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Prevalence of Aspergillus and extremely resistant bacterial superinfection among sever Covid-19 patients

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Abstract

Background and objectives: Co-infections involving bacteria or viruses alongside severe acute respiratory syndrome coronavirus-2 have been documented in numerous studies. The understanding of Aspergillus coinfection in individuals suffering from coronavirus disease 2019 was found to be constrained. This study aims to explore the updated information about coronavirus 2019 associated with pulmonary aspergillosis.

Methods: This study was conducted at coronavirus disease-19 hospital and intensive care unit of Sulaimani and San private clinic and laboratories, from jan-2021 to feb-2022. Seventy-five patients were enrolled, including patients who were intubated on a mechanical ventilator and continuous positive airway pressure mask treatment. The intubated patients were screened after 2-3 days. Non-intubated patients on continuous positive airway pressure mask; sputum study was performed if evidence of secondary chest infection was present including rising fever, and productive cough, with laboratory markers and radiologic investigation.

Results: Out of 75 patients, 10.7% (n=8) were culture negative, 89.3% (n=67) were secondary microbial infection, 64.0% (n=48) were bacterial infection, 25.3% (n=19) were Aspergillus Species, having invasive pulmonary mold superinfection. The majority (42.1%, n=8) noticed in those aged between 60-70 years. *Aspergillus flavus* was the dominant mold (52.6%, n=10). 63.2% (n=12) of the Aspergillus infection was recorded in those patients who were treated with steroids for 11-20 days, with significant difference (P<0.001).

Conclusion: Increasing age, comorbidity like diabetes and extended course of corticosteroid were linked to a higher incidence of invasive pulmonary aspergillosis. Extremely resistant Acinetobacter and E. coli superinfection were estimated as the most common bacterial element in sever coronavirus-2019 pneumonias.

Keyword: Aspergillus, Co-infection acute respiratory distress syndrome, Invasive pulmonary aspergillosis

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Introduction

The COVID-19 pandemic, caused by the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) virus, originated in Wuhan, China in December 2019. It has since spread globally, affecting all continents and leading to the implementation of quarantine and lockdown measures in densely populated.[1] The majority of individuals with COVID-19 may experience either no symptoms or just mild symptoms, such as a fever and dry cough. However, in severe situations, particularly among older individuals with hypertension and diabetes, SARSCoV-2 can lead to the development of severe acute respiratory syndrome (SARS).[2] Severe acute respiratory syndrome coronavirus 2 primarily infects the lungs and causes pneumonia, but data has shown that it can also damage several other organs such as the cardiovascular, immunological, neurological, and gastrointestinal systems.[3] Published reports indicate that a subset (7.2%) of individuals with COVID-19 may have co-infection with other microbial infections. This co-infection contributes to the severity of these cases and poses challenges in terms of diagnosis, prognosis, and therapy. Approximately 2-5% of COVID patients require mechanical ventilation, which puts them at a higher risk of developing secondary infections associated with intensive care, such as ventilator-associated pneumonia (VAP) caused by bacteria and fungi. However, the emergence of highly drug-resistant superinfections in intubated patients were observed, including an outbreak of Aspergillus mold co-infection. [4] This polymicrobial infection with a highly resistant profile significantly increases mortality rates and reflects the quality of intensive care in the area.[4] Both clinical trials and metagenomic investigations have shown that COVID-19 patients had additional viruses, bacteria, archaea, and fungi present alongside SARS-CoV-2.[5] Approximately half of the patients who succumbed to COVID-19 experienced subsequent bacterial infections.[6] This exacerbates the pathophysiological development of COVID-19. A comprehensive study of co-infections in COVID-19 is crucial for the efficient management, therapy, and containment of SARS-CoV-2. Hence, it is imperative to enhance the research on co-infection in patients with COVID-19.[7] Moreover, Regarding COVID-19, there are still many unknowns, including useful measures for preventing the spread of the disease, proper collection of clinical specimens, the route of transmission, viral dynamics, and viable medication treatments. Nevertheless, the understanding of co-infections with other respiratory pathogens, such as bacteria, archaea, viruses (except beta coronavirus), and fungi, remains unclear. The doctor should consider the relationship between these secondary pathogens with creating co-infections as a significant issue while managing COVID-19 cases.[8] The Centers for Disease Control and Prevention (CDC) in the United States, specifically the section that focuses on emerging infectious diseases, has officially supported the idea of testing for other respiratory pathogens. This recommendation suggests that finding evidence of another infection could help assess patients who may have COVID-19, especially when rapid testing for the specific virus (SARS-CoV-2) is not readily accessible.[8] This study aims to investigate a secondary invasive fungal infection among sever COVID-19 patient.

