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The prevalence of pulmonary aspergillosis in coronavirus disease 19 (COVID-19) patients in Shebin El-Kom teaching hospital in Egypt

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Abstract

Background

A high rate of invasive fungal infection has been demonstrated among critically COVID-19 ill patients admitted to the ICU, with high odds of mortality. Simple and rapid risk stratification methods are mandatory to recognize severe patients.

Objectives

The aims was to study the prevalence of invasive fungal infection in Corona virus disease 19 (COVID-19) patients, the effect of some inflammatory markers that lead to the development and progression of invasive fungal infection and to assess the value of PCR in early and rapid detection of invasive fungal infection in immune compromised patients with COVID-19.

Methods

This study was conducted at the period from October 2020 to October 2021 on two groups classified as following: Group I: included 120 immuno-compromised inpatients (2-80 years), (68 males and 52 females) from ICUs. Group II: included 40 outpatient's COVID-19 (4 – 56 years). All basic laboratory biomarkers at time of admission were recorded.

Results

This study showed highly significant increase in neutrophil/ lymph, IL6,CRP, D-dimer and malondialdhyde (MDA) in COVID-19 patients in ICU compared with outpatient one with *P* value < 0.001). No significant difference between them in LDH, ferritin and procalcitonine. The most common isolated organisms (167 isolates) from group I (230 samples from 120 patients) were bacterial spp. (111/167, 66.5%)followed by Candida spp. (30, 17.9%), Aspergillus spp. (11, 6.6%) while mucormycosis was 5 isolates (3%) and associated bacterial infection represented 5.9%of all. Out of 120 patients suspected of complaining of BSI 17 (14.1%) of them proved to be fungemia. The most common isolated yeast was Candida spp. (11/120, 9.1%) followed by Aspergillus spp. (6/120, 5%). While out of 20 patients (group I) suspected of complaining of eye infections, mucormycosis was represented by 5/20 (25%). Fungaemia was detected by PCR and blood culture in 50 high risk ICU patients was 22/50 (44%) and 17/50 (34%) respectively. PCR is more sensitive than blood culture, as blood culture failed to detect 5 cases of fungemia with a significant difference (*P*-value <0.05).

Conclusion

Increase in neutrophil/lymph, IL6,CRP, D-dimer and MDA in COVID-19 ICU patients compared with outpatients may be significant biomarkers used to detect severity of disease in ICU patients and monitor treatment. Also decrease in immunity as results of corticostorides admission, lead to presence of fungaemia in some patients in ICU.

Keywords: Candida, COVID-19, CRP, D-dimer, fungaemia, ICU admission, MDA, mucormycosis, Neutrophil-to-lymphocyte ratio, PCT

Introduction

Candida, aspergillus, and mucormycosis are opportunistic fungi mainly affecting immunocompromised patients. A high rate of

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invasive pulmonary aspergillosis (IPA) has been demonstrated in critically ill patients admitted to the ICU with severe influenza [1] and nearly 5% of the affected coronavirus disease 2019 (COVID-19) patients are critically ill, develop an acute respiratory distress syndrome during an ICU stay, and super infections, including IPA, which are well-known complications of severe viral pneumonia in critically ill patients [2].

Many risk factors for COVID-19-associated pulmonary aspergillosis were recognized, including lymphopenia, high levels of systemic pro-inflammatory cytokines, use of steroids among COVID-19 patients, and the preexisting medical history of diabetes [3], as well as an uncontrollable second wave of COVID-19 in India, an outbreak of mucormycosis with a fatality rate of 50%. Many Indian states and union territories have declared an epidemic of black fungus due to its unprecedented emergence, which has adversely affected the already debilitated health system of the country [4].

Bloodstream infection (BSI) with invasive fungal infections caused mainly by Candida spp. and Aspergillus spp. have been assumed increasing importance over the last decades, with a high mortality and morbidity among hospitalized and immunocompromised patients [5].

The use of molecular diagnostic tools to detect fungus from clinical specimens or cultures has been reviewed, and many researchers have reported the usefulness of DNA-based methods for the diagnosis of invasive aspergillosis infections [6]. However, most of these studies were performed on a limited number of patients, and no large prospective clinical trials have yet been reported [7].

