Oral microbial profile in oral cancer patients before and after radiation therapy in a cancer care center – A prospective study

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Abstract

Background: Head and neck cancer is the sixth most common cancer reported worldwide. In many cases, the level of aggressiveness of therapy adopted in cancer patients may cause the alteration in oral microbiota; the emergence of potential pathogens may cause opportunistic infections in already immune-compromised individuals leading to increases in morbidity and mortality. Hence, this study was conducted to assess the oral microbial profile in oral cancer patients before and after radiotherapy.

Materials and Methods: A total of 145 oral swabs were collected before radiotherapy (n = 96), 3 months postradiotherapy (n = 25), 6 months postradiotherapy (n = 12) and controls (n = 12). The samples were inoculated into brain–heart infusion broth and later in different media for bacterial isolation. The isolates were subjected to phenotypic characterization by automatic identification system.

Results: Among the 96 samples studied from the preradiotherapy patient samples, Streptococcus species (n = 28) were the predominant isolate, followed by Staphylococcus species (n = 16), Enterobacter species (n = 6) and Enterococcus species (n = 6). Of the 25 samples studied 3 months after radiotherapy, Klebsiella pneumoniae (n = 4) was isolated and 12 samples studied after 6 months of radiotherapy Candida species (n = 4) and Pediococcus species (n = 3) were isolated. Among the control group (n = 12) screened, Streptococcus acidominimus (n = 3) is the predominant bacteria isolated.

Conclusion: High prevalence of Streptococcus sp. was found in patients of oral cancer before radiotherapy, while Candida albicans and Klebsiella species and Pediococcus species are the significant pathogens isolated in postradiotherapy cancer patients.

Keywords: Oral microbial profile, oral microbiota, oral squamous cell carcinoma, radiotherapy

INTRODUCTION

Head-and-neck cancer is the world’s sixth most common type of cancer. More than 600,000 new patients are diagnosed and about 350,000 individuals die from this disease worldwide each year.1 Occurrence of oral cancer is on the rise, causing major global health problem. Incidence

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Submitted: 14-Jul-2019, Accepted: 14-Oct-2019, Published: 08-May-2020
rate of oral cancer is unevenly distributed geographically, India and France showing the highest.\textsuperscript{[3]} Increased risk of oral cancer is associated with poor oral hygiene along with the use of alcohol and tobacco.\textsuperscript{[3]} Microbial flora on the oral mucosa seems to differ between healthy and malignant sites.\textsuperscript{4,5} Role of specific bacterial infection causing or promoting cancer is well-known in cases of Helicobacter pylori with gastric cancer and other cancers of colon, gall bladder, prostate and lung.\textsuperscript{6-8} Rich and varied community of microbes resides on exposed surfaces (e.g., gut, skin and mouth) which outnumber the cells in human body by ten times.\textsuperscript{9} Changes in microbes composition and overgrowth of certain pathogen are associated with chronic periodontitis, which is a risk factor for oral cancer.\textsuperscript{10} Despite the availability of various successful treatment modalities such as surgery, radiation, chemotherapy or combination for oral cancer, local and systemic immunity reduced in treated patients due to swallowing dysfunction, hyposalivation and variation in quality, quantity, complexity of oral microbes leading to imbalance in oral ecosystem.\textsuperscript{11} Increased levels and changes in the composition of microbes in the oral cavity are reported in precancerous conditions and cancers.\textsuperscript{12} The predominant and abundant phyla reported in oral cancer belong to Firmicutes, Proteobacteria, Bacteroidetes, Actinobacteria and Fusobacteria.\textsuperscript{13-15} In healthy individuals, Streptococcus species are the frequently observed oral microbe and less frequently seen are Prevotella, Veillonella, Neisseria and Haemophilus.\textsuperscript{14,15} Infections result when there is disruption of balance between bacterial load and compromise in immune status, allowing certain bacteria and Candida species to multiply and overwhelm other resident microorganisms. Radiotherapy for oral cancer causes alteration in oral microbiota which may lead to the emergence of potential pathogen subsequently leading to other systemic problems.\textsuperscript{16} After radiotherapy, the patient may acquire drug-resistant opportunistic infections from altered microbes in the oral cavity which may cause systemic complications and high morbidity. As there are no sufficient microbial studies to precisely infer the presence or absence of certain microorganisms and its impact on immunocompromised patients, there is need to study the exact role played by the oral microbes and its impact on pre- and post-cancer treatment. Hence, this present study was conducted to identify microbial profile in oral cancer patients undergoing radiotherapy at tertiary care cancer center, Northern Kerala.

