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STUDY OF COVID-19 ASSOCIATED FUNGAL LUNG INFECTION AND ANTI FUNGAL SUSCEPTIBILITY PATTERN WITH RESPECT TO CANDIDA SPECIES

Medical Microbiology

Dr Saurabh Mitra* (MBBS,MD), Assistant Professor, Department of Microbiology, Murshidabad Medical College and Hospital, West Bengal. *Corresponding Author

Dr Priyanku Sharma BDS, MSc, PGDBI, FID, Scientist-B (Medical), Virus Research and Diagnostic Laboratory, Department of Microbiology, Murshidabad Medical College and Hospital, West Bengal.

ABSTRACT

Introduction: A major healthcare threat emerged in the form of corona virus towards the end of 2019 (COVID-19). Apparently,the novel corona virus (SARS-CoV-2) was capable of spreading and dysregulating the immune mechanisms. The likelihood of developing pulmonary candidiasis wasmore in COVID-19 positive patients,who werehospitalized for long duration of time. **Aim:** This study aims to report the candidal lung co infection in COVID-19 patients,who were admitted in critical care unit and to rule out the anti fungal susceptibility pattern in respect with Candida species found in those patients. **Material and Methods: Study Design:** Short term hospital based observational study. **Study Population:** Patients with positive reports for COVID 19, diagnosed by RT PCR method, admitted in the critical care unit (CCU) of a medical college and hospital. In this short term hospital based observational study Broncho Alveolar Lavage (BAL) samples,of COVID-19 positive patients, who were diagnosed by RT PCR method and admitted to respiratory care unit and followed up in critical care unit of a medical college and hospital, who were suspected as pulmonary candidiasis by chest computed tomography (CT) were collected aseptically followed by ICMR guidelines .Species identification was performed by VITEK-2 automated system and also anti fungal susceptibility testing of Fluconazole, Voriconazole, Amphotericin B, Caspofungin, Micafungin and Flucytosine was performed. **Results:** Among 350 patients, who were admitted with COVID-19, 18 patients were micro biologically diagnosed as pulmonary candidiasis. The predominant species was Candida albicans followed by Candida glabrata, Candida tropicalis and Candida parapsilosis. In anti fungal susceptibility test 1 isolate displayed multi drug resistance and another4 isolates showed resistant and intermediate pattern of drug sensitivity. **Conclusion:** This study clarifies some concern regarding the occurrence of pulmonary candidiasis in COVID-19 patients. As the study was done from microbiological aspect, this study will help to understand the anti fungal drug susceptibility pattern and further management of pulmonary candidiasis in COVID-19 patients. Though further studies should be conducted to design an appropriate prophylaxis programme and improve management of pulmonary candidiasis in critically ill COVID-19 patients.

KEYWORDS

COVID-19, Candida species, pulmonary candidiasis, Anti fungal susceptibility, Critical Care

INTRODUCTION:

The human upper respiratory tract serves as the primary point of entry for infectious microorganisms transmitted through droplets or aerosols,such as the severe acute respiratory syndrome corona virus 2 (SARS-CoV-2) that emerged in 2019 [1].The mortality rate has been found to differs greatly from country to country[1]. Various factors have contributed to morbidity and mortality in COVID-19 patients, the prevalence and role of bacterial and fungal co-infections particularly in patients suffering from acute respiratory distress syndrome (ARDS) has been a significant contributor. Inadequate attention was given to the prevalence of fungal infections in patients suffering from COVID-19, several factors may have contributed to the rise in fungal infection
a) prolonged hospitalization in intensive care unit (ICU),
b) broad-spectrum antibiotic and corticosteroid usage
c), intubation[2,3,4,5].

