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Comparative Study of Oral Swab and Saliva Flora in Patients with Oral Cancer during Chemo- Radiotherapy

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Abstract:-

BACKGROUND: Patients of oral cancer receiving Chemo-radiotherapy often develop oral mucosit is due to influence in imbalance of oral flora which colonize over it. These patients are at high risk for oral bacterial and yeast infection.

OBJECTIVES: This study was aimed to evaluate the effect of radiation on oral swab and salivary bacterial and yeast flora in patients with oral cancer under chemo-radiotherapy.

PATIENTS AND METHODS: Swab and saliva samples were collected before RT and post RT from each of the 77 oral cancer patients, who were advised RT and Chemotherapy. Bacterial and yeast culture and growth was done using standard techniques.

RESULTS: Out of 77 patients 89.6% were male and maximum patients were from age group 61-70 years. 64% patients were tobacco chewers. Oropharynx cancer (29.8%) was found to be most common followed by tongue cancer (23.4%).A definitive decrease in *Staphylococcus aureus* and *E. Faecium* was observed in both swab and saliva samples post radiotherapy, *Citrobacter* was found to be significantly decreased in saliva sample post radio therapy while high prevalence of *Candida* colonisation in the saliva sample was observed post RT.

CONCLUSION: The present study showed that changes in the oral and/or systemic environment due to cancer therapy can result in growth of many bacterial and fungal species in oral cavity that can lead to clinical bacterial/fungal infection.

Keywords:- *Chemo-Radiotherapy, Oral Cancer, Oral Microorganisms*

I. INTRODUCTION

Oral cancer ensues with a small, unfamiliar, unexplained growth or sore in the mouthparts that include lips, cheeks, sinuses, tongue, hard and soft palate and the base of the mouth extended to the oropharynx. Worldwide, oral cancer ranks sixth among all types of cancer. India has the largest number of oral cancer cases around 77,000 new cases and 52,000 deaths are reported annually, which is approximately one-fourth of the total burden of oral cancer globally. (1) In the latest national registry program, 2020, the projected incidence of patients with oral cancer in India was 3,77,938. with the number of male patients about three times more (Crude rate 19.2) than that of female patients (Crude rate 7.3) (2). Approximately 70% of the cases are reported in the advanced stages (American Joint Committee on Cancer, Stage III-IV) as compared to 40% in western countries hence the apprehension of oral cancer is considerably higher in India. Because of late-stage detection, the probability of healing is very low and pessimistic; with five-year survival rates of about only 20% (3, 4).

In India, where chewing tobacco is used with betel nuts and reverse smoking (placing the lit end in the mouth) is practiced, there is a striking incidence of oral cancer. (5) Oral cancer is related to these etiological factors from qualitative as well as quantitative points of view. A study on reverse smoking revealed that the use of tobacco in this form conferred 5.19 times higher risk of oral precancerous lesions of the palate than did the use of chewing tobacco. (6)

The national institute of cancer prevention and research statistics has reported that around 2,500 people pass away every day due to tobacco-related diseases in India. (7).

The alteration of the oral micro-flora in Oral squamous cell carcinoma (OSCC) plays a significant role in the prior diagnosis of oral cancer (8, 9). Due to oral cavity infection, these microflorae may be consequently replaced by potentially pathogenic microorganisms which resulted in several histological changes in oral mucosa and salivary glands such as oral mucositis and decreased phagocytic activity of salivary granulocytes. The decreased number of salivary glands facilitates the growth of these micro floras (10). An immune-compromised individual can easily get local and systemic infections caused by several different micro-organisms due weakened immune system by chemotherapy, irradiation, and surgery (11,12). These microorganisms include Staphylococcus aureus, Pseudomonas aeruginosa, and Candida species (13,14). The changes in micro-flora on oral mucosa begin after cancerous alteration, due to changes in pH, irregularity of lesion surface, and broken defines mechanism of the oral mucosa. It remains unclear whether the higher presence of bacteria on the surface of OSCC is caused by patients weakened immune systems, chronic inflammation, easy bacterial retention on irregular surfaces, or just neglect of oral hygiene.

