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Research Article

Histopathological Detection of Post-COVID-19 Fungal Co-infection in the Respiratory Tract

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Abstract

Background: Invasive fungal infections can complicate the clinical course of COVID-19, especially in severely ill patients who require admission to the intensive care unit. **Objective:** To evaluate the histopathological features and severity score of post-COVID-19 fungal co-infection in the respiratory tract in Ninevah Province and correlate the result with age, sex, and diabetes. **Methods:** Thirty patients who presented with post-COVID-19 fungal co-infection from August to May 2022 were involved in this retrospective case series study. Hematoxylin/eosin stain and periodic acid Schiff stain were used to stain the biopsies. Disease severity was assessed using a scoring system that incorporated grades from I to III across multiple histological variables. **Results:** Mucormycosis is the only fungal infection found in the samples received, with female predominance and an age of 54.20 years. Perinasal facial pain and swelling were the most common clinical presentations, and 76.7% were diabetics. Necrosis of $\geq 50\%$ was observed in 63.3% of cases with a severe degree of neutrophilic infiltrate, and angioinvasion in more than 3 vessels in 10 high-power fields was identified in 60% of cases. Histologically, grade II was found in 50% of the cases. **Conclusions:** Post-COVID-19 patients have a significant risk of mucormycosis, in which diabetes and steroid administration were the most important predisposing factors in addition to systemic immune alteration in COVID-19-infected patients. Acute inflammation, tissue necrosis, fungal load, and angioinvasion were proposed.

Keywords: COVID-19, Fungal infection, Histopathology, Mucormycosis, Respiratory tract, Severity score.

الكشف النسيجي المرضي للعدوى الفطرية المصاحبة بعد COVID-19 في الجهاز التنفسي

الخلاصة

الخلفية: يمكن أن تؤدي الالتهابات الفطرية في الجهاز التنفسي إلى تعقيد المسار السريري لـ COVID-19، خاصة في المرضى المصابين بأمراض خطيرة والذين يحتاجون إلى الدخول إلى وحدة العناية المركزة. **الهدف:** تقييم السمات النسيجية المرضية ودرجة شدة العدوى الفطرية المصاحبة بعد COVID-19 في الجهاز التنفسي في محافظة نينوى وربط النتيجة بالعمر والجنس ومرض السكري. **الطرائق:** شارك ثلاثون مريضاً مصاباً بعدوى فطرية مشتركة بعد COVID-19 من أغسطس إلى مايو 2022 في دراسة سلسلة الحالات بأثر رجعي هذه. تم استخدام صبغة الهيماتوكسيلين/الإيوزين وصبغة شيف الحمضية الدورية لتلطيف الخزعات. تم تقييم شدة المرض باستخدام نظام تسجيل يتضمن الدرجات من الأول إلى الثالث عبر متغيرات نسيجية متعددة. **النتائج:** الفطريات المخاطية هي العدوى الفطرية الوحيدة الموجودة في العينات المستلمة، مع غلبة الإناث وبعمر 54.20 سنة. كانت آلام الوجه والتورم في محيط الأنف من أكثر الأعراض السريرية شيوعاً، وكان 76.7% مرضى السكر. لوحظ نخر $\leq 50\%$ في 63.3% من الحالات ذات الدرجة الشديدة من تسلل العدلات، وتم تحديد غزو الأوعية الدموية في أكثر من 3 أوعية في 10 مجالات عالية الطاقة في 60% من الحالات. من الناحية النسيجية، تم العثور على الدرجة الثانية في 50% من الحالات. **الاستنتاجات:** مرضى ما بعد COVID-19 معرضون لخطر كبير للإصابة بالفطريات المخاطية، حيث كان مرض السكري وإعطاء الستيرويد من أهم العوامل المؤهبة بالإضافة إلى التغيير المناعي الجهازى لدى المرضى المصابين بـ COVID-19. وتم افتراض التهاب الحاد ونخر الأنسجة والحمل الفطري والغزو الوعائي كمعامل مسببة.

