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Original article

Microbiological assessment of COVID-19 associated acute invasive fungal rhinosinusitis: a tertiary hospital based study

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ABSTRACT

Background: Acute invasive fungal rhinosinusitis (AIFRS) is an emerging disease on top of SARS CoV-2 virus infection and associated with severe and fatal prognosis especially in the presence of other immunosuppressive conditions. **Aim of study:** Identification of the causative agents of AIFRS in COVID-19 patients and its impact on the survival outcome. **Methods:** This retrospective study was carried out on 70 clinically and radiologically diagnosed AIFRS patients either in concomitant with COVID-19 infection or following recovery. Exudates from the lesions were collected during surgical debridement. Both microbiological and pathological examinations were done to identify the type of the causative fungi followed by assessment of the relation between different type of fungi and the outcome in the affected patients. **Results:** Positive fungal cultures were detected in 54 cases out of 70 where *Mucor* species were the most common isolates (42 patients) and only 12 cases were proven to be *Aspergillus* species. All patients were diabetic and under steroid therapy. Mortality rate was 42.9% and 25% in mucormycosis and aspergillosis respectively. High dose of steroid together with *Mucor* species infection were the most important risk factors in determining the prognosis and the outcome of the infection. **Conclusion:** Microbiological diagnosis together with other methods plays an important role in accurate and rapid diagnosis of AIFRS in association with COVID-19 for proper management and improving the outcome.

Introduction

Coronavirus disease 2019 (COVID-19) is a major wide world public health problem with high mortality and morbidity rates. The lungs are the main target of infection by COVID-19 but its complications may involve different organs. Alteration of cytokine release and T-helper cell responses usually occur with COVID-19 and cause suppression of the immune response with subsequent different forms of bacterial and fungal co-infection. Acute invasive fungal sinusitis is one of these serious infections that may occur with COVID-19 [1-3]. Although acute invasive fungal rhinosinusitis (AIFRS) is rare, it has an aggressive course with high mortality rate [4]. Acute invasive fungal rhinosinusitis occurs on top of an attack of sinusitis that lasts for one month or less followed by detection of fungal hyphae in the sinonasal mucosa, submucosa, vasculature or bone [5,6].

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As a result of down regulation of normal immune response, the fungal spores will settle on the mucosa after being inhaled in the susceptible host with subsequent tissue penetration. Soon after invasion, thrombosis, necrosis and infarction usually take place followed by involvement of the surrounding tissues commonly the bones [7].

Usually, the early manifestations of AIFRS are nonspecific in the form of fever, nasal obstruction, and rhinorrhea, while in advanced cases visual impairment and other neurological insults occur. Sometimes, the orbit is involved and may end up to orbital exenteration. By clinical examination, pale, red or black necrosis is detected in the nasal cavity. As the presence of black eschar is an important finding in AIFRS, it is usually discovered late after invasion, tissue necrosis and vascular thrombosis [8].

Aspergillus species and Zygomycetes are the most common causative agents of AIFRS. Nowadays, mucor species are being isolated from AIFRS patients which shed the light to the term mucormycosis [9].

As immunosuppression is the most common risk factor for AIFRS, the causative pathogen usually differs according to the underlying condition. Zygomycetes are frequently isolated from poorly controlled diabetic patients with ketoacidosis due to acidic environment and high glucose levels in these patients [7]. In contrast, *Aspergillus* species were isolated from patients with hematological malignancies, neutropenia or those under chemotherapy [10].

In small subsets of patients with renal failure or under deferoxamine treatment for iron chelation, *Rhizopus* species were isolated as they depend on deferoxamine as a source of extra iron for their growth [11].

The aim of the present study is to detect different types of fungi involved in AIFRS in COVID-19 patients by microbiological examination and the role of fungal species and other co-morbidities in determining the final outcome in these cases.

Materials and Methods

Study design

A retrospective study that was conducted on 70 patients with AIFRS recently diagnosed with COVID-19, cases were recruited from January 2021 to September 2021. All cases were referred from Otorhinolaryngology department, Faculty of Medicine, Tanta University, Egypt.

