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Original Research Article

A Prospective Study to Identify the Fungal Isolates as well as their Antifungal Susceptibility in Patients from Undergoing Chemo-radiotherapy

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Abstract

Aim: The aim of this study was to identify the fungal isolates as well as their antifungal susceptibility in patients from undergoing chemo-radiotherapy.

Material & Methods: A prospective study was conducted on 100 randomly selected patients of Head and Neck cancer undergoing treatment at Department of Microbiology and Department of Radiotherapy at Patna Medical College and Hospital, Patna for the duration of 1 year.

Results: Majority of the study population belonged to age group of 51-60 years (33%) followed by 61-70 years (21%). The mean age was found to be 54.2 years. There were no cases belonging to age group of less than 20 years. Carcinoma of the oral cavity was the most common tumour present among the members of the study group, with Ca Tongue constituting (28%) and Ca Buccal mucosa (26%) followed by Carcinoma supraglottis (20%). The samples collected are urine, blood and throat swab during 0, 2 and 6 weeks of radiotherapy. 90.5% of the isolates were isolated from throat swab and 9.4% isolates from Urine. There were no isolates in blood samples. The increased incidence of fungal infections in both throat swab and urine at 6th week of radiotherapy compared to before radiotherapy was found to be statistically significant with p value of <0.001 (throat swab) and 0.04 (urine). A wide spectrum of clinical presentation was present in the culture positive cases such as Presence of white patch (52.8%), Redness in oral cavity (20.8%), Dry mouth (11.3%), asymptomatic (9.4%) and Dysphagia (5.7%).

Conclusion: Oral candidiasis is a common fungal infection in patients with cancer on treatment with chemotherapy and/or radiotherapy. Candida albicans and non-Candida albicans differ significantly in their antifungal susceptibility pattern. Non-Candida albicans like Candida krusei are inherently resistant to azoles. Hence, species level identification with in vitro antifungal susceptibility pattern is essential to choose the appropriate antifungal drug and to predict the outcome of therapy.

Keywords: Head and neck malignancies, Fungal infection, Chemoradiotherapy.

Introduction

Cancer or neoplasia is defined as a disorder of cell growth that is triggered by a series of mutation affecting a single cell and its clonal progeny. According to the Global Health Observatory (GHO) data, mortality due to cancers accounts 22% which were caused by non-communicable diseases. [1] Head and neck cancers are the most common malignancy among male (23 %) and second most common malignancy among females (6.2%) in India. The term “Head and Neck” cancer includes cancer of Oral cavity, Pharynx, Larynx, Paranasal sinuses and Salivary glands. Squamous cell carcinoma constitutes 90-95% of Head and neck cancers.[2] Head and Neck Cancers (HNCs) are often treated with Concurrent Chemotherapy and Radiation therapy or chemoradiation also referred to as “Organ preserving therapy”. Chemoradiation therapy can lead to toxicity of the surrounding mucosal areas. Chemotherapy and radiation cause many changes in the body as they destroy malignant cells. One major change is it weaken immune system, makes them particularly susceptible to getting fungal infections.[3,4] Malignancies, radiotherapy and chemotherapy compromises the cell mediated immunity predisposing the person to fungal infection.[3] Worldwide, Candida species are frequently present in oral microbiota in about 50% of population.[5] Therefore, under chemoradiotherapy, the yeast would transform from commensal to pathogen in malignant patient. Incidence of oral candidiasis has to be reported between 7% to 52% among patients (head neck malignancy, solid tumors and hematopoietic malignancy) on radiotherapy or chemotherapy.[6]

Radiotherapy administered to patients with head and neck malignancies makes them particularly susceptible to oropharyngeal candidiasis.[7]