Due to undefined pharmacological treatment for COVID-19, indirect complex effect, invasive therapeutic methods and multi-drug treatment, also presence of some preexisting pathological oral conditions has been aggravated by SARS-CoV-2, particularly in those patients with a compromised immune mechanism, or those subjects receiving long-term pharmacotherapy[5]. Respiratory viral infections are linked to shifts in the microbial composition within the respiratory tract. These changes can subsequently impact the immune response to secondary infections or influence the interactions among microorganisms ,potentially leading to the increased proliferation of pathogenic bacterial and fungal species. With a rise in the number of corona virus disease 2019 (COVID-19) cases worldwide and because of apparently absent cross-protective immunity from related viral infections, high SARS-CoV-2 transmissibility, has facilitated widespread person-to- person transmission and many fold rise of fungal pulmonary infection has followed. [2]. This study has explored the relationship between SARS-CoV-2 infection and the fungal infection in patients, who were admitted in critical care setup.

MATERIALAND METHODS:

Inclusion Criteria:

-COVID 19 positive patients,diagnosed on RTPCR test in the age group of 18-80 years.
-Patients, who were admitted to critical care unit (CCU).
-Patients or their legal guardians, who gave verbal consent and written consent.

Exclusion Criteria:

-Who,did not give verbal consent
-Who has not shown significant chest computed tomography (CT) findings

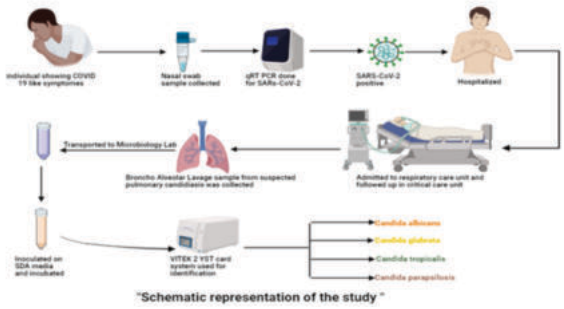

Figure 1: Diagrammatic Representation Of The Whole Study, From SARS-CoV-2 Detection To Pulmonary Candidiasis Species Identification.

Study Design And Population:

Thisobservational short term study was conducted in between March, 2021 to August, 2021. Samples were collected from COVID 19 positive patients (COVID-19 diagnosis was conformed by RT PCR testing and chest computed tomography in patients with symptomes compatible with COVID-19) , who were admitted in respiratory care unit and followed up in CCU. All the samples were collected followed by ICMR guidelines with proper protection and by the trained medical personal. Sample size: 350

Clinical specimens:

Total 350 patients, who matched inclusion criteria,who were admitted to CCU from March,2021 to August,2021 were included in the study and in between them 18 pulmonary candidiasis suspected Broncho Alveolar Lavage (BAL) samples were aseptically collected by the

trained medical personals. Samples were collected separately from ventilated patients by endotracheal suction by the trained medical professionals and sealed and transported in sterile tube on the same day of collection to the microbiology laboratory of the hospital and inoculated on Sabouraud dextrose agar and incubated at 37 degree for 24hr. Regular examinations were carried out on the plates to observe any presence of yeast-like growth. Any plates showing no mycological growth after 10 days of incubation were deemed negative and subsequently discarded.

Identification:
Isolates were identified by VITEK 2 YST card system (Biomerieux, France) following the manufacturer's instructions.

RESULTS:

Species Identified-

A total of 18 cases of pulmonary candidiasis were reported among the study sample (350) with COVID 19 positive history and history of CCU admission during the study period. Most common isolate was Candida albicans(61.11%) followed by non albicans candida species, Candida glabrata(22.22%), Candida tropicalis(11.11%) and Candida parapsilosis(5.55%).[Table 1]

Table 1: Species Isolated From Culture Positive Cases (n=18)

Species Identified	Number Of Isolates	Percentages
Candida albicans	11	61.11%
Candida glabrata	4	22.22%
Candida tropicalis	2	11.11%
Candida parapsilosis	1	5.55%

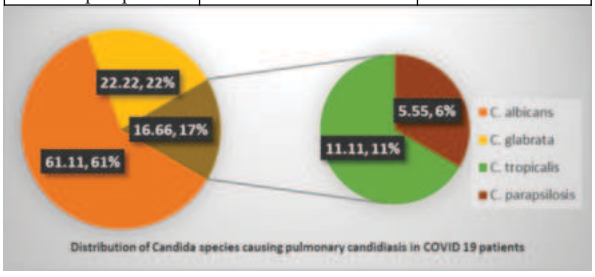

Figure 2: Distribution of candida species in positive clinical isolates (n=18), showing prevalence of Candida albicans with Non Albicans candida species among study population during the study period.