The study was approved by the Ethics and Research Committee, Faculty of Medicine, Tanta University, Egypt. All procedures were carried out under the tenets of the Helsinki Declaration. Written informed consent was obtained from all patients or their relatives. A code number was added to each sample for adequate provision to maintain privacy of participants and confidentiality of data.

All patients with clinically and radiologically diagnosed AIFRS on top of previous or recent COVID-19 infection by positive reverse transcriptase polymerase chain reaction test (RT-PCR) were included in the present study. Patients with AIFRS were presented by one or more of the following presentations like visual loss, diplopia, proptosis, sinusitis, headache, facial cellulitis, blackish eschars over the bridge of nose/palate, fever of long duration and jaw involvement. Cases with bacterial infection and cases with negative fungal cultures were excluded from this study. Thorough examination including history taking (history of coexisting systemic diseases especially diabetes mellitus and steroid intake and its dose during COVID-19 infection), laboratory investigations were performed including complete blood counts, blood glucose level, liver and renal function tests, along with glycosylated hemoglobin (HbA1C), C reactive protein (CRP), D- dimer and serum ferritin.

Diagnosis of AIFRS depended on clinically detected sinonasal infection confirmed by routine endoscopic examination and variable imaging modalities including CT, MRI or both of them. All confirmed cases were subjected to surgical debridement to remove all necrotic tissue until healthy sites were detected followed by administration of antifungal drugs in the form of intravenous infusions of amphotericin B.

Collection and processing of samples

During surgical debridement samples were collected either by scraping or collection of the exudate from the nasal cavity and/or paranasal sinuses, hard palatal lesions, sinus aspirate, biopsy from nasal polyps, sinus mucosa and exenterated orbit. The samples were divided into two separate sterile containers, one with normal saline that was sent immediately for microbiological examination in Microbiology and Immunology Department and the other one with 10% formalin prepared for

histopathological evaluation at Pathology Department in order to detect the causative fungal agents.

Microbiological diagnosis

Direct examination: First crushed smear from area with black coloration was subjected to direct microscopic examination after addition of 10% KOH and examined with low and high power objective lenses (10x and 40x). Aseptate hyphae with wide angle were suspected to be *Mucorales* while narrow angled septate branched hyphae was considered *Aspergillus* and both of them were confirmed by culture [12].

Culture: As detection of fungi from patient samples is the most conclusive method for diagnosis of fungal infection; fungal cultures were done for all samples. Sabouraud Dextrose Agar (SDA) was used after addition of chloramphenicol to prevent bacterial contamination. Two plates were used, in the first plate; cycloheximide was added to prevent growth of environmental fungi and in the other plate cycloheximide was not used to allow opportunistic fungi to grow. The plates were incubated at 25-30˚c at humidified environment for 3 weeks before being reported as a sterile sample.

During the first week, the plates were inspected daily, later on; the plates were examined three times per week. The colonies were identified grossly by their morphology, color and texture. Subsequently, microscopic examination to detect different fungal elements (yeast, spores and hyphae) was done using lactophenol cotton blue preparations (LPCB) [13].

Histopathological diagnosis

Samples were subjected to histopathological confirmation using Harris hematoxylin and eosin (H&E) stain. *Mucor* species were diagnosed when aseptate hyphae irregularly branched or at acute angles, however *Aspergillus* was diagnosed when thin septate hyphae could be detected [14].

Statistical analysis

Data analysis was done by IBM SPSS Statistics for Windows, Version 25.0. (IBM Corp, 2017). Qualitative data were expressed as number and percentage of total. Continuous data were expressed as mean ± SD. Chi-square test was used to compare qualitative data between 2 groups. Fischer exact test was used to compare qualitative data when expected count is less than 5, while continuous data were compared by student t test. Hazard ratio and its 95 % confidence interval were estimated using Cox regression analysis. p value $\leq$ 0.05 is considered statistically significant.

