DOI: 10.7860/JCDR/2023/65554.18373

Original Article

Microbiology Section

Extracellular Enzymatic Activity of *Candida* Species Isolated from Patients with Head and Neck Cancer Undergoing Radiation Therapy and its Correlation with Mucositis: A Cross-sectional Study

NEETHU BABU[1], CHITRALEKHA SAIKUMAR[2], JOMON RAPHAEL CHALISSERY[3], HARISH KUMAR[4]

ABSTRACT

Introduction: The differences in enzymatic activity expressed by various *Candida* species determine their virulence and play a pivotal role in understanding the pathogenesis of candidiasis. Additionally, this knowledge aids in the development of new antifungal drugs that target these enzymes, thereby enhancing therapeutic approaches. Understanding the extracellular enzymatic activity of *Candida* species is crucial for the development of new anticandidial drugs targeting these enzymes.

Aim: To determine the differences in enzymatic activity expressed by various *Candida* species isolated from patients with Head and Neck Cancer (HNC) undergoing Radiation Therapy (RT) and to correlate these differences with the severity of mucositis.

Materials and Methods: This cross-sectional study evaluated the enzymatic activity of *Candida* species, including *C. albicans* and Non *albicans Candida* species (NAC), isolated from HNC patients undergoing RT at the radiation oncology department, Amala Institute of Medical Sciences, Thrissur, Kerala, India. A total of 276 patients were enrolled in the study over a four-year period (January 2019 to December 2022). Extracellular enzymatic activities such as proteinase, phospholipase, haemolysin, and esterase were detected using the plate method, as described previously. Mucositis was graded according to the Radiation Therapy Oncology Group (RTOG) criteria. Data were analysed using Statistical Package for the Social Sciences (SPSS) version 23.0 (IBM, Illinois, US). The Chi-square test was used to analyse the variables, and the unpaired t-test was used to compare enzymatic activity. Spearman's rank correlation was used to identify any correlation between mucositis and extracellular enzymatic activity.

Results: A total of 97 *Candida* strains (56 *C. albicans* and 41 NAC) were isolated. There was no statistically significant difference between *C. albicans* and NAC species causing infections in men and women (p-value=0.390), as well as in those with diabetes (p-value=0.127) and hypertension (p-value=0.979). Proteinase, haemolytic activity, and esterase production were detected in 88 (90.7%), 84 (86.6%), and 67 (69.0%) isolates, respectively, while phospholipase activity was shown by 18 (18.5%) isolates. There was no statistically significant difference between *C. albicans* and NAC species regarding the mean phospholipase, proteinase, haemolysin, and esterase activity (p-value >0.05). *C. albicans* exhibited high activity for all four enzymes, while a considerable percentage of NAC showed moderate activity. High phospholipase and proteinase activity in *C. albicans* showed a good correlation (r=0.148 and r=0.186, respectively) with mucositis.

Conclusion: *C. albicans* showed high activity for all four enzymes, indicating its virulence. The majority of *C. albicans* strains exhibited proteinase activity, which is associated with the severity of mucosal infections. The phospholipase activity has the potential to play a role in the emergence of drug resistance and should be closely monitored.

Keywords: Candidiasis, *Candida albicans*, Drug resistance, Enzymes, Virulence

INTRODUCTION

Oropharyngeal candidiasis is responsible for considerable morbidity in patients undergoing RT for HNC. *Candida albicans* is the most common species isolated from oral lesions, but recent epidemiological studies have reported NAC species including *C. tropicalis, C. parapsilosis, C. krusei,* and *C. glabrata* repeatedly emerging as human pathogens [1-3]. The virulence of the *Candida* species is attributed to a wide variety of mechanisms, including adherence, biofilm formation, production of extracellular enzymes such as proteinases, phospholipases, esterases, and haemolysins. The expression of virulence factors may vary depending on the infecting species, geographical origin, type of infection, site and stage of infection, and host reaction [4]. Therefore, knowledge of these factors is important to understand the pathogenesis of candidiasis, which in turn is responsible for the virulence of the strain [4,5].