Aim

(1) To study the prevalence of fungemia in immuno compromised patients and to evaluate the incidence and risk factors with severe COVID-19.
(2) To study the effect of some inflammatory markers that lead to the development and progression of fungemia in COVID-19 patients.
(3) To assess the value of PCR in early and rapid detection of fungemia in immunocompromised patients.

Patients and methods

This study was conducted in the period from October 2020 to October 2021 on patients admitted to different wards and units of Shebin El-Kom Teaching Hospital. The purpose and nature of the study were explained to all participants and written voluntary consents were obtained before their participation. Approval was taken from the research committee of the General Organization of Teaching Hospitals and Institutions (GOTHI) with approval number HSH00034.

The study included two groups classified as follows:

Group I: included 120 immunocompromised inpatients (68 males and 52 females) from ICUs (renal and hepatic including ICU COVID-19, burn and diabetes mellitus unit ranging in age from 2 to 80 years; mean: 38.63). Samples were obtained from each patient including blood for complete blood picture chemistry, culture, and PCR, urine, and eye swab.

Group II: included 40 outpatients who were confirmed as COVID-19 (4–56 years) (mean: 24.78), had lower respiratory tract infection, and come to the outpatient clinics.

All patients were subjected to full history taking and physical examination and computed tomography chest and nasal swab for detection of COVID-19.

(1) Samples:
   (a) Blood samples were taken for complete blood count, malondialdehyde (MDA), interleukin 6 (IL-6), C-reactive protein (CRP), D-dimer, ferritin, procalcitonin, blood for cultures, and PCR (for only 50 high-risk patients) for invasive fungal infections.
   (b) Eye swab and urine samples.
   (c) Nasal swab for confirmation of COVID-19 by PCR.
(2) Culture: all samples were cultured on blood agar, MacConkey (24–48 h at 37°C), and Sabouraud dextrose agar and were incubated aerobically (24–72 h at 37°C).
   (a) Bacterial growth on MacConkey and blood agar was further identified conventionally.
   (b) Fungal growth on Sabouraud dextrose agar was identified by their colony morphology. Colonies suspected to be Candida were identified morphologically by Gram stain and germ tube test. Diagnosis of aspergillosis and mucormycosis is offered by detection of a hyphae process and isolation of the organism from clinical samples. The hyphae are characteristically formed as a V-shaped branched, septated hyphae that branches at a 45 and 90° angle for aspergillosis and mucormycosis, respectively [8].
   (c) Candida isolates (as positive control) were stored in distilled water at room temperature and subcultured on Sabouraud dextrose agar 48 h before further study [9,10].
(3) Detection of COVID-19 by ID now COVID-19 (Abbott Diagnostics Scarborough, Inc. 10 South gate Road Scarborough, Maine 04074 USA), which is an automated assay that utilizes isothermal nucleic acid amplification technology for the qualitative detection of SARS-CoV-2 viral nucleic acids [11].
(4) Detection of fungal DNA by PCR (Gene Amp PCR system 9700 Perkin-Elmer).
   (a) PCR was performed on DNA extracted from serum samples that were obtained from inpatients, controls (negative controls), and Candida as positive controls.
   (b) Extraction of Candida DNA was done as described by Malke *et al*. [12].
      (i) The DNA of Candida and Aspergillus was extracted from serum samples by heat shock method described by Kaucner and Stinear [13]. The method of DNA

amplification was carried out as described by Henry *et al*. [14] and Wahyuningsih *et al*. [15]. Oligonucleotide primers were derived from rRNA genes of fungi and can be used for universal fungi PCR. Forward primer ITS3 (5'-GCA TCG ATG AAG AAC GCA GC-3') corresponds to the 5.8 S rRNA gene, and reverse primer ITS4 (5'-TCC TCC GCT TAT TGA TAT GC-3') corresponds to the 28 S rRNA gene of fungi. Primers [15] were purchased from (Qiagen, Operon (Germany) Taq PCR master mix kit (250 U) (Qiagen). Agarose gel electrophoresis was carried out as per Hassab and Elhassanee [16].
ITS2 amplicons from Aspergillus species ranged in size from 565 to 613 bp [14].
ITS2 amplicons from Candida spp. ranged in size from 300 to 400 bp [15].

(5) Biochemical assay.