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INTRODUCTION

SARS-COV-2, the coronavirus disease 2019 (COVID-19) infectious agent, causes a respiratory illness with a wide range of severity, from an infection without symptoms or with mild symptoms to serious cases of pneumonia that progress to respiratory failure and necessitate mechanical ventilation, which may be invasive or not [1,2]. Among many causes leading to morbidity and mortality in COVID-19 patients, opportunistic bacterial or fungal infections could deteriorate the status of patients and lead to adult

respiratory distress syndrome [3]. There are several histochemical stains that can be used to identify fungi in tissue sections. In most laboratories, hematoxylin and eosin (H&E) is the first stain used on all tissue sections because it perfectly illustrates host inflammatory reaction, shows the Splendore-Hoepli reaction, and shows whether fungi are pigmented or not. The existence of necrosis, granuloma, and/or pyogranuloma should always prompt consideration for the presence of invasive fungi [4]. Due to the limitations of the H&E stain, additional "special stains" that react with the majority of fungal genera should be used for the best

visualization of fungi in tissue sections, like Gomori's methenamine silver (GMS), the periodic acid-Schiff reaction (PAS), and Gridley's fungus (GF) procedure, which are helpful for displaying by far the majority of fungal elements in tissue sections [5]. The only way to determine a reliable *in situ* causative diagnosis in formalin-fixed biopsies is to use immunohistochemistry in conjunction with *in situ* hybridization techniques when no usual or characteristic morphological hall indicators are present [6]. An infection with SARS-COV-2 has profound effects on the immune system, causing an inflammatory storm and leading to an increase in neutrophils and a decrease in lymphocytes (particularly CD4⁺ and CD8⁺ T-cells). Since neutrophils are responsible for maintaining an individual's immune competence, an increase in neutrophil counts during SARS-COV-2 suggests that the immune response to fungal infection will be significantly more robust than usual. However, because of a paucity of lymphocytes, these patients are more likely to acquire opportunistic infections such as mucormycosis [7]. In severe cases of COVID-19, *Aspergillus* and *Candida* are the most common fungal pathogens responsible for fungal coinfections. *Mucor* and *Cryptococcus* are other opportunistic pathogenic fungi that can cause lung infections and should be taken into account [8]. This study aims to evaluate the histopathological features and severity score of post-COVID-19 fungal co-infection in the respiratory tract in Ninevah Province and correlate the result with age, sex, and diabetes.

METHODS

Study design and setting

A retrospective case series study was conducted from August 2020 to May 2022, with 30 patients who presented with post-COVID-19 fungal co-infection. The data was collected from the histopathology department of Al-Jamhoori Teaching Hospital and some private laboratories in Mosul City.

Inclusion criteria

All cases of post-COVID-19 fungal infection in the respiratory tract were enrolled in this study, whatever the age, sex, and complaint.

Outcome measurement

Biopsies of patients were received fixed in formalin. An adequate representative sample was submitted in capsules, processed, and paraffin-embedded, and slides were prepared and stained with Hematoxylin and Eosin (H & E) stain and with Periodic Acid Schiff (PAS) stain for the histopathological examination. For each case, the H&E and PAS stain slides were reviewed regarding the final diagnosis and the other parameters or risk factors needed in this study. The severity categorization of

mucormycosis infections was evaluated for the four factors listed below [7]: 1) Neutrophilic inflammatory cell infiltrate was evaluated in tissue fragments containing fungal hyphae in a 400x magnification field. 2) Tissue necrosis was described as the presence of non-viable tissue with fungal hyphae. 3) The fungal load was assessed as the number of microscopic fields at X400 showing fungal hyphae. 4) We counted the number of blood vessels in ten X400 microscopy fields. On the basis of the microscopic examination evaluation for every parameter, a consensus score of 1 to 3 or 1 to 2 was assigned, and then the lesion was graded by summing the scores. A lesion with a score of 4-5 was classified as Grade I, while a score of 6-8 was classified as Grade II, and a score of 9-11 was classified as Grade III.

Ethical considerations

This study was conducted based on the principles of ethics that have their origin in the Declaration of Helsinki. The study protocol was approved by the Medical Research Ethics Committee, College of Medicine, University of Mosul, with a reference ID: UOM/COM/MREC/24-25/MAY8 on May 14, 2025.

Statistical analysis

Various clinicopathological variables were analyzed using the SPSS 26 statistical program. A probability of 95% was used to assign a significant end result; a *p*-value < 0.05 is considered significant.