Among the various extracellular enzymes that act as virulence factors of *Candida*, the most important ones are phospholipase and proteinase, as they facilitate *Candida*-host interaction [6,7]. Haemolysin degrades haemoglobin, thereby helping *Candida* acquire iron, which further aids in its persistence and survival [8]. Esterase enzyme degrades ester bonds and assists in tissue invasion. All these enzymes together contribute to the pathogenicity of these yeasts by exacerbating the damage to mucosal surfaces and the immune system, thereby enhancing their dissemination, virulence, and drug resistance [9,10].

Extracellular enzymatic activity is a crucial factor that defines not only the virulence of the infecting *Candida* strain but also provides an indication of the likelihood of rapid resistance development to the commonly used empirical drug fluconazole. Existing information on enzyme activity of *Candida* infecting HNC patients is scarce [11,12]. *C. albicans* and NAC species are clinically indistinguishable, but they vary widely in terms of virulence and drug resistance. The role of various extracellular enzymes in increasing the severity of mucositis is poorly investigated. Mucositis, a major dose-limiting toxicity that

occurs in the majority of patients undergoing RT for HNC, becomes even more complicated with candidiasis, thereby increasing the cost of treatment [13]. There are various studies assessing the extracellular enzymatic activities of *Candida* causing various clinical infections [14-18], but there are not many studies from India on *Candida* causing oropharyngeal infections in HNC patients. The aim of the present study was to determine the difference in enzymatic activity expressed by various *Candida* species isolated from HNC patients undergoing RT. The primary objective of this study was to investigate the difference in the extracellular enzymatic activities of *C. albicans* and NAC species for the determination of their pathogenesis and virulence. The secondary objective of the study sought to establish a correlation between the severity of mucositis in HNC patients undergoing RT and the extracellular enzymatic activities of *Candida* species.

MATERIALS AND METHODS

A cross-sectional study was conducted in the outpatient clinic of Department of Radiation Oncology, Amala Institute of Medical Sciences, Thrissur, Kerala, India on HNC patients in whom *Candida* was isolated. A total of 276 patients were enrolled in the study over a period of four years (January 2019 to December 2022). The study was approved by the institutional research and ethics committee (Ref No. 30/IEC/19/AIMS-11). According to the ethical principles involving research in human subjects, all procedures were performed after obtaining informed and written consent from each subject. Co-morbidities such as diabetes and hypertension were also noted.

Inclusion criteria: Individuals of both genders, aged 18-85 years, undergoing cancer treatment (RT or chemotherapy (CT)/RT) for histologically confirmed carcinoma. All patients who were treated using Intensity Modulated Radiation Therapy (IMRT) technique with a dose ranging from 60 to 70 Gy were included in the study.

Exclusion criteria: Patients with a recent history of candidiasis or currently on antifungals were excluded from the study.

Sample size: Based on previous studies with a prevalence rate ranging from 25-35% [1,19], an average prevalence of 30% was used for sample size calculation.

$n=(Z1-\alpha/2)^2pq/d^2$

P=Prevalence 30%, d=relative precision 20% P, α=significance level 5%.

The minimum sample size required for the study was 224.

Determination of enzyme activity: Oral swabs were collected whenever there were clinical signs and symptoms of candidiasis and processed in the microbiology laboratory according to standard protocol [20]. Species identification was done based on colour in HiCromeTM *Candida* differential agar (HiMedia Laboratories Pvt., Limited, Mumbai, India), germ tube test, and chlamydospore production. The strains were stored in Sabouraud's Dextrose Agar (SDA) slants until further testing.

Determination of phospholipase activity: The method described by Price MF et al., was used with minor modifications. Egg yolk agar medium was used for culture [21]. Basal media was SDA with NaCl and CaCl to which 10% sterile egg yolk emulsion was added. *Candida* isolates were freshly subcultured on SDA for 24 hours, and a 10 µL suspension of 10^7 yeast cells/mL in Phosphate Buffered Saline (PBS) was plated on the surface of egg yolk medium and left to dry at room temperature. The plates were then incubated at 37°C for 72 hours. The phospholipase activity of the isolates was interpreted as positive when a precipitation zone was visible around the growth. The value of phospholipase activity (Pz) is determined by the ratio of the diameter of the colony plus the precipitation zone to the diameter of the colony. A Pz value of 1 means the test strain is negative, and a value of 0.69 or lower indicates very high phospholipase production [21]. Each *Candida* isolate was tested twice.

