Original Article

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Opportunistic Microorganisms in Oral Cavity According to Treatment Status in Head and Neck Cancer Patients

Microbiology Section

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ABSTRACT

Introduction: Cancer patients receiving chemotherapy and/or radiation therapy are prone to many predisposing factors like immunosuppression, imbalance in the oral flora, hypo-salivation and local tissue damage. Therefore, considered to be at higher risk for oral bacterial and fungal infection than the general population.

Aim: To study oropharyngeal flora in head and neck cancer patients under treatment and to correlate their incidence according with Chemotherapy cycles and Radiochemotherapy.

Materials and Methods: Total 110 patients were selected for study, those were further divided into two groups, group I under Chemotherapy (CT) – 55 patients and group II under Radiochemotherapy (RCT) – 55 patients and 50 healthy individuals were taken as control. Saliva sample was collected from control and study group and inoculated on Blood agar, MacConkey agar and Sabouraud's Dextrose Agar (SDA). The identification of bacterial and fungal isolates was done by standard microbiological methods and result was calculated according to cycles of Chemotherapy and Radiochemotherapy combined. Significant differences between patients were tested using the Chi-square test or Fisher's exact test. A p-value less than 0.05 was considered as statistically significant.

Result: There were 149 culture isolates from 110 patient in which Gram Negative Bacilli (GNB) found in 63.6%, *Candida* spp. in 50%, *Staphylococcus aureus* in 8% and Normal commensal of oral cavity in 13.6% patients in study group and this was higher than control group and this difference was statistically significant in relation to all isolates individually. Relatively more microorganism were isolated during RCT (56%) in compare to CT alone (44%), among GNB- *Pseudomonas* (27.7%,32.3%) and *Klebsiella* (25%,29.4%) were most frequently isolated during CT, RCT respectively. *Candida* spp. were more commonly isolated from patient on RCT (63.6%) than CT (36.3%) when compared to control group (20%) among which *C. tropicalis* was more prevalent species. Both GNB & *Candida* spp. were more commonly isolated in later chemotherapy cycles (CT4, CT5 CT6).

Conclusion: Colonisation of Gram negative bacilli & *Candida* spp. is directly related to number of chemotherapy cycles and combined cancer therapy. Hence, prophylactic medication for these two organisms should be incorporated along with cancer therapy.

Keywords: Chemotherapy, Germ tube test, Oral cavity, Radiochemotherapy, Saliva

INTRODUCTION

In oral cavity infection the oral micro flora may be subsequently replaced by potentially pathogenic microorganisms, such as pathogenic Gram positive cocci, Gram negative bacilli & *Candida* spp. There are some histological changes in oral mucosa and salivary glands such as oral mucositis, reduced phagocytic activity of salivary granulocytes and reduced amount of salivary glands facilitate their growth [1]. Opportunistic infections are common in individuals with poor health (an immunocompromised host) and are caused by several different microorganisms, among them representative microorganisms include *Staphylococcus aureus, Pseudomonas aeruginosa*, and *Candida* species [2,3]. The colonisation of bacterial and fungal infections in cancer patients varies from institution to institution and depends on use of prophylactic medication and hospital environment. The Bacterial infections are more common during the early phases of a neutropenia whereas, fungal infections occur during the latter part of cancer treatment patients with prolonged period of neutropenia [4]. Most of these organisms become drug-resistant, which has resulted in difficulties in curing the related infectious diseases. This is of utmost importance as most postoperative infections in head and neck have an endogenous source, mainly from the oropharynx [5]. Thus, the study was done to observe the change in oropharyngeal flora in head and neck cancer patients under treatment and to correlate their prevalence according with Chemotherapy cycles and Radiochemotherapy.

MATERIALS AND METHODS

The present prospective case control study was carried out in the Department of Microbiology at Dr. SN Medical College, Jodhpur, from January 2015 to July 2015 after ethical clearance by local ethical committee. Study group comprised of total 110 patients of head and neck tumours of age 20-80 years, 55 patients on Chemotherapy (CT) and 55 on Radiochemotherapy (RCT). The number of patients was decided according to prevalence of hospital admission of head and neck cancer patient for cancer therapy. Fifty normal healthy attendants of patients were taken as control group whose age and sex were matched with study groups and were apparently healthy without cancer and any systemic diseases. Detailed history was taken about Patient name, age, gender, residence, complain, duration of complain, site and stage of cancer, type of cancer therapy (Chemotherapy, Radiotherapy, or both), duration of therapy, cycle of Chemotherapy, HIV status, history of diabetes, steroid or other drug use, any associated illness, TLC and DLC etc.

Exclusion criteria: The patients with other risk factors for change in oral flora such as diabetes/HIV, recent usage of corticosteroids/antibiotics/immunosuppressive drugs and patients using intra oral prostheses were excluded from the study.

Sample collection & processing: Saliva sample was collected from control and study group after taking consent by oral rinse with phosphate buffer saline for 1 minute in a sterile plastic container.

