



FUNGAL CO-INFECTIONS ASSOCIATED WITH COVID-19 AND POST COVID - 19 CASES : A DIAGNOSTIC AND CLINICAL PERSPECTIVE

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ABSTRACT

The human-to-human transmitted disease, coronavirus disease 2019 (COVID-19), caused by severe acute respiratory syndrome coronavirus 2 (SARSCoV-2), has been an emergency global pandemic. It is important to diagnose and treat fungal co-infections associated with COVID and Post COVID 19 patients at an early stage. Early and efficient diagnosis will assist the clinicians and laboratory experts in the management of aspergillosis, candidiasis and mucormycosis, also helps to reduce mortality and morbidity. Hence the study was conducted. The present study is a prospective analytical study and was conducted between May 2021 to Dec 2021 for a period of 8 months at Department of Microbiology, Bowring and Lady Curzon Medical College, Bangalore. The clinical specimens like nasal swab, tissue bits and nasal crust submitted to Microbiology laboratory were included in the study. Various clinical specimens were received for identification, of which nasal swabs constituted a major sample with 569 (55.78%) followed by tissue bits 435(42.65). Occasional samples of nasal crust 6(0.59) and sputum 10(0.98) were also processed. Among these samples, 126(28.97%) organisms isolated from tissue bits and 118 (20.97%) from nasal swabs. The samples were received more often in the month of June comprising 395 (38.73%), in July 212 (20.78%) and 123 (12.06%) in May corresponding to the covid peak waves after months. Of the 1020 specimens processed in the laboratory, 24.51% (250) of specimens yielded fungal isolates. The most common fungal species isolated is Aspergillus species, 132 (52.8%) and Mucor species 50 (20%) followed by Candida species of 58(23.2%). Even 8 Rhizopus and one Cryptococcus also isolated. The Candida species isolated were further speciated, C. glabrata(12), C. albicans (11) and C. tropicalis (10) constituted 20.69%, 18.97% and 17.24% respectively. However 19 of 58 isolates couldn't be speciated further. C.parasitosis and C. krusei were also isolated each in 5.17%.

KEYWORDS : Fungal co- infections, aspergillosis, COVID 19, mucormycosis

INTRODUCTION:

The human-to-human transmitted disease, coronavirus disease 2019 (COVID-19), caused by severe acute respiratory syndrome coronavirus 2 (SARSCoV-2), has been an emergency global pandemic [1, 2]. Like SARS-CoV and Middle East Respiratory Syndrome Coronavirus (MERSCoV), SARS-CoV-2 is responsible for lower respiratory infection and can cause Acute Respiratory Distress Syndromes (ARDS) [3]

Epidemiology of invasive fungal infection is based on the risk factors which are diverse and multifactorial in every host.[4] Majority of the risk factors are neutropenia, intake of immunosuppressive drugs, diabetes mellitus, prolonged broad spectrum antibiotic use, central venous catheters and other indwelling lines, break in the mucosal barrier may be due to chemotherapy. [5]

Looking back on SARS in 2003, we found the incidence of fungal infection in SARS patients was 14.8 to 27%, which was even higher in severely ill ones, up to 21.9 to 33%. Meanwhile, fungal infection happen to be the main cause of death for SARS patients, accounting for 25 to 73.7% in all causes of deaths [6, 7].

Hence it is important to diagnose and treat fungal co-infections associated with COVID and Post COVID 19 patients at an early stage. Early and efficient diagnosis will assist the clinicians and laboratory experts in the management of aspergillosis, candidiasis and mucormycosis, also helps to reduce mortality and morbidity[8].

MATERIAL AND METHODS:

Study Design:

The present study is a prospective analytical study and was conducted between May 2021 to Dec 2021 for a period of 8 months at Department of Microbiology, Bowring and Lady Curzon Medical College, Bangalore. The clinical specimens like nasal swab, tissue bits and nasal crust submitted to Microbiology laboratory were included in the study.

Procedure: [9]

KOH mount was prepared and examined microscopically to identify fungal elements like aseptate or septate hyphae with or without branching or for budding yeast like structures.

To isolate the causative agents, samples were inoculated on to Sabouraud's dextrose agar (SDA), blood agar/chocolate agar and

incubated at 37 °C and SDA at room temperature and were examined periodically for any growth and colony morphology observed. Gram stain done for yeast or yeast like growth and Lacto phenol Cotton Blue (LPCB) mount done for mycelial growth to study their morphology. Candida species identification done by growth on CHROME agar.

Statistical Tool For Analysis:

Data were entered on Microsoft excel and analyzed using SPSS version 22.

RESULTS:

A total of 1020 samples were received for testing during the study period from May 2021 to December 2021.

Table 1: Distribution of clinical specimens.