Determination of proteinase activity: Bovine Serum Albumin (BSA) media was used following the method described by Rüchel R et al., [22]. A solution containing 0.04 g $MgSO_4.7H_2O$, 0.5g K_2HPO_4, 1 g NaCl, 0.2 g yeast extract, and 4 g glucose was prepared. The pH was adjusted to 3.5. The solution was sterilised by filtration and then mixed with 140 mL of melted agar and poured into petri dishes. The formation of a transparent halo around the yeast colonies indicated the production of proteinase. *Candida* strains were inoculated in triplicate, and the plates were incubated at 37°C for seven days. Proteinase activity (Prz) was measured and calculated according to the method described by Price MF et al., in terms of the ratio of the diameter of the colony to that of the colony plus the precipitation zone [21].

Determination of haemolytic activity: The method by Luo G et al., was followed for haemolysin detection, and the haemolytic index was calculated [23]. SDA with 3% glucose and 7% fresh blood was used as the medium. *Candida* isolates were freshly subcultured on SDA for 24 hours, and a cell suspension of 10^8 cells/mL was prepared. A 10 µL aliquot was taken from this suspension, and a 5 mm inoculum was made on the surface of the agar plate. The plates were then incubated at 37°C in 5% CO_2 for 48 hours. The presence of a distinct translucent halo and/or a greenish-black ring around the inoculum site indicated positive haemolytic activity when viewed with transmitted light. Haemolysis tests were repeated three times, and the results represent mean±SD values.

The diameters of the zone of haemolysis and the colony were measured to evaluate the intensity of haemolys in production exhibited by different *Candida* strains. The diameter of the translucent radial zone of haemolysis was divided by the diameter of the colony size, and this ratio was used as the haemolytic index [23].

Determination of esterase activity: Tween 80 opacity medium was used for the assay according to the previously described method [24]. The medium was prepared with 10 g peptone, 5 g NaCl, 0.1 g $CaCl_2$, 15 g agar, and 1000 mL distilled water, with the pH adjusted to 6.8. It was autoclaved at 121°C for 15 minutes, allowed to cool to about 50°C, mixed with 5 mL of preautoclaved and cooled Tween 80, and dispensed into sterile petri dishes. An overnight culture of each *Candida* isolate grown on SDA was transferred to the Tween 80 opacity medium and spread over a circular inoculation site of approximately 10 mm diameter. The inoculated agar plates were incubated aerobically at 35°C and examined daily for upto 10 days. All strains were assayed in duplicate. Esterase activity on the test plates was detected by observing halos of precipitation around the inoculum under transmitted light.

Grading of mucositis was performed by an oncologist based on RTOG [25] as Grade-1 (mild), Grade-2 (moderate), Grade-3 (severe), and Grade-4 (life-threatening).

STATISTICAL ANALYSIS

The data were analysed using SPSS (v23, IBM, Illinois, US). The demographic variables of patients with *C. albicans* and NAC were analysed using the Chi-square test. Unpaired t-test was used to analyse the difference in mean enzyme activity between the two groups. The correlation between mucositis and extracellular enzyme activity was analysed using Spearman rank correlation.