Patients and methods

The cross-sectional study was conducted at COVID-19 hospital, COVID-19 intensive care unit of Sulaimani and San private clinic and laboratories, from Jan-2021 to Feb-2022. Seventy-five patients were enrolled, with a mean age of 57.2±24.6 years (18-88 years), including both genders, the majority were intubated on mechanical ventilator and a group of them on continuous positive airway pressure (CPAP) mask treatment. The intubated patient was screened after 2-3 days of intubation or CPAP mask based on evidence of secondary infection including laboratory markers such as C- Reactive protein (CRP), procalcitonin, Complete blood count (CBC) and radiological examination, a sample collected was blind endotracheal aspirate (ETA) using mucus collector. While non-intubated patients were on CPAP masks sputum studies were performed if evidence of secondary chest infection was present including rising fever, and productive cough, with laboratory markers and radiologic investigation. The samples were sent to the laboratory for immediate processing as the following investigation done on either ETA or sputum samples: aerobic culture on blood, chocolate, McConkey for cultivable bacterial agents; anaerobic culture; fungal special culture SDA media and identification system using ViteK-II and LiofilChem API; Gram stain; Acid-fast stain as well as LPB- Lactophenole blue and Calcoflour white fluorescent stain. All grown microbial agent of ETA considered significant and there is no risk of contamination science the samples were collected from tracheal bronchial tree using mucus extractor. While for sputum specimens high power fill examination criteria applied to access sputum quality. The inclusion criteria were: both gender male and female, older than 18 years old, all conditions including chronic illness and comorbidities, COVID-19 PCR test positive on NPS-nasopharyngeal swab, Intubated and non-intubated but on CPAP mask patients. The patients younger than 18 years, old were excluded in this study. This study was ethically considerate by the Ethical committee of the college of medicine/University of Sulaimani, No:145; 18/12/2022. For this study the patients were verbally consented. The Statistical Package for the Social Sciences (SPSS) version 26 was used to analyze the data. The data was expressed as numbers and percentages, p value of ≤ 0.05 was considered as significant. This study was approved by Ethical committee of College of Medicine, University of Sulaimani.

Results

The culture result was completed in most at 2 days, some take up to 3 days in polymicrobial infection, and culture for fungal elements was kept for a total of 7-10 days. Sputum quality assessment was done as high-power field examination criteria applied to exclude oral contamination of the sputum for those patients on CPAP masks while every microbial agent from the ETA sample was considered invasive. Based on our finding, 10.7% (n=8) were culture negative (we consider them as progressive viral pneumonia and cytokine storm responsible for sudden severe deterioration and extensive consolidation) despite few variances’ Streptococcal oral floral agent from sputum samples. On the other hand, 89.3% (n=67) were showing secondary microbial infection, mostly (48.0%, n=64) were bacterial especially multidrug-resistant and extremely drug-resistant gram-negative bacteria while gram positives were considerably implicated in the sample. Common bacterial agents were Klebsiella pneumoniae, Acinetobacter baumannii, Pseudomonas, E. coli, Staphylococci and Enterococci. According to the colonial gross morphology Figure (1), color and microscopic characteristics Figure (2); a very significant number of Aspergillus Species were identified (25.3%, n=19), causing invasive pulmonary mold superinfection. The majority was noticed in those patients aged between 60-70 years (42.1%, n=8) followed by 50-60 years (31.6%, n=6). Based on our finding Aspergillus flavus was the dominant mold infection among severe COVID-19 pneumonia cases, estimated as 52.6% (n=10), then Aspergillus fumigatus (36.8%, n=7), whereas Aspergillus niger, was recorded only 10.5% (n=2) of the total Aspergillus infection. Indeed, four candida monomicrobial infection were implicated in only two cases. Our results revealed that those COVID-19 patients aged between 50 and 60 years old were more suffering from bacterial superinfection (35.4%, n=17) as the aforementioned identified bacterial agents, followed by those younger than 40 years (27.0%, n=13). Of note, 30.7% (n=23) of the patients were DM, 12.0% (n=9) were HTN and the other 57.3% (n=43) were not associated with chronic disease. Very interestingly, of 19 ASP patients, 78.9% (n=14) were DM, meanwhile, 15.8% (n=4) were associated with HTN and only 5.3% (n=1) had no chronic disease. Those with chronic comorbidities like diabetes and hypertension showed heavier fungal burden and earlier recovery of the mold element in their specimen, Table (1).

