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Acute Invasive Fungal Rhino-sinusitis: Clinical, Microbiological and Pathological Diagnosis

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Authors' contributions

This work was carried out in collaboration between all authors. Authors SE, SAR and NTAEK designed the study, wrote the protocol and wrote the first draft of the manuscript. Authors NTAEK, SMEA, NSG and HS managed the analyses of the study and managed the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

Background: Acute invasive fungal rhinosinusitis (AIFRS) is a challenging disorder that is diagnosed frequently in immunocompromised patients with very rapid progression.
Objective: to estimate the burden of AIFRS in Mansoura University Hospitals and to assess the mycological and clinicopathological profile of the disease.
Methods: Specimens were subjected to microbiological and histopathological examinations. Data about demographic characters, underlying diseases, presenting symptoms, signs, surgical interventions, and complications were collected.
Results: Twenty-two patients were diagnosed as AIFRS. Patients were 15 males and 7 females with age ranged from 14 to 55 years. The disease was prevalent among immunocompromised patients (100%). Hematological malignancies were the most common underlying conditions

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(68.18%), The remaining patients had disorders associated with immunosuppression as solid organ transplantation (18.18%) and non-insulin-dependent diabetes mellitus (13.64%). Culture results were positive only in 15 patients (68.18%). *Aspergillus* species (8/15) were the most common isolated organisms followed by *Zygomycetes* (7/15).
Conclusions: AIFRS continues to present a challenge to the otolaryngologist who must be highly suspicious at risk patient populations. Histopathological examination had high sensitivity. The isolated organisms were *Aspergillus* species and *Rhizopus*.

Keywords: Sinusitis; fungal sinusitis; immunocompromised hosts.

1. INTRODUCTION

Acute invasive fungal rhinosinusitis (AIFRS) is serious lethal form of fungal sinusitis that frequently affects severely immunocompromised patients. It occurs in patients with hematologic malignancies, aplastic anemia and uncontrolled diabetes mellitus; patients under chemotherapy, patients with organ/ bone-marrow transplantation are exclusively susceptible [1].

The illness has an aggressive course, as the fungus rapidly grows through sinus tissue and bone and spreads into the nearby areas of the brain and eye through bone erosion; it also tends to hematogeneously spread to multiple organs [2].

Early presenting manifestations are often nonspecific and may include fever, nasal obstruction, and rhinorrhea. Symptoms, such as vision changes, paresthesias, and other cranial neuropathies, often represent late findings in patients with more advanced disease. Nasal cavity examination reveals ischemic nasal mucosa; pale to red to black necrotic areas encompassing the turbinates or septum. Although the emergence of a black eschar is considered almost pathognomonic for AIFRS, it is usually a late discovery due to vascular thrombosis and tissue necrosis [3].

Multiple fungal organisms have been reported as causative agents in AIFRS. Members of *Aspergillus* species and *Mucoraceae* genera are the most frequent causative agents, but other kinds of fungi may also act as the etiologic agents [4]. The spores are settled on the mucosa of a susceptible host, then penetrate into the tissue, permitting angioinvasion to happen. The invasion leads to thrombosis, secondary ischemic infarction and hemorrhagic necrosis, where the fungus flourishes in this milieu and spreads lengthwise-injured vessels [5].

Because of its invasiveness potential, proper, fast and correct diagnosis based on strong criteria, will help to begin the treatment as fast as possible for better prognosis of this disease [6]. Diagnosis of AIFRS is complicated. It can be achieved directly or indirectly. It should be based on clinical examination and investigation, of which the most important is the histopathological proof of fungal presence and isolation or identification of fungi microbiologically [7].

Histopathological examination of tissues and the mucus is done for detection of fungal elements, inflammatory cells and any specific reactions. It can be made using: Hematoxylin- Eosin (HE), Periodic Acid-Schiff (PAS) or Gomori Methionine Silver (GMS) impregnation that can distinguish between fungal morphology. It is fast and relatively inexpensive techniques which often bring positive diagnosis or, at least, raise the suspicion of diagnosis [8].

Mycological examination is also an essential step in the analysis. It can be performed by microscopy, culture, or non-cultural techniques. The utility of immunofluorescence techniques as well as antigen and antibody detection and molecular methods in the diagnosis of fungal infections was strongly confirmed by many studies [9].

Combined aggressive surgical and medical therapy are required. Repeated surgery may be compulsory to eliminate all devitalized tissue. Debridement of all dead tissues is essential to prevent proliferation of the fungus in the necrotic tissue. Anti-fungal agents and drugs that help to restore the immune status of the patient are crucial to improve the survival but the most important is the treatment of the underlying etiology [10].

The objective of this study was to estimate the burden of AIFRS in Mansoura University Hospitals and to assess the mycological and clinicopathological profile of the disease.