Candida species are very frequently present in the oral flora under normal conditions, though, in certain physiological and pathological conditions, the yeast may

perhaps transform from a commensally to a pathogen, predominantly in patients reported to have malignancies. While in patients treated with ionizing radiation, up to 86% oral mucosa yeast colonization was reported (15-17) Several studies with saliva samples have demonstrated high levels of colonization of OSCC by facultative oral streptococci (18, 19), whereas viable bacteria have also been isolated from both superficial and deep section of OSCC (20, 21), suggesting that the tumor microenvironment is apt for the survival of bacteria. The major objective for the management of malignancy is to provide treatment as early as possible to evade recurrence and metastasis of the primary lesion. (22) The mode of early treatment includes surgery, chemotherapy to radiation therapy (RT) (23) This study aimed to evaluate the effect of radiation on the oral swabs and salivary bacterial and yeast flora in patients with oral cancer under chemo-radiotherapy.

II. MATERIALS AND METHODS

This is an observational and comparative study conducted by the 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). Consent from all the patients was taken during this research. A detailed history was taken including Patient name, gender, age, address, type and duration of complaint, site, and stage of cancer, type and duration of therapy, a cycle of Chemotherapy, HIV status, history of diabetes, consumption of tobacco or related products, steroid or other drug use, any related illness, TLC and DLC, etc. The patients with additional risk factors for change in oral flora such as current and history of usage of drugs/corticosteroids/antibiotics/immunosuppressive drugs and patients using dentures were excluded from the study.

III. SAMPLE COLLECTION

At every visit, two samples (swabs and saliva) were collected from each patient. The swab sample was collected by sterile cotton bud stick by collecting as much ooze as possible from the growth of cancer or ulcer or any other inflamed or ooze-containing area for bacteriological examination. Saliva was also collected from the same patients in the sterile container. All these samples were collected from patients before and after radiotherapy and were transported to the microbiology lab within 2 hours at room temperature.

All patients were given symptomatic treatment when required. Oral hygiene measures comprising betadine gargles high protein diet and plenty of fluids were routinely recommended to all patients. One hour prior to swab and saliva collection, the patients were not allowed to eat, drink, smoke, or use oral hygiene products.

Bacterial and yeast culture and growth: The oral swab and saliva sample collected was spread on MacConkey Agar and blood agar plate (HI Media/Oxoid Thermo Fisher Scientific). DNase Agar and Arabinose agar base for subculture were used to differentiate between Staphylococcus and Enterococcus. Subsequently, the subculture plates were incubated under aerobic conditions at 37ºC for 24- 48 hours.

Using the standard departmental protocol, isolated bacterial strains were subjected to various biochemical tests namely, Urease, citrate, methyl red, indole, and sulfur indole motility which were specific for oral bacteria such as Lactose fermenter and non-lactose fermenter. Thus, these evaluations enabled the identification of the oral bacteria and yeast flora in the study subjects. The other biochemical tests done in this experiment were: Gram staining, catalase test, oxidase test, hemolysis in blood agar plate (BAP), and growth in bile aesculin agar.

IV. RADIOTHERAPY PROCEDURES

Treatment comprised a dose of 66 GY of radiation therapy (RT) delivered over approximately 6 to 7 weeks, along with concomitant chemotherapy whenever and as required. RT was administered 5 days a week. The patients were evaluated before the start of RT (week 0), and stated as pre-RT and 6 to 7 weeks after completion of RT and stated in the text as post-RT. All patients were given symptomatic treatment when required. Complete blood count (CBC) liver function test (LFT), and Kidney function test (KFT) were evaluated prior to chemotherapy and radiation was given as per clinical needs.

V. STATISTICAL ANALYSIS

Data was entered and analysed using statistical software (SPSS 21.0 for window) used to evaluate and summarized data of the study subjects. An association between two different sample variables was analysed by Fisher exact test and P-value <0.05 was statistically significant.

VI. RESULTS

77 patients of histopathologically proven oral squamous cell carcinoma under chemo-radiotherapy were included in the study of which 69(89.6%) were Male and 08(10.4%) were female. Figure 1 showed the age-wise distribution of the patients. The patients were between 30 years to 80-year age group and a maximum (of 22) of 21 males in the age group 61-70years and minimum (4) in the age group 71-80 years, a maximum (of 3) female patients in the age group 51-60 years. Some patients are diagnosed as diabetic along with cancer. Table 1 shows the number of diabetics patients was 14 (36%) and tobacco chewer patients 49 (64%). Figure 2 demonstrates the Site of Lesions of cancer patients. The maximum lesion was at Oropharynx (23), followed by the base of the tongue (18), larynx (12), soft plate (7), pharynx, buccal mucosa, and upper alveolar region (4 patients each) Tongue and lateral border of the tongue (2 each) and throat (1). Table 2 depicts Bacterial and yeast type representation in OSCC group pre and post RT. The percentage of oral swab flora (bacterial flora) *Staphylococcus aureus* present was11.9 in patients pre-RT while 5.19 in post-RT while in saliva percentage was 10.39 pre-RT and 6.49 in post-RT. The percentage present of *E.coli* in the swab was 9.09 pre-RT which increased to 11.69 post-RT, and the saliva sample to it showed an