Candida species are very often present as commensal in oral microbiota in about 50% of worldwide population. However, under immunosuppressive treatment (chemoradiotherapy), the yeast may transform from commensal to a pathogen, in patients with malignancies. This change usually constitutes an opportunistic infection by normally harmless micro-organisms because of local (i.e., mucosal), or systemic factors altering host immunity, in this case chemoradiotherapy. Oral mucosal colonization and infection are common in patients receiving radiation therapy for head and neck malignancies. One of the prime causes for this is thought to be the resultant xerostomia due to destruction of glandular tissue by radiation.[8] One of the acute side effects of head and neck radiotherapy is oral mucositis, one of the closest differential diagnosis of oral candidiasis, which is marked by erythema and oral ulcerations. The high prevalence of candidiasis during head and neck radiotherapy, combined with difficulties in differentiating infection and mucositis and the potential role of candidiasis in increasing the severity of mucositis has led to consider the need for timely diagnosis and management.[9,10] Cancer patients should be evaluated microbiologically and clinically for the presence of Candida in the oral cavity. Infection and oral colonization are common in patients receiving radiation therapy for neck and head cancer. One of the major causes of this is thought to be the resultant xerostomia due to destruction of glandular tissue by radiation.[3] Candida albicans is the most preponderant organism causing fungal infections in patients with head and neck malignancies receiving radiotherapy, but since last decade there is emergence of Non-albicans Candida. Hence rapid diagnosis followed by appropriate management may reduce the severity and morbidity of fungal infections in these patients. Therefore, the aim of this study was to identify the fungal isolates as well as their antifungal susceptibility in patients from undergoing chemo-radiotherapy.

Material & Methods

A prospective study was conducted on 100 randomly selected patients of Head and Neck cancer undergoing treatment at Department of Microbiology and Department of Radiotherapy at Patna Medical College and Hospital, Patna for the duration of 1 year.

Inclusion Criteria:

- Newly diagnosed and histopathologically confirmed cases of Head and
- Neck cancer undergoing Chemoradiation.

Exclusion Criteria:

- Patients with fungal infections before start of chemoradiotherapy
- Patients who had taken anti-fungals within two weeks of the study period
- Patients with immunocompromised conditions like HIV infection and on immunosuppressive therapy
- Patients with comorbidities such as Diabetes
- Patients who were not be willing for the study

Sample Collection

Throat swabs were collected from the Head and Cancer patients on Chemoradiation at 0 week (start of radiotherapy), at 4 weeks (during radiotherapy) and at 7 weeks (at the end of radiotherapy). Two sterile cotton swabs were used to collect material from the lesions present in the buccal mucosa, tongue, gingiva and the palate.

Transportation

The sample collected was transported to the laboratory within two hours since over-growth of the slower growing pathogenic fungi by contaminating bacteria or fungi is common. It is important to transport a sample to the lab as soon as possible.

Processing

Mycological study was carried out on throat swab sample. From the two cotton swabs collected, one swab was used for gram staining and the others were used for inoculation into culture media. Direct microscopic examination was carried out for detection of fungal elements. For this KOH (10%) and Gram stain was used. For culture samples was inoculated on Sabouraud's Dextrose Agar (SDA). Culture tubes were examined for growth every 2-3 days. Identification was done by direct microscopy from culture growth by using Lacto- Phenol Cotton ablue (LPCB) mount preparation and Gram stain. Yeast like candida was identified by microscopy examination by LPCB mount preparation. For candida species germ tube test was followed by culture on cornmeal agar. This was followed by sugar assimilation and sugar fermentation test. After completion of treatment, evaluation was done immediately after 1 month (during last sampling) and after 6 weeks of completion of radiotherapy (for response assessment). Clinical examination, ENT examination with CT/MRI of head and neck were done 6 weeks after the completion of treatment.

Statistical Analysis

The results were evaluated according to CLSI guidelines, 2012. In all cases, patients were evaluated weekly for response of tumour and toxicities [The Response Evaluation Criteria in Solid Tumours (RECIST) Criteria and Common

Terminology Criteria for Adverse Effects (CTCAE) version 4.0].

Results

Table 1: Demographic characteristics of the study population

Age (Years)	Male	Female	Total no ofcases n=100
< 20	-	-	-
21-30	2	-	2(2%)
31-40	6	4	10(10%)
41-50	19	9	28(28%)
51-60	24	9	33(33%)
61-70	17	4	21(21%)
>70	4	2	6(6%)

Majority of the study population belonged to age group of 51-60 years(33%) followed by 61-70 years(21%).The mean age was found to be 54.2 years. There were no cases belonging to age group of less than 20 years.