MATERIALS AND METHODS

This prospective study was carried out in a randomly selected cohort of total 96 oral cancer patients before radiation and 25 cases were followed up after 3 months and 12 cases after 6 months of radiation along with 12 controls at cancer center, Thalassery, Kerala, from June 2017 to October 2018. The study was approved by the Institutional Review Board No - 1617/IRB-IEC/13/MCC/06-03-2017/12 and also ethical clearances were obtained from the ethical clearance committee before the study. All patients were informed about the study, counseled and informed consent was obtained before taking the samples. Demographic details such as age, gender and habits were collected as per the standard pro forma.

Inclusion criteria

All consenting patients suffering from oral cancer and those who underwent radiotherapy were included in this study, irrespective of age and gender. Participants having good oral health without a history or symptoms of oral cancer and precancersous lesions were taken as the control group.

Exclusion criteria

The patients suffering from diabetes mellitus, infected by human immunodeficiency virus, hepatitis and autoimmune diseases were excluded after obtaining the laboratory data from the case files.

Sample collection and processing

Oral swabs were collected from areas such as buccal mucosa, tongue, alveolar ridges, floor of the mouth and various regions in the oral cavity by scrubbing the lesions using a dry sterile cotton swab. The oral samples were immediately taken to microbiology laboratory and put in brain–heart infusion broth. The broth was incubated aerobically for overnight at 37°C. Then, it was inoculated into culture media such as MacConkey agar, blood agar and chocolate agar from the broth and incubated at 37°C. The colony characters on the grown culture plates and morphology of the microorganism by Gram staining was observed. The mixed cultures grown on the primary culture plate were subcultured into blood agar and chocolate agar separately, and individual type of colonies were subjected for rapid phenotypic identification by automated bacterial identification system (Phoenix TM – Becton Dickinson). It is a fully automated system for rapid identification of bacteria and antimicrobial susceptibility testing.

RESULTS

A total of 145 oral swabs were collected from oral cancer patients. Of which, 96 were collected before radiotherapy, 25 were collected 3 months and 12 were collected 6 months of follow-up after radiotherapy along with 12 controls collected from persons with good oral hygiene and no symptoms of oral diseases. Loss of follow-up of patients was due to patient death (n = 11), loss of communication (n = 25),
clinical deterioration \( (n = 11) \) and patient subjected to only surgery without radiotherapy \( (n = 24) \).

A total of 96 cases of oral squamous cell carcinoma (OSCC) studied during this period, 72.9% (70 cases) occurred in males and 27.1% (26 cases) in females, with a male-to-female ratio of 2.7:1 [Table 1]. The mean age distribution of cases was 56.5 years (range: 32–83 years), with highest 28.1% (27 number) diagnosed in the fifth and sixth decades of life followed by 20.8% (20 number) in the fourth decade. Among 70 male patients studied, 31.4% were diagnosed in the fifth, 28.6% in the sixth decade and 8.9% in the third decade of life. Of 26 female patients studied, 26.9% were diagnosed in their sixth decade, 19.2% each in fifth and seventh decades and 7.7% each in third and eighth decades of life [Table 1].

Of 96 patients, 77 (80.2%) were with a positive history of habits which included smoke and smokeless tobacco consumption, alcohol consumption and combination of both the habits, whereas 19 patients (19.7%) were without any habits or familial history of cancer or cancer-related death. Of 70 male patients, 61 (87.14%) had a positive history of habits, whereas 9 (12.85%) were without any habit history. In case of the female patients, 16 of 26 (61.53%) were with a positive habit history and 10 (38.46%) were without any habit history.