Results

Seventy cases of clinically and radiologically diagnosed AIFRS associated with COVID-19 infection were admitted in Tanta University Hospitals as shown in **figure (1)**, from these, the type of causative fungus was detected in only 54 cases by microbiological examination. The demographic and clinical data including age, sex, associated comorbidities and risk factors are illustrated in **table (1)**. *Mucor* species were the most predominant pathogen reported in the present study representing (77.8%). However, *Aspergillus* species were isolated only in (22.2%) as shown in **figure 2 (A, B, C and D)** and **figure 3 (A,B)**. Furthermore, most of AIFRS patients were detected after previous COVID-19 infection (63%) while (37%) developed fungal infection in concomitant with COVID-19 infection.

Diabetes mellitus (DM) with glycosylated HB level more than 8% and high dose of corticosteroid intake were the most common predisposing factors to AIFRS in patients with COVID-19 infection in this study.

Regarding the complications and the extension of fungal infection, it is observed that visual loss, cavernous sinus thrombosis, orbital invasion with subsequent exenteration and cranial nerve involvement were the most common complications occurred on top of AIFRS representing (70.4%, 40.7%, 24% and 25.9%) respectively. Along the 6 months period of our study, death occurred in (38.9%) of cases and this is shown in **table (2)**.

Mucormycosis was significantly associated with elevated level of glycosylated HB more than 8% confirming the role of immunosuppression in occurrence of *Mucor* spp. infections. Moreover, mucormycosis resulted in more complications and poorer prognosis including visual loss (73.8%), cavernous sinus thrombosis (45.2%), orbital invasion (26.2%) and cranial nerve involvement (21.4%). Death was reported in (42.9%) of cases with mucormycosis as shown in **table (3)**.

Regarding the relation between different hazards and the prognosis of AIFRS, the high dose of steroid intake was associated with higher prevalence of death. This result was statistically significant (p value 0.03). On the other hand,

infection with *Mucor* spp was associated with higher death rate (18 cases) but this result was statistically non significant (*p* value 0.07). As regards other clinical and sociodemographic factors, the results were statistically non significant including age, sex, comorbidities, glycosylated HB and other complications as illustrated in **table (4)**.

Table 1. Clinical, sociodemographic data, associated comorbidities, causative agents and risk factors of AIFRS patients with COVID-19 infection.

Variable	**N (%)** **N= 54**
Age Mean ± SD	48.1 ± 16.5
<60 years	38(70.4)
≥ 60 years	16(29.6)
Sex	
Male	30(55.6)
Female	24(44.4)
Comorbidities	
No	13(24.1)
Yes *	41(75.9)
Hypertension	27(50)
Renal	10(18.5)
Hepatic	2(3.7)
COPD	14(25.9)
COVID-19 PCR	
Recent positive	20(37)
Previous positive	34(63)
Glycosylated HB% Mean ± SD	10.1 ± 2.1
≤ 8	14(25.9)
>8	40(74.1)
Steroid dose (mg)	
20 or 30	26(48.1)
40	28(51.9)
Systemic antifungal	
No	4(7.4)
Yes	50(92.6)
Pathogen	
Mucor spp	42(77.8)
Aspergillus spp	12(22.2)

*: data are not mutually exclusive.

Table 2. Complications, clinical extension and outcomes for the studied AIFRS patients.

Complications	**N (%)** **N= 54**
Visual loss	38(70.4)
Cavernous sinus thrombosis	22(40.7)
Orbital invasion	13(24.1)
Cranial nerve involvement	14(25.9)
Outcome	
Died	21(38.9)
Survived	33(61.1)

Table 3. Sociodemographic and clinical profile of mucormycosis and aspergillosis patients

