Effect of Chemo-radiotherapy on Salivary Flora of Oral Cancer Patients

Reeta Maurya¹², Madhup Rastogi³, Manodeep Sen²*, Ajai Kumar Singh⁴ and Somali Sanyal¹

¹Amity Institute of Biotechnology, Amity University, Lucknow - 226 010, Uttar Pradesh, India.
²Department of Microbiology, Dr. Ram Manohar Lohia Institute Medical Sciences, Lucknow - 226 010, Uttar Pradesh, India.
³Department of Radiation Oncology, Dr. Ram Manohar Lohia Institute Medical Sciences, Lucknow - 226 010, Uttar Pradesh, India.
⁴Department of Neurology, Dr. Ram Manohar Lohia Institute Medical Sciences, Lucknow - 226 010, Uttar Pradesh, India.

Abstract

Management of cancers of oral cancers has remained a major challenge in India and globally. Radiotherapy and chemotherapy are mostly employed for treatment which inflicts changes in oral mucosa and makes it vulnerable for bacterial colonization and eventual infections. This study aims at evaluating the changes in oropharyngeal flora (bacteria and yeast) in oral cancer patients treated by a combination of chemo-radiotherapy with the control groups comprising of non-cancerous patients living in the same environment. This prospective evaluation included Seventy-seven patients with oral squamous cell carcinomas in the study group. Whereas the control group comprised of twenty-five non-cancerous patients. Saliva samples were collected from patients with oral carcinomas and those of the control group for bacteriological examination, and were transported within 2 hours to the laboratory and immediately inoculated and incubated. The oral microflora samples collected were evaluated for the presence of bacteria in saliva in both study and control group of patients. We evaluated the change in salivary oral flora during chemo-radiotherapy treatment. A statistically significant increase in growth of normal as well as abnormal oral flora was observed post-radiation. Escherichia coli showed a significant decrease in post-RT and also near to significant in control. Various changes in salivary oral flora were observed during the course of chemo-radiotherapy in study and controls groups. This shows that there are some sensitive spots in the oral cavity where the occurrence of oral cancer is more.

Keyword: Oral cancer, Radiation, Oral flora

*Correspondence: sen_manodeep6@yahoo.com; +91 9839446858

(Received: June 10, 2021; accepted: August 03, 2021)
INTRODUCTION

Incidence of Oral cancer is reported to be the sixth common cancer in our country and has remained a major challenge health challenge inspite of various progress made in management. It continues to have a five year mortality rate of about 50%\(^1\). There has been quantum jump both in radiotherapy technology and chemotherapy but cure and survival have not changed very significantly. The high prevalence of oral cancer in India and other south east Asian countries is attributable to rampant use of tobacco in its diverse variety and betel quid chewing and other contributory epidemiological factors\(^2\). There is a noticeable shift in the age of occurrence of oral squamous cell carcinoma (OSCC) in India as the younger populations are attracted towards heavy use of tobacco products in its various forms.

The recent studies have indicated that prevalence of oral squamous cell carcinoma (OSCC) has significantly increased in younger aged population. This phenomenal increase is due to enhance use of tobacco and its various derivatives at younger age and its prevalence is on ascendance. High morbidity observed in the OSCC may be associated with delayed diagnosis and lack of awareness\(^3\). Cancer has emerged as the second biggest killer only after the cardiac related death; it is found that cancer related fatality rate in high income group countries is above 20% of all death while in low income countries it is 10%. It is anticipated that by 2020 new cancer cases added annually will be 15 million and death may be 10 million per annum. The incidence of cancer is on ascendance both in high and low income countries due to various epidemiological factors and us tobacco being one of the main contributory factor\(^4\). Studies have indicated that about 43% cancers related death are associated with tobacco consumption, use of alcohol and unhealthy food habits\(^5\). Radiotherapy continues to be the main stay of treatment in oral cancers and it causes modification of the oral mucosal barrier inclining it to colonization and oral infection.

Cancer treatment in the past two decades has undergone spectral changes. There has been vast improvement in technology and innovations in surgical, chemotherapy and radiotherapy practices which have contributed to a significant improvement in overall survival in various subsets of cancer patients particularly involving the oropharynx.

Secretion of Saliva in oral cavity is a vital natural fluid required for various functions. It is very important ingredient in speech, flavor, food digestion, antiviral and antibacterial protection, and overall oral healthiness\(^6\,7\). Saliva is without difficulty and readily available, and its collection is graceful and non-invasive. Thus, saliva has been a conventional medium for various research studies including bacterial and yeast manifestations\(^8\).