RESULTS

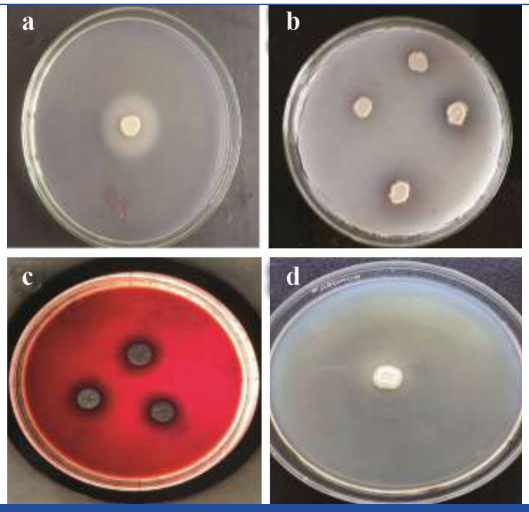
Candidiasis was confirmed in 97 (35.1%) out of 276 patients who were undergoing RT for HNC. The mean age of the patients was 65±11 years. Out of the 97 (35.1%) *Candida* species isolated, *C. albicans* was the predominant one, with 56 (57.7%) cases, and NAC species accounted for 41 (42.3%) cases. Among a total of 79 (81.4%) men with *Candida* infection, *C. albicans* was isolated from 44 (55.6%) and NAC species from 35 (44.4%). In women with oropharyngeal candidiasis, *C. albicans* was isolated from 12 (66.7%) cases and NAC from 6 (33.3%) cases, out of a total of 18 (18.6%)

women. There was no statistically significant difference between the two groups causing infections in men and women (p-value=0.390), as well as those with diabetes (p-value=0.127) and hypertension (p-value=0.979) [Table/Fig-1].

Characteristics	*C. albicans*	Non *albicans Candida* (NAC) species	p-value
Gender			
Women	12 (66.7%)	6 (33.3%)	0.390
Men	44 (55.6%)	35 (44.4%)	
Diabetic			
Yes	20 (48.8%)	21 (51.2%)	0.127
No	36 (64.3%)	20 (35.7%)	
Hypertension			
Yes	22 (57.9%)	16 (42.1%)	0.979
No	34 (57.6%)	25 (42.4%)	

[Table/Fig-1]: Demography of Head and Neck Cancer (HNC) patients with candidiasis.
p-value >0.05=Not significant (Chi-square test)

Extracellular enzymes, including phospholipase, proteinase, esterase, and haemolytic activity, were shown by most of the *Candida* species isolated [Table/Fig-2]. However, phospholipase activity was exhibited by only a few strains. Proteinase and haemolytic activity were detected in 88 (90.7%) and 84 (86.6%) isolates, respectively, while phospholipase activity was shown by only 18 (18.5%) isolates [Table/Fig-3].



[Table/Fig-2]: Photographs of extracellular enzyme activity of *Candida* species isolated: (a) Phospholipase; (b) Proteinase; (c) Haemolysin; and (d) Esterase.

Out of the total 88 *Candida* strains that were proteinase producers, the majority (50, 56.8%) were *C. albicans*, with a mean Prz of 0.87±0.05. The NAC strains (38, 43.1%) showed a slightly higher mean Prz value (0.88±0.04), but there was no statistical significance (p-value=0.346). The same was the case with haemolysin and esterase production, with 52/84 (61.9%) and 42/67 (62.6%) strains isolated being *C. albicans*. The mean haemolytic activity of *C. albicans* and NAC were 0.82±0.06 and 0.83±0.07, respectively. Phospholipase production was detected in very few strains (18, 18.5%), of which the majority were *C. albicans* (11, 61.1%) with a mean Pz value of 0.82±0.09. The mean Pz of NAC (0.85±0.09, n=7, 38.9%) was slightly higher than that of the *C. albicans* strains, but without statistical significance (p-value=0.465) [Table/Fig-3]. A higher degree of enzyme activity (4+ and 3+) was shown by a greater number of *C. albicans* strains than NAC. However, there was no statistically significant difference between the organisms with respect to the mean phospholipase, proteinase, haemolysin, and esterase activity (p-value >0.05). The results are shown in [Table/Fig-3-5].

Grade-2 mucositis was recorded in 36 (37.1%) of the patients with candidiasis, and 39 (40.2%) showed Grade-3 mucositis. 22 (22.7%) patients developed grade 1 mucositis and grade 4 mucositis was not reported in any of the patients. Spearman's rank correlation test was done to assess the correlation. A weak positive correlation was noted between mucositis and extracellular enzymes, such as phospholipase and proteinase, in *C. albicans*, which was not shown by NAC [Table/Fig-6].