Figure (1): Gross morphology of Aspergillus flavus mold colonies.

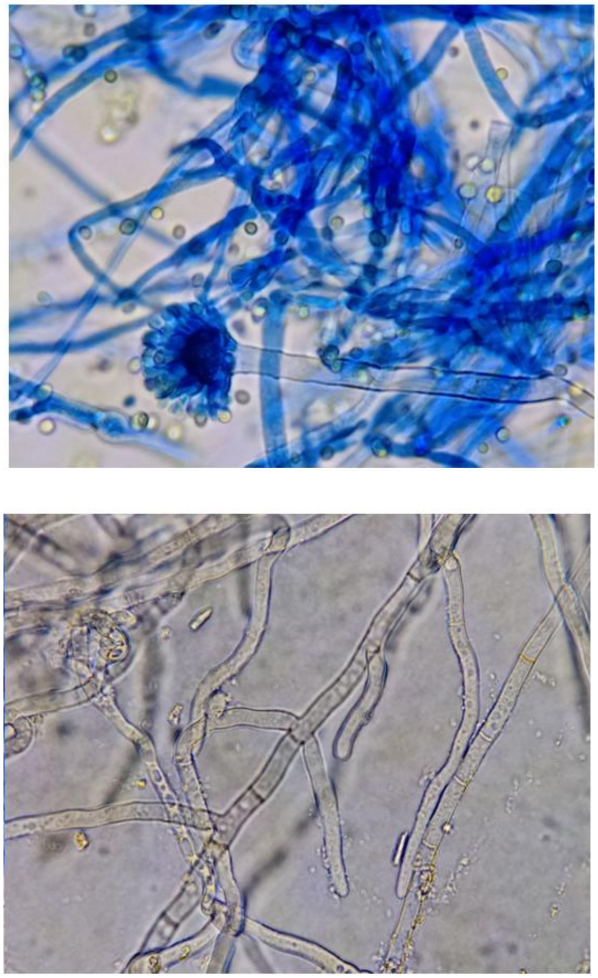

Figure (2): Microscopic characteristic of Aspergillus hyphae and spores.

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We found that the yield of Aspergillus true infection is more sensitive and reliable from blind endotracheal aspirate in mechanically intubated patients rather than sputum samples in patients with CPAP masks as some patient sputum revealed few Aspergilli mold and we were doubtful from oral floral contamination of dirty oral hygiene as were expected in sever COVID pneumonia patient on CPAP mask.

Table (1): Characteristics of the patients

Characteristics	Culture results						
	Sterile (n=8, 10.7%)	Bacterial (n=48, 64.0%)	ASP (n=19, 25.3%)	A. flavus (n=10, 52.6%)	A. fumigatus (n=7, 36.8%)	A. niger (n=2, 10.5%)	Total (n=75, 100.0%)
Age (Mean ± SD= 57.2 ± 24.6 year)							
<40	2 (25.0%)	11 (22.9%)	1 (5.3%)	1 (10.0%)	0 (0.0%)	0 (0.0%)	14 (18.7%)
40-50	2 (25.0%)	6 (12.5%)	2 (10.5%)	1 (10.0%)	1 (14.3%)	0 (0.0%)	10 (13.3%)
50-60	1 (12.5%)	17 (35.4%)	6 (31.6%)	3 (30.0%)	2 (28.6%)	1 (50.0%)	24 (32.0%)
60-70	2 (25.0%)	7 (14.6%)	8 (42.1%)	5 (50.0%)	2 (28.6%)	1 (50.0%)	17 (22.7%)
>70	1 (12.5%)	7 (14.6%)	2 (10.5%)	0 (0.0%)	2 (28.6%)	0 (0.0%)	10 (13.3%)
Comorbidity							
DM	2 (25.0%)	7 (14.6%)	14 (78.9%)	6 (60.0%)	7 (100.0%)	1 (50.0%)	23 (30.7%)
HTN	2 (25.0%)	3 (6.3%)	4 (15.8%)	3 (30.0%)	0 (0.0%)	1 (50.0%)	9 (12.0%)
No	4 (50.0%)	38 (79.2)	1 (5.3%)	1 (10.0%)	0 (0.0%)	0 (0.0%)	43 (57.3%)

The values were expressed as n (%); n: number; %: percentage; DM: diabetes mellitus; HTN: Hypertension; ASP: Aspergillus; A. flavus: Aspergillus flavus; A. fumigatus: Aspergillus fumigatus; A. niger Aspergillus niger.

