DOI: 10.7860/JCDR/2023/66418.18509

Original Article

Microbiology Section

A Study on Subsite-specific Prevalence of Candidiasis in Head and Neck Cancer Patients and its Antifungal Susceptibility Pattern: A Cross-sectional Study

NEETHU BABU[1], CHITRALEKHA SAIKUMAR[2], JOMON RAPHAEL CHALISSERY[3]

ABSTRACT

Introduction: The incidence of candidiasis can vary across various subsites within the head and neck region and is associated with various co-morbidities and risk factors. The increase in the incidence of resistant Non-*albicans Candida* (NAC) species among these patients and the limited number of available antifungal agents make treatment difficult. A better understanding of the subsite-specific prevalence of candidiasis and its antifungal susceptibility is crucial in enhancing effective control and treatment.

Aim: To determine the subsite-specific prevalence of candidiasis among Head and Neck Cancer (HNC) patients undergoing radiotherapy.

Materials and Methods: A cross-sectional study was conducted on patients undergoing therapy for head and neck malignancies at the Department of Radiation Oncology, Amala Institute of Medical Sciences, Thrissur, Kerala, India over a four-year period (January 2019 to December 2022). A total of 276 patients aged 18 to 85 years with squamous cell carcinoma were included. Oral samples were collected from patients who developed candidiasis, and co-morbidities and risk factors were documented. *Candida* species were isolated and identified. Antifungal susceptibility was determined using the VITEK system, and fluconazole susceptibility was compared with the standard disc diffusion method. Data were entered into an Excel sheet and analysed using Statistical Package for the Social Sciences (SPSS) software.

Results: Pharynx was the most frequent site of head and neck malignancy, accounting for 104 cases (37.7%), followed by the oral cavity with 83 cases (30.1%). Among patients with malignancies in the pharyngeal region, a high rate of *Candida* infection was observed in 42 (43.3%) out of 97 cases. *Candida* species isolated included *C. albicans* (56, 57.7%), *C. tropicalis* (26, 26.8%), *C. krusei* (8, 8.3%), *C. glabrata* (3, 3.1%), and *C. parapsilosis* (4, 4.1%). Sixty-five patients (23.5%) had diabetes, which was statistically significant (p-value <0.05). All *C. albicans* strains were sensitive to fluconazole. The Minimum Inhibitory Concentration (MIC) of voriconazole was very low for all tested *Candida* species.

Conclusion: Patients with pharyngeal and oral cavity carcinomas are at an increased risk of developing candidiasis during radiotherapy. Diabetes is significantly associated with candidiasis. While *C. albicans* was the most common species isolated, a significant number of NAC species were also identified. Voriconazole exhibited low MIC values for *C. krusei* and *C. glabrata*, suggesting its potential as an alternative treatment option. Obtaining VITEK system identification and susceptibility reports is advisable for borderline values that may not be evident using conventional disc diffusion methods.

Keywords: *Candida albicans*, *Candida glabrata*, Fluconazole, Pharynx, Radiotherapy, Voriconazole

INTRODUCTION

Candidiasis, a fungal infection caused by the *Candida* species, is a common complication in individuals with HNC [1-3]. The prevalence of candidiasis can vary among different subsites within the head and neck region, including the oral cavity, pharynx, larynx, nasal area, and the salivary glands [4]. Complications associated with candidiasis, such as pain, swallowing difficulties, and compromised nutrition, can significantly impact the well-being of HNC patients [5]. Oral candidiasis is frequently observed in individuals with diabetes, as *Candida* thrives in an environment promoted by heightened blood sugar levels. Despite diabetes being closely associated with systemic problems like hypertension and kidney disease in immunocompromised patients, the direct association with causing candidiasis is evident primarily among individuals with diabetes [6-8]. Tobacco use is associated with a high rate of Oropharyngeal Candidiasis (OPC), and alcohol consumption has been linked to the development of cancer of the oral cavity and pharynx [9-11]. By understanding the cancer subsites with a higher prevalence of candidiasis, healthcare professionals can focus on improving oral health, swallowing function, and overall quality of life in the most affected areas [12,13].