DISCUSSION

The probable diagnosis of oropharyngeal candidias is based solely on signs and symptoms which can be misleading, as these yeasts are found in the oral cavity of the majority of HNC patients undergoing RT. In this study, the overall prevalence of oropharyngeal candidiasis, both clinically and microbiologically confirmed, was 97 (35.1%). Such a prevalence rate was consistent with previous reports [1,26,27]. Clinical as well as microbiological correlation is essential in the diagnosis of candidiasis, as it is difficult to differentiate it from mucositis in HNC patients. This will also help in the early detection and treatment to avoid premature termination of RT. The main aim of the present study was to determine the difference in enzymatic activity expressed by *C. albicans* and NAC species. Various extracellular enzymes are secreted by *Candida*, which act as potential virulence factors. The study results show that *C. albicans* (56, 57.7%) was the most frequently isolated *Candida* species with a high degree (4+, 3+, and 2+) of enzymatic activity, which accounts for its virulence. Similar results were also reported by Maheronnaghsh M et al., and Tsang CS et al., [28,29]. In their studies, high enzymatic activity was shown by the strains isolated from patients compared to those from control groups, indicating a significant association between the degree of enzyme production and strain virulence.

Isolate	Phospholipase			Proteinase			Haemolytic activity		
	n*(%) 18 (18.5%)	Mean±SD	p-value	n*(%) 88 (90.7%)	Mean±SD	p-value	n*(%) 84 (86.6%)	Mean±SD	p-value
C. albicans	11 (61.1%)	0.82±0.09	0.465	50 (56.8%)	0.87±0.05	0.346	52 (61.9%)	0.82±0.06	0.478
NAC†	7 (38.9%)	0.85±0.09		38 (43.1%)	0.88±0.04		32 (38.1%)	0.83±0.07	

[Table/Fig-3]: Enzyme production.
n*=No: of isolates; †NAC: Non albicans candida; species. p-value >0.05=Not significant. (Student t-test); (N=97)

Pz*/Prz† value	Degree	*C. albicans*		Non *albicans Candida* (NAC) species	
		Phospholipase	Proteinase	Phospholipase	Proteinase
<0.69	++++	1 (9.1%)	3 (6%)	1 (14.3%)	1 (2.6%)
0.70-0.79	+++	4 (36.4%)	1 (2%)	0	1 (2.6%)
0.80-0.89	++	4 (36.4%)	28 (56%)	4 (57.1%)	16 (42.1%)
0.90-0.99	+	2 (18.1%)	18 (36%)	2 (28.6%)	20 (52.6%)
1.00	-	0	0	0	0
Total		11	50	7	38

[Table/Fig-4]: Distribution of Phospholipase/Proteinase activity values among *C. albicans* and Non Albicans *Candida* (NAC) species.
Pz*-Phospholipase activity; Prz†-Proteinase activity

Esterase activity	*C. albicans*	Non-*albicans Candida* (NAC) species	p-value
Positive 67 (69.1%)	42 (62.6%)	25 (37.3%)	0.141
Negative 30 (30.9%)	16 (53.3%)	14 (46.7%)	

[Table/Fig-5]: Esterase activity of *C. albicans* and Non-*albicans Candida* (NAC) species.
p-value=0.141=Not significant. (Student t-test)

C. albicans				Non *albicans Candida* (NAC) species		
Correlation between mucositis and	Spearman's rank correlation	95% CI*	p value	Spearman's rank correlation	95% CI*	p-value
Phospholipase	0.148	0.101-0.248	0.306	-0.292	-0.210-0.320	0.099
Proteinase	0.186	0.146-0.210	0.346	-0.169	-0.134-0.189	0.348
Haemolytic activity	-0.159	-0.113-0.69	0.270	-0.196	-0.116-0.210	0.275

[Table/Fig-6]: Correlation between mucositis and enzyme activity of *C. albicans* and Non albicans *Candida* (NAC) species.
*Confidence Interval; p-value >0.05=Not significant

