical documentation of radiation-induced toxicities.

Exclusion Criteria

- Patients with recurrent head and neck malignancies.
- Prior radiotherapy to the head and neck region.
- Active systemic fungal or bacterial infections before radiotherapy.
- Human immunodeficiency virus infection or severe immunosuppression.
- Incomplete medical or microbiological records.

Data Collection

Data were retrieved from hospital electronic medical records and patient files. Variables collected included age, sex, tobacco and alcohol use, primary tumor site, TNM stage, radiation dose, chemotherapy details, oral hygiene status,

baseline oral microbial profile, and treatment-related toxicities.

Oral Microbiological Assessment

Baseline oral swab specimens were cultured using standard microbiological techniques. Isolated microorganisms were identified by conventional biochemical methods. Microbial findings were categorized into commensal flora, pathogenic bacteria, fungal colonization (*Candida* species), and mixed microbial dysbiosis.

Outcome Measures

Radiation-induced toxicities were assessed according to the **Common Terminology Criteria for Adverse Events (CTCAE) Version 5.0**, including:

- Oral mucositis
- Xerostomia
- Dysgeusia
- Oral candidiasis
- Secondary bacterial infection

Patients were categorized into mild toxicity (Grade 0–I) and severe toxicity (Grade II–III).

Statistical Analysis

Data were analyzed using SPSS version 26.0. Continuous variables were expressed as mean $\pm$ standard deviation, while categorical variables were summarized as frequencies

and percentages. Student's t-test and Chi-square test were used to compare continuous and categorical variables, respectively. Variables showing $p < 0.10$ on univariate analysis were entered into multivariate logistic regression to identify independent predictors of severe radiation-induced toxicity. Odds ratios (ORs) with 95% confidence intervals (CIs) were calculated. A p -value < 0.05 was considered statistically significant.

Ethical Considerations

The study was approved by the Institutional Ethics Committee of Burdwan Medical College and Hospital. Patient confidentiality was maintained throughout the study by anonymizing all clinical records. The study was conducted in accordance with the ethical principles outlined in the Declaration of Helsinki.

Results

A total of **79 patients** who underwent radiotherapy for head and neck malignancies were included in the study. The mean age was **56.8 $\pm$ 10.9 years** (range: 32–78 years), with a male predominance (73.4%). The majority of patients had Stage III–IV disease (65.8%) and received concurrent chemoradiotherapy (60.8%).

Table 1. Baseline Demographic and Clinical Characteristics (n = 79)

Variable	Frequency (%)
Age Group (years)	
<50	21 (26.6)
50–59	28 (35.4)
≥ 60	30 (38.0)
Gender	
Male	58 (73.4)
Female	21 (26.6)
Primary Tumor Site	
Oral cavity	28 (35.4)
Oropharynx	20 (25.3)
Larynx	18 (22.8)
Hypopharynx	13 (16.5)
AJCC Stage	
Stage I–II	27 (34.2)
Stage III–IV	52 (65.8)
Concurrent Chemotherapy	
Yes	48 (60.8)
No	31 (39.2)

Most patients were older than 50 years (73.4%), and males constituted nearly three-fourths of the study population. Oral cavity carcinoma was the most common primary site, followed by oropharyngeal carcinoma. Advanced-stage disease (Stage III–IV) was observed in 65.8% of patients.

Table 2. Distribution of Radiation-Induced Oral Toxicities

Toxicity	Grade 0–I	Grade II–III	p-value
Oral mucositis	37 (46.8)	42 (53.2)	0.001
Xerostomia	44 (55.7)	35 (44.3)	0.012
Dysgeusia	49 (62.0)	30 (38.0)	0.036
Oral candidiasis	57 (72.2)	22 (27.8)	0.021

Grade II–III oral mucositis was the most frequent adverse event, affecting more than half of the patients. Xerostomia, dysgeusia, and oral candidiasis were also commonly observed. Oral mucositis showed the strongest statistical association with treatment-related toxicity ($p=0.001$).

Table 3. Baseline Oral Microbial Profile

Oral Microbiota	Frequency (%)
Predominant commensal <i>Streptococcus</i> spp.	31 (39.2)
<i>Candida</i> spp. colonization	24 (30.4)
Gram-negative bacilli	16 (20.3)
Mixed microbial dysbiosis	8 (10.1)

Commensal *Streptococcus* species were the predominant oral flora in 39.2% of patients, whereas 30.4% demonstrated baseline *Candida* colonization. Mixed microbial dysbiosis was identified in 10.1% of cases.