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increase in the almost same pattern, was 11.69 pre-RT and increased to 14.29 post-RT which was found to be statistically significant. On the other hand, we observed the percentage of *Citrobacter* in swabs was 10.39 pre-RT which increases to 12.99 post-RT, while in saliva it was 10.39 pre-RT and showed a significant decrease to 2.56 % post-RT. *E. faecalis* was showing no difference in the swab samples of the patients both pre- and post-RT (15.58% in both conditions) while in saliva samples there was an increase in presence from 12.9 % pre-RT to 16 % post-RT. *E. faecium* showed a percent decrease during pre-RT to post-RT in both samples and in swabs, it decreased from 14.29 to 7.79 whereas in saliva from 16.80 to 10.39. *Candida albicans* showed a slight percent decrease (from 1.3 to 1.2) in swab sample while a non-significant increase was observed (from 0.00 to 2.56%) in pre-and post-RT. Though the changes were there in the presence of flora pre-and post-RT in both the samples most of them were statistically non-significant except the presence of E. coli and Citrobacter saliva samples where the difference was found to be statistically significant ($P< 0.05$).

VII. DISCUSSION

This observational and comparative study is based on an assessment of microorganisms of the oral cavity isolated as an outcome of pre- and post-Radio-chemotherapy for oral cancer patients. In our study, most of the patients were in the age group of 61-70 years (28.57%) and the majority (89.6%) of study subjects were male. This finding is in accordance with the study conducted by Panghal M et al., with the dominance of male patients (24). Almost similar results were obtained in several studies where most of the subjects were male but belong to the age group of 51-60 years (25). Oropharynx cancer (29.8%) was found to be most common in our study which was also observed by Kamath et al. in their study, followed by tongue cancer (23.4%), while Panghal M et al., found tongue cancer most common in their study (24,25]. The major factor of oral cell cancer is Chronic inflammation (26, 27), while drinking and smoking are the co-promoters (28)

The effect of chemoradiotherapy on oropharyngeal flora was noted pre-radiotherapy and post-radiotherapy. It was observed that there was a definitive alteration in oropharyngeal flora post-radiotherapy and this finding was in accordance with the study done by Mehrotra R (29). We found a definitive decrease in *Staphylococcus aureus* and *E. faecium* post-radiotherapy. This decrease was prominent in both swab and saliva samples. The earlier studies also showed a significant decrease in normal commensally in cancer patients compared to controls post-radiation therapy (30, 31). Bacteria have the capability to attack different epithelial cells through penetration; it affects colonization and thus persuades inflammation which may probably correlate to the progression of cancer (32). These cancer patients are colonized with a wide variety of Gram-positive, Gram-negative, aerobic, anaerobic, and mycotic pathogens. In the present study, *Citrobacter* was isolated and it was found to be significantly decreased in saliva samples post-radiotherapy. Pushkar et al in their findings illustrated that certain bacterial species or phylotypes spotted in their study might play a role in triggering chronic inflammation in the oral cavity and probably be linked to different stages of cancer (12). This perhaps is due to a disrupted oral mucosal surface which possibly serves as a point of entry to the regional lymph nodes permitting the invasion of bacteria (34, 35). This specifies that although the bacterial species were commensals of the oral cavities when their balance is disturbed, they may become pathogenic.

In accordance with our study Yamashita K et al., also found *Staphylococcus aureus* and *Candida spp.* as common isolates (36). *Candida species*, also present as commensals in oral microbiota in roughly 50% of the global population, and their presence is not considered a disease, but when *Candida species* after chemoradiotherapy transforms into pathogen it attacks host tissues and results in oral candidiasis. We found a high prevalence of Candida colonization in the saliva sample of the patients' post-RT this might be due to the reduced immunity of the patients following therapy (37). High occurrence colonization of *Candida spp*. was also reported in several studies (38). While the incidence of colonization of *Candida spp*. was reported to be more in the Chemo-radiotherapy group compared to Chemotherapy alone, Different studies reported a wide variation in the epidemiology of oral candidiasis (38)

The predominant species isolated which chiefly affects oral mucosa is *C. albicans*. In our study we found Candida albicans isolated from patients undergoing Chemo-radiotherapy which agreed with the studies conducted by Shaheen et al.