Variables	Mucormycosis N=42 (%)	Aspergillosis N= 12 (%)	*p* value
Age			
<60 years	29 (69)	9 (75)	0.9
≥ 60 years	13 (31)	3 (25)	
Sex			
Male	25 (59.5)	5 (41.7)	0.3
Female	17 (40.5)	7 (58.3)	
Comorbidities			
No	9 (21.4)	4 (33.3)	0.5
Yes	33 (78.6)	8 (66.7)	
Hypertension	25 (59.5)	2 (16.7)	0.009
Renal	6 (14.3)	4 (33.3)	0.2
Hepatic	1 (2.4)	1 (8.3)	0.4
COPD	11(26.2)	3 (25)	0.9
COVID-19 PCR			
Recent positive	15 (35.7)	5 (41.7)	0.7
Previous positive	27 (64.3)	7 (58.3)	
Glycosylated HB%			
≤ 8	8 (19)	6 (50)	0.05
>8	34 (81)	6 (50)	
Systemic antifungal	39 (92.9)	11 (91.7)	0.9
Steroid dose (mg)			
20 or 30	20 (47.6)	6 (50)	0.9
40	22 (52.4)	6 (50)	
Visual loss			
No	11 (26.2)	5 (41.7)	0.3
Yes	31 (73.8)	7 (58.3)	
Cavernous sinus thrombosis			
No	23 (54.8)	9 (75)	0.3
Yes	19 (45.2)	3 (25)	
Orbital invasion			
No	31 (73.8)	10 (83.3)	0.7
Yes	11 (26.2)	2 (16.7)	
Cranial nerve involvement			
No	33 (78.6)	7 (58.3)	
Yes	9 (21.4)	5 (41.7)	0.3

Outcomes			
Died	18 (42.9%)	3 (25%)	0.3
Survived			
	24 (57.1%)	9 (75%)	

Table 4. Univariate Cox proportional hazards regression of the association between invasive fungal sinusitis survival and clinical and sociodemographic factors.

Variable	Hazard ratio (95 % CI)	*p* value
Age <60 years	1.6(0.5-4.8)	0.4
Sex (Female)	1.1(0.5-2.7)	0.8
Comorbidities	1.1(0.3-3.2)	0.9
COVID-19 PCR (Previous positive within 2 months)	1.8(0.7-4.9)	0.2
Glycosylated HB**%** > 8	1.5(0.5-4.1)	0.5
Pathogen Mucourmycosis	3.6(0.8-15.6)	0.07
Systemic antifungal	1.2(0.2-9.1)	0.9
Steroid dose (40mg)	2.7(1.1-7.1)	0.03
Visual loss	2.2(0.5-9.9)	0.3
Cavernous sinus thrombosis	0.9(0.4-2.2)	0.8
Orbital invasion	0.7(0.2-1.9)	0.4
Cranial nerve involvement	1.8(0.7-4.8)	0.3

Figure 1. A 72 years old Male patient with uncontrolled diabetes mellitus after 3 weeks of COVID-19 infection **(A)** intraoral photograph showing massive palatal necrosis **(B)** multiple coronal cuts of a CT scan of paranasal sinuses showing opacification of ethmoid, frontal and maxillary sinuses **(C)** multiple coronal cuts of an MRI with contrast on paranasal sinuses showing mixed intensities of maxillary and ethmoid sinuses **(D)** intraoperative view after total maxillectomy **(E)** resection specimen showing evident necrosis of the maxilla.