More than 90% of the strains isolated in the present study were proteinase producers, with 50 (56.8%) being *C. albicans*. Although the mean proteinase activity of NAC species was slightly higher than that of *C. albicans*, the majority of them had low degrees of activity. A study by Nawaz A et al., showed that NAC species, particularly *C. tropicalis*, exhibited high proteolytic activity [30]. It has been proven that *C. albicans* proteinases play a role in augmenting pathogenesis. The breakdown of humoral host defense mechanisms caused by the action of *C. albicans* proteinases may render hosts more vulnerable to microbial infections and aggravate infectious diseases in compromised hosts. Studies have described that Secreted Aspartyl Proteinases (SAP) damage surface proteins and degrade locally protective IgA and C3 components, thus facilitating tissue invasion [17,31,32]. In this study, a high number of *C. albicans* strains were proteinase producers and showed high activity. A positive correlation was observed between mucositis and proteinase production in the present study. Ilkhanizadeh-Qomi M et al., in a similar study on oral cancer patients, showed that *C. albicans* strains were more potent producers of proteinase and phospholipase with a high level of gene expression [32]. The SAP highly contributes to the damage to mucosal surfaces, as shown by the high level of expression of the Sap genes in their study, which clearly indicates the relationship between virulence and enzymatic activity. Jahanshiri Z et al., also demonstrated a high level of Sap gene expression in HNC patients, which again indicates its role in increasing the severity of mucositis in these patients [17]. The initiation of mucositis occurs concurrently with the administration of RT. Almost 90% of HNC patients develop mucositis, which may affect their quality of life, treatment costs, and prognosis if treatment breaks occur [1,2,19]. Extracellular phospholipase lyses host cells to facilitate adhesion and penetration. The phospholipase enzyme breaks down the phospholipids of the cell membrane, causing cell lysis. Direct host cell damage and lysis have been proposed as major mechanisms contributing to microbial virulence. In the present study, *C. albicans* was found to be the most frequent phospholipase producer. This finding was similar to that reported earlier by Ilkhanizadeh-Qomi M et al., [32]. Only 4 (12.5%) *C. albicans* isolates in their study lacked phospholipase activity, while in this study, only 11 (19.6%) of the *C. albicans* strains showed phospholipase activity. Pandey N et al., reported a decrease in phospholipase activity compared to other enzymes in their study on *Candida* isolates from the blood of intensive care patients [18]. The strains in their study showed 84.72%, 55.69%, and 37.97% proteinase, phospholipase, and esterase activities, respectively. NAC species were the most prevalent in their study, but there was no significant difference in enzyme production compared to *C. albicans*, which was consistent with the findings of the present study. Erum R et al., in their study on *Candida* infections in surgical sites and Jahanshiri Z et al., in a study on *C. tropicalis* from oral cancer patients showed that a high level of enzymatic activity is directly associated with the severity of infection and azole resistance [33,34].

In this study, 52/56 (92.8%) of the *C. albicans* strains showed haemolytic activity, while with NAC species, it was 32/41 (78%). The mean haemolytic activity of NAC species was slightly higher than that of *C. albicans*, but a high degree of haemolytic activity was shown by *C. albicans*. Similar results of high haemolysin production were reported by Pandey N et al., in their study [18]. Duval C et al., demonstrated in their study that haemolysin enzymes in *C. albicans* could cause iron release from red blood cells, and these enzymes can cause sustained adhesion of the yeast cell to the host, ultimately leading to infection [35]. They showed that the pathogenicity of *Candida* species is directly dependent on the activity level of the enzyme. Nouraei H et al., in their study on candidemia patients, reported a significant difference in the enzymatic activity of *C. albicans* and NAC species [36]. Furthermore, very high levels of phospholipase, proteinase, and haemolytic activity were shown by *C. albicans*, which was similar to the present study. The strength of this study lies in the uniform patient selection, with all patients receiving RT using the IMRT technique in the head and neck region.

Limitation(s)

The major limitation of this study was the lack of comparison regarding the enzymatic activity of *Candida* isolates from the oral cavity of non immunocompromised individuals. Further molecular studies are needed to assess the expression level of these genes and compare it with the pathogenicity and virulence of these *Candida* strains.