Table 4. Association Between Oral Microbiota and Grade II–III Oral Mucositis

Oral Microbiota	Mild Mucositis	Severe Mucositis	p-value
Predominant commensal flora	24	7	0.002
<i>Candida</i> spp.	8	16	
Gram-negative bacilli	4	12	
Mixed dysbiosis	1	7	

Patients with *Candida* colonization, Gram-negative bacteria, and mixed microbial dysbiosis experienced significantly higher rates of Grade II–III oral mucositis compared with patients having predominantly commensal flora ($p=0.002$).

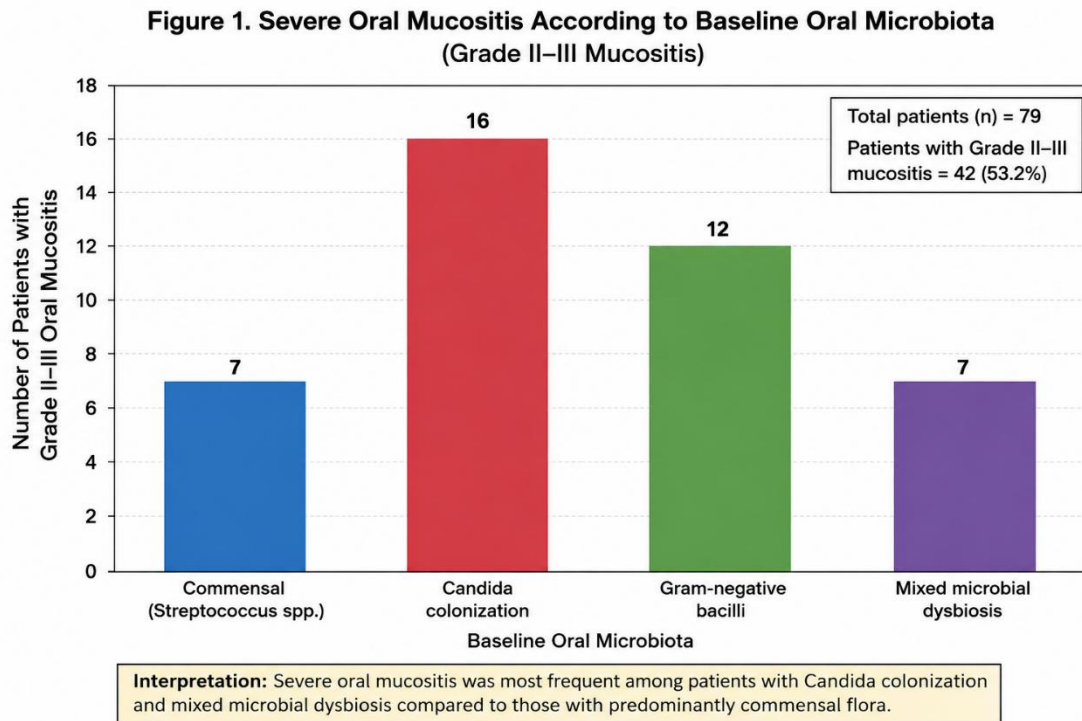


Table 5. Predictors of Grade II–III Radiation-Induced Oral Mucositis (Multivariate Logistic Regression)

Variable	Adjusted OR	95% CI	p-value
<i>Candida</i> colonization	2.91	1.18–7.20	0.019
Gram-negative bacterial colonization	2.54	1.05–6.14	0.038
Mixed microbial dysbiosis	3.42	1.48–7.92	0.003
Concurrent chemoradiotherapy	2.12	1.01–4.49	0.046
Stage III–IV disease	1.74	0.82–3.68	0.148

Multivariate logistic regression demonstrated that mixed microbial dysbiosis was the strongest independent predictor of Grade II–III oral mucositis (adjusted OR 3.42, 95% CI 1.48–7.92; $p=0.003$). Baseline *Candida* colonization, Gram-negative bacterial colonization, and concurrent chemoradiotherapy were also independently associated with severe radiation-induced oral toxicity.

Table 6. Relationship Between Oral Microbiota and Other Radiation-Induced Toxicities

Baseline Oral Microbiota	Xerostomia (%)	Dysgeusia (%)	Oral Candidiasis (%)	p-value
Predominant commensal flora	29.0	22.6	9.7	0.008
<i>Candida</i> spp.	58.3	45.8	54.2	
Gram-negative bacilli	50.0	37.5	31.3	
Mixed microbial dysbiosis	75.0	62.5	62.5	

Patients with baseline microbial dysbiosis demonstrated significantly higher frequencies of xerostomia, dysgeusia, and oral candidiasis than those with predominantly commensal oral microbiota ($p=0.008$). These findings suggest that baseline alterations in the oral microbial

ecosystem may predict multiple radiation-induced oral complications.