2. SUBJECTS AND METHODS

2.1 Study Design and Setting

A retrospective study was done from January 2014 to May 2016. All cases were referred from ICUs in Mansoura University Hospitals and Specialized Medical Centers.

2.2 Case Definition

Cases were patients with AIFRS. Patient with positive fungal culture, or positive histopathological smear, or both together with the onset of symptoms less than one month was considered a case of AIFRS [3].

2.3 Data Collection

Cases were identified by ENT surgeons through clinical examinations of suspected patients. A collection of data included demographic data, underlying diseases, presenting symptoms, nasal tissue cultures, nasal tissue pathological examinations, radiological findings, surgical interventions, morbidity, and mortality.

Surgical, pathological and histological slides reports were revised. Fungal cultures results were correlated with the histopathological findings.

2.3.1 Collection and processing of samples

Sinus aspirate or tissue biopsy from nasal polyps and sinus mucosa were collected during standard paranasal surgical treatment. They were all received in two sterile containers, one container with normal saline for microbiological study and the other with 10% formalin for histopathological study. Tissue samples were cut into small pieces using sterile scissors.

2.3.2 Histopathological diagnosis

All specimens were subjected to histopathological examination [11] using Harris hematoxylin and eosin (H&E), modified Giemsa stain (GMS) and Periodic acid–Schiff (PAS) stains. Masson Fontana (MF) stain was done wherever necessary. Thin slender septate dichotomously branching hyphae at acute angle were classified as probable *Aspergillus* species. Broad, hyaline aseptate hyphae branching irregularly or at right angles were classified as mucormycetes.

2.4 Microbiological Diagnosis

Each sample was subjected to direct light microscopy using 10% potassium hydroxide (KOH) and fluorescence microscopy after digestion with a mixture of KOH and calcofluor white (Becton Dickinson, USA). Slender septate hyphae with acute angle branching were conveyed as *Aspergillus* species; broad aseptate hyphae with right-angled branching as *Zygomycetes*. The remaining portions of the samples were homogenized and inoculated on two sets of Sabouraud dextrose agar (SDA) tubes; one set containing chloramphenicol and cycloheximide and the other set without cycloheximide. One tube of each set was incubated at 37°C and the other set at 22°C. The cultures were examined daily for first week and twice a week for next three weeks before reporting as sterile. Each sample was cultured triple and the result was considered positive only when all tubes showed the same fungal growth [12]. The resulting fungal isolates were identified macroscopically by colonial morphology and microscopically by lactophenol cotton blue preparations. Negative culture documented after 4 weeks of incubation. Only samples that were positive by both microscopy and culture were included in this analysis.

Antifungal susceptibility testing for filamentous isolates was done according to CLSI guidelines [13]. The sensitivity was tested to amphotericin B, voriconazole, itraconazole, ketoconazole and posaconazole.

2.5 Statistical Analysis

Statistical analysis was done using SPSS software (version 17.0, SPSS Inc., Chicago, IL, USA). The correlation between two variables was evaluated using chi-square, Fisher's exact and Student t tests. For all statistical tests, $p \leq 0.05$ was considered to indicate a significant difference.

3. RESULTS

Between January 2014 and May 2016, twenty-two patients were enrolled in the study; they were classified and diagnosed as AIFRS. Their ages ranged from 14 to 55 years (mean age 45.7 years ± S.D. 13.9). Patients included in the study were 15 males and 7 females with a ratio of 2:1 (Table 1).

Our patients showed variant immunocompromised conditions. Fifteen patients had an underlying hematologic malignancy (68.18%), of them 8 (36.36%) patients had acute leukemia, 5 (22.72%) patients had non-Hodgkin lymphoma and 2 (9.09%) patients had multiple myeloma. The remainder had immunosuppression in the form of history of solid organ transplantation (4 patients; 18.18%) (2 liver and 2 kidney transplant), and non-insulin-dependent diabetes mellitus (3 patients, 13.64%) (Fig. 1).

Patients with AIFRS showed wide range of symptoms. At the time of diagnosis, fever was the predominant presenting symptom (11 patients, 50%) followed by facial pain (10 patients, 45.45%), purulent rhinorrhea (9 patients, 40.90%), watery rhinorrhea (7 patients, 31.81%), headache (6 patients, 27.27%), visual loss (5 patients, 22.72%), diplopia (5 patients, 22.72%), and maxillary toothache (5 patients, 22.7%). Other symptoms as stuffiness (2 patients, 9.09%), postnasal drip (2 patients, 9.09%), cough (1 patient, 4.54%), change of conscious (1 patient, 4.54%), and hyposmia (1 patient, 4.54%), were less common presentations (Fig. 2).

On nasal endoscopy, the most common findings were black crustations and mucosal necrosis (Figs.3,5,6,7). They were seen in all patients (22 patients, 100%). Other findings seen, were pus in middle meatus (11 patients, 50%) and perforation of septum (5 patients, 22.72%).