Patients with a positive habit history 24 (31.46%) and 25 (32.46%) were diagnosed in the fifth and sixth decades of life, respectively, whereas seven patients (36.8%) without a habit history were diagnosed during the same period. A total of 28 cases (29.1%) occurred below 50 years, among them 18 (64.3%) cases were with a positive habit history and 10 (35.7%) cases were without a habit history. The mean age of patients with and without a history of habits was 52.1 years for males and 50.8 years for females with no significant difference [Table 2].

Most common habit among the male patients was tobacco smoking and alcohol consumption, combination of both accounted for 32.78% of the total cases followed by simultaneous use of chewing tobacco (smokeless), smoking and alcohol intake that accounted for 29.5% of the total cases. While in females, the most significant habit was the tobacco (smokeless) form which accounted for about 87.5% [Table 3].

The oral swabs collected from the participants before radiation therapy \( (n = 96) \) showed the growth of *Klebsiella pneumoniae* (12; 12.5%) as the predominant organism in Gram-negative bacilli group followed by *Escherichia coli* (6; 6.2%), *Pseudomonas aeruginosa* (6; 6.2%), *Pasteurella species* (5; 5.2%) and others. Among Gram-positive organisms grown, *Streptococcus* species (28; 29%) was predominant followed by *Staphylococcus* species (7; 7.2%), *Staphylococcus aureus* (6; 6.2%) and others. The oral swabs collected after 3 months of radiation therapy \( (n = 25) \) revealed *K. pneumoniae* (4; 16%) as the predominant Gram-negative organism followed by *P. aeruginosa* (1; 4%) and *Acinetobacter baumannii* (1; 4%) while among Gram-positive organisms, *Streptococcus* species (4; 16%) was predominant followed by *Enterococcus faecalis* (4; 16%). Oral swabs \( (n = 12) \) collected 6 months after radiation therapy showed predominantly *Candida* species \( (n = 5; 42\%) \), followed by *Pediococcus* species \( (n = 3; 25\%) \). Oral swabs collected for control samples \( (n = 12) \) showed *Streptococcus* species \( (n = 8; 67\%) \) as the predominant species followed by *Leuconostoc* species and *Candida* species \( (n = 3; 25\%) \) [Tables 4-6].

**DISCUSSION**

OSCC can be located anywhere in the oral tissues, including the lip, floor of the mouth, buccal mucosa, gingival, palate or in the tongue. The majority of squamous cell carcinoma usually arise from preexisting “potentially
In our study, 8 OSCC occurred below 40 years, among them four cases of the tongue OSCC noted corroborating the findings reported earlier by Selvamani et al.,[17] where they have recorded the prevalence of tongue cancers in the Indian population to the extent of 12% in the age group of 31–40 years, and also a review by Sarkaria and Harari identified 14 reports with three or more patients younger than 40 years of age with OSCC of the tongue.[19]

Gender differences in the occurrence of OSCC generally show male preponderance over female, and our study also showed similar trend with a male-to-female ratio of 2.7:1. The male-to-female ratio varies widely by geographic location worldwide, 1.45:1 in Japan, 1.5:1 in Pakistan, 1.65:1 in Yemen and 10.5:1 in Taiwan patient population, and a reverse trend has been observed in the Thailand population with a male:female ratio of 1.45:1 in Japan, 1.5:1 in Pakistan, 1.65:1 in Yemen and 10.5:1 in Taiwan patient population, and a reverse trend has been observed in the Thailand population with a male:female ratio of 1.45:1 in Japan, 1.5:1 in Pakistan, 1.65:1 in Yemen and 10.5:1 in Taiwan patient population.