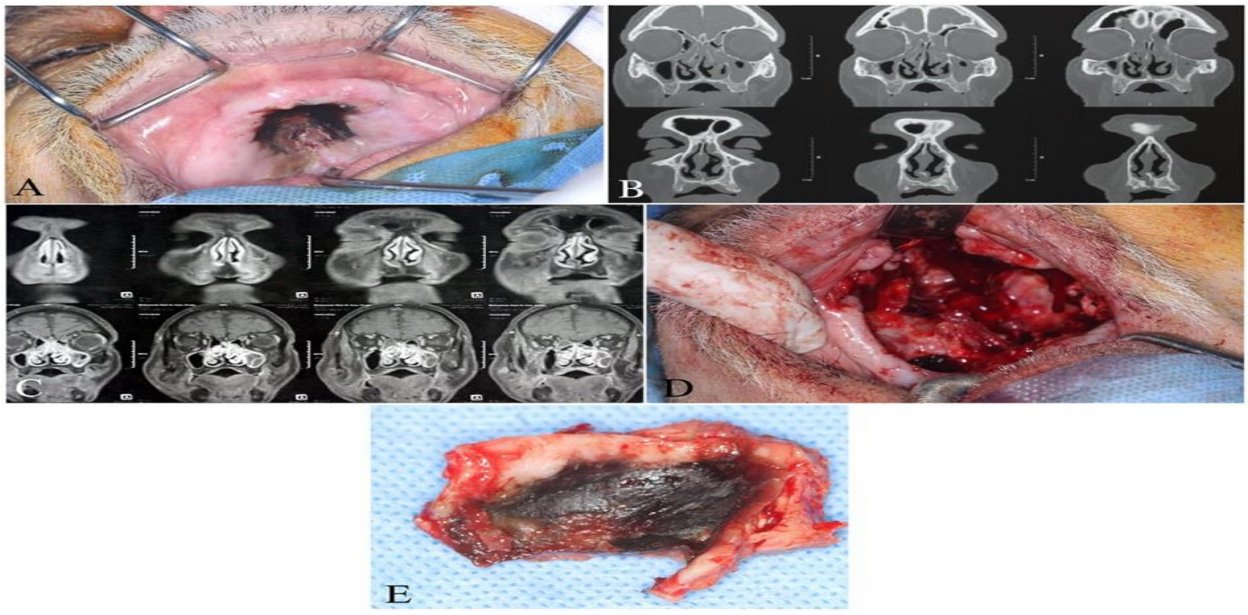


Figure 2. (A): *Zygomyces* spp on SDA showing showing wooly gray- white colored colonies with black dots. **(B):** *Aspergillus* spp on SDA showing granular dark greenish colonies with white margin. **(C):** *Mucor* spp with lactophenol cotton blue staining showing thick, non septate hyphae with conidia (magnification, ×40). **(D):** *Aspegillus* spp with LPCB staining showing septate hyphae with chains of conidia (magnification, ×40).