Over the past few years, there has been a significant rise in the occurrence of infections among immunocompromised patients caused by NAC species. This increase is either equivalent to or surpassing the prevalence of the widely recognised pathogen, *C. albicans* [4,14]. Azole antifungal drugs are most commonly used in the treatment of oral *Candida* infections due to their efficacy, oral bioavailability, and tolerability. However, NAC species are more resistant to fluconazole, the most commonly used azole group of antifungal drugs, which makes treatment challenging [15,16]. As the availability of antifungal medications is limited, addressing resistant *Candida* infections is progressively becoming more challenging. There is a scarcity of information regarding the distribution of candidiasis in specific subsites within the head and neck region and the effectiveness of the various available antifungal agents against *C. albicans* and NAC strains among HNC patients in this region, where there is a rise in the burden of HNC [17,18].

A better understanding of the subsite-specific prevalence of candidiasis and its antifungal susceptibility is crucial in enhancing effective control and treatment. The aim of the present study was to identify the subsite-specific prevalence of candidiasis among HNC patients undergoing treatment. The primary objective of the

study was to find its association with co-morbidities and risk factors and to detect the antifungal susceptibility of the isolates. The secondary objective of the study was to determine the distribution of *Candida* species among HNC patients, determine the Minimum Inhibitory Concentration (MIC) of six antifungal agents against the *Candida* isolates, and compare fluconazole susceptibility using the standard disc diffusion method to provide insights into the reliability of the standard approach in determining fluconazole susceptibility in HNC patients.

MATERIALS AND METHODS

A cross-sectional study was conducted at the Department of Radiation Oncology, Amala Institute of Medical Sciences, Thrissur, Kerala, India, on patients undergoing Radiation Therapy (RT) for head and neck malignancies. The study was conducted over a four-year period from January 2019 to December 2022.

The study protocol was presented to the Institutional Ethics Committee (IEC), and approval was obtained (Ref No. 30/IEC/19/AIMS-11). All participants were provided with information about the study and gave their written consent.

Inclusion criteria: Patients with proven squamous cell carcinoma of the head and neck region were included in the study.

Exclusion criteria: Patients who were on antifungal therapy and below 18 years of age were excluded from the study.

Sample size: The study aimed to investigate candidiasis among 276 patients with head and neck malignancies, showing a prevalence ranging from 25-35% [3,19]. Therefore, using an average prevalence (P) of 30%, precision (d) as 20% of prevalence, and a significance level (α) of 5%, the minimum sample size was calculated as 224.

$$n=\frac{(Z_{1-\alpha}1^{2})^{2}\,pq}{d^{2}}$$

Study Procedure

Swabs were collected from the oropharyngeal region and sent to the microbiology laboratory for preliminary isolation and identification. KOH mounts were prepared to check the presence of yeasts and pseudohyphae. Samples were cultured on Sabouraud's dextrose agar and Hichrome agar (HiMedia Laboratories Pvt., Limited., Mumbai, India) plates and incubated at 37°C for 24-48 hours. Gram staining was performed for the preliminary identification of *Candida* [20]. Fresh subcultures were then performed on SDA plates to obtain pure cultures for VITEK® 2 Systems identification and MIC detection. Additionally, fluconazole susceptibility was determined using the conventional disc diffusion method. Underlying conditions such as diabetes and hypertension were observed, and factors contributing to the risk, such as tobacco and alcohol use, were documented.

Identification and susceptibility testing using the VITEK® 2 Compact System were conducted following the manufacturer's instructions. Inoculum was prepared in sterile saline provided by the manufacturer, with a turbidity range between 1.80-2.20 McFarland standard, as per bioMérieux VITEK® 2 DensiCHEK™ instrument. VITEK® 2 YST cards were used for identification testing, while VITEK® 2 AST-YS08 cards were used to determine the susceptibility of the yeasts to antifungal agents. The VITEK® 2 AST-YS08 card contained different concentrations of various antifungal agents, including amphotericin B, caspofungin, flucytosine, micafungin, voriconazole, and fluconazole. MIC values were interpreted according to the breakpoints stated in CLSI M27-M44S [21].

Fluconazole susceptibility was tested and interpreted using the standard disc diffusion method, following CLSI M44-A2 guidelines [22]. Fluconazole discs (25 µg) were obtained from HiMedia Labs, India. Mueller-Hinton agar supplemented with 2% glucose was used for sensitivity testing. Microbial Type Culture Collection (MTCC) 3017 from the Institute of Microbial Technology, Chandigarh, India, was used as the control strain.