Histopathologically, all patients exhibited necrotic sinonasal mucosa with the presence of angioinvasive fungal forms. Aseptate fungal hyphae were present in 9 samples (40.91%) indicating positive results for *Zygomycetes*, while 13 samples (59.09%) showed septate dichotomously branching hyphae indicating positive results for *Aspergillus* spp. (Table 2, Fig. 8).

Fungal culture results were positive only in 15 (68.18%) patients with most common isolated organisms were *Aspergillus* spp. (8 patients) followed by *Rhizopus* spp. (7 patients). Seven patients had negative fungal cultures.

By broth microdilution method, all *Aspergillus* isolates were sensitive to voriconazole and posaconazole while all *Rhizopus* isolates showed sensitivities to both amphotericin B and posaconazole (Table 3).

The AIFRS patients presented with many complications. Preseptal cellulitis was the most common, developed in 10 patients (45.45%), while orbital cellulitis and orbital abscess developed in 5 patients (22.72%) separately. Cavernous sinus thrombosis and intracranial involvement were also detected in 4 patients (18.18%) for each (Fig. 4).

Table 1. Different predisposing factors associated with *Aspergillus and Rhizopus* species infection in paranasal sinusitis

	***Aspergillus* positive (8)**	***Rhizopus* positive (7)**	**Odds ratio**	**$\chi 2$**	***P* value**
Gender (male: female)	5:3	5:2	0.67	0.1339,1	0.714
Smoking	5	5	0.67	0.1339,1	0.714
Acute leukemia (8)	3	3	1.25	0.04464,1	0.832
Non H. Lymphoma (5)	2	2	0.833	0.02435,1	0.876
Multiple Myeloma (2)	1	0	2.64	0.8296,1	0.362
Kidney transplant (2)	1	0	2.64	0.8296,1	0.362
Liver transplant (2)	0	2	0.129	2.637,1	0.104
DM (3)	1	0	2.64	0.8296,1	0.362

Table 2. Differences between culture and histopathology data regarding diagnosis of both *Aspergillus* and *Rhizopus* species

	Histopathology	**Culture**	**Odds ratio**	**$\chi 2$**	**P value**
Aspergillus	13	8	0.3956	2.277,1	0.13
Rhizopus	9	7	1.484	0.3929,1	0.53

Table 3. Antifungal susceptibility testing of *Aspergillus* and *Rhizopus* species

Antifungal	*Aspergillus* (n = 8)		*Rhizopus* (n = 7)	
	Sensitive	Resistant	Sensitive	Resistant
Amphotericin B	6	2	7	-
Itraconazole	6	2	6	1
Voriconazole	8	-	4	3
Ketoconazole	7	1	6	1
Posaconazole	8	-	7	-

