



A case study of fungal diversity and virulence factors in COVID-19 patients at Al-Muthanna Hospital in Iraq

Huda R. Hashim*, Wissam J. Kazem, Ali K. Kadom



Department of Biology, College of Basic Education, University of Al-Muthanna, Iraq.

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ABSTRACT

This study examined the presence and diversity of fungal taxa in the eyes and noses of COVID-19 patients. We collected sixty samples from COVID-19 patients and recovered about 30 fungal isolates. Six species of fungi were identified as *Aspergillus niger* (40%), *A. flavus* (23.33%), *A. parasiticus* (13.33%), *Alternaria alternata* (10%), *Fusarium oxysporum* (10%), and *Candida albicans* (3.33%), respectively. We initially isolated *A. niger* from the pulmonary system. Its virulence factors were more prominent than those of other taxa isolated from the eyes, suggesting a significant risk to the patients. We studied the serum immunoglobulin (IgG and IgM) levels of COVID-19 patients and controls. The results showed that a week after infection, the IgG level was 12.74 AU/ml, significantly higher than the healthy control, which ranged from 12–15 AU/ml for negative and 0.73 for positive. During the first week of infection, IgM reached 3.1 AU/ml, and in the fourth week, IgG rose to 53.63 AU/ml, whereas IgM levels fell to 0.73. These findings provide valuable information on COVID-19 patients' immune responses and how they evolve over time. Our study also compared COVID-19 patients' WBC levels to those of the control group. The median was 40, with 10% of patients having low WBC counts and 50% having high ones. Lymphocyte counts differed significantly between 47.5% (low count) and 17.5% (high count). Patients had normal neutrophil counts, with 5% having low counts and 45% having high counts, like the control group. Monocyte, eosinophil, and basophil counts were likewise similar to those in the control group.

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Introduction

On 30 January 2020, the World Health Organization (WHO) declared the COVID-19 outbreak as the sixth public health emergency of international concern, following H1N1 (2009), polio (2014), Ebola in West Africa (2014), Zika (2016), and Ebola in the Democratic Republic of the Congo (2019). In March 2020, WHO declared COVID-19 a pandemic, when over 118,000 cases in over 110 countries around the world suffered from it (El-Maradny et al. 2020; Saied et al. 2021).

The severe acute respiratory syndrome coronavirus creates plans for the care and prevention of fungal infections in COVID-19 patients (Rasmussen & Jamieson 2021). SARS-CoV-2 has recently been responsible for a previously unheard-of coronavirus disease epidemic (COVID-19) over the world (Arora et al. 2021). Since it first appeared, COVID-19 has quickly spread across continents, infecting millions of individuals and causing serious problems for international healthcare systems. Recent findings have shown an unexpected and concerning development in addition to the well-

*Corresponding author Email address hudaraheem@mu.edu.iq (Huda Raheem Hashim)



documented respiratory complications and fungal infections in COVID-19 patients (Huang et al. 2020).

Healthcare professionals and academics are gravely concerned about the prevalence of fungal infections in COVID-19 patients. Understanding the connection between fungal infections and SARS-CoV-2 is crucial to providing comprehensive care and lowering death rates, even though the predominant focus has been on viral transmission and the management of COVID-19. In order to create effective solutions to address this new problem, this study intends to investigate the prevalence, risk factors, and probable processes behind fungal infections in COVID-19 patients, Blot and colleagues published criteria 8 years ago for defining invasive aspergillosis in critically ill patients (Blot et al.2012). To categorize a case as hypothetical for immunocompetent patients, hyphae in respiratory samples had to be directly examined; otherwise, all of the instances that were presented would have been excluded. Since then, the criteria for diagnosing COVID-19-related aspergillosis have changed, but they still resemble those recommended by the authors. These criteria include *Aspergillus* spp. cultivated from BAL (without direct examination) or a galactomannan index of 0.5 or above on serum or BAL. On the other hand, it could be risky to diagnose invasive aspergillosis in an immunocompetent person based just on one positive lung specimen culture or one galactomannan indicator (Fekkar et al,2020; Hashim et al. 2024).

To comprehend the interaction between viral infection and fungal co-infections, as well as their impact on the immune responses and clinical consequences of patients our study aims to assess the diversity and frequency of fungal taxa obtained from the eyes and nasal passages of COVID-19 patients in comparison to healthy individuals. In addition to aforementioned goal the study targeted the analyze of the pathogenic characteristics of the recovered fungi and quantify the levels of immunoglobulins (IgG and IgM) in individuals with COVID-19.

