PREVALENCE OF CANDIDAL SPECIES IN SALIVA BEFORE AND AFTER ORAL CANCER THERAPY

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Abstract

Aims: To investigate the prevalence of oral candida species in patients with head and neck cancer before and after radiation therapy.

Material and Methods: A total of 219 subjects was screened initially.180 patients were lost for follow-up due to peak Covid -19 pandemic. Thirty- nine subjects who received radiation therapy (RT) for the treatment of head and neck cancer were examined for signs of oral candidiasis before and one month after the treatment. The stimulated saliva was collected from the subjects in a sterile bottle. The samples were plated onto the HiChrome agar for Candida. The inoculated plates were incubated at 37ºC for 48 hours. The species was the identified by seeing the color of the colonies grown in Chrom Agar. Mucositis and dysphagia were also evaluated in each subject and their association with candidiasis were also analyzed.

Results: Among the thirty-nine samples studied Candida was present in 53.8% of cases pre and 69.2% post treatment respectively. C. albicans (35.9%) was the predominant isolate followed by C. krusei (23.1%) and in the second visit. Eventhough there was an increase in C. albicans (53.8%), C. kruseui (28.2%), two more variants (C. tropicalis and C. mucor) were present. Subjects were also graded for mucositis and dysphagia; it was found that most of them had grade 1 mucositis (61.5%) and it was noted that higher proportion of the candida (70%) was found in the second visit. While grading dysphagia majority had grade 2(35.9%) and grade 2 dysphagia showed highest percentage having Candida (40.7%).

Conclusion: The study revealed an increase incidence of Candida species in saliva in the patients who are undergoing radiotherapy for oral cancer. At the end of radiotherapy there was an increase in the growth of Candida-albicans species and non - albicans candida.

Clinical Significance: Although all forms of candida are closely related they differ in antifungal susceptibility patterns. Candida Species identification through antifungal susceptibility testing is essential for selecting the appropriate medication and predicting the treatment outcome.

Keywords: Oral Candida, Oral cancer, Radiation therapy.

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1. Introduction

Many cancer patients undergoing therapy often develop mucositis due to changes in the tissue microenvironment. To treat this condition, azole derivatives are commonly used, but they only eliminate the albican species of Candida. Unfortunately, other species like C. glabrata and C. tropicalis can predispose areas with chronic mucositis into areas of primary carcinoma. It's important to note that all forms of Candida are closely related, but they differ in antifungal susceptibility patterns. Additionally, C. albicans infection itself can cause tumor progression and metastasis. Neutropenia, an adverse effect of both radiation and chemotherapy in cancer patients, can also act as a predisposing factor of systemic candidiasis. This can cause a further delay in treatment, leading to an increase in cancer cell growth and tumor size, which can ultimately affect the quality of life and lead to increased morbidity and mortality. If not treated promptly, systemic candidiasis can evade the underlying mucosa and vessels, causing even more harm. Identifying the different species of Candida will help us choose appropriate treatment and predict the outcome of therapy. Therefore, understanding the mechanisms underlying systemic candidiasis in susceptible patients is crucial to developing novel strategies for prevention and treatment of the disease, both clinically and microbiologically.The present study aims to evaluate the Candida species in patients undergoing cancer therapy. By doing so, we hope to gain a better understanding of the disease and improve treatment outcomes for these vulnerable patients.

2. Materials and methods

The study enrolled 39 patients who had been diagnosed with oral squamous cell carcinoma in the oral cavity and referred for concurrent cancer radiotherapy between 2019 and 2021. Prior to conducting the study, approval was

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obtained from the institutional ethics board (**Protocol ref no.19053**), and written consent was obtained from each patient after explaining the procedure involved. The study collected comprehensive data on each patient's demographics, habits, and clinical history, including cancer type, histopathological grading, and treatment specifics, from their medical records. Furthermore, an oral screening was performed using a mouth mirror and optimal lighting both before and one month after the final radiation fraction. Patients who were already undergoing antifungal treatment were excluded from the study to ensure accurate results. All patients with head and neck cancers received radiation therapy with a dose of 70 Gy in 35 fractions over 7 weeks. Typically, radiation was administered for five days per week, with a daily dose of 2 Gy. Patients were instructed to chew on a sterile rubber band for one minute to stimulate salivary flow. Following this, 10 ml of saliva was collected in a sterile bottle and immediately transported to the Microbiology Department for culture. To identify the presence of Candida, 100μl of the sample was plated onto HiChrome agar (Himedia, Mumbai) and spread in four directions using a plastic spreader. The inoculated plates were then incubated at 37ºC for 48 hours. The species of Candida was identified by observing the color of the colonies grown in Chrom Agar (C. albicans-Green, C. glabrata-Creamy white, C. tropicalis-Bluish white, C. kruseii-Pinkish purple). The growth was quantified as CFU/ml. The patients were also evaluated and graded for dysphagia and mucositis according to National Cancer Institute Common Toxicity Criteria (NCI-CTC) version 2.0. Statistical analysis SPSS Statistics version 25 was used for statistical analysis. The colony counts of the candida species was compared pre radiation and post radiation therapy using Mann Whitney U test or Paired t test based on the normality distribution of the data. The type species change over the time period will be compared using McNemars Chi square test.