CONCLUSION(S)

Although all the strains were pathogenic, the enzyme activity shown by them varied. *C. albicans* was the predominant *Candida* species isolated from HNC patients undergoing RT. Majority of the *C. albicans* strains isolated from these patients showed proteinase activity, haemolytic activity, and esterase activity. They also showed a high degree of all four enzymatic activities, indicating their virulence. Phospholipase activity, which has a potential role in the emergence of drug resistance, was detected in very few strains and needs to be closely monitored. The majority of the *C. albicans* strains were proteinase producers, highlighting their role in increasing the severity of mucosal infections in HNC patients. A positive correlation was observed between mucositis and proteinase as well as phospholipase activity with *C. albicans*.

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] 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 HNC undergoing radiotherapy. Current Medical Mycology. 2021;7(1):44.
[2] 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 Medicine. 2023;19(1):7.
[3] Monsen RE, Kristoffersen AK, Gay CL, Herlofson BB, Fjeld KG, Hove LH, et al. Identification and susceptibility testing of oral candidiasis in advanced cancer patients. BMC Oral Health. 2023;23(1):01-09.
[4] Staniszewska M. Virulence factors in *Candida* species. Current Protein and Peptide Science. 2020;21(3):313-23.
[5] Lopes JP, Lionakis MS. Pathogenesis and virulence of *Candida albicans*. Virulence. 2022;13(1):89-121.
[6] Richardson JP, Ho J, Naglik JR. *Candida*-epithelial interactions. Journal of Fungi. 2018;4(1):22.
[7] Nikou SA, Kichik N, Brown R, Ponde NO, Ho J, Naglik JR, et al. *Candida albicans* interactions with mucosal surfaces during health and disease. Pathogens. 2019;8(2):53.