Nature of sample	Total (%)	Positive	Percentage Positivity
Nasal swab	569(55.78)	118	20.74
Tissue bits	435(42.65)	126	28.97
Nasal crust	6(0.59)	2	33.33
Sputum	10(0.98)	4	40.00
Total	1020	250	24.51

Various clinical specimens were received for identification, of which nasal swabs constituted a major sample with 569 (55.78%) followed by tissue bits 435(42.65). Occasional samples of nasal crust 6(0.59) and sputum 10(0.98) were also processed. Among these samples, 126(28.97%) organisms isolated from tissue bits and 118 (20.97%) from nasal swabs.[table 1]

The samples were received more often in the month of June comprising 395 (38.73%), in July 212 (20.78%) and 123 (12.06%) in May corresponding to the Covid peak waves after months. (Table 2)

Table 2: Month wise distribution of clinical specimens

Month	Nasal swab	Tissue	Sputum	Nasal crust	Total samples (Percentage)
May	103	17	3	0	123(12.06)
June	256	134	5	0	395(38.73)
July	100	110	0	2	212(20.78)
August	29	35	2	1	67(6.57)
September	4	30	0	2	37(3.63)
October	20	49	0	0	69(6.76)
November	30	40	0	1	71(6.96)

December	27	20	0	0	47(4.61)
Total	569	435	10	6	1020

Of the 1020 specimens processed in the laboratory, 24.51% (250) of specimens yielded fungal isolates. The most common fungal species isolated is *Aspergillus* species, 132 (52.8%) and *Mucor* species 50 (20%) followed by *Candida* species of 58(23.2%). Even 8 *Rhizopus* and one *Cryptococcus* also isolated.[Table 3]

Table 3: Profile of fungal isolation (n=250)

Isolate	Numbers	Percentage
<i>Aspergillus</i> species	132	52.8
<i>Candida</i> species	58	23.2
<i>Mucor</i> species	50	20.0
<i>Rhizopus</i> species	8	3.2
<i>Cryptococci</i>	1	0.4
<i>Penicillium</i>	1	0.4
Total	250	100

The *Candida* species isolated were further speciated, *C. glabrata* (12), *C. albicans* (11) and *C. tropicalis* (10) constituted 20.69%, 18.97% and 17.24% respectively. However 19 of 58 isolates couldn't be speciated further. *C. parapsilosis* and *C. krusei* were also isolated each in 5.17%.

The 132 *Aspergillus* isolated were further speciated based on macroscopic morphology and microscopic features. *A. fumigatus* (64 out of 132) was the most frequently isolated species contributing to 48.48%, followed by *A. flavus* (47) and *A. niger* (15) at 35.61% and 11.36% respectively.

DISCUSSION:

The second wave of SARS- CoV 2 across the globe has presented a formidable challenge to clinicians and health professionals. [10] COVID-19 infection, also brought many risk factors like lung injury, immunosuppression, requirement for oxygen therapy, use of monoclonal antibodies, extensive steroid therapy, etc. which are known predisposing factors for fungal infections. [11]

Hence, the victims of COVID-19 are more prone to fungal infections, especially among those requiring intensive care unit admissions. Aspergillosis, invasive candidiasis and mucormycosis are the most common associates. The occurrence of these coinfections was rising and alarming. [12] In the present study, majority of the suspected individuals were males forming 67%, similar male preponderance was noted by others. [13]

Kundu R et al in their review noted COVID-19-associated candidiasis (CAC) could be a superficial or invasive infection and also found it to range from 0.7% to 23.5%. They also found *C. albicans* to be the commonest species followed by *C. auris*, *C. glabrata* and others. [11]

Sonal Vijay in a multicentric study between June to August 2020 found *Candida* species among 6% (61/1006) of admitted patients. They also reported *Aspergillus* species 0.04% (16/354). However, here the most samples were collected between May 2022 to July 2022. *Aspergillus* species, 132 (52.8%) and *Mucor* species 50 (20%) followed by *Candida* species of 58(23.2%) to be most common fungal isolates. [14] Several studies from Asia and Europe have reported up to 28% of IPA in patients with severe influenza. *Aspergillus fumigatus* being the etiological agent in most infections, followed by *Aspergillus flavus*. Similar species distribution was found with 48.48% of *Aspergillus fumigatus* followed by 35.61% *Aspergillus flavus*. [15]

Kundu R reported Covid Associated Mucormycosis (CAM), a potentially fatal infection known to cause tissue gangrene due to its angioinvasive nature due to vessel thrombosis. The hot and humid climate serves as an icing on the cake with huge load of *Mucor* spores in environment. Raised serum ferritin levels, steroid therapy and hyperglycemia predisposes these individuals to develop the disease. Also noted a prevalence of 0.27% among hospitalized patients, which later raised to 2.1 fold during September to December 2020 compared to same period in 2019. [16]

CONCLUSION:

Fungal co-infections are frequent in the COVID-19 patients; its awareness is important for proper diagnosis and subsequently for efficient treatment of the fungal co-infections in reducing morbidity and mortality. Categorically, these patients can develop fungal infections throughout any stage of this disease. Challenges do exist

especially related to diagnosis of secondary infections in COVID-19. It is the right time to develop a standardized methodology for diagnosing these infections. There is a need for clinicians to be aware of complications such infections can cause, and also, there is need to strengthen Microbiology laboratories. There is a need to formulate effective practical guidelines for emerging disease prevention.

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