Blood samples were collected, centrifuged at 3000 rpm for 10 min at 4°C, and serum samples were rapidly separated and aliquoted. Determination of random blood glucose was done at once from serum samples, and then stored at −20°C until the measurements of CRP, IL-6, MDA, procalcitonin, and ferritin. The level of cytokine IL-6 was determined in serum using the enzyme-linked immunosorbent assay kit (Ray Bio Rat IL-6 enzyme-linked immunosorbent assay kit). MDA level, as a marker of lipid peroxidation, was determined according to the method of Kei [17] using Biodiagnostic Company Kits, Egypt. Determination of ferritin and procalcitonin according to electrochemiluminescent immunoassay using Cobas Roche 6000 instruments. Roche's technology for immunoassay detection is based on the use of a ruthenium complex and tripropylamine. The chemiluminescence reaction for the detection of the reaction complex is initiated by applying a voltage to the sample solution resulting in a precisely controlled reaction. ECL technology can accommodate many immunoassay principles while providing superior performance (*http://www.cobas.com*).

Determination of CRP using Roche diagnostic kits by (Cobas Integra 400 plus instrument) was done using the Tina-quant technique. With the introduction of the Tina-quant CRP Gen. 3 and Gen. 4 using dual-radius-enhanced latex (DuREL) technology, another part of the blood was taken on EDTA for the determination of complete blood count, which was done

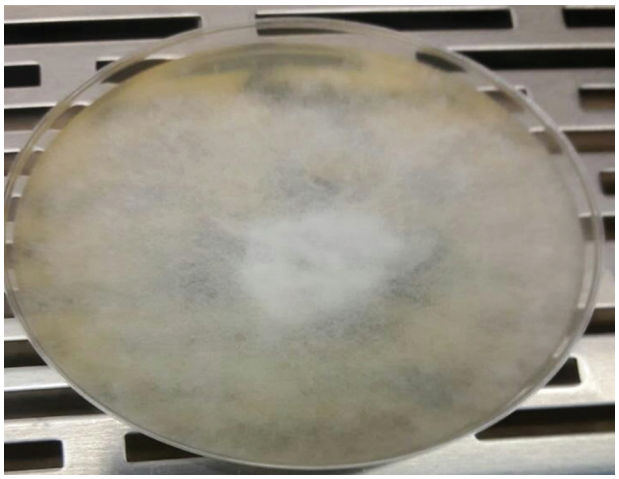

Figure 1: *Aspergillus niger* colonies on Sabouraud dextrose agar.

Table 1: Biochemical parameter results between studied groups

	Neutrophil/lymph		IL-6 (pg/ml)		Procalcitonin (ng/ml)		Ferritin (ng/ml)	
	Group I	Group II	Group I	Group II	Group I	Group II	Group I	Group II
Mean±SD	16.34±14.12	8.47±6.84	26.66±7.55	8.4±1.64	1.45±4.97	0.036±0.020	148.12±103.59	160.93±77.420
P	0.001*		0.0001*		0.074		0.474	
	MDA (μmol/l)		CRP (mg/dl)		D-dimer (μg/ml)		LDH (IU/l)	
	Group I	Group II	Group I	Group II	Group I	Group II	Group I	Group II
Mean±SD	3.12±1.06	1.01±0.42	56.45±41.4	14.91±9.06	2.17±1.67	0.46±0.36	329.86±146.87	289.45±92.51
P	0.0001*		0.0001*		0.0001*		0.105	

CRP, C-reactive protein; IL-6, interleukin 6; LDH, lactate dehydrogenase; MDA, malondialdehyde. *P* value between groups by analysis of variance test. *Significant if *P* value less than or equal to 0.05.

Table 2: Distribution of the isolated organisms among 230 clinical specimens collected from group I

Source of specimens	Number of specimens	Candida isolates [*n* (%)]	Aspergillus isolates [*n* (%)]	Mucormycosis [*n* (%)]	Associated bacteria [*n* (%)]	Bacteria isolates [*n* (%)]	No growth [*n* (%)]
Blood culture	120	11 (9.1)	6 (5)	0	4 (3.3)	58 (48.3)	45 (37.5)
Urine	44	10 (22.7)	0	0	3 (6.9)	18 (41)	16 (36.4)
Eye swab	20	0	0	5 (10)	0	15 (75)	0
Skin swab	46	9 (19.6)	5 (10.9)	0	3 (6.5)	20 (43.5)	12 (26.9)
Total number of specimens	230	30 (13)	11 (4.7)	5 (2.2)	10 (4.3)	111 (48.3)	73 (31.7)
Total number of isolates	167	30 (17.9)	11 (6.6)	5 (3)	10 (5.9)	111 (66.5)	–

by CELL-DYN Ruby Hematology Analyzer by Abbott (Ruby; Abbott Company).