Overall, the results indicate that oral microbial dysbiosis—particularly *Candida* colonization and mixed microbial communities—is strongly associated with severe radiation-

induced oral toxicities and may serve as a clinically useful predictive biomarker in patients undergoing radiotherapy for head and neck cancer.

Discussion

In patients with head and neck tumours undergoing radiation therapy at Burdwan Medical College and Hospital, the current retrospective study assessed the function of oral microbiota as predictive biomarkers for radiation-induced toxicities. The results show a strong correlation between the development of radiation-induced oral problems, including oral mucositis, xerostomia, dysgeusia, and oral candidiasis, and the baseline oral microbial composition. Compared to patients with primarily commensal oral flora, those with baseline microbial dysbiosis, particularly *Candida* species colonisation and Gram-negative bacterial overgrowth, had a considerably higher risk of developing severe treatment-related toxicities(4). The most common hazard found was oral mucositis, which affected 53.2% of patients with Grade II–III severity. This result is in line with earlier studies showing that between 40 and 70 percent of patients undergoing radiation therapy for head and neck tumours have moderate-to-severe mucositis, especially when concomitant chemotherapy is administered. Direct epithelium destruction, reactive oxygen species production, inflammatory cytokine activation, and disruption of mucosal integrity are the causes of radiation-induced mucosal injury. According to the current study, changes in the oral microbiota may exacerbate these pathogenic pathways by encouraging secondary infection, poor epithelium regeneration, and prolonged inflammation(5).

The substantial correlation between baseline *Candida* colonisation and severe oral mucositis was one of the study's most noteworthy discoveries. Compared to patients with primarily commensal flora, those with pre-radiation *Candida* species had significantly higher rates of Grade II–III mucositis. Fungal colonisation has been linked to delayed mucosal healing, higher inflammatory responses, and increased susceptibility to opportunistic infections after radiation, according to similar findings from recent microbiome investigations. By lowering salivary antimicrobial proteins, changing oral pH, and weakening local immunological defence systems, radiation-induced salivary gland dysfunction fosters the growth of fungi. Additionally, the study showed that enhanced radiation-induced toxicity was independently linked to Gram-negative bacterial colonisation (6). By activating Toll-like receptors and nuclear factor kappa B (NF- κ B), these bacteria create endotoxins and other virulence factors that can trigger inflammatory pathways, increasing cytokine release and exacerbating mucosal damage. Furthermore, with an adjusted odds ratio of 3.42, mixed microbial dysbiosis was found to be the best independent predictor of severe

mucositis. This discovery highlights that radiation-associated tissue damage may be primarily caused by disruption of the entire microbial ecology rather than the existence of a specific pathogen (7).

Microbial dysbiosis was substantially linked to xerostomia, dysgeusia, and oral candidiasis in addition to oral mucositis. Significant changes in microbial diversity are caused by salivary gland irradiation, which also lowers salivary flow, diminishes mechanical oral cavity cleaning, and modifies the content of oral secretions. These changes decrease beneficial commensal species like *Streptococcus* and *Actinomyces*, which both support the preservation of oral mucosal homeostasis, while favouring opportunistic pathogens. Treatment-related problems may become more likely if these beneficial microorganisms are lost. Another independent predictor of severe oral toxicity was found to be concurrent chemoradiotherapy. Combined therapy increases myelosuppression and epithelial damage, which increases microbial colonisation and decreases the ability of the mucosa to regenerate(8). As a result, during treatment, patients undergoing concomitant chemotherapy need especially careful oral monitoring. The above results provide credence to the expanding theory that oral microbiota could function as a non-invasive prognostic biomarker for radiation-induced damage. Microbiological profiling, in contrast to traditional clinical risk indicators, makes it possible to detect high-risk patients before the start of radiation therapy. Individualised preventive measures, such as strict oral hygiene practices, prophylactic antifungal therapy, selective antibiotic interventions, salivary replacements, probiotics, prebiotics, or microbiome-modulating medicines, may be beneficial for these patients. Early intervention may increase overall treatment compliance, improve nutritional status, and decrease treatment interruptions (9).