RESULTS

This study analyzed a total of 30 biopsies from a patient with post-COVID-19 fungal infection. All the biopsies show evidence of mucormycosis fungal infection as seen in Figure 1 which show broad aseptate ribbon like hyphae with 90° angles branching in a nasal septum biopsy.

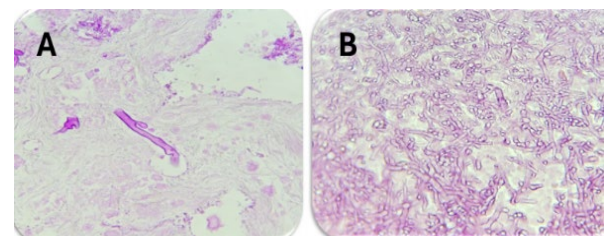


Figure 1: Broad aseptate hyphae of mucormycosis; **A)** with PAS stain (400X), and **B)** with H&E stain (400X).

In Figure 1A, PAS stain (400X) the color of the hyphae appears magenta and in Figure 1B with H & E stain (400X) the color of the hyphae appears pale eosinophilic. A female predominance of 17 cases (56.7%) was observed, while the affected male represents 13 cases (43.3%) with a female-to-male ratio of 1.3:1. The average age of patients was 54.20 ± 13.66

years; the highest frequency was 17 cases (56.7%) in the 51–70-year age group, as shown in Table 1.

Table 1: Age distribution of mucormycosis patients

Age category (year)	Cases n(%)
< 30	2(6.7)
30-50	8(26.7)
51-70	17(56.7)
> 70	3(10)
Total	30(100)

Regarding the clinical presentation, in 12 cases (40%) the clinical presentation was perinasal facial pain and swelling, as shown in Table 2. About the relation with diabetes, the majority, 23 cases (76.7%), were diabetic, while 7 cases (23.3%) were without a history of diabetes. Regarding the site of the biopsy, 17 (56.7%) out of 30 biopsies were from the nose and palate, 11 (36.7%) from the maxilla and maxillary sinus, 1 (3.3%) from the mandibular bone, and 1 (3.3%) from the sphenoid sinus.

Table 2: The clinical presentation of mucormycosis, degree and neutrophilic infiltrates score at X400 power field, degree of tissue necrosis, and severity score distribution in post-COVID-19 patients

Categories	Result n(%)	Score
<i>Clinical presentation</i>		
Perinasal facial pain and swelling	12(40)	-
Nasal pain with stuffiness	7(23.3)	-
Black discoloration of the palate	6(20)	-
Vision loss and periorbital cellulitis	3(10)	-
Mass in the maxillary sinus	1(3.3)	-
Facial numbness	1(3.3)	-
Total	30(100)	-
<i>Severity</i>		
Mild	3(10)	1
Moderate	10(33.3)	2
Sever	17(56.7)	3
Total	30(100)	4
<i>Degree of necrosis</i>		
≥ 50 %	19(63.3)	2
< 50 %	11(36.7)	1
Total	30(100)	-
<i>Grade</i>		
Grade I	2(6.7)	-
Grade II	15(50)	-
Grade III	13(43.3)	-
Total	30(100)	-

All the cases received steroids as a part of COVID-19 treatment. The degree of neutrophilic infiltrate was assessed in tissue fragments having fungal hyphae. Neutrophil infiltration was mild in 3 cases (10%), moderate in 10 cases (33.3%) as seen in Figure 2A which shows fungal hyphae along with clusters of neutrophilic infiltrates, and severe in 17 cases (56.7%) as seen in Figure 2B which shows inflammatory cell infiltrates with neutrophilic predominance. None of the cases showed a complete absence of inflammation around the fungal hyphae (Table 2). All cases showed tissue necrosis as eosinophilic amorphous material with fungal hyphae in one or all necrotic tissues. The degree of tissue necrosis was assessed as the percentage of the area involved; in 19 cases (63.3%), the necrosis was

≥50% of the tissue (score 2), while in 11 cases (36.7%), the necrosis was <50% of the tissue (score 1). The fungal load was high in necrotic areas.

