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Significance of *Candida species* in patients with coronavirus disease

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Abstract

Background: Respiratory fungal infection is a severe clinical condition especially in immune-compromised individuals. Secondary fungal infections as complications of severe coronavirus disease 2019 are on the rise.

Objectives: This study was carried out to isolate the *Candida species* from coronavirus disease 2019 patients, identify different species of *Candida* and to show the significance of *Candida species* in coronavirus disease 2019 patients attending a dedicated hospital for coronavirus disease management.

Methods: A hospital-based descriptive cross-sectional study was done in a period between 2nd Jestha to 23rd Ashad 2077 (16th May to 7th July 2021) in Nepalgunj Medical College, Kohalpur, Banke. The study included 100 Coronavirus disease 2019 patients. Sputum samples were taken and subjected to direct microscopy, cultured onto Sabouraud Dextrose Agar. *Candida species* were determined by standard microbiological methods. Informed consent was taken prior to participation in study. Data were analysed for simple descriptive analysis using Microsoft Office Excel.

Results: Out of 100 sputum samples, 78 (78%) men and 22 (22%) women were included; *Candida species* were isolated from 36 (36%) patients. This study shows *Candida species* isolated higher in male and from 31-60 years of age group.

Conclusion: We recommend that the investigation and identification of *Candida* may be routinely followed in the microbiology laboratories.

Key words: Candida; Coronavirus disease 2019; Hospital-based.

INTRODUCTION

World Health Organisation (WHO) announced new virus outbreak on 30 December 2019 and named it coronavirus disease 2019 (COVID-19) on 11 February 2020.[1] Respiratory fungal infection is a severe clinical condition especially in immunocompromised.[2] Yeast-like fungus *Candida* produces pseudomycelia in culture and tissue.[3] *Candida* is commonly found as normal flora throughout body.[4] Most abundant species is *Candida albicans*.[5] Isolation of *Candida species* from respiratory tract secretions is frequent in non-immunocompromised, mechanically ventilated patients. Studies have reported *Candida species* in sputum of 20-55% of patients receiving antibiotics.[6] *Candida species* is most common cause of invasive fungal infections, with incidence of 72.8 cases per million inhabitants per year.[7] Five main species of *Candida*: *C. albicans*, *C. parapsilosis*, *C. glabrata*, *C. tropicalis,* and *C. krusei* can cause >90% of invasive fungal infections, in both intensive care unit (ICU) and non-ICU patients.[8] *Candida* pneumonia is rare lung infection with high morbidity and mortality, observed as part of disseminated *Candida* infection and associated with predisposing clinical circumstances: long-term antibiotic use, haematologic malignancy or severe immunosuppressive states. Majority of *Candida*

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pneumonia cases are secondary to haematological dissemination of *Candida species*.[9] Hence, this study was carried out to isolate the various *Candida species* from COVID-19 patients.

METHODOLOGY

This was a hospital-based descriptive cross-sectional study conducted at Nepalgunj Medical College over a period of two months (2nd Jestha 2077 to 23rd Ashad 2077 (16th May 2021 to 7th July 2021). Nepalgunj Medical College, Kohalpur, Banke, Nepal was converted into COVID-19 dedicated hospital during the second wave of COVID-19 pandemic in Nepal on 2078 Baisakh 18 BS (2021 May 1 AD). A total of 100 patients infected with COVID-19 were enrolled in this study by convenience sampling. The study was approved by institutional ethical committee (Ref. 764/077-078) and informed consent was obtained from all the participants and their relatives.

Sputum sample were collected in a leak proof container, tightly sealed, and transported in leak proof plastic zip bag. Patient name, hospital id number, date and time of collection were written on sample container properly and details of patients were recorded. The sputum sample were transferred to the microbiology laboratory and processed immediately. Samples were processed following the standard protocol of biosafety. The sputum smears were, prepared, fixed by heat, and stained by Gram's stain. The smears were examined under light microscope using oil immersion lens. Gram stain was used to observe for presence of budding yeast cells and pseudohyphae. The direct wet mount microscopy using 10% potassium hydroxide (10% KOH) solution and Lacto Phenol Cotton Blue (LPCB)[10] mount was done by placing the samples on slide and adding 10% KOH and LPCB. Cover slips were placed over slide and examined under light microscope using low power lens followed by high power lens. These direct wet mount microscopy tests were done to determine the presence of budding yeast cells and pseudohyphae in the sample.

Sputum sample was streaked on Sabouraud Dextrose Agar (SDA) (HiMedia, India) and incubated at 37°C for 48 hours. After 48 hours, the colony characteristic was recorded. At the same time, Gram's stain, 10% KOH, and LPCB mount was carried out. A small portion of an isolated colony from SDA was suspended in test tube containing 0.5 ml human serum. Then test tube was incubated at 37°C for four to six hours.[11] Afterwards a drop of yeast suspension was placed on the slide covered by cover slip. Finally examination was done under light microscope for the formation of Germ tube. Colonies suggestive of *Candida albicans* were confirmed by this germ tube test.

Data were entered in Microsoft Excel Sheet and analysed. Descriptive findings have been presented as frequencies and percentages.

RESULTS

A total of 100 patients were included in the study. The participants were divided on the basis of different age groups. Maximum patients (64, 64%) were in the age group of 31-60 years (Table 1).