In the present study, we observed that 50% of female cases showed involvement of the buccal mucosa as predominant site for OSCC in smokeless tobacco users and also the most common site observed among the female patients, similar to the study done by Singh et al.[25] In this study, 47.14% cases could be attributed to the consumption of malignant lesions or disorders and from normal-appearing epithelium.[16] Although the causes for OSCC could be multifactorial, most common etiology is related to tobacco or its product in both smoke and smokeless forms. The increased consumption of tobacco has become a multidimensional problem and a threat to the health of the youth in India.[17]

Although it is recognized that oral cancer is more prevalent among elderly age groups, the data trend in recent years indicate the increased incidence rates of oral cancer in younger age groups, and in the present study, similar trend was noted. Many recent studies have reported the prevalence rate of 4%–6% of oral cancers in the younger populations <40 years.[10,17] In our study, 8 OSCC occurred below 40 years, among them four cases of the tongue OSCC noted corroborating the findings reported earlier by Sarkaria and Harari identified 14 reports with three or more patients younger than 40 years of age with OSCC of the tongue. [19]
alcohol and smoking, whereas Ajay et al. reported similar association in 28.1% of patients.\cite{26}

The human body is inhabited by over 100 trillion microbial cells living in association with their host.\cite{27} Microorganism at certain body sites has long been believed to be involved in immune modulation, disease progression and health preservation. Some of these microorganisms have been implicated in oral diseases such as caries and periodontitis, which are among the most common bacterial infections in humans.\cite{14} Microorganisms and their products, including endotoxins (lipopolysaccharides), enzymes (e.g., proteases, collagens, fibrinolysin and phospholipase) and metabolic by-products (e.g., hydrogen sulfide, ammonia and fatty acids), are toxic to host cells and may directly induce mutations or alter signaling pathways that may affect cell proliferation and/or survival of epithelial cells.\cite{28} A bacterial infection can play a role in initiating or promoting cancer is a well-known fact with respect to the association of Helicobacter pylori with gastric cancer, and other cancers of gallbladder, colon, lung and prostate have been associated with particular bacterial infections.\cite{12,29} Hence, the question arises whether there may be shifts in the composition of the normal oral flora and/or chronic microbial infection can support or causes oral cancer.

Although primary causes of OSCC are tobacco and alcohol, recently, the malignancy was even seen in individuals who do not have the habit of smoking and drinking, suggesting that other factors such as poor oral hygiene and chronic periodontitis may also play a role in increasing the risk of oral cancers. Numerous microorganisms, residing in the

### Table 5: Distribution of Gram-positive organisms in cancer patients at different intervals

| Total number of organisms | Before radiation (n=96), n (%) | After radiation 3 months (n=25), n (%) | After radiation 6 months (n=12), n (%) | Control (n=12), n (%) |
|---------------------------|-------------------------------|--------------------------------------|--------------------------------------|---------------------|
| Streptococcus acidomininus | 8 (8.3)                       | -                                    | -                                    | 5 (42)              |
| Streptococcus sanguinis    | 5 (5.2)                       | -                                    | -                                    |                     |
| Streptococcus anginosus    | 5 (5.2)                       | -                                    | -                                    |                     |
| Streptococcus sobrinus     | 3 (3)                         | -                                    | -                                    | 3 (25)              |
| Streptococcus porcinus     | 2 (2.08)                      | -                                    | -                                    |                     |
| Streptococcus galactiae    | 2 (2.08)                      | -                                    | -                                    |                     |
| Streptococcus gordonii     | 1 (1.04)                      | -                                    | -                                    |                     |
| Streptococcus pneumoniae   | 1 (1.04)                      | -                                    | -                                    |                     |
| Streptococcus mutans       | 1 (1.04)                      | 1 (4)                                | -                                    |                     |
| Streptococcus oralis       | -                             | 1 (4)                                | -                                    | 2 (16)              |
| Staphylococcus aureus      | 6 (6.2)                       | 1 (4)                                | 1 (8.3)                              |                     |
| Staphylococcus haemolyticus| 1 (1.04)                      | -                                    | -                                    |                     |
| Staphylococcus lugdunensis | 1 (1.04)                      | -                                    | -                                    |                     |
| Staphylococcus saprophyticus| 1 (1.04)                     | -                                    | -                                    |                     |
| Staphylococcus epidermidis | 1 (1.04)                      | -                                    | -                                    |                     |
| Staphylococcus pettenkoferi| 1 (1.04)                      | 1 (4)                                | -                                    |                     |
| Staphylococcus capitis     | 1 (1.04)                      | -                                    | -                                    |                     |
| Staphylococcus auricularis | 1 (1.04)                      | -                                    | -                                    |                     |
| Staphylococcus cohnii      | -                             | 1 (4)                                | -                                    |                     |
| Staphylococcus kloosi      | -                             | -                                    | 1 (8.3)                              |                     |
| Staphylococcus hominis     | -                             | -                                    | 1 (8.3)                              |                     |
| Enterococcus raffinosus    | 1 (1.04)                      | -                                    | 1 (8.3)                              |                     |
| Enterococcus avium        | 1 (1.04)                      | -                                    | -                                    |                     |
| Enterococcus faecalis     | 4 (4.1)                       | 4 (16)                               | -                                    |                     |
| Aerococcus viridans       | 1 (1.04)                      | -                                    | -                                    |                     |
| Leuconostoc mesenteroides | -                             | 1 (4)                                | -                                    | 2 (16)              |
| Pediococcus species       | -                             | -                                    | (25)                                 |                     |