[8] Furlaneto MC, Góes HP, Perini HF, Dos Santos RC, Furlaneto-Maia L. How much do we know about hemolytic capability of pathogenic *Candida* species? Folia Microbiologica. 2018;63:405-12.
[9] Millet N, Solis NV, Swidergall M. Mucosal IgA prevents commensal *Candida albicans* dysbiosis in the oral cavity. Front Immunol. 2020;11:555363.
[10] Deorukhkar SC, Roushani S. Virulence traits contributing to pathogenicity of *Candida* species. J Microbiol Exp. 2017;5:00140.
[11] El-Houssaini HH, Elnabawy OM, Nasser HA, Elkhatib WF. Correlation between antifungal resistance and virulence factors in *Candida albicans* recovered from vaginal specimens. Microb Pathog. 2019;128:13-19.
[12] Mashaly GE, Zeid MS. *Candida albicans* genotyping and relationship of virulence factors with fluconazole tolerance in infected pediatric patients. Infect Drug Resist. 2022:2035-43.
[13] Pulito C, Cristaudo A, Porta CL, Zapperi S, Blandino G, Morrone A, et al. Oral mucositis: The hidden side of cancer therapy. J Exp Clin Cancer Res. 2020;39:01-05.
[14] Pramodhini S, Srirangaraj S, Easow JM. Candiduria-study of virulence factors and its antifungal susceptibility pattern in tertiary care hospital. J Lab Physicians. 2021;13(03):231-37.
[15] Pawar MY, Hatolkar SM, Misra RN. Phenotypic and molecular detection of virulence factor genes SAP4 and PLB in *Candida albicans* isolates from the Western part of India. Med J Armed Forces India. 2022;78(3):271-76.
[16] Mohammadi F, Charkhchian M. Phenotypic and genotypic characterization of virulence markers and antifungal susceptibility of oral *Candida* species from diabetic and non-diabetic hemodialysis patients. BMC Oral Health. 2023;23(1):1-1.
[17] Jahanshiri Z, Manifar S, Hatami F, Arastehnazar F, Shams-Ghahfarokhi M, Razzaghi-Abyaneh M. Genotyping of *Candida albicans* isolates from oropharyngeal candidiasis in head and neck cancer patients in Iran: Molecular epidemiology and SAP2 gene expression. Journal de Mycologie Médicale. 2019;29(4):310-16.
[18] Pandey N, Gupta MK, Tilak R. Extracellular hydrolytic enzyme activities of the different *Candida* spp. isolated from the blood of the Intensive Care Unit-admitted patients. J Lab Physicians. 2018;10(04):392-96.
[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? Med. 2018;97(44).
[20] Walsh TJ, Hayden RT, Larone DH. Larone's medically important fungi: A guide to identification. John Wiley & Sons; 6th Edition 2018 Jun 1.
[21] Price MF, Wilkinson ID, Gentry LO. Plate method for detection of phospholipase activity in *Candida albicans*. Sabouraudia. 1982;20(1):07-14.
[22] Rüchel R, Tegeler R, Trost M. A comparison of secretory proteinases from different strains of *Candida albicans*. Sabouraudia. 1982;20(3):233-44.
[23] Luo G, Samaranayake LP, Yau JY. *Candida* species exhibit differential in vitro hemolytic activities. J Clin Microbiol. 2001;39(8):2971-74.
[24] Rudek WA. Esterase activity in *Candida* species. J Clin Microbiol. 1978;8(6):756-59.
[25] Riesenbeck D, Dörr W. Documentation of radiation-induced oral mucositis. Scoring systems. Strahlenther Onkol. 1998;174:44-46.
[26] 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.
[27] Saito H, Shodo R, Yamazaki K, Katsura K, Ueki Y, Nakano T, et al. The association between oral candidiasis and severity of chemoradiotherapy-induced dysphagia in head and neck cancer patients: A retrospective cohort study. Clin Transl Radiat Oncol. 2020;20:13-18.
[28] Maheronnaghsh M, Fatahinia M, Dehghan P, Mahmoudabadi AZ, Kheirkhah M. Comparison of virulence factors of different candida species isolated from the oral cavity of cancer patients and normal individuals. Jundishapur J Microbiol. 2019;12(5):e91556.
[29] Tsang CS, Chu FC, Leung WK, Jin LJ, Samaranayake LP, Siu SC. Phospholipase, proteinase and haemolytic activities of *Candida albicans* isolated from oral cavities of patients with type 2 diabetes mellitus. J Med Microbiol. 2007;56(10):1393-98.
[30] Nawaz A, Mäkinen A, Pärnänen P, Meurman JH. Proteolytic activity of non-albicans *Candida* and *Candida albicans* in oral cancer patients. New Microbiol. 2018;41(4):296-301.
[31] Zhou Y, Cheng L, Lei YL, Ren B, Zhou X. The Interactions between *Candida albicans* and mucosal immunity. Frontiers in Microbiology. 2021;12:652725.
[32] Ilkhanizadeh-Qomi M, Nejatbakhsh S, Jahanshiri Z, Razzaghi-Abyaneh M. Aspartyl Proteinase and phospholipase activities of *candida albicans* isolated from oropharyngeal candidiasis in head and neck cancer patients. Jundishapur J Microbiol. 2020;13(9):e105200.
[33] 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 Microbiol. 2020;20(1):01-02.
[34] 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. Iranian J Microbiol. 2023;15(1):163.
[35] Duval C, Macabiou C, Garcia C, Lesuisse E, Camadro JM, Auchère F. The adaptive response to iron involves changes in energetic strategies in the pathogen *Candida albicans*. Microbiologyopen. 2020;9(2):e970.
[36] Nouraei H, Pakshir K, ZareShahrabadi Z, Zomorodian K. High detection of virulence factors by *Candida* species isolated from bloodstream of patients with candidemia. Microb Pathog. 2020;149:104574.

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, Thrissur, 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, Amala Nagar, Thrissur, Kerala, India.
4. Professor and Head, Department of Microbiology, School of Medical Education, Kottayam, 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, Thrissur-680555, Kerala, India.
E-mail: joeraphael@gmail.com

PLAGIARISM CHECKING METHODS: [Jain H et al.]
- Plagiarism X-checker: May 23, 2023
- Manual Googling: Jun 05, 2023
- iThenticate Software: Jun 16, 2023 (14%)

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: **May 23, 2023**
Date of Peer Review: **Jun 03, 2023**
Date of Acceptance: **Jun 17, 2023**
Date of Publishing: **Sep 01, 2023**