In this study, 78 (78%) men and 22 (22%) women were included. Out of 100, *Candida spp.* isolated from 36 samples where 28 were sample from male patients and eight from female patients (Table 2). There were only *Candida albicans* isolated from all the samples.

Table 1: Agewise distribution of the study subjects, n (%)

Age in years	Number of patients	*Candida* isolated
0-30	14	4 (4)
31-60	64	20 (20)
61-90	22	12 (12)
Total	**100**	**36 (36)**

Table 2: *Candida species* isolated in relation to gender, n (%)

Sex	Number of patients	*Candida* isolated
Male	78	28 (28)
Female	22	8 (8)
Total	**100**	**36 (36)**

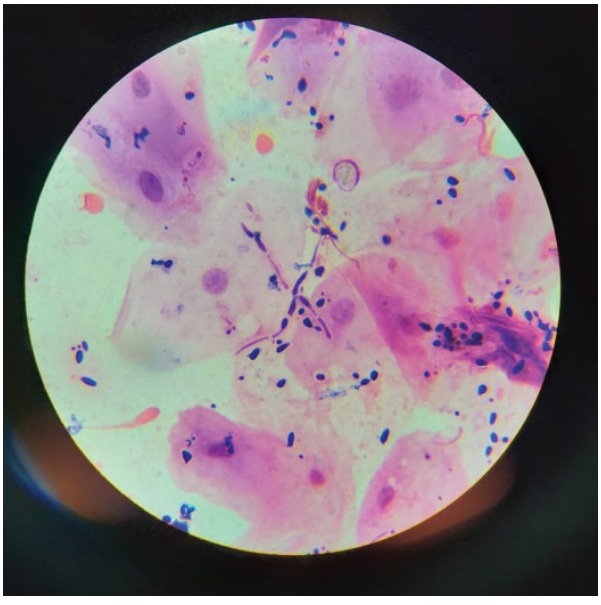

Figure 1: Candidial cells with psudohyphae in Gram stain

Figure 2: ***Candida species*** **grown on Sabouraud dextrose agar**

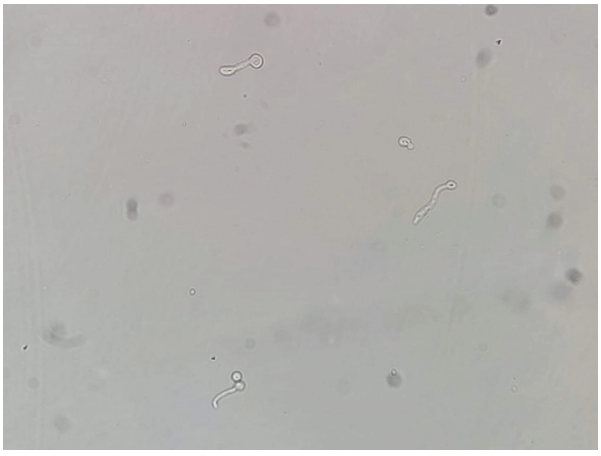

Figure 3: **Germ tube**

DISCUSSION

While in the beginning of the COVID-19 pandemic, superinfections had been rarely reported. Reports about secondary fungal infections as complications of severe COVID-19 are on the rise. In COVID-19 patients with acute respiratory distress syndrome, COVID-19 associated pulmonary aspergillosis and COVID-19 associated candidiasis has been described to complicate the clinical course.[12]

It is established that *Candida albicans* can convert into a disease-causing pathogen from a commensal, when there is a change in the host environment.[13] In this study it was observed that *Candida species* were isolated from 36 (36%) patients where 28 (28%) were male and 8 (8%) were female. All the isolates were identified as *Candida albicans* on the basis of Germ tube test.

In this study, number of study subjects was divided into three age groups: 0-30 years, 31-60 years, and 61-90 years. *Candida albicans* isolated from different age groups respectively were 4 (4%) in 0-30 years, 20 (20%) in 31-60 years, and 12 (12%) in 61-90 years. Most of the studies related to *Candida* associated with COVID-19 found in search engines are case study articles. There are some review articles stating the yeast infections among critically ill COVID-19 patients.

The United State of America state's health department reported three cases in 2021 July in a hospital's dedicated COVID-19 unit, additional screening found that 52% of 67, COVID-19 patients in the unit were colonised with the yeast. Current study shows 36% which when compared to study of Kuehn et al. is less.[14]

A review study by Arastehfar et al. stated that invasive yeast infections among critically ill COVID-19 patients *C. albicans* (19/43; 44.1%) were shown to be the most prevalent yeast species, followed by *C. auris* (10/43; 23.2%); *C. glabrata, C. parapsilosis, C. tropicalis, and S. cerevisiae* (2/43; 4.6% each); and *C. krusei,* and *Rhodotorula spp.* (1/43; 2.3% each).[15] In current study only *Candida albicans* was isolated. When compared to study by Arastehfar et al., the isolation of *Candida albicans* in the previous study was higher than current study.[15]

CONCLUSION

The presence of *Candida species* in pathological samples should not be clinically ignored because it could probably be associated with a more severe illness. *Candida albicans* was found to be the predominant species isolated in the present study. Males were seen more infected than females. Several more studies are required to understand the impact of *Candida species* on respiratory infection, development, and patients' outcomes and consequently the possible protective role of antifungal agents' administration.

It is concluded that complete identification of pathogens in all the microbiology laboratories is highly recommended in order to study the emergence and spread in the hospital and community.

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