Blood agar, MacConkey agar and SDA with gentamycin were used as primary culture media. The Blood agar, MacConkey agar plates were incubated at 37°C at for 24 hours and SDA plates on 25°C for 7 days. The identification of bacterial isolates was done by standard microbiological methods [6]. The creamy white, smooth, pasty colonies on SDA agar were further subjected to HiCrome *Candida* Differential Agar (HiMedia, M-1297A) [7], Germ Tube test, Carbohydrate Fermentation Test, and Corn Meal Tween 80 agar streak culture to identify various species of *Candida.*

STATISTICAL ANALYSIS

Statistical analysis were performed using the software SPSS Incs v.13, Chicago, IL, USA. The frequency of detection of the microorganisms was computed for each subject. Significant differences between patients were tested using the Chi-square test or Fisher's-exact test. Differences of p<0.05 was considered statistically significant.

RESULT

Total patient were 110 in which male were 80 (72.8%). The Mean age of patients was 51-60 years (32.7%). Total 62% of the patients were rural and 52.7% from IPD.

The most common cancer was larynx (25.5%) followed by pharynx (20%) in head and neck region in present study group and most common histopathology of head and neck cancer was moderately differentiated squamous cell carcinoma (47.2%).

Out of 110 patients, 71% had single organism while remaining patients have poly-microbial aetiology. Among 149 culture isolates from 110 Patient, GNB was found in 70 patients i.e., 63.6%, *Candida spp.* in 50%, *Staphylococcus aureus* in 8% and Normal commensal of oral cavity in 13.6% patients in study group. This was higher than control group and this difference was statistically significant in relation to all isolates individually [Table/Fig-1].

GNB is almost equally isolated from both group whereas, *Candida* spp. were more common during Radiochemotherapy (63.6%) but results were non-significant expect for *Candida* spp. which was significantly high in RCT group of patients. Among GNB *Pseudomonas* (27.7%, 32.3%) and *Klebsiella* (25, 29.4%) were most frequently isolated during CT, RCT respectively. Statistical Analysis showed that isolation of *Acinetobacter, Citrobacter, Pseudomonas, Klebsiella* were significantly high in study group as compared to control group, (Study vs. Control group, p-value <0.0001). However, the results were non-significant when compared CT vs. RCT groups. *Candida* spp. was more commonly isolated from patient on RCT (63.6%) than CT (36.3%) as compared to control group (20%) among which *C. tropicalis* was more prevalent species [Table/Fig-2]. In later Chemotherapy cycles (CT4, CT5, CT6), 64% and in early CT cycles (CT1, CT2, CT3) 36% microorganism were isolated. Normal commensal were common in early cycles of Chemotherapy. In early CT cycles percent of GNB and *Candida* spp. were 44.4%,30% whereas in the later cycles it was 55.6%, 70% respectively; this

Organism	Study Group			Control
	CT	RCT	Total patient (110)	
GNB	36	34	70 (63.6%)	1 (2%)
GPC-*Staphylococcus aureus*	3	6	9 (8.1%)	0
Candida species	20	35	55 (50%)	10 (20%)
N.C. (Normal Commensal)	10	5	15 (13.6%)	39 (78%)
Total isolates	69	80	149	50

Organism	p-value	
	CT vs. RCT	Study vs. control
GNB	0.842	<0.0001
GPC-*Staphylococcus aureus*	0.489	0.002
Candida species	0.007	0.0004
N.C. (Normal Commensal)	0.266	<0.0001

[Table/Fig-1]: Distribution of micro organism in study group and control group in relation with patient no. {CT= Chemotherapy; RCT= Radiochemotherapy; GPC= Gram positive cocci; GNB= Gram negative bacilli; N.C. =Normal commensal; p-value <0.05 is significant, chi-square test was used to calculate p-values}

Organism	CT1	CT2	CT3	CT4	CT5	CT6	Total isolates during CT	Total isolates during RCT	p-value
Total GPC (9)									
Staphylococcus aureus (9)	0	0	1	1	1	0	3	6	0.489
Total GNB (70)									
Acinetobacter (10)	0	0	1	1	2	1	5	5	0.0
Citrobacter(14)	1	0	1	1	2	1	6	8	0.779
Esch.coli (2)	0	0	0	1	0	1	2	0	0.496
Enterobacter (4)	0	0	2	1	1	0	4	0	0.11
Klebsiella (19)	1	1	2	1	2	2	9	10	0.800
Pseudomonas (21)	1	1	2	2	1	3	10	11	0.808
Total GNB (70)	3	2	8	7	8	8	36 (51.4%)	34 (48.5%)	0.865
Total Candida sp. (55)									
C. albicans(6)	0	0	0	1	1	0	2	4	0.678
C. dublin-iensis(5)	0	0	0	0	1	0	1	4	0.363
C. glabrata(6)	0	0	0	0	2	0	2	4	0.678
C. guiller-mondii(3)	0	0	0	0	0	1	1	2	0.558
C. krusii(4)	0	1	0	0	0	1	2	2	0.00
C. parap- sillosis(5)	0	0	1	1	1	0	3	2	0.647
C. pseudo-tropicalis(5)	0	0	1	1	0	1	3	2	0.647
C. tropicalis (21)	1	1	1	1	1	1	6	15	0.050
Total *Candida* (55)	1	2	3	4	6	4	20 (36.3%)	35 (63.6%)	0.007
Grand Total (134)	4	4	12	12	15	12	59 (44%)	75 (56%)	0.066
Normal commensal									
Normal Commensal (15)	5	1	4	0	0	0	10	5	0.143

[Table/Fig-2]: Distribution of micro organism in study group according to treatment status of cancer. {CT1 to CT6= Chemotherapy Cycle No. 1 to 6; RCT= Radiochemotherapy; GPC= Gram Positive Cocci; GNB= Gram Negative Bacilli}
(The value in the bracket denotes the total no. of isolates.