Materials and Methods

Sampling and isolation of fungi

A total number of 60 samples were collected from Al-Shaheed Youssef Najim Hospital's, in Muthanna Governorate. Samples of blood, nasopharyngeal and eye fluids were collected in compliance with COVID-19's aseptic and barrier procedures (Abed Alah et al.2021). One drop of whole blood was required for the "on-site" testing according to standard operating procedure (SOP) of Abbott PanbioTMCOVID-19 rapid

diagnostic test kit according to Ahmed et al. (2020); Chamieh et al. (2021) and Zhang et al. (2021).

The two experimental groups were divided into equal and random subgroups. The control group comprises 10 individuals who are in good health and 10 persons who have been infected with the Corona virus. The blood samples from all experimental groups were collected via venous access and serum were separated by centrifugation at 2500 rpm for 5 minutes. All blood samples were analysed to assess the levels of immunoglobulin G (IgG) and immunoglobulin M (IgM) (Yel et al. 2015).

For isolation of fungi, five plates of Sabouraud's Dextrose Agar (SDA) were used for each sample. Inoculated plates were incubated for two up to seven days at 28°C and 37°C, respectively.

Phenotypic identification of fungi

Phenotypic identification of recovered microfungi was primarily based on the relevant identification keys for *Aspergillus* (Abdel-Azeem et al. 2020), dematiaceous hyphomycetes (Ellis 1971, 1976). *Fusarium* (Leslie and Summerell, 2006), different taxa (Domsch et al., 2007) and *Alternaria* (Simmons, 2007) Taxonomic position, assignments and name corrections of all recovered taxa were checked against the Index Fungorum website database (<https://www.indexfungorum.org/Names/Names.asp>).

Examination of virulence factors

Isolates of *Aspergillus niger* as the most frequent taxon recovered from patients with covid19-infected noses and eyes were examined for several virulence factors. Various parameters were chosen for virulence factors analysis, including biofilm formation (Jain et al., 2022), lipase production using phenol red and T80 agar plates (Hashim et al., 2018), amylase production through the starch hydrolysis technique (Sharma et al., 2011), proteolytic activity using the biuret method (Eggins & Pugh, 1962), and phospholipase activity as described by Birch et al. (1996).

Results and Discussion

The isolation results from COVID-19-infected noses and eyes revealed 30 isolates from fungi, belonging to six species, from 60 samples from the noses and eyes of COVID-19 patients. Of these, 40 samples showed a positive result of 66.67%, while 20 samples showed a negative result of 33.34%, as presented in table (1). This result aligns with the findings of Abed Ali & Hashim's COVID-19 study (2020).

Table (1) Isolation results from the nose and eyes.

| Growth | Types of swabs | | | | |
|----------|----------------|--------|------|--------|-------|
| | Nose | % | Eyes | % | Total |
| Positive | 26 | 86.67% | 14 | 46.67% | 66.67 |
| Negative | 4 | 13.34% | 16 | 53.33% | 33.33 |

Based on phenotypic identification, we identified six species of fungi, with *Aspergillus niger* exhibiting the highest percentage of isolated appearances (40%) among the four genera. According to table (2), *Alternaria alternata* had the greatest appearance percentage (10%), *Fusarium oxysporum* and *Candida albicans* had the lowest (3.33%), and *Aspergillus flavus* had the highest appearance percentage (23.33%) and the lowest (13.33%), respectively. This outcome coincided with Hashim et al. (2024).

We examined *Aspergillus niger* isolates recovered from the nose and eyes of COVID-19-infected individuals for several virulence traits using relevant techniques, as indicated in tables 3 and 4.

Despite the fact that both species contained virulence factors and posed a serious threat, *A. niger* isolated from the respiratory system had more pronounced virulence factors than other species isolated from the eyes.

The mean immunoglobulin (IgG) and (IgM) concentrations in serum from control and Corona virus patient groups were shown in table (5). After one week of infection, he found a significant increase in IgG concentration (12.74) (AU/ml) compared to the average value for the control (Negative>12-15> positive) (AU/ml), as shown in table (5). The IgM level was greater during the one week following infection (3.1) (AU/ml) than it typically was for the control (Negative>12-15> positive) (AU/ml).

Table (2) Recovered taxa frequency of occurrence.

| Isolated taxa | Appearance % | Frequency % |
|-----------------------------|--------------|-------------|
| <i>A. niger</i> | 12 | 40 |
| <i>A. flavus</i> | 7 | 23.33 |
| <i>A. parasiticus</i> | 4 | 13.33 |
| <i>Alternaria alternata</i> | 3 | 10 |
| <i>Fusarium oxysporum.</i> | 3 | 10 |
| <i>Candida albicans</i> | 1 | 3.33 |

Table (3) Virulence factors of *Aspergillus niger* isolated from respiratory system

| Isolate | Biofilm | Lipase | α -amylase | Phospholipase |
|---------|---------|--------|-------------------|---------------|
| 1 | + | - | + | + |
| 2 | + | + | - | + |
| 3 | + | - | + | + |
| 4 | + | + | + | - |
| 5 | + | - | - | + |
| 6 | + | + | + | + |