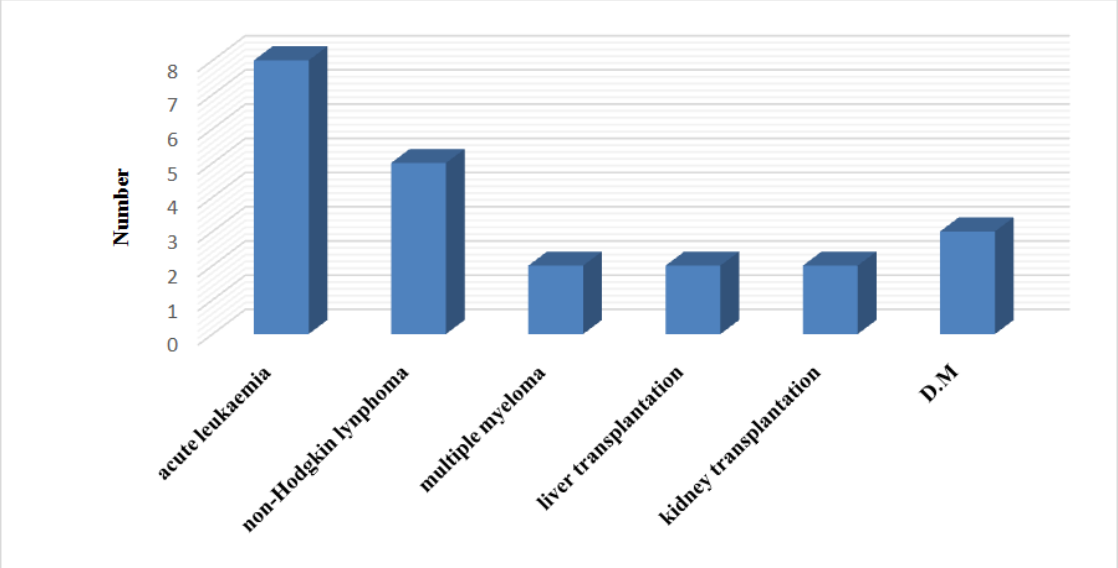

Fig. 1. Systemic diseases associated with AIFRS

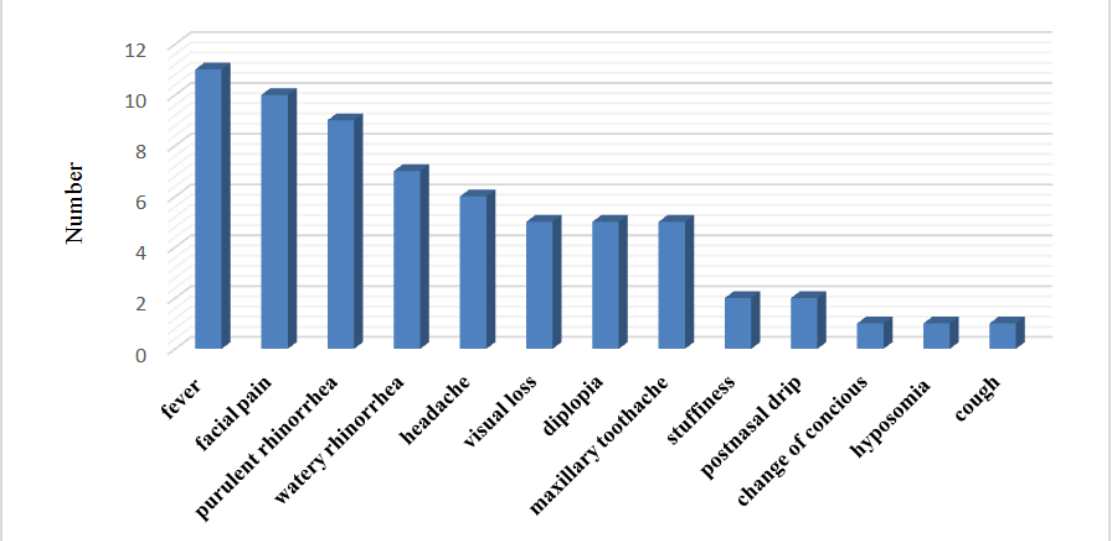

Fig. 2. Presenting symptoms in patients with AIFRS

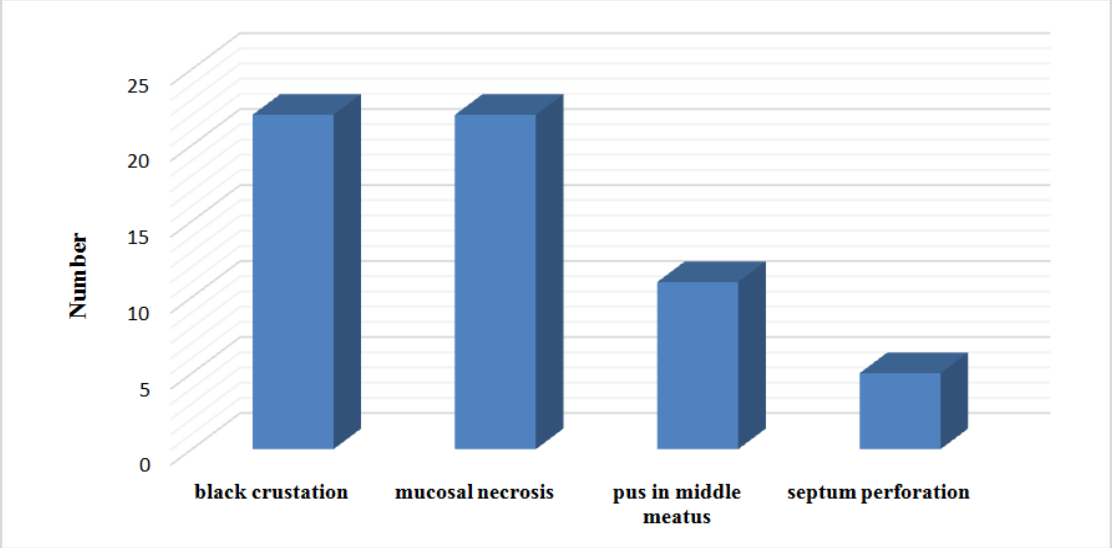

Fig. 3. Endoscopy finding in patients with AIFRS.