Table 2: Distribution of Study Population Based On Site of Tumour

Site of Tumour	N	Males	Females
Carcinoma Oral cavity			
Carcinoma buccal mucosa	26	16	10
Carcinoma Tongue	28	20	8
Carcinoma oropharynx			
Carcinoma Tonsillar fossa	3	2	1
Ca Hypopharynx			
Carcinoma pyriform sinus	4	3	1
Carcinoma posterior pharyngeal wall	2	1	1
Carcinoma Larynx			
Carcinoma supraglottis	20	17	3
Carcinoma glottis	7	5	2
Carcinoma Maxilla	6	5	1
Carcinoma submandibular gland	4	3	1

Carcinoma of the oral cavity was the most common tumour present among the members of the study group, with Ca Tongue constituting (28%) and Ca Buccal mucosa (26%) followed by Carcinoma supraglottis (20%).

Table 3: Fungal infection in throat, urine and blood samples

Samples taken	**Samples positive for fungal infection**		
Before radiotherapy	***Throat swab***	***Urine***	***Blood***
	7	Nil	Nil
At 2 Weeks of radiotherapy	18	1	Nil
At 6 weeks of radiotherapy	23	4	Nil
TOTAL	**48(90.5%)**	**5(9.4%)**	**Nil**
P value	**<0.001**	**0.04**	**-**

The samples collected are urine, blood and throat swab during 0, 2 and 6 weeks of radiotherapy. 90.5% of the isolates were isolated from throat swab and 9.4% isolates from Urine. There were no isolates in blood samples. The increased incidence of fungal infections in both throat swab and urine at 6th week of radiotherapy compared to before radiotherapy was found to be statistically significant with p value of <0.001 (throat swab) and 0.04 (urine).

Table 4: Spectrum of clinical presentation in culture positive patients

Clinical Presentation	No of positive cases	Percentage
Dysphagia	3	5.7
Asymptomatic	5	9.4
Dry mouth	6	11.3
Redness in oral cavity	11	20.8
Presence of white patch in oral cavity	28	52.8
TOTAL	**53**	**100**

A wide spectrum of clinical presentation was present in the culture positive cases such as Presence of white patch (52.8%), Redness in oral cavity (20.8%), Dry mouth(11.3%), asymptomatic (9.4%) and Dysphagia (5.7%).

Table 5: Correlation of dosage of radiotherapy with occurrence of infections

Week of assessment	Fractions ofradiotherapy undergone	Dose of radiation exposed	No of culture positivecases	Percentagen=53
0 week	Nil	Nil	7	13.2%
2 weeks	10	20 Gy	19	35.8%
6 weeks	30	60 Gy	27	50.9%

The average dose of radiation which on exposure caused infections was found to be 37.7 Gy.

Table 6: Antifungal susceptibility to Fluconazole, Amphotericin B, Itraconazole

SPECIES	FLUCONAZOLE			AMPHOTERICIN B		ITRACONAZOLE		
	S N (%)	SDD N (%)	R N (%)	S N (%)	R N (%)	S N (%)	SDD N (%)	R N (%)
C.albicans (14)	9 (64.3)	2 (14.2)	3 (21.4)	14 (100)	-	10 (71.4)	2 (14.3)	2 (14.3)
C.tropicalis (20)	15 (75)	1 (5)	4 (20)	20 (100)	-	15 (75)	1 (5)	4 (20)
C.krusei (4)	-	-	4 (100)	4 (100)	-	4 (100)	-	-
C.glabrata (8)	-	6 (75)	2 (25)	8 (100)	-	5 (62.5)	1 (12.5)	2 (25)
C.kefyr (2)	2 (100)	-	-	2 (100)	-	2 (100)	-	-
TOTAL (48)	26 (54.2)	9 (18.8)	13 (27.1)	48 (100)	-	36 (75)	4 (8.3)	8 (16.7)

In this study, 54.2% of Candida isolates were susceptible, 18.8% susceptible dose dependent and 27.1% resistant to Fluconazole. 100% of candida species in this study were susceptible to Amphotericin B.75% of Candida isolates were sensitive, 8.3% susceptible dose dependent and 16.7% were resistant to Itraconazole.

Discussion

The oral mucosa acts as an important physiological barrier providing innate immunity and prevents local and systemic invasion by microorganisms. Cytotoxic radiotherapy induced mucosal injury of oral cavity causes salivary hyposecretion which in turn can contribute to local invasion by colonizing microorganisms.[11] Oral candidial colonisation and infection are the most common fungal infections in head and neck cancer patients.[12] Candidiasis is caused by various species of yeast-like fungi which belong to the genus Candida and Candida albicans is the representative species.