The chemical compositions of various types of saliva vary, so it is important to distinguish the specific type of saliva to be used for the study\(^9\,10\). To improve the prognosis for oral cancer including oral cancer, investigators have carried out studies using salivary flora\(^11\). Saliva is the most recurrent body fluid to identify oral cancer\(^12\).

The present study was planned to analyze alteration in oropharyngeal flora in patients with oral cancer undergoing chemo-radiotherapy and which possibly will be the source of a clinically feasible process of screening and monitoring of non-invasive oral cancer.

METHODOLOGY

This is an observational prospective analytical case-control study conducted between July 2017 to June 2019 in the Department of Microbiology and department of Radiation oncology, a tertiary care center in Lucknow. This study was duly permitted by the Institutional Ethics Committee (IEC No. 11/17) as a prospective randomised study. All the patients who participated in the study were provided detailed information regarding the study and their informed written consent was attained before taking the samples. Enumeration details such as age, gender along with habits of the patients were collected as for every the customary performa. Every patient with a new primary oral cancer was included in the study as a case and those not suffering from cancer but affected by some other disease were taken as controls. Oral/Oropharyngeal saliva was collected from both cases and controls and processed for aerobic bacterial culture as per standard protocol. The age inclusion criteria in the study was patients above the age of 18 years, while those who had received prior radiotherapy and who are considered to meet the criteria for
mental incapacity. Carcinoma in Situ where there is no evidence of invasion (these patients were only eligible if a clinical diagnosis of oral cancer has been made) Patients who have already commenced their oral cancer treatment or had any prior treatment for malignancy were excluded from this prospective revision.

Seventy-seven patients (69 males and 8 females): mean age 54 years (range 31-78 years) of oral cancer who were clinically and histopathologically proven and undergo chemo-radiotherapy were included in this study. Radiotherapy (RT) Treatment protocol comprised of a total dose of 66.0 Gy delivered over 45 days in 33 equivalent fractions, with a fraction size of 2Gy per day from 1-5 days. The RT was delivered by Elekta Infinity high energy linear accelerator unit (Elekta UK make). The patients undergoing RT also received a well-tapered course of chemotherapy in dissimilar settings viz. in neoadjuvant (taxane and cisplatin) and contemporaneous settings (cisplatin, 40 to 50 days) as per institutional protocol. Complete Blood counts and blood biochemistry were evaluated before the start of chemotherapy with particular prominence on kidney function test (KFT) and Karnofsky performance scale (KPS>70) and it was monitored every week. The fusion of chemotherapy and radiation was delivered as per medical needs and institutional protocol for oral cancer patients.

Every time patients were explained about saliva assortment and samples were obtained using a customary gathering method which comprised of spitting into a 50 ml sterile falcon tube for 2–3 min. The quantity of saliva obtained was between 5-10 ml. These samples were collected in three phases before the beginning of chemotherapy (RT), during treatment (after 14 days of starting RT) and after completion of RT (after 42-45 days). The oral cancer patients undergoing chemo-radiotherapy at the department of Radiation Oncology, RMLIMS Lucknow participated in the study. Saliva control sample were collected from disease patients between 10 am and 12 pm, who were not suffering from any cancerous disease, Saliva was transported to microbiology lab within 2 hours at room temperature. It was ensured that patients from whom the saliva was taken were fasting and had not consumed any tobacco products.

The oral saliva sample thus collected from oral cancer patients (study group) and healthy patients (control group) was extend on MacConkey Agar and blood agar plate (HI Media/Oxoid Thermo Fisher Scientific) as per departmental evaluation protocol. DNase Agar and Arabinose agar base for culture were worn to differentiate between *Staphylococcus* and *Enterococcus*. Later, the culture plates were incubate under aerobic conditions at 37°C intended for 24-48 hours.

Isolated bacterial strains were subjected to different biochemical tests specific for oral bacteria such as lactose fermentor and non-lactose fermentor using the departmental protocol. These tests were chosen to obtain accurate information on bacterial manifestations (Urease, Citrate, Methyl red, Indole and Sulfur Indole Motility). The evaluation parameters were chosen carefully based on evidence which are particular for oral bacteria such as (Lactose fermentor and non-lactose fermentor). It is imperative to mention that these assessments enabled the clear-cut detection of the oral microflora in the patients under revision. It is pertinent to mention that biochemical tests used in the evaluations of the saliva samples in the experimental set up were Gram staining, catalase test, oxidase test, hemolysis in blood agar plate (BAP), and growth in bile aesculin agar.