3. Results

Thirty–nine patients completed the study. These patients originally constituted 219 who were screened initially however, 180 patients were lost for follow-up due to peak Covid -19 pandemic and a 154 had developed comorbidities and had failed for follow-up and 26 had died.
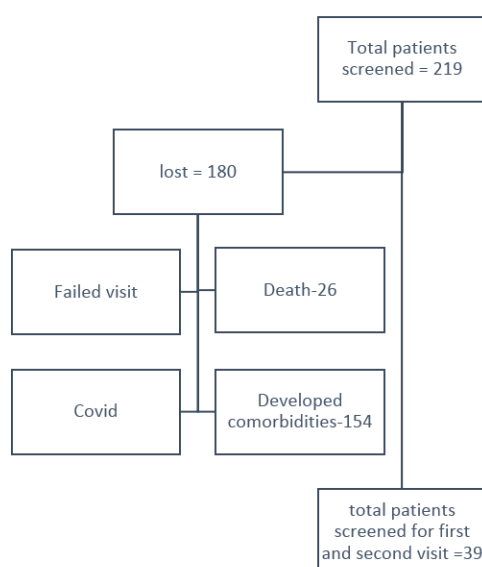
The demographic characteristics of the patients are listed in Table 1. Most of the patients were males (79.5%) and most were between 41-70 years old.

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Predominantly patients consumed tobacco in smokeless form, and around 48.7% had a combination of tobacco and alcohol. In our study around 66.7% of the patients were in stage 4(TNM Staging). The presence of Candida among the different histopathological grading was not significant. Candida was present in 53.8% of cases pre-operatively and 69.2% post-operatively. The various species of Candida identified in the first visit were C. albicans (35.9%), C.kruseuii (23.1%), and C.glabrata (2.6%). Gram negative bacilli were seen in 10.3% of the individuals post radiation. In the second visit C.albicans (53.8%), C.kruseuii (28.2%), and C.tropicalis (5.1%), C.mucor (2.6%) were observed. An increase in C.albicans and C.kruseii was seen in the second visit along with two more variants (C.tropicalis and C.mucor) (Table 2,3) The patients were also graded for mucositis; it was found that most of them had grade 1 mucositis (61.5%) while grading dysphagia majority had grade 2(35.9%).

Grades of mucositis did not show any association in first or second visit with the presence of candida colonization. However, the presence of dysphagia showed higher colonization of candida in second visit with grade 2,3 and 4 dysphagia showing 40.7%, 22.2% and 3.7% patients showing colonization with p value of 0.045. similar association of dysphagia was not seen in first visit. (Table 4) In the second visit grade one mucositis showed higher proportion of Candida (70%).On comparison of dysphagia it was seen that first visit did not show any significant difference but grade 2 dysphagia showed highest percentage of 40.7 having Candida. (Table 4).

4. Discussion

Candida is a normal commensal found in the oral cavity. However, in individuals with certain habits or chronic systemic diseases, Candida can become pathogenic and cause oral candidiasis. This condition is particularly prevalent among oral cancer patients undergoing treatment, as their immune systems are often compromised. In fact, studies have shown that Candida colonization tends to increase as radiation treatment progresses. Under normal circumstances, the T cells and epithelium work together to suppress the virulence of harmful organisms and maintain the integrity of the mucosa. However, in head and neck cancer patients, particularly those undergoing treatment, the mucosal barrier can break down, leading to the colonization of Candida. This, in turn, is associated with a higher incidence of morbidity and mortality. Many cancers are often diagnosed in later stages, as was found in our study where approximately 66.7% of patients were in stage 4, similar to the findings reported by Seoane-Romero JM et al in 2012. This high prevalence of late-stage cancer may be attributed to delays in diagnosis.[7] Such delays may be caused by patients failing to recognize the signs and symptoms of the disease, or by physicians failing to recognize and investigate the disease in a timely manner. The isolation of Candida species from saliva is a non-invasive method that is well-tolerated by oral cancer patients. However, due to the growing drug resistance of various fungal organisms, it is recommended to conduct an antifungal susceptibility test before initiating treatment for a better prognosis. In our study, we found that 53.8% of patients had Candida present before treatment, and this prevalence increased to 69.2% after radiotherapy.