STATISTICAL ANALYSIS

The data were analysed using SPSS software (v23, IBM, Illinois, US). Continuous measurements were reported as the mean±Standard Deviation (SD). Categorical measurements were presented as numerical counts along with their respective percentages. The association between co-morbidities and risk factors with candidiasis was determined using the Chi-square test.

RESULTS

The study included 276 patients with HNC. The age range was 31 to 85 years, with an average age of 65. The majority of the patients were men, accounting for 231 (83.7%) cases, with an average age of 65. Women comprised 45 (16.3%) of the patients, with an average age of 65 years. In the current study, the pharynx was the most frequent site of head and neck malignancy, with 104 (37.7%) cases. This was followed by the oral cavity with 83 (30.1%) cases, the larynx with 74 (26.8%) cases, the nasal cavity and paranasal sinuses with 8 (2.9%) cases, and the salivary glands with 7 (2.5%) cases of parotid malignancies observed [Table/Fig-1].

Characteristics	N (%)
Gender	
Women	45 (16.3)
Men	231 (83.7)
Age (Mean±SD) 65±11 years	
Site of malignancy	
Oral cavity	**83 (30.1)**
Buccal mucosa	15 (18.1)
Tongue	46 (55.4)
Floor of mouth	6 (7.2)
Hard palate	3 (3.6)
Lip	3 (3.6)
Alveolus	9 (10.9)
Maxilla	1 (1.2)
Pharynx	**104 (37.7)**
Nasopharynx	10 (9.6)
Oropharynx	46 (44.2)
Hypopharynx	48 (46.2)
Larynx	**74 (26.8)**
Vocal cords and mucosa	48 (64.9)
Supraglottic	4 (5.4)
Subglottic	22 (29.7)
Nasal cavity and paranasal sinuses	**8 (2.9)**
Salivary glands (parotid)	**7 (2.5)**

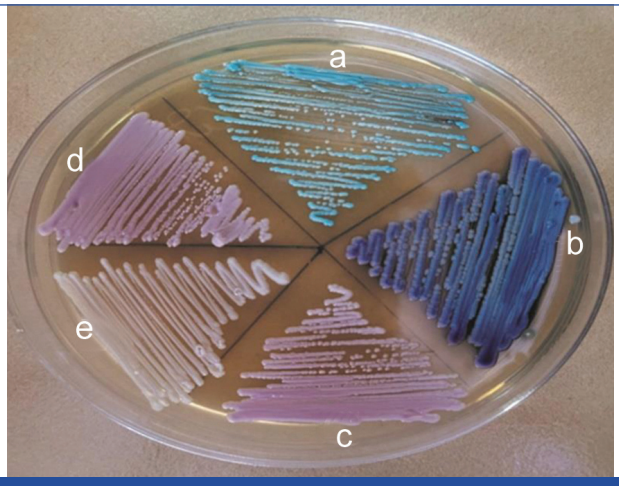
[Table/Fig-1]: Demography of Head and Neck Cancer (HNC) patients (n=276).

Candidiasis was identified in 97 (35.1%) out of 276 patients, with the most prevalent species being *C. albicans* in 56 (57.7%) cases. Other NAC species, are shown in [Table/Fig-2].

Candida species	N (%)
C. albicans	56 (57.7)
C. tropicalis	26 (26.8)
C. krusei	8 (8.3)
C. glabrata	3 (3.1)
C. parapsilosis	4 (4.1)

[Table/Fig-2]: *Candida* species isolated in Head and Neck Cancer (HNC) patients (n=97).

Various *Candida* colonies of *C. albicans*, *C. tropicalis*, *C. krusei*, *C. glabrata*, and *C. parapsilosis* were seen on the Hichrome agar plate, as shown in [Table/Fig-3].



[Table/Fig-3]: *Candida* colonies on Hichrome agar plate. a) *C. albicans*; b) *C. tropicalis*; c) *C. krusei*; d) *C. glabrata*; and e) *C. parapsilosis*.

In the study, 65 (23.5%) patients were diabetic, and among them, 41 (63.1%) had candidiasis. The association between diabetes and oral candidiasis (OPC) was found to be statistically significant (p=0.0001). However, there was no significant association with risk factors like tobacco and alcohol (p-value >0.05) [Table/Fig-4].