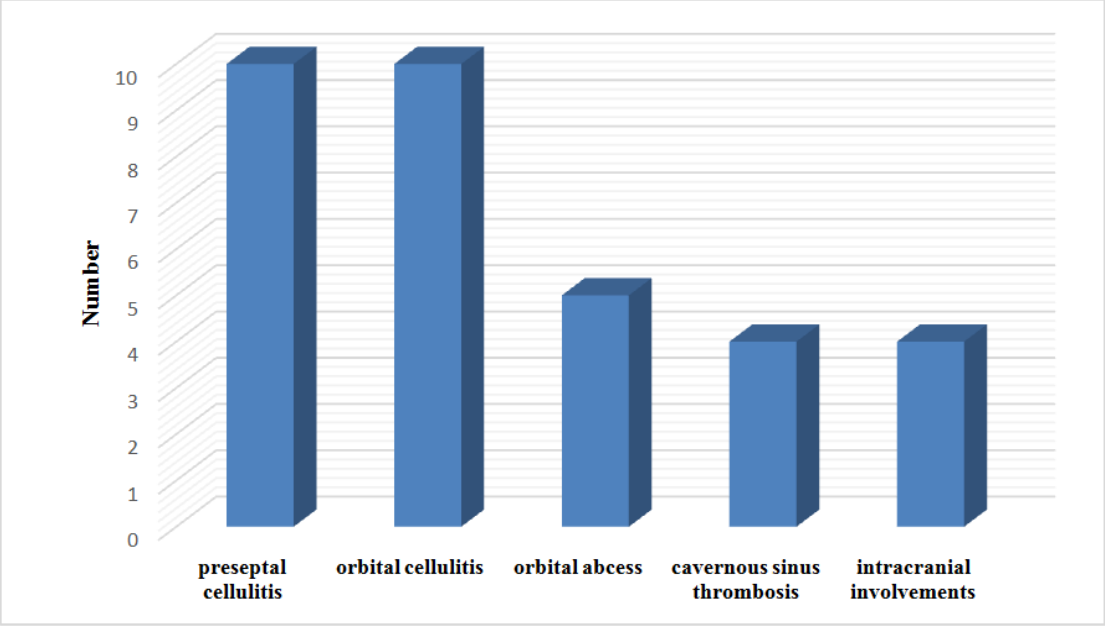

Fig. 4. Complications associated with AIFRS

4. DISCUSSION

AIFRS is a potentially opportunistic infection. This type of infection occurs usually in immunocompromised patients, as those receiving immunosuppressive therapy, bone marrow transplants, organ transplants, HIV-infected patients, the corticosteroid-dependents and diabetics with protein malnutrition. It is extremely rare in immunocompetent individuals [14,15].

Tissue IFRS is classified according to the clinical condition, immune status, histopathology, and fungus infection into: invasive forms (with three subtypes: acute invasive fungal rhinosinusitis AIFRS, chronic invasive fungal rhinosinusitis CIFRS and granulomatous invasive fungal

rhinosinusitis CGFRS) and non-invasive forms (fungus ball, allergic fungal rhinosinusitis). AIFRS is referred to fulminant, or necrotizing and would refer to disease of less than 4 weeks' period in immunocompromised patients [16].

AIFRS has the uppermost morbidity and mortality rate. The majority of patients have already a poor physical condition, because of the previous diseases or treatment related. Immunocompromised patients have twice the mortality rate, in comparison with immunocompetent patients. The prognosis of AIFRS in the absence of treatment is very poor; it is rapidly fatal in 50% to 80% of untreated patients [17].

In our study, patients with AIFRS had age range between 14:55 years with mean age of 45.7 and sex ratio (M: F) was 2:1. This was relatively similar to Montone et al. [18] who found M: F ratio 1.5: 1 but mean age was 54 years. In another studies, the prevalence of the disease was high among female patients with (M: F ratio of 1: 1.3 and 1: 1.25 respectively [19,20].

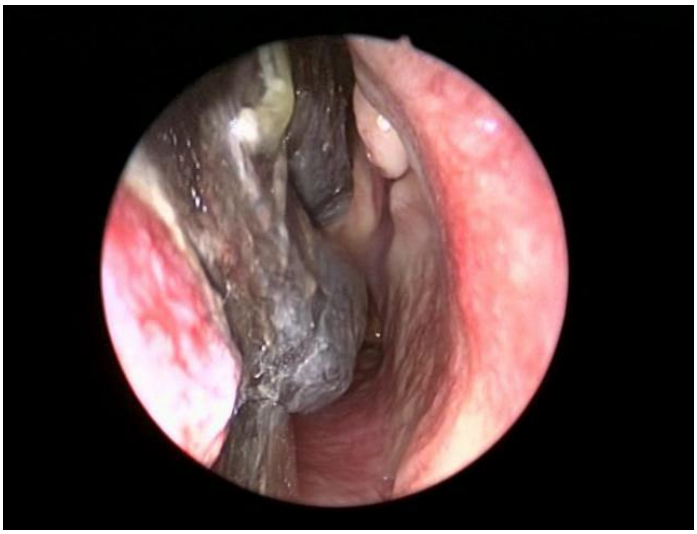

Fig. 5. Endoscopic view of nasal cavity showing mucosal necrosis and black crusts