*RT: Radiation therapy, *sp: Species

### Table 6: Microbial profile of oral cancer patients with complete follow-up (n=12)

| Patient ID | Before RD | Follow-up |
|------------|-----------|-----------|
|            | 3 months after RD | 6 months after RD |
| R1         | Staphylococcus sp. | -         |
| R2         | Pseudomonas sp. Neisseria sp. | Pasteurella sp. | Klebsiella sp. |
| R3         | Staphylococcus sp. | - | Pediococcus sp. |
| R4         | Streptococcus sp. Bacillus sp. | Enterococcus sp. | Candida sp. |
| R5         | Enterococcus sp. | - | Bacillus sp. |
| R6         | Bacillus sp. Acinetobacter sp. | Staphylococcus sp. | Candida sp. |
| R7         | Staphylococcus sp. Enterococcus sp. | Staphylococcus sp. | Staphylococcus sp. |
| R8         | Bacillus sp. Citrobacter species | Streptococcus sp. | Enterococcus sp. |
| R9         | Staphylococcus sp. | - | Pediococcus sp. |
| R10        | Bacillus sp. Pasteurella sp. | - | Candida sp. |
| R11        | Pseudomonas sp. Escherichia sp. | - | Staphylococcus sp. |
| R12        | Bacillus sp. Pasteurella sp. | Streptococcus sp. | Candida sp. |

*RT: Radiation therapy, *sp: Species
oral region, such as bacteria, viruses and fungi, may affect the development and progression of OSCC. In the present study, we found Pseudomonas, Neisseria, Staphylococcus, Streptococcus, Enterococcus, Bacillus, Citrobacter, Pasterella, Pseudomonas and E. coli in oral cancer patients. Similar findings were observed by Nagy et al. in their study where aerobic bacterial species Haemophilus, Enterobacteriaceae and Streptococcus and anaerobic bacterial species such as Veillonella, Fuso bacterium, Prevotella, Porphyromonas, Actinomyces and Clostridium were detected more frequently in oral cancers than in normal oral mucosa.

Binder Gallimidi et al. reported that chronic infection of Porphyromonas gingivalis and Fuso bacterium nucleatum promotes chemically induced OSCC in mice. Streptococcus thermophilus and Streptococcus mitis hidden in deep periodontal pockets exhibit alcohol dehydrogenase activity and produce acetaldehyde, a known carcinogen derived from alcohol, suggesting one mechanism whereby the pathogenesis of oral cancer may occur. These findings concur with the present study where Staphylococcus, Streptococcus and Enterococcus seen in almost all patients.