Total number of patients (n=276)	Candidiasis		p-value
	Yes	No	
Diabetic n=65 (23.5%)	41 (63.1%)	24 (36.9%)	0.0001
Non diabetic n=211 (76.4%)	56 (26.5%)	155 (73.5%)	
Tobacco users n=63 (22.8%)	28 (44.4%)	35 (55.5%)	0.078
Tobacco non users n=213 (77.2%)	69 (32.4%)	144 (67.6%)	
Alcohol consumers n=18 (6.5%)	5 (27.7%)	13 (72.2%)	0.49
Alcohol non consumers n=258 (93.5%)	92 (35.6%)	166 (64.3%)	

[Table/Fig-4]: Association between diabetes and risk factors with Candidiasis. p-value <0.05=Significant. (Chi-square test)

Among the patients with malignancies in the pharyngeal region, a high rate of *Candida* infection was observed, specifically in 42 (43.3%) out of 97 patients, including 5 (11.9%) out of 42 in the nasopharynx, 22 (52.4%) out of 42 in the oropharynx, and 15 (35.7%) out of 42 in the hypopharynx. Additionally, 37 (38.1%) out of 97 patients with oral cavity malignancies were found to have *Candida* infection. A total of 16 (16.5%) out of 97 patients with cancer in the laryngeal region had *candidiasis* [Table/Fig-5].

Candida albicans strains isolated were sensitive to fluconazole by the standard disc diffusion test and to all six antifungal agents tested using the VITEK® 2 Compact System. *C. tropicalis* and *C. parapsilosis* strains showed 100% susceptibility to fluconazole by the disc diffusion method, as well as to all six antifungals by the VITEK® 2 Compact System. *C. krusei* strains are inherently resistant to fluconazole, and *C. glabrata* strains show acquired resistance. The MIC to voriconazole was very low for all the *Candida* species tested. All the strains were sensitive to micafungin and amphotericin B. Out of the eight *C. krusei* strains, 2 (25%) showed intermediate susceptibility, and 2 (25%) were resistant to Caspofungin, and all of them were resistant to Flucytosine by the VITEK® 2 Compact System [Table/Fig-6].

DISCUSSION

The occurrence of oral candidiasis is prevalent in individuals with diabetes, and it has been observed that higher blood sugar levels create a favourable environment for *Candida* growth. The interplay between hyperglycaemia, impaired immune response, dry mouth, and altered microbial balance contributes to the increased susceptibility to oral candidiasis in individuals with diabetes [23,24]. In the present study, a significant association between diabetes and HNC was found. Out of a total of 65 (23.5%) diabetic patients, 41 (63.1%) had candidiasis during the course of treatment. Gong Y et al., and Abhinav RP et al., in their studies have found evidence indicating an increased risk of developing oral cancer in patients with diabetes and also a higher mortality rate among them [25,26]. Proper management of blood sugar levels, along with appropriate antifungal therapy, is crucial in preventing and treating oral candidiasis in diabetic patients.

Subsite	*C. albicans* n=56 (57.7%)	*C. tropicalis* n=26 (26.8%)	*C. krusei* n=8 (8.3%)	*C. glabrata* n=3 (3.1%)	*C. parapsilosis* n=4 (4.1%)
Oral cavity n=37 (38.1%)	**24**	**10**	**2**		**1**
Buccal mucosa	5	3			
Tongue	14	5	1		1
Floor of mouth	1				
Hard palate/soft palate	1	1			
Lip	1				
Alveolus	2	1	1		
Maxilla					
Pharynx n=42 (43.3%)	**23**	**11**	**4**	**2**	**2**
Nasopharynx	3	2			
Oropharynx (including tonsils)	11	5	3	2	1
Hypopharynx (pyriform fossa)	9	4	1		1
Larynx n=16 (16.5%)	**8**	**4**	**2**	**1**	**1**
Vocal cord	3	2		1	1
Supraglottic	1				
Subglottic	4	2	2		
Nasal cavity and paranasal sinuses n=1 (1.03%)	**1**				
Salivary glands (parotid) n=1 (1.03%)		**1**			

[Table/Fig-5]: Subsite wise distribution of various *Candida* species causing infection (n=97). n=number of isolates