Anti Fungal Susceptibility Test And Minimal Inhibitory Concentration (MIC)-

Anti fungal susceptibility patterns of isolates to three classes of anti fungal drugs, that is azoles (fluconazole, voriconazole), polyenes (amphotericin B) and echinocandins (caspofunginand micafungin), were assessed.AFST was performed following the Clinical Laboratory Standards Institute (CLSI) guidelines [7]. Minimal inhibitory concentrations (MIC) were determined and interpreted based on the available clinical break points and epidemiological cut-off values according to the CLSI guidelines [8].

Anti fungal susceptibility and MIC data for culture positive isolates against 6 antifungals are detailed in Table 2.

Table 2: MIC Distribution Among Culture Positive Isolates (Candida albicans and non albicans candida species)

	ISOLATES	AZOLES	ECHINOCANDINS		POLYENES	PYRIMIDINE ANALOGUE
	FLC	VRC	CAS	MFG	AMB	FC
Ca1	64	8	4	4	16	64
Ca2	1	0.12	0.25	0.06	2	64
Ca3	1	0.12	0.25	0.06	0.5	1
Ca4	1	0.12	0.25	0.06	1	1
Ca5	1	0.12	0.25	0.06	1	1
Ca6	1	0.12	0.25	0.06	0.5	1
Ca7	1	0.12	0.25	0.06	0.25	1
Ca8	1	0.12	0.25	0.06	0.25	1
Ca9	1	0.12	0.25	0.06	0.5	1
Ca10	1	0.12	0.25	0.06	0.25	1
Ca11	1	0.12	0.25	0.06	1	1
Cg1	64	1	0.25	0.06	16	16
Cg2	32	0.25	0.25	0.06	0.5	32
Cg3	8	0.12	0.25	0.06	0.5	1
Cg4	10	0.12	0.25	0.06	0.25	1
Ct1	4	0.12	0.25	0.06	0.25	1
Ct2	1	0.12	0.25	0.06	1	2
Cp1	1	0.12	1	1	1	2

(Ca=Candida albicans, Cg= Candida glabrata, Ct= Candida tropicalis, Cp= Candida parapsilosis, FLC= Fluconazole, VRC=Variconazole, CAS=Caspofungin, MFG=Micafungin, AMB=AmphotericinB, FC=Flucytosine)

(Resistant and Intermediate parts are mentioned in bold anditalic)

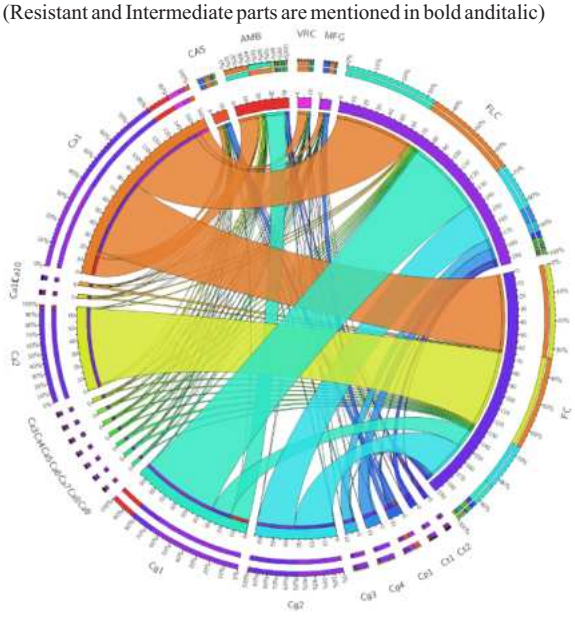

Figure 3 : Cricos plot displaying MIC distribution among 18 clinical isolates of Candida albicans (Ca) and non albicans candida species,Candida glabrata (Cg), Candida tropicalis (Ct), Candida parapsilosis (Cp) against 6 anti fungal drugs, Fluconazole(FLC), Variconazole(VRC), Caspofungin(CAS), Micafungin (MFG), Amphotericin B (AMB), Flucytosine(FC). In this Cricos plot peripheral part showing isolates and anti fungal drugs and internal part representing relation in between them.