Data were analyzed using IBM SPSS software package, version 20.0. (IBM Corp., Armonk, New York, USA). Quantitative data were described using mean and SD. The *t* test (analysis of variance) was used for normally distributed quantitative variables to compare between more than two groups and post-hoc test (Tukey) for pairwise comparisons.

Results

The biochemical parameters between COVID-19 outpatients (group II) and COVID-19 patients in the ICU (group I) (Table 1) showed a highly significant increase in neutrophil/lymph, IL-6, CRP, D-dimer, and MDA in COVID-19 patients in the ICU compared with the outpatient one. There was no statistically significant difference between them in lactate dehydrogenase, ferritin, and procalcitonin.

The most commonly isolated organisms (Table 2) were bacterial spp. (111/167, 66.5%) followed by Candida spp. (30 isolates, 17.9%) and Aspergillus spp. (11 isolates, 6.6%) (Figs. 1, 2), while mucormycosis (five isolates), 3%; Figs. 3, 4). The table also shows that the associated bacterial infection represented 5.9% of all isolates. In fungemia: out of 120 patients suspected of complaining of BSI, 17 (14.1%) of them proved to be fungemia. The most common isolated yeast was Candida spp. (11/120, 9.1%) followed by Aspergillus spp. (6/120, 5%).

In mucocutaneous infections (Table 3) in groups I and II: Candida spp. represented 19/90 (21.1%) and 5/40 (12.5%) while Aspergillus spp. represented 5/90 (5.6%), and 0% in both groups, respectively, while out of 20 patients (group I) suspected of complaining of eye infections, mucormycosis was represented by 5/20 (25%).

N.B: all Aspergillus isolates were *Aspergillus fumigatus* except four (two isolates were *Aspergillus flavus* and two isolates were *Aspergillus niger*).

Table 4 shows that there was no significant correlation detected between fungemia and sex or age.

Table 5 shows the most common risk factors in COVID-19 patients in the ICU (group I), as we reported that all cases took antibiotics and corticosteroids, and the most common and the risky risk factor is hypertension as there is a clinical significance between hypertension and Candida infection, aspergillums, and mucormycosis infections

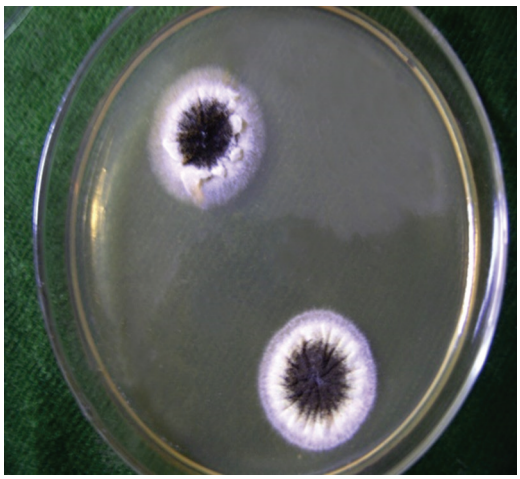

Figure 2: *Aspergillus niger* colonies on Sabouraud dextrose agar.

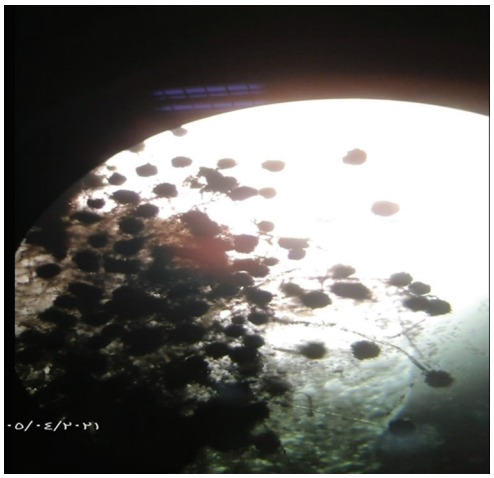

Figure 4: Mucormycosis microscopic appearance on Sabouraud dextrose agar.