Organism	Strains	MIC value						Interpretation					
		Flu	Vor	Cas	Mic	AmB	Flucy	Flu	Vor	Cas	Mic	AmB	Flucy
C. albicans	Strain 1	≤0.5	≤0.12	≤0.12	≤0.06	1	≤1	S*	S	S	S	S	S
	Strain 2	1	≤0.12	≤0.12	≤0.06	1	≤1	S	S	S	S	S	S
	Strain 3	≤0.5	≤0.12	≤0.12	≤0.06	1	≤1	S	S	S	S	S	S
C. tropicalis	Strain 1	2	≤0.12	≤0.12	≤0.06	0.5	≤1	S	S	S	S	S	S
	Strain 2	2	≤0.12	0.25	0.12	0.5	≤1	S	S	S	S	S	S
	Strain 3	1	≤0.12	≤0.12	≤0.06	0.5	≤1	S	S	S	S	S	S
C. krusei	Strain 1	-	≤0.12	0.25	0.12	0.5	32	R‡	S	S	S	S	R
	Strain 2	8	≤0.12	1	0.12	1	8	R	S	R	S	S	R
	Strain 3	8	≤0.12	0.25	0.12	1	8	R	S	I†	S	S	R
C. glabrata	Strain 1	-	≤0.12	0.5	≤0.06	≤0.25	≤1	R	S	R	S	S	S
	Strain 2	-	≤0.12	0.5	≤0.06	0.5	≤1	R	S	R	S	S	S
	Strain 3	-	≤0.12	≤0.12	≤0.06	0.5	≤1	R	S	S	S	S	S
C. parapsilosis	Strain 1	≤0.5	≤0.12	0.25	0.5	0.5	≤1	S	S	S	S	S	S
	Strain 2	2	≤0.12	0.25	2	0.5	≤1	S	S	S	S	S	S
	Strain 3	≤0.5	≤0.12	0.25	0.5	0.5	≤1	S	S	S	S	S	S

[Table/Fig-6]: Antifungal susceptibility of representative *Candida* species by VITEK system.
MIC: Minimum inhibitory concentration; Flu: Fluconazole; Vor-Voriconazole; Cas: Caspofungin; Mic: Micafungin; AmB: Amphotericin B; Flucy: Flucytosine; *Sensitive, †Intermediate, ‡Resistant

According to a study conducted by Bagnardi V et al., it has been demonstrated that even small amounts of alcohol consumption are linked to the development of oral cavity and pharynx cancer, as well as oesophageal cancer [11]. In the present study, the highest incidence of cancer was observed in the pharyngeal region, with 104 (37.7%) cases, followed by cancer in the oral cavity with 83 (30.1%) cases. This finding was comparable to a recent study by De Vasconcellos Ferreira PM et al., where the oral cavity accounted for 28 (42.4%) cases and the pharynx accounted for 23 (34.8%) cases [27]. However, in the current study, no significant association was found between known risk factors such as tobacco and alcohol consumption. Various studies have indicated a notable increase in the incidence of oropharyngeal cancer in several countries, suggesting that the positive trends in oral and pharyngeal cancer mortality and incidence in certain countries worldwide can be attributed to changes in tobacco and alcohol consumption among men over the past few decades [9,10,28].

In this particular study, *C. albicans* was identified as the most commonly isolated *Candida* species, followed by NAC species such as *C. tropicalis*, *C. krusei*, *C. glabrata*, and *C. parapsilosis*. Similar findings were reported in studies conducted by Wu J et al., where 53.2% of the isolates were *C. albicans* followed by NAC species like *C. tropicalis*, *C. krusei*, and *C. parapsilosis*, and E Silva VC et al., where *C. albicans* was the most prevalent species isolated [29,30]. A study conducted in the southern part of India also reported findings consistent with the pattern of isolation observed in this study, with *C. albicans* being the most prevalent fungal species isolated. NAC species isolated in their study were *C. tropicalis*, *C. krusei*, *C. glabrata*, and *C. parapsilosis* [31]. However, a separate study conducted in the eastern part of India revealed that NAC species were more prevalent, with *C. parapsilosis* being the most frequently identified species in that particular region [32].