Research on the biological processes that connect radiation damage with microbial dysbiosis is still ongoing. Bacterial translocation, local immune activation, and host-microbe interactions are all affected by radiation-induced epithelium disruption. A vicious cycle of inflammation and dysbiosis is created when inflammatory mediators generated during mucosal damage further alter the oral microbial ecology. The current study's findings are supported by advanced molecular techniques including metagenomic analysis and 16S ribosomal RNA sequencing, which have shown decreased microbial diversity and the growth of opportunistic pathogens during radiation therapy. This study's investigation of clinically significant radiation-induced toxicities and baseline microbiological testing in an actual tertiary care context are among its merits. The reliability of the results was increased by using multivariate regression analysis and standardised CTCAE grading. However, it is important to recognise some limits. Potential confounding factors such as nutritional status, dental

hygiene habits, antibiotic exposure, smoking history, and eating habits were difficult to control for due to the retrospective design. Unlike next-generation sequencing techniques, conventional culture-based microbiological methods might not have detected the entire oral microbial community. Additionally, the single-center design and somewhat small sample size may restrict generalizability to larger populations(10).

Notwithstanding these drawbacks, the work offers insightful initial proof of the prognostic function of oral microbiota in radiation-induced toxicities. To validate these results and develop microbiota-guided preventative interventions in standard oncology practice, more multicenter prospective trials using longitudinal microbial profiling, salivary biomarkers, and thorough microbiome sequencing are needed (11).

Generalizability

The findings of this study may apply to adult patients with histologically confirmed head and neck malignancies receiving definitive radiotherapy, particularly in tertiary-care settings with access to baseline oral microbiological assessment. However, the generalizability of the findings is limited by the retrospective design, relatively small sample size, single-center setting, and use of conventional culture-based microbiological methods. Differences in patient characteristics, tumor types and stages, treatment protocols, oral hygiene practices, antibiotic exposure, dietary habits, and local microbial ecology may influence the observed associations. Therefore, the findings should be interpreted cautiously when applied to other populations and healthcare settings. Larger multicenter prospective studies using standardized microbiome sequencing and longitudinal microbial assessment are needed to determine whether these findings can be generalized to broader head and neck cancer populations.

Conclusion

The current study shows that in patients receiving radiation therapy for head and neck malignancies, changes in the oral microbiota are strongly linked to the onset and severity of radiation-induced oral toxicities. Severe oral mucositis, xerostomia, dysgeusia, and oral candidiasis were strongly correlated with baseline colonisation with *Candida* species, Gram-negative bacteria, and mixed microbial dysbiosis. The most powerful independent predictor of Grade II–III oral mucositis among these variables was mixed microbial dysbiosis.

These results imply that evaluating the oral microbial profile before starting radiation therapy may offer useful predictive data for identifying patients who are more likely to experience treatment-related side effects. Early identification of high-risk individuals would make it easier

to implement focused preventive measures, such as improved oral hygiene, nutritional support, antimicrobial stewardship, and microbiota-modulating interventions, which would improve patient outcomes and minimise treatment interruptions.

Larger prospective multicenter investigations employing cutting-edge molecular microbiome sequencing techniques are necessary to confirm these findings and pinpoint particular microbial signatures predictive of radiation harm, notwithstanding the positive results. The formulation of individualised supportive care plans in radiation oncology may be made possible by the integration of oral microbiota studies with clinical, molecular, and immunological indicators. In the end, patients with head and neck cancer receiving radiation therapy may benefit from microbiota-based risk classification in terms of overall therapeutic results, treatment adherence, and quality of life.

Limitations

- The retrospective study design may have introduced selection and information bias.
- The sample size was relatively small (79 patients), limiting statistical power for subgroup analyses.
- Oral microbiota was assessed using conventional culture-based techniques rather than high-throughput sequencing methods, potentially underestimating microbial diversity.
- Molecular characterization of bacterial and fungal species was not performed.

Recommendations

Based on the findings of this study, baseline oral microbiological assessment may be considered as a potential component of risk stratification for patients undergoing radiotherapy for head and neck malignancies. Patients identified as having significant microbial dysbiosis may benefit from closer oral monitoring and appropriate supportive care during treatment. However, microbiota-directed interventions should not be routinely adopted solely based on the present findings. Future multicenter prospective studies with larger sample sizes, longitudinal microbial profiling, and high-throughput microbiome sequencing are recommended to validate the identified microbial associations and determine their clinical utility in predicting radiation-induced toxicities.

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List of Abbreviations

AJCC American Joint Committee on Cancer
CTCAE Common Terminology Criteria for Adverse Events
CI Confidence Interval
IEC Institutional Ethics Committee
NF- κ B Nuclear Factor Kappa B
OR Odds Ratio
SPSS Statistical Package for the Social Sciences
TNM Tumor, Node, Metastasis

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This study received no specific funding from any public, commercial, or not-for-profit organization.

Conflict of Interest

The authors declare that they have no conflict of interest.

Data Availability

The data supporting the findings of this study are not publicly available because they contain patient-related information and are subject to institutional and ethical restrictions. De-identified data may be made available from the corresponding author upon reasonable request and subject to appropriate institutional approval.

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