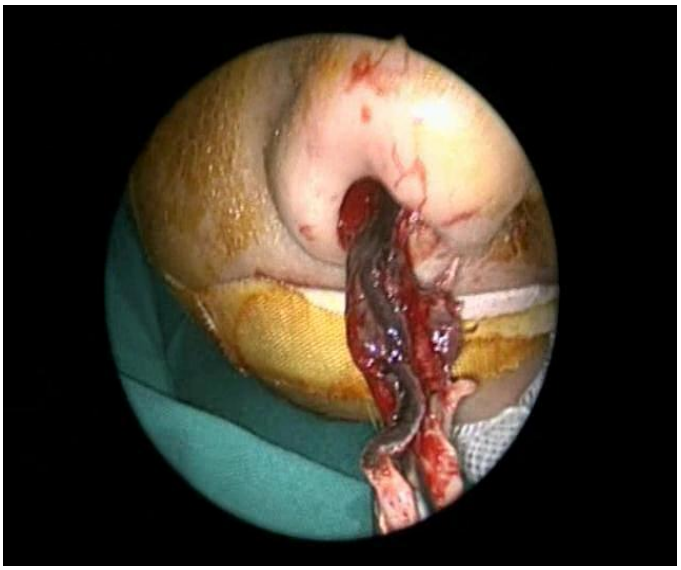

Fig. 6. The crust is removed outside the nose

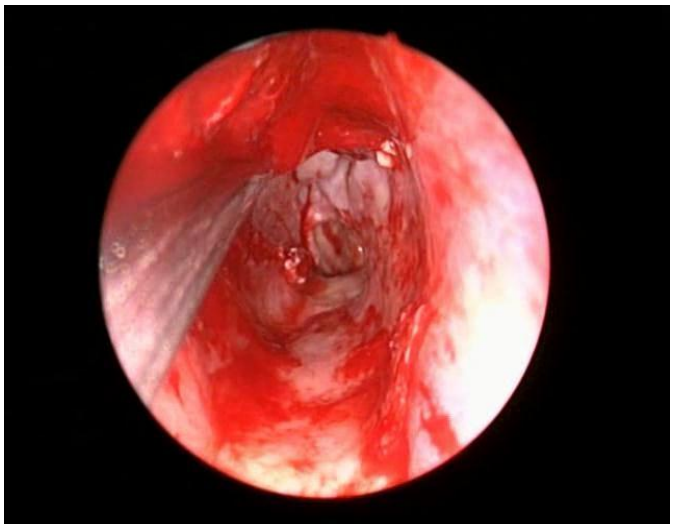

Fig. 7. Endoscopic view of the nasal cavity, raw areas with bleeding after removing the crust

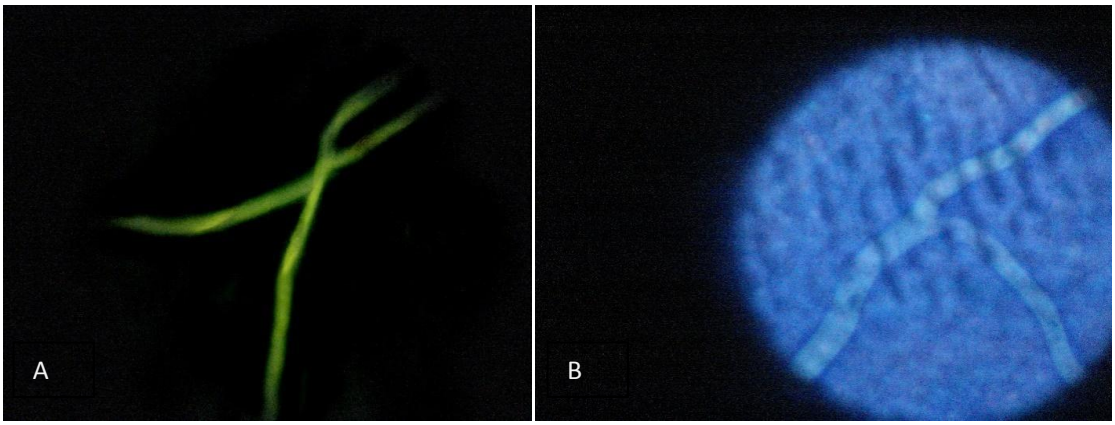

Fig. 8. Fungal hyphae seen by calcofluor stain (A) and lactophenol cotton blue stain (B)

In this study, patients diagnosed with AIFRS had an underlying immunosuppression states. Hematologic disorders (15 patients, 68.18%) were the most common. This is consistent with other reports in which lymphoma, acute myeloid leukemia, and myeloma were the most common hematological malignancies in patients with AIFR [21]. Higher incidence was reported by Pagella et al. [22] as they found that hematological malignancies represented the principal comorbidity (100%). Also Montone et al. [18] from USA found hematological disorders were more commonly associated with AIFRS patients (84%). In other studies, lower incidence was found such as Badiee et al. [23] who found that 55.55% of patients with AIFRS showed hematologic malignancies, and Nucci et al. [24] who reported that the rate of invasive fungal infections in these patients was 30.5%.