In the present study, all the *C. albicans* strains isolated were susceptible to fluconazole by both disc diffusion and the automated method. Generally, *C. albicans* exhibits good susceptibility to commonly used antifungal agents such as fluconazole, itraconazole, and voriconazole. However, there have been reports of increased resistance to azole antifungals, especially in certain geographic regions or among patients with prior antifungal exposure. NAC species, such as *C. glabrata*, *C. tropicalis*, *C. krusei*, and *C. parapsilosis*, have shown varying patterns of antifungal susceptibility. *C. glabrata* is known to have higher acquired resistance to azole antifungals, while *C. krusei* is often intrinsically resistant to fluconazole. The VITEK® 2 compact system indicated that both of these strains in the present study were resistant to fluconazole. Voriconazole, a triazole antifungal drug, exhibits enhanced antifungal activity compared to fluconazole and is effective against all *Candida* species, including *C. krusei* and *C. glabrata*, which are inherently resistant to fluconazole, as well as fluconazole-resistant *C. albicans* strains [33,34]. In this study, all the strains of *C. krusei* and *C. glabrata* exhibited significantly low (MIC values for voriconazole). Therefore, voriconazole can be considered as a viable alternative drug option for the treatment of infections caused by these specific *Candida* species. Normally, *C. tropicalis* and *C. parapsilosis* show good susceptibility to azole antifungal drugs, although there have been reported cases of resistance. In the current study, the *C. tropicalis* strains were found to be pan sensitive, but their susceptibility to fluconazole was at the borderline level as indicated by the MIC values. All *C. parapsilosis* strains were sensitive to all the tested drugs, which was in concordance with the results of the study by Erum R et al., [35].

Limitation(s)

The study included patients exclusively from a single centre. Nevertheless, considering the limited availability of related studies in the current literature, these findings may still hold relevance in the management of OPC in HNC patients undergoing RT.

CONCLUSION(S)

Patients receiving RT for pharyngeal and oral cavity carcinoma are at an increased risk of developing candidiasis during the course of their treatment. There is a notable association between diabetes and the risk of *Candida* infection in HNC patients. However, this study did not find an association between alcohol and tobacco use and the occurrence of oral cancer, which could possibly be attributed to changes in lifestyle. Although *C. albicans* was the most commonly found *Candida* species, a considerable number of NAC species were also identified. All the *C. albicans* strains showed sensitivity to fluconazole. Voriconazole exhibited very low MIC values for *C. krusei* and *C. glabrata*, suggesting it can be considered as an alternative option for treating infections caused by these strains.

It is advisable to obtain VITEK system identification and susceptibility reports whenever possible, as they can provide insight into borderline values that may not be evident when using conventional disc diffusion methods.

Acknowledgement

The authors are grateful to Dr. Ajith TA, Professor, Department of Biochemistry, Amala Institute of Medical Sciences, for his valuable help during the preparation of this manuscript.