Our study revealed that the most prevalent candida species was albicans, followed by kruseii, which is consistent with the findings of Suryawanshi H et al. (2012). However, it is important to note that non-albican species such as Kruseii and

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glabrata are known for their drug resistance.[2] our research showed that during the initial visit, 23.1% of patients had kruseii and 2.6% had glabrata. Furthermore, we observed an increase in c.kruseii to 28.2%. The remaining species identified were tropicalis (5.1%), c.mucor (2.6%), and gram-negative bacilli. These findings highlight the importance of monitoring and addressing drug-resistant candida species in clinical settings.

Oral mucositis is a condition that occurs when radiation destroys the epithelial cells, leading to desquamation and ulceration. Additionally, when it affects the salivary glands, it can result in xerostomia and later mucositis. In a recent study, it was found that 61% of patients experienced grade 1 mucositis, which is similar to the findings of Phongsuphot, K. et al in 2021. However, this differs from the study conducted by Elting LS et al in 2007, where the majority of patients experienced grade 3-4 mucositis. It is crucial to promptly treat mucositis as it can significantly impact the quality of life and result in treatment delays, discontinuation, and hospitalization. Dysphagia, a prevalent side effect of head and neck cancer therapy, affects over 50% of patients undergoing treatment and can lead to severe malnutrition. However, this issue can be mitigated by providing patients with the necessary nutrition and fluids through the use of nasogastric tubes (NG) and percutaneous endoscopic gastrostomy tubes (PEG).[15]Our study found that the majority of patients experienced grade 2 dysphagia, requiring a pureed, soft, or liquid diet. Dysphagia, the most common side effect of head and neck cancer therapy is almost seen in more than 50% of the patients undergoing treatment which can result in severe malnutrition. This can be avoided by providing them with nutrition and fluids required through nasogastric tube (NG) and the percutaneous endoscopic gastrostomy tube (PEG).[15]In our study most of the patients had grade 2 dysphagia

wherein they required a pureed, soft, or liquid diet. Upon comparing the incidence of mucositis and dysphagia during initial and follow-up visits, it was found that there was no significant difference in the occurrence of mucositis between the two visits. During the first visit, both Candida-positive and Candida-negative cases were observed at a rate of approximately 61%. However, during the second visit, a higher proportion of Candida-positive cases (70%) were observed in patients with grade one mucositis. While the exact role of Candida in mucositis is not yet fully understood, previous research has suggested that Candida colonization is more likely to occur in areas of mucosal inflammation, which can lead to discomfort for the patient.

Candida infection is a known aggravating factor for dysphagia, as it can destroy the mucosa. In addition to candida infection, inflammation of the mucosa can cause submucosal pharyngeal constriction, leading to dysphagia in patients undergoing cancer treatment. When comparing dysphagia, it was observed that the first visit did not show any significant difference. However, grade 2 dysphagia showed the highest percentage of 40.7% in patients with Candidal infection.

5. Conclusion

Identification of various Candida species, along with antifungal susceptibility testing, is necessary to select the appropriate antifungal drug and predict the outcome and prognosis of therapy. Additionally, physicians should prescribe prophylactic antifungals as soon as patients develop signs and symptoms of burning sensation, xerostomia, or ulceration in oral cancer patients.

6. References

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		Count	Column N %
Age Categories	<40	3	7.7%
	41-50	12	30.8%
	51-60	9	23.1%
	61-70	11	28.2%
	>70	4	10.3%
Sex	Male	31	79.5%
	Female	8	20.5%
Smoking	No	26	66.7%
	Yes	13	33.3%
Smokeless	No	17	43.6%
	Yes	22	56.4%
Alcohol	No	19	48.7%
	Yes	20	51.3%
TNM STAGING	Stage I	2	5.1%
	Stage II	7	17.9%
	Stage III	4	10.3%
	Stage IV	26	66.7%

Table 1: Basic characteristics of the patients

Table -2: Association of the grades of OSCC and Salivary gland tumours with candida prevalence in patients undergoing radiotherapy

	Categories	N	Histopathological Diagnosis				Chi square	P value
			Well Differentiat	Moderately Differentiated SCC (N (%))	Poorly Differentiate	Salivary Gland		