Radiation therapy standard protocols play an important role in the treatment of patients with head-and-neck cancer but may result in short- and long-term side effects. Possible side effects include mucositis, osteoradionecrosis, taste loss, hyposalivation, radiation caries, periodontal disease, trismus and so on. Hyposalivation and the loss of the protective effects of saliva may predispose changes in the oral microbiome, with a shift to periodontal disease-associated flora. Kamath et al. studied radiotherapy effect on oropharyngeal flora in head-and-neck cancer and concluded that Streptococcus pneumoniae was significantly decreased at the end of irradiation, while there was an increase in S. aureus, Pseudomonas, Bacteroides and Candida species. In the present study, we found that before initiation of radiation therapy, there was increased number of Staphylococcus, Streptococcus, Pseudomonas, Bacillus, Enterococcus, Neisseria, E. coli, etc., but after 3 months of radiation, either decrease or no counts of Pseudomonas were noted; Staphylococcus, Streptococcus and Enterococcus could still identified in culture. Newer entities of microbes like Actinobacter, Pasterella, Pediococcus and Leuconostoc were observed after radiotherapy, However these micro organism were not observed prior to initiation of radiation therapy [Table 6].

A study by Sharma on salivary bacteria linked to oral cancers found that Prevotella melanogena, Leptotrichia buccalis, Capnocytophaga gingivalis, Eubacterium salicreum and S. mitis were significantly higher in patients with radiotherapy than controls. Eliasson et al. studied dental plaque pH and microorganisms during hyposalivation in ten patients after 3–5 years of radiotherapy with equal controls and concluded that when compared with their controls, the irradiated group displayed increased numbers of Lactobacilli and Candida species due to reduced buffering capacity of saliva. Similar findings were noted in the study done by Srithavaj and Thaweboon and Al -Nawas and Grötz where they noted increased in the Lactobacilli and Streptococci count. In the present study, we too observed increase in Candida after 6 months of radiation therapy along with Staphylococcus, Bacillus, Enterococcus, Pediococcus and other microorganisms such as Klebsiella, Staphylococcus kloasi and Staphylococcus hominis [Table 6]. Streptococci produce short-chain organic acid from carbohydrates lowering the pH of their local environment which may contribute to the acidic and hypoxic microenvironment of tumors. Bacterial colonization may induce alterations in microenvironment and promote chronic inflammation. The pathogenesis of approximately 15%–20% of human tumors is correlated with infection-triggered inflammations. Evidence also support the hypothesis of E. coli inhibiting apoptosis and thus promoting carcinogenesis. E. coli releases a range of virulence factors, including cytotoxic necrotizing factor type 1 which prevents apoptosis in epithelial cells by activating a cell signaling cascade and promoting the expression of antiapoptotic members of the Bcl-2 gene family.

Increase in oral Candida colonization in patients who received radiation for head-and-neck carcinoma was seen in the present study as reported in other studies. It is reported that Candida strains, by producing nitrosamine compounds, may directly or in concert with other carcinogens be able to activate specific proto-oncogenes, thus initiate oral cancer. It was found that Candida species associated with advanced precancerous lesions have relatively higher potentials for producing N-nitroso-benzylmethylamine, a compound able to induce carcinoma compared to strains associated with normal mucosa.

**CONCLUSION**

The present study shows that oral cancers occur commonly in the fifth and sixth decades with male preponderance and associated with smoking and drinking habits. There is a wide variation in the microbial flora of oral cancer patients’ pre- and post-radiation therapy at different intervals. Gram-positive organisms are isolated more frequently than that of Gram-negative organisms. The high prevalence of streptococcus sp. was found in patients of oral cancer before radiotherapy and Candida albicans and Klebsiella species and Pediococcus species are the significant
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pathogens isolated in postradiotherapy. Further studies are required to investigate the impact of alteration in microbial flora during the treatment of cancers. The continued surveillance and prompt examination, testing and careful monitoring of the imbalance in microbial ecosystem of the oral cavity and early management of complications are of prime importance in the long-term care of the patients receiving radiation treatment to reduce morbidity and give good quality of life.

Financial support and sponsorship

Nil.

Conflicts of interest

There are no conflicts of interest.

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