REFERENCES

[1] Bensadoun RJ, Patton LL, Lalla RV, Epstein JB. Oropharyngeal candidiasis in head and neck cancer patients treated with radiation: Update 2011. Support Care Cancer. 2011;19(6):737-44.
[2] Deng Z, Kiyuna A, Hasegawa M, Nakasone I, Hosokawa A, Suzuki M. Oral candidiasis in patients receiving radiation therapy for head and neck cancer. Otolaryngology- Head and Neck Surgery. 2010;143(2):242-47.
[3] Kermani F, Sadeghian M, Shokohi T, Hashemi S, Moslemi D, Davodian S, et al. Molecular identification and antifungal susceptibility testing of Candida species isolated from oral lesions in patients with head and neck cancer undergoing radiotherapy. Curr Med Mycol. 2021;7(1):44-50.
[4] Kibwana UO, Manyahi J, Kamori D, Mushi M, Mwandigha AM, Majigo M. Predominance of non-*Candida albicans* species oral colonisation among patients on anticancer therapy: Findings from a cross-sectional study in Tanzania. BMJ Open. 2023;13(4):e070003.
[5] Keziah SV, Dharman S, Maragathavalli G. Assessment of onset and progression of chemoradiotherapy induced oral complications in head and neck cancer patients-A prospective study. J Indian Academy of Oral Med Radiol. 2022;34(3):246-53.
[6] Zomorodian K, Kavoosi F, Pishdad GR, Mehriar P, Ebrahimi H, Bandegani A, et al. Prevalence of oral *Candida* colonization in patients with diabetes mellitus. J Mycol Med. 2016;26(2):103-10.
[7] Belazi M, Velegraki A, Fleva A, Gidarakou I, Papanaum L, Baka D, et al. Candidal overgrowth in diabetic patients: Potential predisposing factors. Mycoses. 2005;48(3):192-96.
[8] Mohammadi F, Javaheri MR, Nekoeian S, Dehghan P. Identification of *Candida* species in the oral cavity of diabetic patients. Curr Med Mycol. 2016;2(2):01-07.
[9] Khalili P, Movagharipoor A, Sardari F, Movaghari Pour F, Jamali Z. Oral candidiasis and cigarette, tobacco, alcohol, and opium consumption in Rafsanjan, a region in the southeast of Iran. BMC Oral Health. 2023;23(1):01-08.
[10] Chauhan R, Trivedi V, Rani R, Singh U. A study of head and neck cancer patients with reference to tobacco use, gender, and subsite distribution. South Asian J Cancer. 2022;11(01):046-51.
[11] Bagnardi V, Rota M, Botteri E, Tramacere I, Islami F, Fedirko V, et al. Alcohol consumption and site-specific cancer risk: A comprehensive dose-response meta-analysis. Br J Cancer. 2015;112(3):580-93.
[12] Cohen EEW, LaMonte SJ, Erb NL, Beckman KL, Sadeghi N, Hutcheson KA, et al. American Cancer Society head and neck cancer survivorship care guideline. CA Cancer J Clin. 2016;66(3):203-39.
[13] Shabestari SB, Shirinbak I, Azadarmaki R. A comprehensive look at oromaxillofacial and laryngopharyngeal cancers. Cancer Genetics and Psychotherapy. 2017:531-87.
[14] Tarapan S, Matangkasombut O, Trachootham D, Sattabanasuk V, Talungchit S, Paemuang W, et al. Oral *Candida* colonization in xerostomic postradiotherapy head and neck cancer patients. Oral Diseases. 2019;25(7):1798-808.
[15] Jahanshiri Z, Manifar S, Hatami F. Evaluation of virulence factors and azole resistance mechanisms of *Candida* tropicalis isolates from head and neck cancer patients with OPC. Iran J Microbiol. 2023;15(1):163-73.
[16] Singh A, Singh PK, de Groot T, Kumar A, Mathur P, Tarai B, et al. Emergence of clonal fluconazole-resistant *Candida parapsilosis* clinical isolates in a multicentre laboratory-based surveillance study in India. J Antimicrob Chemother. 2019;74(5):1260-68.
[17] Ganesan S, Sivagnanaganesan S, Thulasingam M, Karunanithi G, Kalaiarasi R, Ravichandran S, et al. Diagnostic delay for head and neck cancer in South India: A mixed-methods study. Asian Pacific Journal of Cancer Prevention. 2020;21(6):1673-78.
[18] Kulkarni MR. Head and neck cancer burden in India. Int J Head Neck Surg. 2013;4(1):29-35.
[19] Kawashita Y, Funahara M, Yoshimatsu M, Nakao N, Soutome S, Saito T, et al. A retrospective study of factors associated with the development of oral candidiasis in patients receiving radiotherapy for head and neck cancer: Is topical steroid therapy a risk factor for oral candidiasis? Medicine (Baltimore). 2018;97(44):e13073.
[20] Walsh TJ, Hayden RT, Larone DH. Larone's medically important fungi: A guide to identification. John Wiley & Sons; 2018 Jun 1.
[21] CLSI. Performance Standards for Antifungal Susceptibility Testing of Yeasts. 3rd ed. CLSI supplement M27M44S. Clinical and Laboratory Standards Institute; 2022.
[22] Clinical and Laboratory Standards Institute. M44- method for antifungal disk diffusion susceptibility testing of yeasts, 3rd edition, 2018. Available at: https://clsi.org/media/2634/m44ed3_sample.pdf.
[23] Ramos-Garcia P, Roca-Rodriguez MD, Aguilar-Diosdado M, Gonzalez-Moles MA. Diabetes mellitus and oral cancer/oral potentially malignant disorders: A systematic review and meta-analysis. Oral Diseases. 2021;27(3):404-21.
[24] González-Moles MÁ, Ramos-García P. State of evidence on oral health problems in diabetic patients: A critical review of the literature. J Clin Med. 2021;10(22):5383.
[25] Gong Y, Wei B, Yu L, Pan W. Type 2 diabetes mellitus and risk of oral cancer and precancerous lesions: A meta-analysis of observational studies. Oral Oncology. 2015;51(4):332-40.
[26] Abhinav RP, Williams J, Bennett C, Livingston P, Jebarani S, Pradeepa R, et al. Frequency and association of self-reported oral cancer among individuals with type 2 diabetes at a tertiary care diabetes centre in South India-A retrospective study. J Diabetes Complications. 2022;36(3):108129.
[27] De Vasconcellos Ferreira PM, Gomes MD, Almeida AC, Cornélio JS, Arruda TJ, Mafra A, et al. Evaluation of oral mucositis, candidiasis, and quality of life in patients with head and neck cancer treated with a hypofractionated or conventional radiotherapy protocol: A longitudinal, prospective, observational study. Head Face Med. 2023;19(1):7.
[28] Chang CP, Siwakoti B, Sapkota A, Gautam DK, Lee YC, Monroe M, et al. Tobacco smoking, chewing habits, alcohol drinking and the risk of head and neck cancer in Nepal. Int J Cancer. 2020;147(3):866-75.
[29] Wu J, Gan C, Li J, Liu Y, Chen Z, Zhang Y, et al. Species diversity and antifungal susceptibilities of oral yeasts from patients with head and neck cancer. Infect Drug Resist. 2021;14:2279-88.
[30] E Silva VC, de Cássia Oliveira V, de Sousa ÁF, Bim FL, Macedo AP, de Andrade D, et al. Prevalence and susceptibility profile of *Candida* spp. isolated from patients in cancer therapy. Arch Oral Biol. 2020;119:104906.
[31] Krishnan NA, Kurup S, Nair AH, Kumar V. *Candida* quantification and species identification in patients undergoing postoperative head and neck radiotherapy and combination of chemo-radio therapy. Annals of the Romanian Society for Cell Biology. 2021;25(5):4891-900.
[32] Prakash V, Singh RK, Saurabh K, Kumar V, Kumari R, Kumar S, et al. Spectrum of chemo-radiotherapy induced fungal infection in head and neck cancer patients at tertiary care centre of Eastern India. Oral Oncology Reports. 2023;6:100039.
[33] Golestannejad Z, Khozeimeh F, Dehghan P, Najafizade N, Faghihian E, Kheirkhah M, et al. Comparison of the antifungal effect of voriconazole and fluconazole on oral candidiasis before and during radiotherapy. Dental Research Journal. 2022;19(1):99.
[34] Partha AD, Widodo AD, Endraswari PD. Evaluation of fluconazole, itraconazole, and voriconazole activity on *Candida albicans*: A case control study. Ann Med Surg (Lond). 2022;84:104882.
[35] Erum R, Samad F, Khan A, Kazmi SU. A comparative study on production of extracellular hydrolytic enzymes of *Candida* species isolated from patients with surgical site infection and from healthy individuals and their co-relation with antifungal drug resistance. BMC Microbiology. 2020;20(1):01-02.