The present study showed that changes in the oral and/or systemic environment due to cancer therapy can result in the growth of many bacterial and fungal species in the oral cavity which can lead to clinical bacterial/fungal infection.

VIII. CONCLUSION

According to the findings of our study, there is an association between the colonization of oral flora and radiotherapy/ chemo-radiotherapy. Bacterial and yeast colonization enhances during radiotherapy and peaks at 6-7 weeks in patients undergoing chemo-radiotherapy in oral/oropharyngeal cancer. There was a significant increase observed in *E Coli* and a non-significant increase in *C. albicans* in the saliva samples of patients post-RT. Evaluation and Screening of radiotherapy patients for bacterial and yeast infections can prevent infections in such patients. Tobacco chewing, smoking, intake of alcohol, and non-maintenance of oral hygiene may possibly be the reasons directly responsible for developing oral/ oropharyngeal cancer in India. Awareness concerning the causes and fatalities of oral/oropharyngeal cancer needs to be reached among people.

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Variables	Study cases	Percentage
Tobacco Chewer	49	64%
Non-chewer	28	36%
Diabetics	14	18%
Non-Diabetics	63	82%

Table 1: Number and percentage wise distribution of OSCC patients of different variables

	Swab Sample			Saliva Sample		
Organism	**Pre RT**	**Post RT**	**P-value**	**Pre RT**	**Post RT**	**P-value**
Staphylococcus aureus	**11.69%**	**5.19%**	NS	**10.39%**	**6.49%**	NS
Escherichia coli	9.09%	11.69%	NS	11.69%	14.29%	S
Citrobacter	10.39%	12.99%	NS	**10.39%**	**2.56%**	S
E. faecalis	**15.58%**	15.58%	NS	12.90%	16%	NS
E. faecium	**14.29%**	**7.79%**	NS	**16.80%**	**10.39%**	NS
Candida albicans	1.3%	1.2%	NS	-	2.56%	NS

Table 2: Bacterial and yeast type representation in both swab and saliva samples (in percentage) of OSCC patients pre and post RT

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P-value <0.05 was considered to be statistically significant.