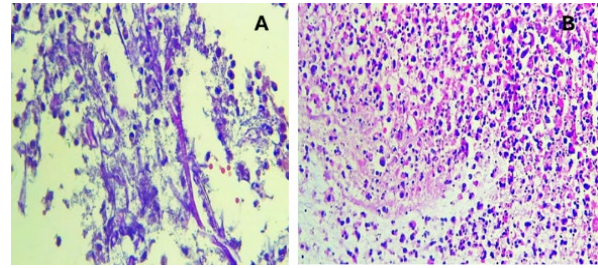


Figure 2 (A-B): Neutrophilic infiltrate associated with fungal hyphae with H & E stain (400X).

The fungal load in areas of necrosis was scored 3 in 22 cases (73.3%), scored 2 in 5 cases (16.7%), scored 1 in 2 cases (6.7%), and scored 4 in 1 case (3.3%) (Table 2). Regarding the angioinvasion, in 12 cases (40%), the angioinvasion was detected in less than 3 vessels in 10 high-power fields and regarded as score 1, while in 18 cases (60%), the angioinvasion was equal to or more than 3 vessels in 10 high-power fields and regarded as score 2 as seen in Figure 3 (A & B), which shows fungal hyphae invade the vessels walls.

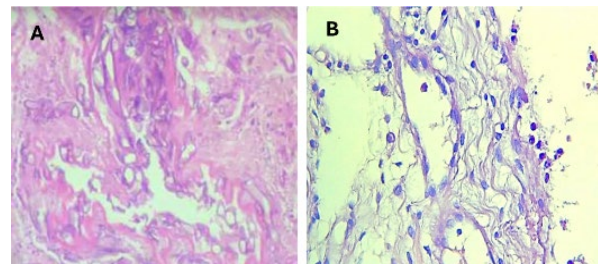


Figure 3 (A-B): Mucormycosis fungal hyphae with angioinvasion with H&E (400X).

According to the histology criterion, the severity score proposed grade I: 4-5, grade II: 6-8, and grade III: 9-11. Grade III infection was detected in 13 cases (43.3%) of the cases, grade II in 15 cases (50%) of the cases, and grade I in 2 cases (6.7%) of the cases (Table 5). The Splendore–Hoeppli phenomenon was detected in one case. Regarding the association between fungal load and neutrophilic infiltrates, the majority of cases with severe neutrophilic infiltrate (14 cases; 82.4%) had a fungal load score of 3, as shown in Table 3.

Table 3: Correlation between fungal load and neutrophilic infiltrate

Fungal load	Neutrophilic infiltrate n(%)			Total
	Mild	Moderate	Sever	
<3	2(6.7)	0(0.0)	0(0.0)	2(6.7)
3-5	0(0.0)	3(30)	2(11.8)	5(16.7)
6-8	1(33.3)	7(70)	14(82.4)	22(73.3)
>8	0(0.0)	0(0.0)	1(5.9)	1(3.3)
Total	3(100)	10(100)	17(100)	30(100)

There is no significant association between fungal load and degree of tissue necrosis; in score 3 fungal load (fungal hyphae in 6-8 high-power fields), there are 15

cases in which the tissue necrosis was more than 50% with a p -value of 0.378, as shown in Table 4.

Table 4: The association between fungal load and degree of tissue necrosis in the sample cases

Fungal load	Degree of tissue necrosis		Total	p -value
	> 50 %	< 50 %		
<3	0	2	2	
3-5	3	2	5	0.871
6-8	15	7	22	0.378
>8	1	0	1	0.456
Total	19	11	30	

There is no significant association between diabetes and the severity score; 11 diabetic patients (47.8%) were grade II with a p -value of 0.679, as shown in Table 5.

Table 5: Correlation between diabetes and the severity score

Grade	Diabetic cases n(%)	p -value
Grade I	2(8.7)	0.437
Grade II	11(47.8)	0.679
Grade III	10(43.5)	0.978