Generally, an extended course of steroid use of more than 10 days was linked to a higher incidence of invasive PA, 63.2% (n=12) of the Aspergillus infection was recorded in those patients who were treated with steroids for 11-20 days, after that, the dominant prevalence was observed in 21-30 days of steroid using duration (21.1%, n=4) followed by 31-40 days (10.5%, n=2), then less than 10 days (5.3%, n=1). p<0.001, Table (2).

Table (2): Comparison of duration of using steroid drugs to the culture results

Steroid day	Culture results			
	Culture sterile (n=8, 10.7%)	Bacterial infection (n=48, 64.0%)	Aspergillus positive (n=19, 25.3%)	Total (n=75, 100.0%)
1-10 days	0 (0.0%)	9 (18.8%)	1 (5.3%)	10 (13.3%)
11-20 days	6 (75.0%)	24 (50.0%)	12 (63.2%)	42 (56.0%)
21-30 days	1 (12.5%)	13 (27.1%)	4 (21.1%)	18 (24.0%)
31-40 days	1 (12.5%)	2 (4.2%)	2 (10.5%)	5 (6.7%)

The values were expressed as n (%); n: number; %: percentage.

After investigation for the source of Aspergillus infection, it was found that water was used for flowmeter noninvasive oxygen mask was the major source of infection as non-sterile water was dispended into the flowmeter bottles as a humidifier for Oxygen therapy early before CPAP and intubation. As shown in Table (3), among 48 bacterial infections, it has been found that 32 (66.7%) COVID-19 patients had monomicrobial bacterial superinfection, 6 (12.5%) were polymicrobial mold and bacteria, and 10 (20.8%) were polymicrobial bacterial mix, Notably, among bacterial agent Acinetobacter baumannii and E. coli were the most commonly implicated agent in the superinfection. On the other hand, among 19 Aspergillus positive patients, 13 (68.4%) were monomicrobial and 6 (31.6%) were polymicrobial Aspergillus with bacteria, $p<0.001$.

Table (3): Secondary microbial super infection among critically ill COVID-19 patients

Secondary microbial super infection	Culture results			
	Culture sterile (n=8, 10.7%)	Bacterial infection (n=48, 64.0%)	Aspergillus positive (n=19, 25.3%)	Total (n=75, 100.0%)
Monomicrobial	0 (0.0%)	32 (66.7%)	13 (68.4%)	43 (57.3%)
Polymicrobial Aspergillus + Bacteria	0 (0.0%)	6 (12.5%)	6 (31.6%)	12 (16.0%)
Polymicrobial Bacteria + Bacteria	0 (0.0%)	10 (20.8%)	0 (0.0%)	10 (13.3%)

The values were expressed as n (%); n: number; %: percentage; bacterial infection species were XDR-Acinetobacter, XDR- E. coli, MDR- Klebsiella, MDR-Pseudomonas, Enterococci, Kocuria kristinae, Stenotrophomonas, while aspergillus species were A. flavus, A. fumigatus and A. niger.

Discussion

Among late 2019, the global COVID-19 pandemic created serious public health problems with higher morbidity and mortality rates, especially among older and medically ill people. Patients may experience different COVID-19 symptoms. Acute respiratory distress syndrome, pneumonia, and multi-organ failure may occur in extreme situations. [9] In this study, we found very interesting and extremely significant invasive infections in severely or critically ill patients with COVID-19 as the morbidity and mortality were highly increased in those patients with secondary IPA or polymicrobial infection. The most significant finding in our study is that 5.38% of the severe COVID pneumonia patients were superinfection by Aspergillus mold with difficulty to treat from some factors such as availability of anti-microbial agent, organ function impairment, including liver function and renal function and finally the expense of the medication. As in our study we found Aspergillus mold fungus to be the dominant mold infection, in addition, Aspergillus flavus was found to be the most common mold in contrast to Aspergillus fumigatus and Aspergillus Niger. Aspergillosis, caused by Aspergillus fumigatus, is a frequent fungal infection that can lead to secondary lung infection in highly immunocompromised patients.[10] Previous studies did not recognize that influenza and COVID-19 viral infections might raise the risk of aspergillosis. However, Fekkar found that 20-30% of severely ill or ventilated