**Statistical analysis**

The data generated by microbiological analysis were tabulated and subjected to statistical examination with the help of appropriate bio-statistical (SPSS 21.0 for Windows) tools for elucidation of the statistical conclusion. P-value was used to ascertain the significant conclusion. The results were considered statistically significant depending on p value. P-value < 0.05. Fisher’s exact test was utilised to obtain the P-value of the experimental evaluation of the saliva.

**OBSERVATION AND RESULTS**

Salivary samples were obtained from 77 oral cancer patients with histologically proven squamous cell carcinoma (Study group) who underwent chemo-radiotherapy were included in this evaluation. 25 normal subjects from whom saliva samples were collected served as the control group who were not suffering from any
malignancy. Thus, the study and control group were in 3:1 ratio and male to female ratio was 89.6% and 11.3% respectively.

The risk factors include 64% of patients as tobacco chewers while 36% were diabetic

The distribution of the oral cancer patients included in the study were 30% (23/77) cancers of the oropharynx, 23% (18/77) base of tongue, 10.25% (8/77) lateral border of tongue and 10.25% (8/77) tongue, 11.68% (9/77) soft palate, 5.19% (4/77) buccal mucosa, 5.19% (4/77) upper alveolar region and 3.8% (3/77) throat (Table 2).

The evaluation of salivary flora was done pre-RT, during RT and post RT to get the pattern of incidence of the bacterial manifestations. It was found that oral bacterial flora, *Staphylococcus aureus* was present in saliva was 10.39% in pre-RT, and it increased to 14.29 during RT and decreased to 6.49 in post-RT. It was inferred that the difference was non-significant when compared with the control group. The percentage of *S. aureus* of cases was compared with that of the control group. The percentage of *Escherichia coli* was 11.69 in pre-RT, 15.58 during RT and subsequently it decreased to 5.19 in post-RT and difference among them was not significant. A decrease in the percentage of *E. coli* was observed during different stages of RT and was found to be significant when compared with the control group. Sample evaluations revealed that incidence of *Citrobacter* bacteria decreased from 10.39% in pre-RT to 2.60% each during RT and post RT samples. This difference may be considered close to significant (P-value 0.06) while *Citrobacter* has no significant difference at pre during and post RT from control. The presence of *E. faecalis* increased from 12.99% in pre-RT to 14.29 during RT and then to 15.58 in post-RT. The difference observed among the three groups was found to be non-significant when compared with control. The percentage of *E. faecium* which was 16.88 in both pre RT and during RT decreased to 10.39 in post-RT samples. And here also the difference observed among the groups as compared to control was found to be non-significant.

*Candida non-albicans* was not present in pre–RT samples while during RT the percentage of the organism was 1.30 which increased to 2.60 post- RT, and the difference among the groups was non-significant. Inter sub group comparison for incidence of bacterium revealed that the groups namely pre-RT, during RT and post- RT from control. The percentage of *E. faecium* which was 16.88 in both pre RT and during RT decreased to 10.39 in post-RT samples. And here also the difference observed among the groups as compared to control was found to be non-significant. *Candida non-albicans* was not present in pre–RT samples while during RT the percentage of the organism was 1.30 which increased to 2.60 post- RT, and the difference among the groups was non-significant. Inter sub group comparison for incidence of bacterium revealed that the groups namely pre-RT, during RT and post- RT from control. The percentage of *E. faecium* which was 16.88 in both pre RT and during RT decreased to 10.39 in post-RT samples. And here also the difference observed among the groups as compared to control was found to be non-significant. *Candida non-albicans* was not present in pre–RT samples while during RT the percentage of the organism was 1.30 which increased to 2.60 post- RT, and the difference among the groups was non-significant. Inter sub group comparison for incidence of bacterium revealed that the groups namely pre-RT, during RT and post- RT from control. The percentage of *E. faecium* which was 16.88 in both pre RT and during RT decreased to 10.39 in post-RT samples. And here also the difference observed among the groups as compared to control was found to be non-significant. *Candida non-albicans* was not present in pre–RT samples while during RT the percentage of the organism was 1.30 which increased to 2.60 post- RT, and the difference among the groups was non-significant. Inter sub group comparison for incidence of bacterium revealed that the groups namely pre-RT, during RT and post- RT from control. The percentage of *E. faecium* which was 16.88 in both pre RT and during RT decreased to 10.39 in post-RT samples. And here also the difference observed among the groups as compared to control was found to be non-significant.