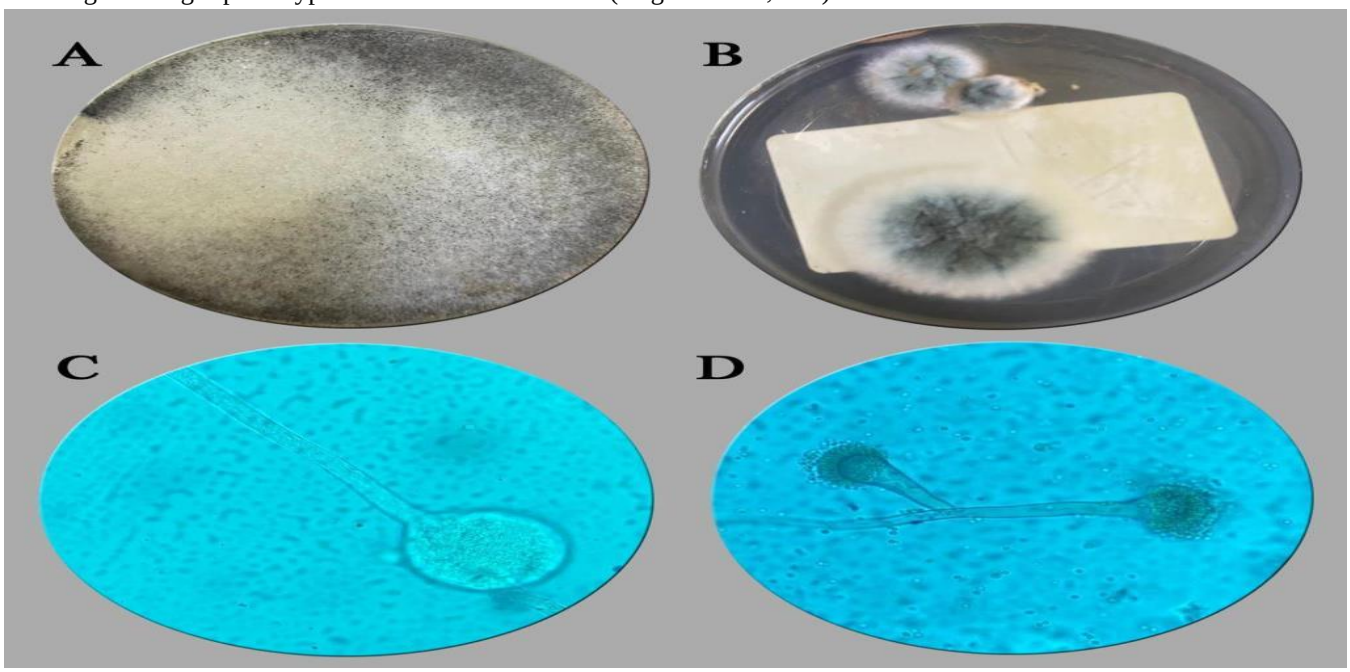
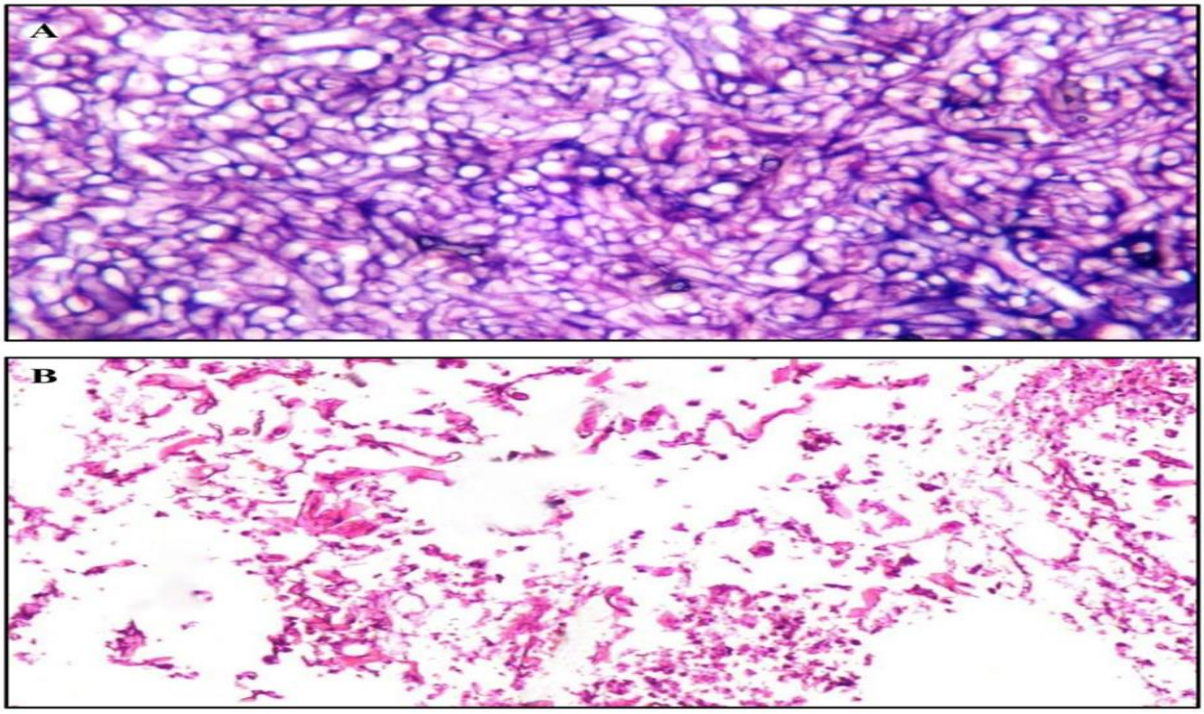


Figure 3. *Aspergillus* ball in the nasal cavity (H&E×200). **(B):** Mucormycosis hyphae within the nasal cavity (H&E×200).