Organism	Early CT cycles (CT1, CT2, CT3)	Later CT cycles (CT4, CT5, CT6)	Total isolate (CT)	Total isolate (RCT)
Total GPC (9)				
Staphylococcus aureus	1	2	3	6
Early vs. Late CT p-value = 1.000				
Total GNB (70)				
Acinetobacter	1	4	5	5
Citrobacter	2	4	6	8
E.coli	0	2	2	0
Enterobacter	2	2	4	0
Klebsiella	4	5	9	10
Pseudomonas	4	6	10	11
Total	13(36.1%)	23(63.8%)	36	34
Early vs. Late CT p-value = 0.345				
Total *Candida* spp.(55)				
C. albicans	0	2	2	4
C. dubliniensis	0	1	1	4
C. glabrata	0	2	2	4
C. guilliermondii	0	1	1	2
C. krusii	1	1	2	2
C. parapsillosis	1	2	3	2
C. pseudo tropicalis	1	2	3	2
C. tropicalis	3	3	6	15
Total	6(30%)	14(70%)	20	35
Early vs. Late CT p-value = 0.565				
Grand Total(134)	23(39%)	36(61%)	59(44%)	75(56%)
Early vs. Late CT p-value = 0.385				

[Table/Fig-3]: Distribution of Organism in Study Group According To Cycles of Chemotherapy; {CT1 to CT6= Chemotherapy Cycle No. 1 to 6; CT = Chemotherapy; RCT= Radiochemotherapy; GPC= Gram Positive Cocci; GNB= Gram Negative Bacilli}.

shows that GNB & *Candida* spp. were more commonly isolated in later chemotherapy cycles but results were non-significant with CT cycles [Table/Fig-3].

DISCUSSION

This prospective case control study based on evaluation of micro-organism of oral cavity isolated as outcomes of various cancer treatment procedures (Chemotherapy, Radiochemotherapy) for head and neck cancer patients. In study group, majority patients were in the age group of 51-60 years (32.7%) and majority of study subjects were male patients (72.8%). This is in accordance to study done by Kamath MP et al., where majority of the subjects were male (75.6%) falling in age group of 51-60 years [8]. Similar results were also seen in a study conducted by Panghal M et al., in which male cases dominated [9]. Majority of patients were of carcinoma of larynx (25.5%) followed by pharynx (20%) in head and neck region, this finding is similar to study done by Rad M et al., Dambroso DA et al., [10,11]. Oropharynx cancer was more common in a study done by Kamath MP et al., and tongue cancer in a study by Panghal M et al., [8,9].

The study done by Kamath MP et al., also showed that normal commensal was significantly reduced in cancer patients compared to controls due to radiation therapy [8]. These cancer patients colonised with a wide variety of Gram positive, Gram negative, aerobic, anaerobic and mycotic pathogens. In present study, GNB isolated in 63.6% patients which is accordance to study done by Panghal M et al., [9] but this finding is in contrast to study done by Yamashita K et al., who found *Staphylococcus aureus* and *Candida* spp. commonly isolated [12].

Another salient feature of the study was the colonization of *Candida* spp. in study group (50%) as compared to control group (20%). Yogitha PPV et al., showed 38% were culture positive for *Candida* spp. in study group and 18% in control group [13]. This could be due to reduced immunity of the patients due to chemotherapy. Bakki SR et al., also found a high prevelance of *Candida* colonisation in the study group (38%) as compared to control group (22%) [14]. Prevalance of colonization of *Candida* spp. were more in the RCT group (63.6%) than CT alone (36.3%), The epidemiology of oral candidiasis shows a wide variation (from 17 to 52.5%) in different studies [15-19].

Among *Candida* spp., *C. tropicalis* was most common isolate i.e., 38% which correlates with studies done by Yogitha PPV et al., and Bakki SR et al., [13,14] and contrast with the study done by Safdar A et al., Shaheen et al, and Suryawanshi H et al, reported *C. albicans* 67.3%, 78%, 78.57% respectively to be the predominant species isolated from patients undergoing RCT or CT [20-22]. 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.

LIMITATION

The sample size was small as the duration of the study was short (only 6 months) and change in the anaerobic oral micro flora was not studied as there is no anaerobic setup in the institute.

CONCLUSION

According to this study, colonisation of Gram Negative Bacilli & *Candida* spp. has linear correlation with immunosuppression and this is directly related to number of Chemotherapy cycles. Hence, it would be advisable that in patients with head and neck malignancy prophylactic medication for these two organisms should be incorporated for treatment algorithm.

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