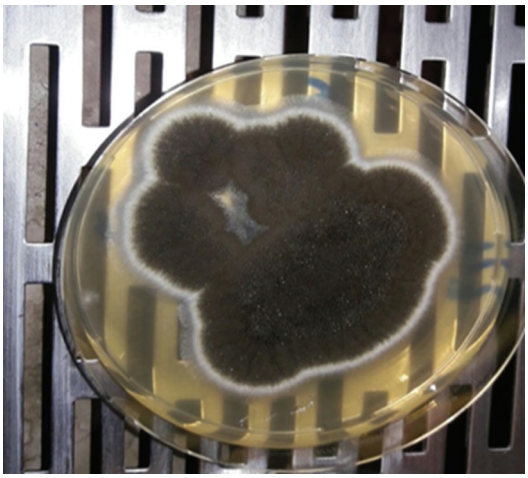

Figure 3: Mucormycosis colonies on Sabouraud dextrose agar.

Figure 5: Ethidium bromide-stained agarose gel detection of PCR products obtained with primers ITS3 and ITS4 and DNAs (aspergillus) from different serum samples. Lane M: molecular weight marker (100 bp ladder). Lane 1, 2, 3, 4, 5, 6, 9: negative samples. Lane 7, 8: positive samples (aspergillus) showing amplified 565–613 bp segments. Lane 10, 11: positive and negative controls, respectively.

Table 3: Isolation rate of fungi among the studied groups

Groups	*Candida* spp. [*n* (%)]	*Aspergillus* spp. [*n* (%)]	Mucormycosis [*n* (%)]	Total [*n* (%)]
Group I blood=120	11 (9.1)	6 (5)	0	17 (14.2)
Eye swab=20	0	0	5 (25)	5 (25)
Others=90	19 (21.1)	5 (5.6)	0	24 (26.7)
Group II=40	5 (12.5)	0	0	5 (12.5)
Total=270	35 (13)	11 (4.1)	5 (1.9)	51 (18.9)

Table 4: Demographic characteristics of patients

Parameters	Fungemia				Total [*n* (%)]	*P*
	Positive	%	Negative	%		
Sex♂	8	6.6	60	50	68 (56.7)	0.32
♀	9	7.5	43	35.8	52 (43.3)	
Age (years)						
<18	3	2.5	11	9.2	14 (11.7)	0.411
>18	14	11.7	92	76.6	106 (88.3)	
Total	17	14.2	103	85.8	120 (100)	

with a *P* value of 0.0001, 0.0001, and 0.020, respectively. Moreover, diabetes mellitus is the next risk factor as there is a clinical significance between diabetes, Candida infection, and mucormycosis with a *P* value of 0.0001. We reported a clinical significance between chronic respiratory illness, cardiac disease, and renal failure with conidial infection with *P* value of 0.0001. In addition, there is a clinical significance between chronic respiratory illness and cardiac disease with candidemia. No clinical significance was reported between fungal infection and hepatitis C, invasive ventilator support. Detection of fungemia in 50 ICU patients in Table 6 shows that 22/50 (44%) were detected by PCR (Figs. 5, 6) compared with 17/50 (34%) detected by blood culture. PCR is more sensitive than blood culture (Table 7), as blood culture failed to detect five cases of fungemia with a significant difference ($P < 0.05$).

Discussion

The number of patients obtaining COVID-19 is dramatically increasing globally affecting the efficiency of health-care systems particularly, ICU bed availability. Therefore, early detection of severe cases is mandatory for rapid triaging of patients. While the clinical presentation, associated comorbidities, extent of radiological infiltration, and the blood oxygen saturation of COVID-19 patients may indicate the need for their admittance to ICUs, several laboratory parameters may facilitate the assessment of disease severity.

This study included 160 patients, group I 120 patients (68 males and 52 females) from ICUs (renal and hepatic), burn and diabetes mellitus units ranging in age from 2 to 80 years (mean: 38.63) with a significantly higher frequency of fever, dyspnea, and cough as well as concomitant comorbid conditions and the other group II including 40 COVID-19 outpatients (4–56 years) (mean: 24.78), having lower respiratory tract infections and attending the outpatient clinics.