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			ed SCC (N (%))		d SCC (N (%))	Tumour (N (%))		
CANDIDA 1ST VISIT	No	18	8 (50)	7 (35)	1 (100)	2 (100)	4.596	0.204
	Yes	21	8 (50)	13 (65)	0 (0)	0 (0)		
CANDIDA 2ND VISIT	No	12	5 (31.2)	6 (30)	0 (0)	1 (50)	0.799	0.85
	Yes	27	11 (68.8)	14 (70)	1 (100)	1 (50)		
First Visit Albicans	Absent	25	10 (62.5)	12 (60)	1 (100)	2 (100)	1.844	0.605
	Present	14	6 (37.5)	8 (40)	0 (0)	0 (0)		
First Visit Kruseii	Absent	30	12 (75)	15 (75)	1 (100)	2 (100)	0.975	0.807
	Present	9	4 (25)	5 (25)	0 (0)	0 (0)		
First Visit Glabrata	Absent	38	16 (100)	19 (95)	1 (100)	2 (100)	0.975	0.807
	Present	1	0 (0)	1 (5)	0 (0)	0 (0)		
Second Visit Albicans	Absent	18	6 (37.5)	10 (50)	0 (0)	2 (100)	3.792	0.285
	Present	21	10 (62.5)	10 (50)	1 (100)	0 (0)		
Second Visit Kruseii	Absent	28	10 (62.5)	16 (80)	1 (100)	1 (50)	2.21	0.53
	Present	11	6 (37.5)	4 (20)	0 (0)	1 (50)		
Second Visit Glabrata	Absent	39	16 (100)	20 (100)	1 (100)	2 (100)	.	.
	Present	0	0 (0)	0 (0)	0 (0)	0 (0)		

Second Visit Tropicalis	Absent	37	16 (100)	18 (90)	1 (100)	2 (100)	2.003	0.572
	Present	2	0 (0)	2 (10)	0 (0)	0 (0)		
Second Visit C. mucor	Absent	38	16 (100)	19 (95)	1 (100)	2 (100)	0.975	0.807
	Present	1	0 (0)	1 (5)	0 (0)	0 (0)		
MUCOSITIS	Grade 0	13	5 (31.2)	6 (30)	1 (100)	1 (50)	4.675	0.862
	Grade 1	24	10 (62.5)	13 (65)	0 (0)	1 (50)		
	Grade 2	1	0 (0)	1 (5)	0 (0)	0 (0)		
	Grade 3	1	1 (6.2)	0 (0)	0 (0)	0 (0)		
	Grade 4	0	0 (0)	0 (0)	0 (0)	0 (0)		
DYSPHAGIA	Grade 0	8	3 (18.8)	3 (15)	1 (100)	1 (50)	11.291	0.504
	Grade 1	8	3 (18.8)	4 (20)	0 (0)	1 (50)		
	Grade 2	14	4 (25)	10 (50)	0 (0)	0 (0)		
	Grade 3	8	5 (31.2)	3 (15)	0 (0)	0 (0)		
	Grade 4	1	1 (6.2)	0 (0)	0 (0)	0 (0)		

Table 3: Prevalence of Candida colonization in first and second visit

		First Visit	Second Visit
CANDIDA presence	No	18 (46.2%)	12 (30.8%)
	Yes	21 (53.8%)	27 (69.2%)
Albicans	Absent	25 (64.1%)	18 (46.2%)

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	Present	14 (35.9%)	21 (53.8%)
Krusei	Absent	30 (76.9%)	28 (71.8%)
	Present	9 (23.1%)	11 (28.2%)
Glabrata	Absent	38 (97.4%)	39 (100%)
	Present	1 (2.6%)	0 (0%)
Gram Negative Bacilli	Absent	35 (89.7%)	38 (97.4%)
	Present	4 (10.3%)	1 (2.6%)
Tropicalis	Absent	0 (0%)	37 (94.9%)
	Present	0 (0%)	2 (5.1%)
C. mucor	Absent	0 (0%)	38 (97.4%)
	Present	0 (0%)	1 (2.6%)

Table -4 Comparison of Candida Presence and Dysphagia with first visit

	Categories	N	CANDIDA 1ST VISIT		CANDIDA 2ND VISIT	
			No (N (%))	Yes (N (%))	No (N (%))	Yes (N (%))
MUCOSITIS	Grade 0	13	6 (33.3)	7 (33.3)	7 (58.3)	6 (22.2)
	Grade 1	24	11 (61.1)	13 (61.9)	5 (41.7)	19 (70.4)
	Grade 2	1	1 (5.6)	0 (0)	0 (0)	1 (3.7)
	Grade 3	1	0 (0)	1 (4.8)	0 (0)	1 (3.7)
	Grade 4	0	0 (0)	0 (0)	0 (0)	0 (0)
	Chi square (P value)		2.025 (0.567)		5.251 (0.154)	
DYSPHAGIA	Grade 0	8	4 (22.2)	4 (19)	6 (50)	2 (7.4)
	Grade 1	8	5 (27.8)	3 (14.3)	1 (8.3)	7 (25.9)
	Grade 2	14	6 (33.3)	8 (38.1)	3 (25)	11 (40.7)
	Grade 3	8	3 (16.7)	5 (23.8)	2 (16.7)	6 (22.2)
	Grade 4	1	0 (0)	1 (4.8)	0 (0)	1 (3.7)
	Chi square (P value)		2.067 (0.723)		**9.744 (0.045)**	

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Fig. 1. Growth of different fungal colonies from salivary samples (A) Candida albicans and Krusei (B) Candida Tropicalis

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