Discussion

Acute invasive fungal rhinosinusitis is an aggressive infection notably found in immunocompromised patients with subsequent serious morbidity and high mortality. COVID-19 patients are at an increased risk of secondary fungal infections including AIFRS most probably due to their immunological impairment [15].

Different fungal species have been reported as causative agents in AIFRS, particularly, *Rhizopus*, *Mucor*, *Rhizomucor*, and *Aspergillus* [16], however, other fungi were reported in other studies [17].

In the present study, the mean age of AIFRS patients was 48.1 and (70.4%) of patients were less than 60 years old with higher prevalence of AIFRS in male patients (55.6%) which was in concordance with **Elmorsy et al.** [13] who detected that the mean age was 45.7 and male to female ratio was 2:1. Also males were more predominant in **Montone et al.** [18] study but mean age was 54 years.

The most important predisposing factors for COVID-19 associated AIFRS are uncontrolled diabetes mellitus, the irrational use of corticosteroids leading to uncontrolled blood glucose level, extensive use of broad-spectrum antibiotics and the presence of other co-morbidities [19].

All COVID-19 associated AIFRS patients in the present study were diabetics. Uncontrolled diabetes mellitus with poor glycemic control was detected in (74.1%) with HbA1c level above 8%. This is quite similar **Ismaiel et al.** [20] who reported DM in (44.4%) of post-COVID-19 AIFRS. In addition, a study conducted by **Bakhshaee et al.** [16] reported DM as the most common underlying disorder in AIFRS patients (50%).

Corticosteroids have been used in patients of COVID-19 infection; they have been proved to be life-saving by reducing cytokine storm, but the misuse of these corticosteroids has been reported as one of the most common predisposing factors for invasive fungal infection in COVID patients [21]. Regarding our study**,** all patients had a history of corticosteroids intake during the course of their COVID- 19 illness. Twenty-eight cases (51.9%) received higher steroid dose 40 mg while 26 cases (48.1 %) received less steroid dose 20 to 30 mg. The use of these corticosteroids may exacerbate acute invasive fungal infections by suppressing the immune system, this agrees with **Moorthy et al.** [21] that observed a significant correlation between post COVID-19 AIFRS and steroid intake.

Furthermore, **Mehta and Pandey** [22] concluded that widespread use of steroids and broad-spectrum antibiotics as part of the treatment of COVID-19 infection is associated with secondary invasive fungal infections. Furthermore, **Turner et al**. [10], **Kursun et al.** [23] and **Kermani et al**. [24] observed the most common risk factors for invasive fungal sinusitis in non COVID-19 patients were the diabetes mellitus followed by hematological malignancies and chronic renal insufficiency.

As regarding the time of occurrence of AIFRS, this study detected that (63%) of patients showed AIFRS after COVID -19 recovery while (37%) of patients had AIFRS in concomitant with COVID-19 infection. Similar to our study, **Sen et al.** [25] reported that AIFRS developed within 14 days after COVID-19 diagnosis in (56%) of patients and only (44%) of patients developed AIFRS after COVID-19 recovery. Also **Abdelsamie et al.** [26] and **Sharma et al.** [27] demonstrated that AIFRS usually occurs after COVID-19 recovery. Furthermore, **Baghel et al.** [28] found that only (8.06%) developed symptoms after COVID-19 recovery.

During this study, fungal culture results were positive only in 54 (77.1%) out of 70 samples. *Mucor* species were the most common findings representing 77.8%; followed by *Aspergillus* species (22.2%). Negative fungal cultures were obtained in 16 samples and this may be due to the fragile nature of fungal hyphae that led to their damage during sample homogenization [29].

These findings are in coincident with those detected by **El-Kholy et al.** [30] who demonstrated *Mucor* and *Aspergillus* species in (77.8%) and (30.6%) of samples respectively. Similar to our study, **Moorthy et al.** [21] reported Mucormycosis in 16 patients, aspergillosis in one patient and mixed infection in one patient. Besides, the study done by **Sebastian et al.** [15] revealed three cases of COVID-19 associated AIFRS in which one caused by *Aspergillus* and the others caused by Zygomycosis.