The pathologic processes involved may range from inflammation and irritation to acute suppuration and granulomatous tissue formation. In the past, Candida albicans infections were more common but the current trend shows a predominance of Candida non -albicans infections. The frequently isolated Candida Non-albicans species are Candida tropicalis, Candida glabrata , Candida krusei, Candida famata, Candida parapsilosis and Candida haemulonii. Oral candidiasis , also known as the “Oral thrush” is the most common clinical presentation produced by Candida species. It usually manifests as a white patch which can be localised to tongue or can involve the buccal mucosa, gums and palate as well.

Majority of the study population belonged to age group of 51-60 years(33%) followed by 61-70 years(21%).The mean age was found to be 54.2 years. This male preponderance was similar to that reported by Raj Sharma et al[13] and Shoba Rani Bakki et al.[14] There were no cases belonging to age group of less than 20 years. This correlated well with a previous study by Mridula Madiyal et al[15] and another study by P.V.V.Yogitha et al.[16] Carcinoma of the oral cavity was the most common tumour present among the members of the study group, with Ca Tongue constituting (28%) and Ca Buccal mucosa (26%) followed by Carcinoma supraglottis (20%). The samples collected are urine, blood and throat swab during 0, 2 and 6 weeks of radiotherapy. 90.5% of the isolates were isolated from throat swab and 9.4% isolates from Urine. There were no isolates in blood samples. The increased incidence of fungal infections in both throat swab and urine at 6th week of radiotherapy compared to before radiotherapy was found to be statistically significant with p value of <0.001 (throat swab) and 0.04 (urine).

The average dose of radiation which on exposure caused infections was found to be 37.7 Gy. This correlated well with a previous study done by Madiyal et al[15] who has given a mean of 41.2 whereas another study by Martha C.Dahiya et al[17], has shown a lower dose of radiation of 28.6Gy. Moreover Jham et al[18], has observed infections with a mean time of onset at the 16th fraction which coincides with the current study. A

wide spectrum of clinical presentation was present in the culture positive cases such as Presence of white patch (52.8%), Redness in oral cavity (20.8%), Dry mouth(11.3%), asymptomatic (9.4%) and Dysphagia (5.7%) which was in concordance with a study by Abirami Lakshmi Jayachandran et al.[19] In this study, 54.2% of Candida isolates were susceptible, 18.8% susceptible dose dependent and 27.1% resistant to Fluconazole. 100% of candida species in this study were susceptible to Amphotericin B.75% of Candida isolates were sensitive, 8.3% susceptible dose dependent and 16.7% were resistant to Itraconazole. This was similar to a study by Abirami Lakshmi Jayachandran et al[19] who has shown 12.7% resistance in C.albicans, 28.5% in C.glabrata. Also, all C.krusei isolates were sensitive to Itraconazole in agreement with this study. Another study by Safdar et al[20] has reported Itraconazole resistance of 21% for C.tropicalis similiar to this study. Amphotericin was found to be the most effective agent with 100% sensitivity against all species of Candida in this study. This is similar to a study by Jaychandran et al.[19] and another study by Dahiya MC et al.[17]

In this study, all the patients were treated with antifungals according to the antifungal susceptibility pattern. Follow-up culture was not done as it was not required after administration of appropriate antifungals both in terms of dose and duration. Clinical resolution was seen in all patients after antifungal treatment.

Conclusion

Oral candidiasis is a common fungal infection in patients with cancer on treatment with chemotherapy and/or radiotherapy. Candida albicans and non-Candida albicans differ significantly in their antifungal susceptibility pattern. Non-Candida albicans like Candida krusei are inherently resistant to azoles. Hence, species level identification with in vitro antifungal susceptibility pattern is essential to choose the appropriate antifungal drug and to predict the outcome of therapy. The relation between microbiological and clinical finding may be help in the correlation of symptoms with detected of candida among patients of cancer. Prophylactic fluconazole in patients receiving concomitant chemoradiotherapy has the potential to reduce new-onset invasive fungal infections and associated morbidity.

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