Figure 6: Ethidium bromide-stained agarose gel detection of PCR products obtained with primers ITS3 and ITS4 and DNAs (candida) from different serum samples. Lane M: molecular weight marker (100 bp ladder). Lane 1, 2, 3, 4, 7, 6, 9: negative samples. Lane 5, 8: positive samples (candida) showing amplified 300 bp segments. Lane 10, 11: positive and negative controls, respectively.

COVID can affect coagulation and hemostasis by different mechanisms including both abnormal bleeding risk and thromboembolism. So, all main coagulation biomarker disturbances were found in COVID-19 cases namely higher serum D-dimer levels [18].

Moreover [19], D-dimer levels correlate with the severity of the disease and are a dependable prognostic indicator for the hospital mortality in the admitted patients with COVID-19. The elevated D-dimer signifies a hyperfibrinolysis state and increased inflammatory burden induced in SARS-COV-2 infection.

This is in agreement with our study, which showed a statistically significant increase in D-dimer results in ICU COVID-19 patients compared with others.

Also, some inflammatory markers showed a highly significant increase in neutrophil/lymph, IL-6, and CRP in COVID-19 patients in the ICU compared with outpatients ($P \leq 0.001$).

This was supported by Elshazli R *et al*. [33] and Tan *et al*. [20], who reported that a high WBC count with lymphopenia could be considered as a differential diagnostic criterion for COVID-19 and also observed higher concentrations of CRP and IL-6 among patients with severe COVID-19 infection in the ICU.

It was supposed that lymphocytes are directly infected and destroyed by SARS-CoV and also due to lymphocyte sequestration in the lung where there is SARS-CoV damage, or cytokine-mediated disruption of lymphocyte trafficking. There may be immune-mediated lymphocyte destruction that lymphocytes especially CD4 are essential to get rid of virally infected cells while with COVID-19 it has been supposed that persistence may be dependent on the ability to change cells killed

Table 5: Risk factors for fungal infections and candidemia, aspergillosis in group I (inpatient group)

Total number	120										
Predisposing factors	Candida infections		*P*	Candidemia		*P*	Aspergillosis		*P*	Mucormycosis		*P*
	Positive [*n* (%)]	Negative [*n* (%)]		Positive [*n* (%)]	Negative [*n* (%)]		Positive [*n* (%)]	Negative [*n* (%)]		Positive [*n* (%)]	Negative [*n* (%)]	
	19 (100)	101 (100)		11 (100)	109 (100)		11 (100	109 (100)		5 (100)	115 (100)	
Antibiotics treatment *n*=120	19 (15.38)	101 (84.16)	–	11 (9.16)	109 (90.8)	–	11 (9.16)	109 (90.84)	–	5 (4.2)	115 (95.8)	–
Steroid *n*=120	19 (15.38)	101 (84.16)	–	11 (9.16)	109 (90.8)	–	11 (9.16)	109 (90.84)	–	5 (4.2)	115 (95.8)	–
DM *n*=35 (29.16%)	14 (40)	21 (60)	0.0001*	11 (31.42)	24 (68.57)	0.063	(22.85)	27 (77.15)	0.244	5 (14.3)	30 (85.7)	0.0001*
Hypertension *n*=30 (25%)	14 (46.66)	16 (53.33)	0.0001*	8 (26.66)	22 (73.33)	0.547	11 (36.7)	19 (63.3)	0.0001*	3 (10)	27 (90)	0.020*
Chronic respiratory illness *n*=26 (21.66%)	15 (57.69)	11 (42.3)	0.0001*	5 (19.32)	21 (80.76)	0.003*	5 (19.3)	21 (80.7)	0.142	–	26 (100)	0.13
Cardiac/vascular disease *n*=16 (13.3%)	10 (62.5)	6 (37.5)	0.0001*	5 (31.25)	11 (68.75)	0. 001*	–	16 (100)	0.175	–	16 (100)	0.814
Renal failure *n*=10 (8.33%)	8 (80)	2 (20)	0.0001*	2 (20)	8 (80)	0.925	–	10 (100)	0.298	–	10 (100)	0.458
Hepatites *n*=12 (10%)	0	12 (100)	0.99	2 (16.66)	10 (83.33)	0.415	–	12 (100)	0.244	1 (8.3)	11 (91.7)	0.072
Invasive Ventilatory support *n*=99 (82.5%)	12 (12.12)	87 (87.87)	0.29	11 (11.1)	88 (88.9)	0.111	11 (11.1)	88 (88.9)	0.111	5 (5.05)	100 (94.95)	0.248

More than one predisposing factor may be present in the same patient. DM, diabetes mellitus. *P* value between groups by analysis of variance test. *Significant *P* value less than or equal to 0.05.