**DISCUSSION**

The aim of the present study is to compare the salivary microbial profiles of patients with oral cancer with those of controls. The results of this study confirmed that association of oral microbial community of cases and controls was remarkably diverse. The seven genera of bacteria

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**Table 1. Distribution of oral cancer (SCC) cases studies**

<table>
<thead>
<tr>
<th>Sex</th>
<th>Age (30-78 years)</th>
<th>Risk Habits</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>N</td>
<td>%</td>
</tr>
<tr>
<td>Male</td>
<td>69</td>
<td>89</td>
</tr>
<tr>
<td>Female</td>
<td>08</td>
<td>11</td>
</tr>
<tr>
<td>Total</td>
<td>77</td>
<td>100</td>
</tr>
</tbody>
</table>

**Table 2. Distribution of alterations noted in oral cancer**

<table>
<thead>
<tr>
<th>Localization</th>
<th>No. of samples (N=77)</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oropharyngeal</td>
<td>23</td>
<td>30</td>
</tr>
<tr>
<td>Base of tongue</td>
<td>18</td>
<td>23.3</td>
</tr>
<tr>
<td>Lateral border of tongue</td>
<td>8</td>
<td>10.25</td>
</tr>
<tr>
<td>Tongue</td>
<td>8</td>
<td>10.25</td>
</tr>
<tr>
<td>Soft palate</td>
<td>9</td>
<td>11.68</td>
</tr>
<tr>
<td>Throat</td>
<td>3</td>
<td>3.8</td>
</tr>
<tr>
<td>Buccal mucosa</td>
<td>4</td>
<td>5.19</td>
</tr>
<tr>
<td>Upper alveolar</td>
<td>4</td>
<td>5.19</td>
</tr>
</tbody>
</table>
### Table 3. Bacterial and yeast oral flora comparison with cases and controls

<table>
<thead>
<tr>
<th>Organism</th>
<th>Pre RT N (%)</th>
<th>During RT N (%)</th>
<th>Post RT N (%)</th>
<th>Control N (%)</th>
<th>Among Cases P-value</th>
<th>Pre RT P-value</th>
<th>During RT P-value</th>
<th>Post RT P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Staphylococcus aureus</em></td>
<td>P+ 8(10.39)</td>
<td>11(14.29)</td>
<td>5(6.49)</td>
<td>2(8)</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td></td>
<td>Ab 69(39.61)</td>
<td>66(85.71)</td>
<td>72(93.51)</td>
<td>23(92)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Citrobacter</em></td>
<td>P+ 8(10.39)</td>
<td>2(2.60)</td>
<td>2(2.60)</td>
<td>-</td>
<td>S</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td></td>
<td>Ab 69(89.61)</td>
<td>75(97.40)</td>
<td>75(97.40)</td>
<td>25(100)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>E.coli</em></td>
<td>P+ 9(11.69)</td>
<td>12(15.58)</td>
<td>4(5.19)</td>
<td>8(32)</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
</tr>
<tr>
<td></td>
<td>Ab 68(88.31)</td>
<td>65(84.42)</td>
<td>73(94.84)</td>
<td>17(68)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>CONS</em></td>
<td>P+ 22(28.57)</td>
<td>20(25.97)</td>
<td>14(14.18)</td>
<td>16(46)</td>
<td>NS</td>
<td>S</td>
<td>S</td>
<td>S</td>
</tr>
<tr>
<td></td>
<td>Ab 55(71.43)</td>
<td>57(74.03)</td>
<td>63(81.82)</td>
<td>9(36)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>E. faecalis</em></td>
<td>P+ 10(12.99)</td>
<td>11(14.29)</td>
<td>12(15.58)</td>
<td>4(16)</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td></td>
<td>Ab 67(87.01)</td>
<td>66(85.71)</td>
<td>65(84.42)</td>
<td>21(84)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>E. faecium</em></td>
<td>P+ 13(16.88)</td>
<td>13(16.88)</td>
<td>8(10.39)</td>
<td>3(12)</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td></td>
<td>Ab 64(83.14)</td>
<td>64(83.14)</td>
<td>69(89.61)</td>
<td>22(88)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Candida non-albicans</em></td>
<td>P+ 0(0)%</td>
<td>1(1.30)</td>
<td>2(2.60)</td>
<td>4(16)</td>
<td>NS</td>
<td>S</td>
<td>S</td>
<td>S</td>
</tr>
<tr>
<td></td>
<td>Ab 77(100)</td>
<td>76(98.70)</td>
<td>75(97.40)</td>
<td>21(84)</td>
<td></td>
<td></td>
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</tr>
</tbody>
</table>

Note: P+ Present  
Ab Absent
namely, *Staphylococcus aureus*, *Citrobacter*, *E. coli*, CONS, *E. faecalis*, *E. faecium* and *Candida non-albicans* indicated significant differences between the cases of oral cancer and control groups.