In this work, three patients (13.64%) diagnosed with AIFRS had diabetes mellitus. Kaur et al. [19] and Micheal et al. [25] had an association of diabetes, as immunosuppressive condition, in 64.2% and 62.7% of AIFRS cases in their studies respectively. Another study conducted in Thailand found that diabetes was associated in 66.6% of their AIFRS cases [20].

In our study, 4 patients (18.18%) with AIFRS had solid organ transplantation (2 liver and 2 kidney transplant, 9.09% for each). Another study reported that liver transplantation and kidney transplantation were associated in 4.7% and 1.3% of their AIFRS cases respectively [26].

Clinical suspicion of fungal rhinosinusitis is difficult to be formulated based only on symptomatology. Symptoms range from non-

specific symptoms to symptoms indicating invasiveness. Symptoms of facial paresthesia and ocular dysfunction are rare, these should alert clinicians, as these symptoms are more likely to be associated with AIFRS. Also the severity of immune-depression appears to be related to the clinical presentation and the rapidity of the disease progression [3].

In this study fever was the predominant presenting symptom (11 patients, 50%) followed by facial pain (10 patients, 45.45%), purulent rhinorrhea (9 patients, 40.90%), watery rhinorrhea (7 patients, 31.81%), headache (6 patients, 27.27%), visual loss (5 patients, 22.72%), diplopia (5 patients, 22.7%), and maxillary toothache (5 patients, 22.7%). Other symptoms as stuffiness (2patients, 9.09%), postnasal drip (2patients, 9.09%), cough (1 patient, 4.54%), change of conscious (1 patient, 4.54%), and hyposmia (1 patient, 4.54%), were less common presentations.

In a study done by Kaur et al. [19], the most frequently reported symptoms were headache (71.4%) and loss of vision (71.4%), afterwards nasal obstruction (57.1%), fever (42.9%) and CNS symptoms (35.7%). Also in another study, headache (59.3%) was the most frequent symptom, then visual loss (47.5%), facial pain (35.6%) and fever (33.9%) [20].

Head and neck examination is an essential step to diagnose AIFRS and should be implemented to all suspected patients along with the endoscopic nasal evaluation, which usually reveals ischemic nasal mucosa in the form of areas of pale and edematous mucosa. Although the emergence of a black eschar is considered almost pathognomonic for AIFRS, it is usually a late discovery due to vascular thrombosis and tissue necrosis. In addition, patients with middle turbinate and/or septal mucosal abnormalities such as pallor and necrosis, are significantly correlated with the presence of AIFRS [3]. The invasiveness potential of the disease may determine affection of the skin, hard and soft palate or intracranial extension, which should exclude the diagnosis of bacterial infection [27].

In our work, mucosal necrosis and black crust/debris were seen in all examined patients (100%), however pus in middle meatus was seen in half of patients (50%). But septal perforation was the least detected sign, it was presented only in 5 patients (22.72%). In a study carried out by Patrascu et al. [6], the most common sign encountered when examining the nose was an ischemic or edematous mucosa, which bleed very little and was painful when performing various invasive maneuvers. Black eschars appeared in the late phases of the disease [6].

Complete history taking, knowledge of any underlying diseases (especially for immunocompromised patients), physical examination, and awareness of these conditions will ultimately lead to diagnosis and rapid treatment of this destructive disease [20].

For diagnosis of IFS, there are some proposed diagnostic criteria: (1) rhinosinusitis confirmed radiographically and (2) histopathological evidence of fungal invasion of the sinus mucosa, submucosa, blood vessels or bones [16].

Adequate quantities of sinus contents and biopsy specimens from the diseased and healthy mucosa as well as bone adjacent to the areas of frank necrosis are needed for diagnosis. Therefore, obtaining appropriate samples during surgery and subsequent processing for histopathologic or microscopic examination to detect fungal elements are essential for the management of AIFRS [15,16].

The diagnosis depends upon direct microscopy, culture and histopathology [28,29,30]. Direct microscopy and culture helps in establishing the etiology, whereas radiological and histopathological findings help to differentiate invasive from non-invasive type [2].

Histopathology remains the benchmark, based on the literature data, that gives the best sensitivity in detecting rhino-sinusal fungal infections. It is rapid and relatively inexpensive technique which often brings positive diagnosis or, at least, raise the suspicion of diagnosis, especially when cultures are not submitted or negative [6]. It detects the presence of necrosis, inflammation, mycelial filaments and tissue reaction which depends on the type of fungus, site involved and immune status of the host. Both GMS and PAS are useful in delineating fungal morphology [31,32].

In this study, histopathological examination displayed necrotic sinonasal mucosa and bone with the presence of angioinvasive fungal forms in 100% cases. These findings were comparable to the histopathological findings reported in different literatures, as hyphal invasion of blood vessels, vasculitis with thrombosis, hemorrhage,

tissue infarction and neutrophilic infiltration that frequently noticed in patients with AIFRS [32]. This is also in accordance with Kaur et al. [19] work in which H&E was 100% sensitive for cases of AIFRS with necrosis of the submucosa, bone and vascular tissues.