Among 11 C albicans isolates 1 isolate (Ca1) [Table 2] displayed multi drugs resistance and corresponding high MIC values. Another 1 isolate (Ca2) [Table 2] ofC albicans showed resistant to FC (MIC >=64) and intermediate to AMB (MIC >=2).In case of C glabrata isolates in between 4 isolates 1 isolate (Cg1) [Table 2] showed resistant to FLC (MIC>=64),AMB (MIC >= 16) and intermediate to FC (MIC >=16) and another 1 (Cg2) [Table 2] of C glabrata showed resistant to FC (MIC >=32). Among 2 C tropicalis isolates 1 isolate (Ct1) [Table 2] displayed intermediate MIC value to FLC (MIC>=4).

DISCUSSION:

In severe COVID-19 infection, oropharyangeal mycobiota diversity can be decreased for months. Nevertheless,the prevalence of Candida species can continue even after the recovery of the mycobiota,with Candida albicans often dominating the mycobiome of many patients *[9].* The overuse of broad-spectrum antibiotics, which was reported during the COVID-19 pandemic, might predispose to breakthrough infections due to multi drug-resistant bacteria and fungi that reside in the patients' flora or are present in the ward and critical care environment [10]. *Candida* co-infection can be either primarily or secondarily acquired, that presented together with or following the onset of COVID-19 disease (super-infection). Fungal co-infections can be severe and may worsen patients' outcomes regarding morbidity and mortality [11],[12],[13].

Studies showed that disturbance of the host defense mechanisms caused by SARS-CoV-2, such as the breakdown of the epithelial barrier, along with other risk factors, promotes colonization and opportunistic infection of Candida species existing in the commensal state of the human micro biome[14].

In this study it was observed thatCandida albicans (61%) and Candida glabrata (22%) were the leading causative agents followed by Candida tropicalis (11%) and Candida parapsilosis(6%).By observing the anti fungal susceptibility patterns,it was clear that all the commonly used anti fungal drugs were active during the pandemic situation.

Limitation of the study:

The limited number of pulmonary candidiasis cases in COVID 19 positive patients and the lack of detailed clinical data due to the nature of the current study were the main limitations of this study. Furthermore, because of the small number of available pulmonary candidiasiscases, statistical assessment of risk factors could not be performed.

Only hospitalized cases, admitted in Critical care unit, were included in this study, hence this may not represent the population as a whole.

Strength Of The Study:

In this study, disease causing species were identified and anti fungal susceptibility test was performed with measuring the MIC range, in respect to commonly used anti fungal drugs,which will be beneficial for future management of this condition in pandemic situation .

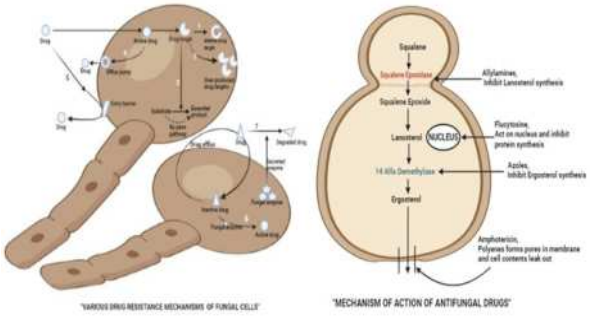

Figure 4: Image Showing Mechanism Of Action Of Various Anti Fungal Drugs And Various Drug Resistance Mechanism Of Fungal Cells.

CONCLUSION:

Almost all isolates weresusceptible to commonly used anti fungal drugs. Anti fungal resistance was not high during COVID 19 period and all commonly used anti fungal drugs were active and was used to manage the pulmonary candidiasis during COVID 19 pandemic situation.

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Conflict Of Interest: There are no conflicts of interest.

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