Table (4) Virulence factors of *Aspergillus niger* isolated from eye

| Isolate | Biofilm | Lipase | α -amylase | Phospholipase |
|---------|---------|--------|-------------------|---------------|
| 1 | + | - | + | + |
| 2 | + | + | - | + |
| 3 | + | - | - | + |
| 4 | + | + | + | - |
| 5 | + | - | + | - |
| 6 | - | + | + | + |

Table (5) IgG and IgM concentrations in sera samples of patients with corona virus in the first week of infection

| Sample | IgG (g/L) | Control (AU/ml) | IgM (g/L) | Control (AU/ml) |
|--------|-----------|--------------------------|-----------|------------------------|
| 1 | 14.1 | Negative<12-15> positive | 3.8 | Negative<1.1> positive |
| 2 | 13.8 | Negative<12-15> positive | 3.0 | Negative<1.1> positive |
| 3 | 11.4 | Negative<12-15> positive | 3.9 | Negative<1.1> positive |
| 4 | 10.5 | Negative<12-15> positive | 2.9 | Negative<1.1> positive |
| 5 | 11.7 | Negative<12-15> positive | 2.7 | Negative<1.1> positive |
| 6 | 12.6 | Negative<12-15> positive | 3.6 | Negative<1.1> positive |
| 7 | 13.6 | Negative<12-15> positive | 3.9 | Negative<1.1> positive |
| 8 | 12.8 | Negative<12-15> positive | 2.5 | Negative<1.1> positive |
| 9 | 14.0 | Negative<12-15> positive | 2.4 | Negative<1.1> positive |
| 10 | 12.9 | Negative<12-15> positive | 2.3 | Negative<1.1> positive |
| Mean | 12.74 | Negative<12-15> positive | 3.1 | Negative<1.1> positive |

An increasingly known method for avoiding host immune response and creating a protected niche is the development of a biofilm lifestyle during fungal infection (Mullins et al.2021). In this situation, the extracellular matrix may be able to prevent the recognition of the fungal cell wall by host cells, controlling the immune reaction (Bourne et al. 2020; Ganesan & Sivanandam 2022). The extracellular matrix is also capable of defending against antimicrobial defenses including defensins, oxidative stress, and NETs. Additionally, biofilm development creates a population that has gathered and may be phagocytosis-resistant (Krakow & Berghella 2020).

The development of biofilms has a significant influence on immunity, but research on the numerous mechanisms behind this control of host response is still in its infancy. Clinical biofilms are likely impacted by a

variety of immune-damaging events since they are heterogeneous structures with varying compositions and designs depending on their environmental habitat (Jain et al.2022). Future research must thus incorporate circumstances that closely reflect the host and animal models of biofilm infection.

The mean immunoglobulin (IgG) and (IgM) concentrations in serum from control and Corona virus patient groups were shown in table (6). After fourth week of infection, he found that the concentration of IgG (53.63) (AU/ml) had significantly increased compared to the normal value for the control (Negative>12-15> positive) (AU/ml). IgM (0.73) (AU/ml) had a greater level during the fourth week following infection compared to the control (Negative>12-15> positive) (AU/ml) average.

Table (6) IgG and IgM concentrations in sera samples of patients with corona virus in the fourth week of infection

| Sample | IgG (g/L) | Control (AU/ml) | IgM | Control (AU/ml) |
|--------|-----------|--------------------------|------|------------------------|
| 1 | 64.5 | Negative<12-15> positive | 1.0 | Negative<1.1> positive |
| 2 | 59.3 | Negative<12-15> positive | 1.1 | Negative<1.1> positive |
| 3 | 54.7 | Negative<12-15> positive | 0.9 | Negative<1.1> positive |
| 4 | 51.9 | Negative<12-15> positive | 0.9 | Negative<1.1> positive |
| 5 | 49.8 | Negative<12-15> positive | 0.7 | Negative<1.1> positive |
| 6 | 61.4 | Negative<12-15> positive | 0.6 | Negative<1.1> positive |
| 7 | 58.7 | Negative<12-15> positive | 0.9 | Negative<1.1> positive |
| 8 | 46.9 | Negative<12-15> positive | 0.5 | Negative<1.1> positive |
| 9 | 45.3 | Negative<12-15> positive | 0.4 | Negative<1.1> positive |
| 10 | 43.8 | Negative<12-15> positive | 0.3 | Negative<1.1> positive |
| Mean | 53.63 | Negative<12-15> positive | 0.73 | Negative<1.1> positive |

The mean WBC level in COVID-19 patients was 40, with 10% exhibiting low WBC counts and 50% exhibiting high WBC counts. The mean lymphocyte

count was 17.5% higher than its peak and 47.5% below its lowest point in history. Both aforementioned

statistics of the infected patients varied greatly from the controls.