Table 6: Molecular detection of fungemia (candidemia and aspergillosis) by PCR compared with blood culture in 50 immunocompromised coronavirus disease 2019 patients

Species	Positive [*n* (%)]	Negative [*n* (%)]	Total [*n* (%)]
PCR	22 (44)	28 (56)	50 (100)
Blood culture	17 (34)	33 (66)	50 (100)

P=0.001.

Table 7: Sensitivity and specificity of blood culture in comparison to PCR for detection of fungemia (candidemia and aspergillosis)

	PCR		Total
	Positive	Negative	
Blood culture			
Positive	17	0	17
Negative	5	28	33
Total	22	28	

Sensitivity=100%. Specificity=84.4%. Positive predictive value=77.3%. Negative predictive value=100%.

by the virus. So, lymphocyte count, especially CD4 lymphocytes, may serve as a clinical predictor of severity and prognosis [21].

In our study, there were no statistically significant differences between the studied groups regarding lactate dehydrogenase, ferritin, and procalcitonin which disagree with Elshazli R *et al.* [33], who showed a higher significance with procalcitonin levels.

Henry *et al.* [21] explained that lack of a statistically significant or even clinically significant difference does not imply a lack of association with the outcome. And he proposed that elevated procalcitonin may be driven by the 50% secondary bacterial infection rate.

Also, we noticed an increase in MDA in COVD-19 ICU patients indicating the presence of lipid peroxidation as a severity of COVID-19 infection and this agreed with Martín-Fernandez *et al*. [22], Blaize *et al*. [24], who found that antioxidant enzymes and oxidative cell damage levels were significantly higher in COVID-19 patients.

It was noted in this study the presence of BSI in some patients. The most commonly isolated organisms were bacterial spp. (111/167, 66.5%), followed by Candida spp. (30 isolates, 17.9%) and Aspergillus spp. (11 isolates, 4.7%), while mucormycosis (five, 25% isolates from 20 patients but represented by 3% of all patients) and the associated bacterial infection represented 5.9% of all isolates.

Bishburg *et al*. [23] reported that Candidemia especially *Candida albicans* is the fourth highest cause of nosocomial BSIs. It was estimated that 33–55% of all episodes of candidemia occur in the ICU.

Blaize *et al*. [24] reported a 19% incidence of invasive aspergillosis among 432 patients admitted to an ICU for influenza-related acute respiratory failure.

Earlier, the anticipated load of mucormycosis in India was about 14 cases per 100 000 populations, which is one of the highest at the global level. However, recently, an alarming increase in the number of COVID-19-associated mucormycosis has been observed in India [25].

In India, more than 45 432 cases and 4252 deaths due to mucormycosis have been reported either among COVID-19 infected patients or in patients who had recovered from COVID-19 with rhinocerebral mucormycosis (77.6%) being the most common type of presentation [22].

Prakash *et al*. [26] found that 18% had DKA and 57% of patients had uncontrolled diabetes mellitus. Similarly, in a data of 465 cases of mucormycosis [27].

Results of this study showed that 82.5% were on invasive ventilator support, 29.16% were diabetic, 25% were hypertensive, and 13.33% had a chronic respiratory illness; the same percentage were cardiac (13.33%), 10% were hepatic, and 8.33% were suffering from renal failure. These were the risk factors presented in ICU COVID-19 patients. But all of them (100%) were managed with corticosteroids and antibiotics.