In non-COVID AIFRS patients, *Mucor* species were the most common isolates as reported by **Kursun et al**. [31] **Bellazreg et al**. [32] and **Kaur et al**. [33] However, **Montone et al.** [18] reported *Aspergillus* spp. were the most common causative agents (49%) followed by *Rhizopus* spp.

(33%). This agrees with another study conducted by **Elmorsy et al**. [13] who found higher predilection of *Aspergillus* species (53.3%) and *Rhizopus* (46.7%).

Microbiological examination is an important step for the diagnosis of AIFRS in detecting the etiologic agents and proper choice of appropriate antifungal drug in order to achieve the best outcomes [34,35].

Beside the underlying medical condition, the extent of fungal infection is another important factor in the prognosis. According to the present study findings, the most common complications occurred on top of AIFRS in COVID-19 patients were visual loss (70.4%), orbital invasion with subsequent orbital exenteration (24%), cavernous sinus thrombosis (40.7%) and cranial nerve involvement (25.9%). In spite of antifungal therapy and surgical debridement, mortality was detected in (38.9%) of cases along the follow up. This agrees with **El-Kholy et al.** [30] in which ophthalmoplegia and visual loss were observed in (63.9%) of their cases and cerebral extension in ten patients while cavernous sinus thrombosis in five patients while thirteen patients (36.11%) died. Moreover, **Moorthy et al**. [21] listed loss of vision in 12 out of the 18 patients and 7 of them underwent orbital exenteration while 9 patients showed intracranial involvement and death occurred in 6 patients.

Regarding our study, *Mucor* spp were isolated from (77.8%) of cases. Patients with mucormycosis showed more complications including visual loss (73.8%), cavernous sinus thrombosis (45.2%), orbital invasion (26.2%) and cranial nerve involvement (21.4%) with mortality rate (42.9%). This is in contrast to the findings of **Sharma et al**. [27] study that was conducted on 23 cases of COVID-19-associated mucormycosis, it reported orbital affection in (43.47%) and intracranial extension in (8.69%) with mortality rate (8.7%) of cases.

During COVID-19 pandemic, similar studies were performed by **Sarkar et al.** [36] **Satish et al.** [37] **Mohammadi et al**. [38] and **Awal et al.** [39] who described case reports of mucormycosis in COVID-19 patients.

Acute invasive fungal rhinosinusitis is a fatal disease; death may be due to the extent of fungal invasion, the complication of underlying COVID-19 infection or the side effects of antifungal drugs, hence, the detection of different hazards which are associated with high mortality rate was very important. This study detected that high dose of steroid intake (40 mg) was a strong hazard element in mortality rate in our patients and the result was statistically significant (*p* value 0.03). Also *Mucor* species infection was considered an important hazard in determining the outcome in these patients (*p* value 0.07). However, intracranial affection increased the death rate to (90%) as reported by **Deutsch et al.** [40]. Also, **Gibertoni et al.** [41] detected that chronic kidney disease associated with significantly increased mortality rate (44.6%) compared to (4.7%) in those without COVID-19 infection. As uncontrolled diabetes was an important risk factor for the development of AIFRS in association with COVID-19 patients, **Zirpe et al.** [42] found that it did not show any significant difference between survivors and non-survivors.

Limitations of our study include that all cases were detected from only one centre, small numbers of cases, short duration of follow up, all underlying risk factors were not available. In addition, fungal serological tests, molecular techniques and antifungal susceptibility tests were not done.

Conclusion

This study may increase the awareness about AIFRS and its associated risk factors especially diabetes and steroid intake. It also highlights the importance of microbiological diagnosis together with other methods in detecting different types of fungi causing AIFRS in COVID-19 patients for proper diagnosis and early treatment to improve the prognosis and avoid fatal complications.

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Declaration of patient consent

The authors certify that they have obtained all appropriate patient consent forms to publish their clinical data in this journal without showing their name or initials.

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