PARTICULARS OF CONTRIBUTORS:
1. Research Scholar, Bharath Institute of Higher Education and Research, Chennai, Tamil Nadu, India; Tutor, Department of Microbiology, Amala Institute of Medical Sciences, Amala Nagar, Kerala, India.
2. Professor and Head, Department of Microbiology, Sree Balaji Medical College and Hospital, Chennai, Tamil Nadu, India.
3. Professor and Head, Department of Radiation Oncology, Amala Institute of Medical Sciences, Thrissur, Kerala, India.

NAME, ADDRESS, E-MAIL ID OF THE CORRESPONDING AUTHOR:
Dr. Jomon Raphael Chalissery,
Professor and Head, Department of Radiation Oncology, Amala Institute of Medical Sciences, Amala Nagar, Thrissur-680555, Kerala, India.
E-mail: joeraphael@gmail.com

PLAGIARISM CHECKING METHODS: [Jain H et al.]
- Plagiarism X-checker: Jul 12, 2023
- Manual Googling: Aug 22, 2023
- iThenticate Software: Sep 02, 2023 (13%)

ETYMOLOGY: Author Origin

EMENDATIONS: 6

AUTHOR DECLARATION:
- Financial or Other Competing Interests: None
- Was Ethics Committee Approval obtained for this study? Yes
- Was informed consent obtained from the subjects involved in the study? Yes
- For any images presented appropriate consent has been obtained from the subjects. NA

Date of Submission: **Jul 07, 2023**
Date of Peer Review: **Aug 11, 2023**
Date of Acceptance: **Sep 05, 2023**
Date of Publishing: **Oct 01, 2023**