In a recent systematic review conducted until April 2021 by Bishburg *et al*. [23] who reported the findings of 41 confirmed mucormycosis cases in people with COVID-19, diabetes mellitus was reported in 93% of cases, while 88% were receiving corticosteroids. Collectively, these findings suggest a familiar connection as a result of this study reported that the most common and the most risk factor is hypertension as there is significance between hypertension and fungal infections with a *P* value of 0.0001, 0.0001, and 0.020, respectively. Also, diabetes is the next risk factor as there is significance between diabetes and candida infection and mucormycosis with a *P* value of 0.0001. We reported significance between that chronic respiratory illness, cardiac disease, and renal failure with conidial infection with a *P* value of 0.0001, in addition to significance between chronic respiratory illness and cardiac disease with candidemia. No significance was reported between fungus infection and hepatitis, invasive ventilator support. Wu *et al*. [28] reported that diabetes is one of the risk factors for COVID-19 severities, which prolongs the hospitalization and recovery period and increases the probability of immunosuppressive medication for patients with moderate to severe forms of COVID-19. Due to these reasons, secondary infection has a significantly higher probability of developing in diabetic COVID-19 patients with or without diabetic ketoacidosis. Nezafati *et al*. [29] reported that mucormycosis is observed to be more common in people with uncontrolled diabetes and hyperglycemic conditions. In a study reported by Corzo-León *et al*. [32], 68% of patients with mucormycosis were diabetic, whereas this study reported 14.3% of diabetic patients with mucormycosis.

All of these complications are caused due to suppression of immunity as a result of corticosteroids intake, antibiotic abuse, and diabetes mellitus. The incidence of fungemia in COVID-19 patients is in agreement with Elshazli R *et al*. [33], as they reported that COVID-19-associated pulmonary aspergillosis has been the predominant fungal disease, adding insult to injury in COVID-19 patients with acute respiratory distress syndrome. This may be due to the virus invasion that results in the release of danger-associated molecular patterns that act as endogenous signals that exacerbate the immune and inflammatory response leading to lung injury. Importantly, danger-associated molecular patterns are known to play a central role in the pathogenesis of fungal diseases.

Moreover, in mild and nonsevere COVID-19 symptoms, the intake of corticosteroids out of panic and anxiety without medical advice leads to an appearance of the black fungus. Intake of corticosteroids for COVID-19 treatment was observed in 76% of mucormycosis cases. Another potential factor of the mucormycosis outbreak among Indian COVID-19 patients is their preexisting diabetes [4].

Lackner *et al*. [30] reported that direct examination of sputum, paranasal sinus secretions, or broncoalveolar lavage fluid is the most rapid approach for the first orientation of diagnosis and has to be considered as evidence of infection in blood cultures that are rarely positive. Laboratory diagnosis is based on conventional methods such as direct examination and culture (direct microscopy of clinical specimens, preferably using optical brighteners in clinical specimens, allows a rapid possible diagnosis of mucormycosis).

The sensitivity and specificity of blood culture method in comparison to PCR for the detection of aspergillosis and candidemia, identified in this work came in agreement with Wahyuningsih *et al*. [15] and Zhou-Xkong *et al*. [31], who reported that the negative outcome of blood cultures was possible due to either the use of suboptimal culture systems or the fact that insufficient numbers of yeast cells were introduced into the bottles, so these results demonstrate the high sensitivity of PCR and the low sensitivity of blood culture and explained that standard culture-based methods are insensitive and slow and to overcome this problem, PCR-based tools have been developed. However, identification of medically important Aspergillus species from short-term culture using nucleic acid sequence analysis of the ITS1 and ITS2 regions in combination with a BLAST bit score is a reliable and efficient method that provides earlier identification than standard culture methods. The identification of rarely encountered opportunistic organisms following sequence analysis should prompt a review of the sequence data and correlation with clinical findings. Investigations are in progress to determine whether the method has utility for the direct identification of fungi in tissue sections where histologic evidence of a fungus exists. Additional studies are needed to demonstrate whether the identification of Aspergillus at the species level will improve patient outcomes through the selection of a more effective antifungal therapy [14].

Successful treatment of mucormycosis requires early diagnosis, reversal of underlying risk factors, prompt administration of antifungal therapy, and surgical debridement when applicable.

Recommendation

Proven IPA is supported by the positive galactomannan test, culture positive, and histopathological evidence. The patient did not respond to voriconazole, and liposomal amphotericin B was added to his antifungal regimen. Further studies are needed to evaluate the prevalence of IPA in immunocompetent patients infected with SARS-CoV-2. Consequently, testing for the incidence of Aspergillus species in lower respiratory secretions and galactomannan test of COVID-19 patients with appropriate therapy and targeted antifungal therapy based on the primary clinical suspicion of IPA are highly recommended [29].

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Conflicts of interest

There are no conflicts of interest.

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