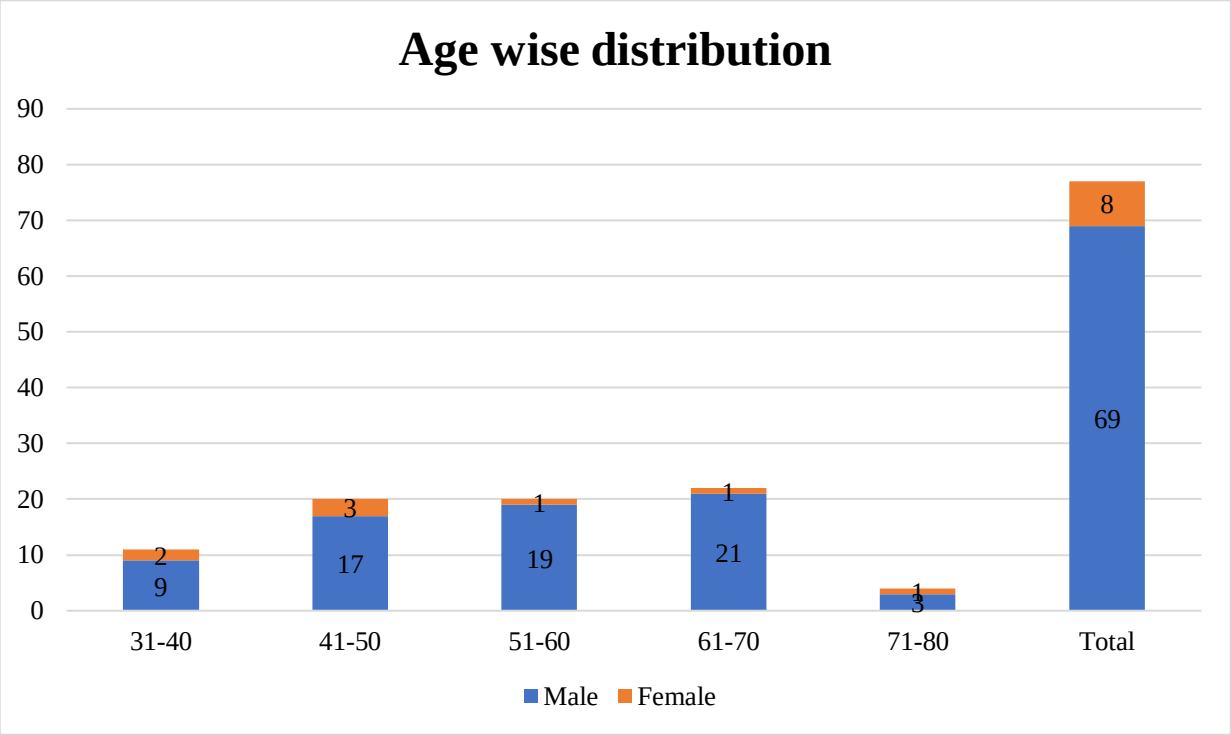

Fig. 1: Age and gender wise distribution (inNumbers) of the OSCC patients

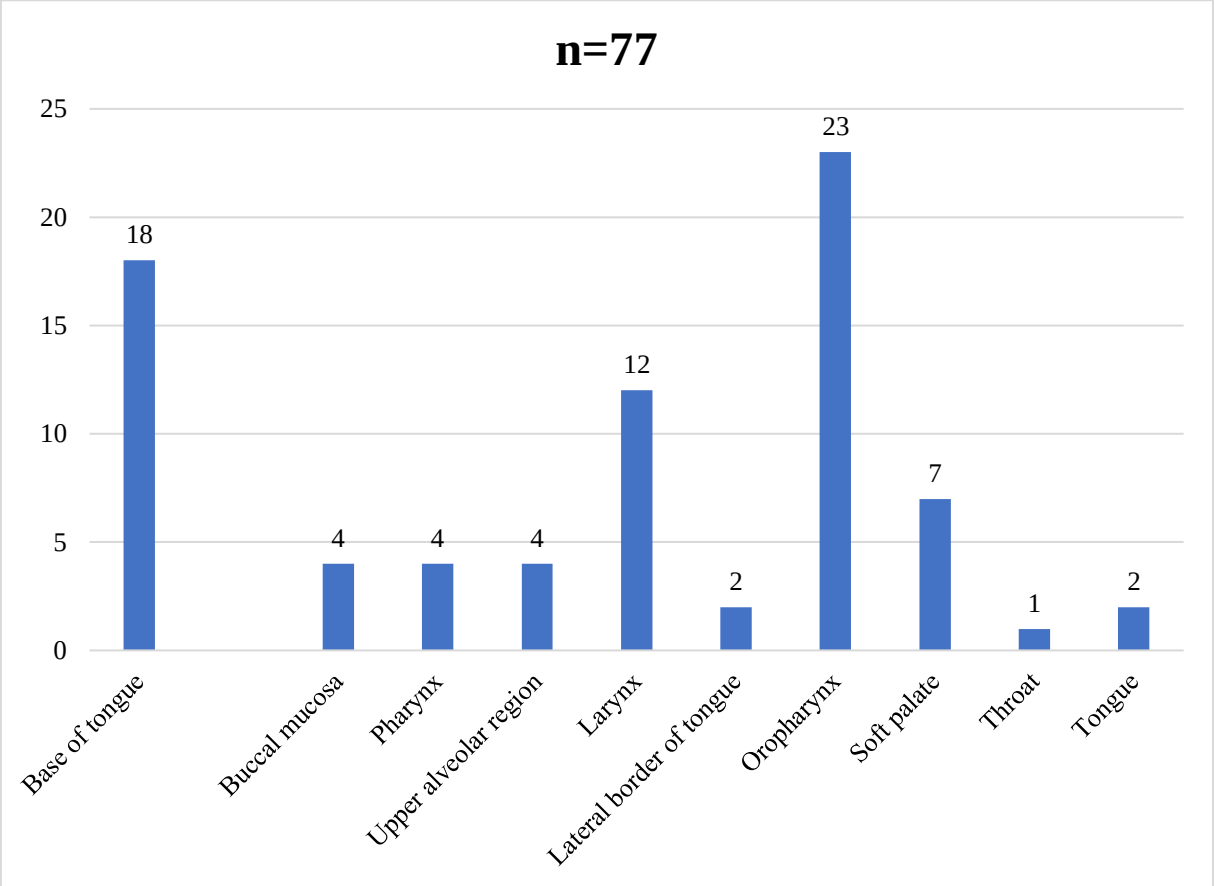

Fig. 2: Demonstrates the Site of Lesion of cancer patients.