The mean neutrophil count ranged from 5% with a low count to 45% with a high count, which was similar to the controls. According to table (7), this also applied to

the quantities of monocytes, eosinophils, and basophils. The mean ratio of neutrophils to lymphocytes was significantly higher than in the controls.

Table (7) Comparison of hematological variable of cases and controls

| Variable | Categories | Groups | | | |
|-------------------|------------|------------------|------------|---------|------------|
| | | Covid19 infected | Percentage | Control | Percentage |
| WBC count | Low | 4 | 10 | 1 | 2.5 |
| | Normal | 16 | 40 | 34 | 85 |
| | High | 20 | 50 | 5 | 12.5 |
| Lymphocyte count | Low | 19 | 47.5 | 4 | 10 |
| | Normal | 14 | 35 | 36 | 90 |
| | High | 7 | 17.5 | 2 | 5 |
| Neutrophils count | Low | 2 | 5 | 4 | 10 |
| | Normal | 20 | 50 | 32 | 80 |
| | High | 18 | 45 | 4 | 10 |
| Monocytes count | Low | 1 | 2.5 | 1 | 2.5 |
| | Normal | 26 | 65 | 33 | 82.5 |
| | High | 13 | 32.5 | 6 | 15 |
| Eosinophil count | Low | 1 | 2.5 | 1 | 2.5 |
| | Normal | 29 | 72.5 | 34 | 85 |
| | High | 10 | 25 | 5 | 12.5 |
| Basophil count | Low | 4 | 10 | 1 | 2.5 |
| | Normal | 27 | 67.5 | 35 | 87.5 |
| | High | 9 | 22.5 | 4 | 10 |

The hematological outcomes from the current research agreed with those of (Sun et al. 2020) who stated that abnormal hematological results are crucial for case diagnosis. The significant WBC count irregularity among the patients in this analysis is similar to the findings of the (Terpos et al. 2020) Research discovered that women who had the COVID-19 disease while pregnant had a lower WBC count.

Contradictory WBC counts during COVID-19 infection may be the cause of the finding due to patients arriving for consultations late 16. The patients in our study showed a very low lymphocyte count, which is consistent with (Afshar et al. 2019). who made the discovery that in early-stage COVID 19 disease in pregnant women, lymphocytopenia is a diagnostic sign. The latest study confirms the results of (Gajbhiye et al. 2021). They found that COVID-19 disease was significantly more common in low-income nations and among pregnant women. The outcomes of the study are

consistent with the patients' higher rate of premature delivery (Mullins et al. 2021). In China, Sun et al. (2020) discovered a lower WBC count in patients with COVID-19 disease, and the current study's significant WBC count anomaly are comparable in their conclusions (Bourne et al. 2021). This finding might be explained by patients arriving for consultations too late, which results in fluctuating WBC levels during COVID-19 infection (Al-Kuraishy et al. 2021; Al-Mosway 2022).

We hope to provide evidence-based solutions for the prevention, early identification, and successful management of fungal infections in people with COVID-19 based on the study's findings. These suggestions will be quite helpful in enhancing patient outcomes and lessening the strain on healthcare infrastructure (Evans et al.2023).

This study is significant because it has the potential to change how COVID-19 patients are treated clinically

throughout the world. By putting light on the intricate interactions between viral infection and fungus co-infections, we seek to increase awareness among policymakers and the medical community and inspire more research into this crucial area of concern.

Conclusion

This extensive scientific investigation significantly elucidates white blood cell counts, immunoglobulin levels, and fungal isolates in COVID-19 patients. The identification of different fungal species and their potential virulence factors underscores the importance of monitoring fungal infections in COVID-19 patients, especially those affecting the respiratory system. The large changes in IgG and IgM levels during the course of the infection emphasize the immune system's dynamic character. The change in WBC counts also imply possible consequences for the severity and development of COVID-19. With a better understanding of the intricate interactions between the immune system and fungi in COVID-19 patients, our findings pave the way for further study and treatment approaches.

Conflict of interest

The authors of this work have no competing interests.

Funding source

Funding for the research was from the College of Basic Education, Al-Muthanna University.

Author contribution

The three researchers worked together to choose the title, formulate the research idea, and initiate the research steps. The research idea stemmed from the problem of secondary fungal infection among individuals infected with the Corona virus.

Ethics Statement

Ethical approval to conduct this study was obtained from the Board of Al-Shaheed Youssef Najim Hospital and the Department of Science, College of Basic Education, Al-Muthanna University, Iraq. Additionally, all patients provided consent prior to being included in this study.

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