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COVID-19 patients develop aspergillosis. This establishes a connection between COVID-19 and pulmonary fungal infections, known as COVID-19 Associated Pulmonary Aspergillosis (CAPA).[11] A study conducted by Lai, 2021 found that Aspergillus species can lead to co-infections in patients with COVID-19, particularly in those with severe or critical illness.[12] It has been observed that Aspergillus fumigatus was the predominant species responsible for co-infection in COVID-19 patients, despite, Aspergillus flavus being the second most prevalent.[12] Furthermore, In our institute, the incidence rate of aspergillosis in COVID-19 patients in the ICU was higher compared to the rate reported in a national survey conducted in Japan (4.1% vs. 0.5%).[13] Our results revealed that those COVID-19 patients aged between 50 and 60 years old were more suffering from bacterial superinfection (35.4%, n=17) as the aforementioned identified bacterial agents, followed by those younger than 40 years (27.0%, n=13). The top common age of severely ill patients who developed IPA are falling in 51–to 60-year-old, then 61–to 70-year-old ranked second, likewise for other ages in less frequent ranges. Study by Ogawa and Chen highlight numerous prominent risk factors, emphasizing old age, hospitalization in the intensive care unit, using mechanical ventilation, kidney failure necessitating hemodialysis, pharmacological immunosuppression (such as steroids or biological therapy), and extended hospital stay.[13, 14] As predicted, those COVID-19 patients with chronic comorbidity like diabetes and hypertension showed heavier fungal burden and earlier recovery of the mold element in their specimen. These results meaning that, comorbidities like DM and HTN were associated with a higher risk of secondary mold infection. Many studies revealed that invasive aspergillosis is most likely to develop following commonly observed in critically ill patients in ICU, solid organ transplant recipients, and patients with comorbidities such as AIDS/HIV, renal failure, COPD, diabetes and malnutrition.[15, 16] The most commonly related risk factor for IPA in our patient group was extended use of corticosteroid therapy mostly intravenous dexamethasone 6mg in some cases 8 mg daily for more than 2 weeks highly correlated with IPA, in our findings using a steroid for about 21-30 days was the highest group of having IPA; hence the highest number of patients fell in this duration of steroid therapy. Whereas, only 1 patient (5.3%) who used steroids for less than 10 days developed IPA.A retrospective cohort study conducted in the United States Guinea identified risk factors associated with the development of invasive pulmonary aspergillosis (IPA), which included the use of corticosteroids and the presence of either respiratory or kidney failure.[16] Additionally, In nonneutropenic individuals, the development of invasive aspergillosis is most usually associated with the continuous administration of corticosteroids, a practice frequently observed in critically sick patients in the intensive care unit.[15] After a field investigation, it has been found that the use of non-sterile tap water for the oxygen flowmeter was another risk and source for IPA in critically ill COVID patients as cultivated Aspergillus in 6 out of 20 bottles of flowmeter humidifier. The latest CDC Advisory Committee Report from 1997 advises using sterile water, rather than distilled water, in bubbling humidifiers.[17] In contrast, a study by Cahill, which aimed to compare bacterial contamination between tap water and sterile water, found that after 5 days, there was greater microorganism growth in the sterile water reservoirs .[18] Additionally, humidifiers that generate water vapor without using the bubble-through method do not aerosolize solutes or microorganisms present in the water, thereby reducing the risk of microbial transmission .[19]

Wenzel et al. also documented that non-sterile tap water might be a safe alternative to sterile water in convection-type humidifiers used for continuous positive airway pressure therapy. [20] A Karnataka minister attributed the surge in Mucor mycosis infections during the COVID-19 pandemic primarily to the use of tap water in humidifiers for oxygen therapy.[19] Among 48 bacterial infections, that 32 (66.7%) COVID-19 patients had monomicrobial bacterial superinfection, 6 (12.5%) were polymicrobial mold and bacteria, and 10 (20.8%) were polymicrobial bacterial mix, Notably, among bacterial agent Acinetobacter baumannii and E. coli were the most commonly implicated agent in the superinfection. SARS-CoV-2 and secondary infections with A. baumannii are critical to COVID-19 development and prognosis, especially in hospitalized patients. There are various COVID-19 diagnosis guidelines, but bacterial coinfections and subsequent infections need more research. [9]

Conclusion

Out of 75 sever COVID-19 patients, 25.3%, (n=19), had Aspergillus spp.-positive respiratory tract cultures. Those had a higher risk of aspergillosis aged between 60-70 years old, also, those with chronic comorbidity like diabetes. Extended course of corticosteroid use was linked to a higher incidence of invasive pulmonary aspergillosis. Extremely resistant Acinetobacter and E. coli superinfection were estimated as the most common bacterial element in sever COVID pneumonias.

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Conflict of interest

The authors declare no conflict of interest regarding the publication of this study.

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