Kang et al have evaluated and published their findings about occurrence of oral microorganisms in the saliva of cancer patients and compared it with healthy subjects. Their study reported presence of oral microorganisms among 3 carcinogenic bacteria, more than 3 periodontopathic bacteria and 4 *Candida species* in the saliva of more than hundred cancer patients and more than 50 healthy subjects. Along with these microorganisms, *Streptococcus mutans*, *Fusobacterium nucleatum* and *Candida albicans* were commonly present and identified in both groups of the patients evaluated. We also found all the oral microbes studied in both the groups except *Citrobacter* which was absent in the control group in our study. They found no significant differences in the incidence of carcinogenic bacteria between the studies along with control groups, whereas they encountered significant differences in the occurrence of *Porphyromonas gingivalis* and *Tannerella forsythia* between the 2 groups (p < 0.05). The incidence of *C. albicans* in study group higher than that of the control group and it was significant (p < 0.05). The occurrence of *Candida* sp. varied from 20 to 70% in cases undergoing RT, and some studies suggest that the prevalence of other species besides *C. albicans* is higher in irradiated patients17,18, while in our study we observed a significant decrease in *C. non albicans* in the RT group as compared to that in control.

Mager et al19 determined the relationship of 40 common salivary bacterial counts between oral carcinoma patients and controls and reported that OSCC patients had the propensity of considerably elevated concentrations of certain bacteria in their saliva. One of the important finding reported was that high salivary counts of *Capnocytophaga gingivalis*, *Prevotella melaninogenica* and *Streptococcus mitis* could be potential diagnostic predictor of OSCC19. Another similar study also found raised concentrations of bacteria within the saliva of oral cancer patients20. Sharma et al21 reported a association among salivary bacteria and oral cancers and found that the percentage of *Prevotellamelaninogenica*, *Leptotrichia buccalis*, *Capnocytopha gagingivalis*, *Eubacterium saburreum* and *S. mitis* were significantly higher in patients undergoing radiotherapy than those of controls21. These findings are similar to our results because we also found an increase in *S. aureus*, *Citrobacter* and *E. faecium* during RT.

CONCLUSION

Among the entire isolated oral flora, *E. coli* and *Citrobacter* showed significant increase during radiotherapy, while *E. coli* was decreased post RT and other oral micro flora namely, *E. faecium* and *S. aureus* showed a non-significant decrease post-RT. When comparing cases with controls, we found that oral flora namely; CONS, *E. coli* *E. faecalis* and *Candida non-albicans* were more in the control group. It is a fact that radiotherapy damages normal tissue which may later recover. Patients undergoing chemo radiotherapy for oral cancer are likely to develop mucositis in various sites such as oral mucosa, the base of the tongue and other parts of the oral cavity. This study also attempts to draw attention to the impact of altered microbial profile in promoting tumors in the oral cavity that may lead to incidence of secondary malignancy in head and neck region. To avoid this condition regular follow-up and careful monitoring should be done.

Oral cancer patients have low immunity and presence and predisposition of oral bacterial colonization before radiotherapy is a major factor for infection. It has been found that in patients with oral malignancy, the standard oral bacterial flora is replaced in an altered pathogenic flora during radiotherapy which results in contamination. It may be pertinent to mention that prior information about predisposition of changes in oral bacterial profile in oral cancer will be useful in anticipation of such infections and their effective prevention/treatment.

ACKNOWLEDGMENTS

None.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.
AUTHORS’ CONTRIBUTION

RM drafted the manuscript, compiled information from the literature, and designed the tables. MR drafted the manuscript and gathered information from the literature. MS supervised and reviewed the manuscript. MS supervised and reviewed the manuscript and designed the tables.

FUNDING

None.

DATA AVAILABILITY

All datasets generated or analyzed during this study are included in the manuscript.

ETHICS STATEMENT

This is an observational and comparative study conducted by Department of Microbiology, Dr. Ram Manohar Lohia Institute of Medical Sciences (RMLIMS) Lucknow, from July 2018 to June 2020 after the approval by the Institutional Ethics Committee (IEC No.11/17). Informed consent was obtained from all the patients.

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