DISCUSSION

Coronavirus disease-associated mucormycosis (CAM) is one of the lethal sequelae of COVID-19 and markedly affects the quality of life [9,10]. The high frequency of mucormycosis may be attributable to various factors, including the existence of a large number of these fungal spores in the hospitals and the environment due to the weather property; the large number of poorly controlled diabetic patients; the huge proportion of undiagnosed diabetic cases; inadequate routine medical check-ups and testing of blood sugar level; newly formed diabetic cases as a result of the "COVID-19 virus" assault on pancreatic islets; and inadequate monitoring of the dosage of the corticosteroids given to treat COVID-19-induced cytokine storm [11,17]. A female predominance in this study (56.7%) was similar to a study done in Mosul and opposed to a study done in India [7]. This may be due to the higher incidence of obesity in females in our community, which in turn increases the risk of diabetes [11]. Regarding age group, the majority (56.7%) was between 50 and 70 years, and the second age group was between 30 and 50 years; in comparison with a study done in India [7], the highest percentage was between 40 and 60 years. This result is attributed to the inadequate prognosis among older age groups infected with COVID-19 and those with other chronic diseases, including cardiovascular disease, elevated blood pressure, and poorly controlled blood sugar levels. In addition, many older adults are given angiotensin-converting enzyme inhibitors and angiotensin II receptor blockers as a treatment for many chronic diseases. According to many researchers, those drugs induce the expression of the angiotensin-converting enzyme-2 receptor, which the SARS-CoV-2 virus utilizes to enter host cells [12]. The most common presentation in the current study is perinasal facial pain

and swelling, accounting for 40% of the total presentation. This result is in agreement with the study done in India [7]. The majority of patients (76%) had diabetes mellitus. This result is similar to a study done in India [7] and Mosul, in which diabetes mellitus was the most common predisposing factor to mucormycosis. The existence of diabetes mellitus is a significant risk factor for mucormycosis, according to a meta-analysis of 851 people with the disease [8]. All patients received steroids as a part of the treatment protocol for COVID-19, which is consistent with the study done in India [7]. Steroids raise random blood sugar in diabetics and may turn pre-diabetics into diabetics. Steroid use also impairs macrophage phagocytic ability, which in turn raises infection risk [13, 18]. The neutrophil infiltrate was severe in 56.7% of the cases, while in a study done in India, the largest percentage of cases (49.4%) had moderate neutrophilic infiltrate [7]. This result may be due to the higher fungal load found in the results of this study. Necrosis of 50% of the tissue (score 2) was detected in 63.3% of the samples. This result is consistent with the study done in India [7]; necrosis is the result of infarction or inflammation. The angioinvasive property of the *Mucor* hyphae causes vessel intima breakdown and thrombus formation, resulting in infarction and hemorrhage [15]. The fungal load in necrotic areas was scored 3 in 73.3%; this result differs from a study done in India, in which, in the highest percentage of cases (38%), the fungal load was scored 2 [7]. This result may be due to the higher age group affected in our study. Patients with a high fungal load often exhibit a condition that promotes fungal growth and are typically immunodeficient [16]. In 60% of cases, angioinvasion was scored 2, which is consistent with a study done in India [7]. Vascular invasion is the common pathological feature of invasive mucormycosis [17]. By adding the above histological parameter, a severity score was proposed; 50% of the cases were Grade II. This result is in agreement with a study done in India [7]. Regarding the correlation between fungal load and degree of tissue necrosis, 83.3% of cases with a high fungal load score (score 3) were associated with necrosis in more than 50%. The p -value was insignificant, which may be attributed to the small sample size. This result is compatible with the one in India [18]. We think that the higher the severity score, the worse the prognosis, but we lack follow-up data on the cases in this study. Nonetheless, this study tries to present insights into an uncommon condition in post-COVID-19-infected individuals for potential prognostic markers on histology.

Study limitations

This study has some limitations; first, the relatively small sample size may limit the statistical result. Second, the retrospective study lacks follow-up, which is why we recommend a larger sample size study with a

prospective design to correlate the results with the prognosis.

Conclusion

There is a high frequency of "mucormycosis in post-COVID-19 patients," in which diabetes and steroid administration were the most important predisposing factors in addition to systemic immune disturbance in COVID-19-infected patients. This study proposes a grading system based on histological parameters (degree of acute inflammation, tissue necrosis, fungal load, and angioinvasion).

Recommendation

After COVID-19, a higher level of suspicion of mucormycosis must be considered, especially in patients who have other risk factors, for early treatment of this fatal fungal infection.

Conflict of interests

The authors declared no conflict of interest.

Funding source

The authors did not receive any source of funds.

Data sharing statement

Supplementary data can be shared with the corresponding author upon reasonable request.

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