In our work, all samples were histopathologically positive as regarding fungal infections. Aseptate fungal hyphae were detected in 40.91% of samples indicating *Rhizopus* infection, while septated dichotomously branching hyphae of *Aspergillus* were detected in 59.09% of samples. Mycological examination is useful and has a certain value, but it involves special conditions for harvesting, transporting and processing in order to obtain positive results [6]. It is an essential step in the analysis and it may be performed with or without coloration with H&E. Multiple fungal species have been identified in patients with AIFRS, in particular, *Aspergillus spp.* and Zygomyces [25]. Other studies reported that other fungi were responsible for AIFRS such as candidiasis which was the third-most prevalent infection in immunocompromised patients [23]. Unique advantage of culture is that it guides the clinicians to properly select antifungal drugs for treatment [33].

In our series, fungal culture results were positive only in 15 (68.18%) patients with the most common cultured organism being *Aspergillus* species (53.3%; 8/15) followed by *Rhizopus* (46.7%; 7/15), while seven patients had negative fungal cultures. Montone et al. [18], found the most common cultured organisms were *Aspergillus* spp. (49%) followed by *Rhizopus spp.* (33%). Also, similar study carried by Badiee et al. [23], who found that 38.8% of the isolates were *Aspergillus spp.*; and another study in south east Asia found a prevalence of 44% [21], while in Europe, this percentage was reported to be higher (61.5%) [34]. However, Kaur et al. [19] and Prateek et al. [28] found *Mucor spp.* (100%) was the commonest isolate among AIFRS cases.

In the present study, we analyzed the correlation and discrepancy between histological and culture diagnosis. There are very few studies that correlated histopathology and culture diagnosis. The discrepancy between histopathological and culture diagnosis was either due to dematiaceous fungi being interpreted as *Aspergillus* species or probable dual infection [30]. In a study performed by kaur et al. [19], culture was positive in 100% of AIFRS. The range of sensitivities in different studies varies widely between 30-50% and 64-100%. Sensitivity rates are related to the type of etiologic agents. Infection with *Mucor* can decrease the sensitivity of culture [35].

Other techniques can be used for the diagnosis of fungal infections, either determine antigens by an immunoassay (ELISA), genomic amplification by molecular techniques (PCR) or serological examination that aims to identify specific immunoglobulins that represent a marker of earlier or present fungal infection. It should be noted that, in order to notice specific serum IgG, two essential conditions are necessary: the fungal antigen must have a long enough contact with the host immune system and the host immune system must be competent [6].

Fine needle aspiration is a simple method that can be beneficial for diagnosis of fungal rhinosinusitis and to exclude malignancy. Preoperative cytological diagnosis precludes the need for biopsy, saves time and helps to plan appropriate treatment [36]. Endonasal approach is suitable for patients diagnosed in the early stages of the disease and provides a less traumatic option in those patients who already have a poor health status [37].

In this study, preseptal cellulitis was the most common complication among patients (10 patients, 45.5%), other complications were orbital cellulitis, orbital abscess (5 patients, 22.7% for each). Less common complications were intracranial involvement and cavernous sinus thrombosis 4 patients, 18.18%) for each. According to Cho et al. [38], cranial neuropathy, visual loss, and orbital pain were the most common complications. Similarly, Suresh et al. [39] found that orbital cellulitis and cranial nerve palsies were the most common complications. It was found that rapid orbital and intracranial spread and a delay in diagnosis and treatment can lead to high mortality rates ranging from 50% to 100% in immunocompromised patients [15].

In the present study, the fungal isolates were tested to determine their sensitivity to some antifungal drugs. We found that all *Aspergillus* isolates were sensitive to voriconazole and posaconazole while all *Rhizopus* isolates were sensitive to both amphotericin B and posaconazole. Different studies found that treatment of AIFRS includes the use of antifungals and aggressive surgical debridement. Surgical debridement serves multiple purposes, including reducing fungal load, providing

specimen for culture, and allowing increased penetration of antifungal medication by removing affected poorly vascularized tissue [28,40]. Open surgery should be favored in the presence of intra-orbital extension, palatinal, and/or intracerebral involvement. Reversing the underlying disease process and immunosuppression is as significant as the surgical and antifungal treatment [37]. The time taken to treat patients suffering from invasive fungal rhinosinusitis is a vital factor in their outcome. Suitable treatment should be administered within 14 days from the start of symptoms [20].

5. CONCLUSION

AIFRS is a challenge to the otolaryngologist especially in immunocompromised patients. The most reliable finding of AIFRS was mucosal necrosis and black crust/debris. The most common cultured organism being *Aspergillus* followed by *Rhizopus* species. Histopathology plays a major role in the diagnosis of infections due to filamentous fungi, especially when cultures are not submitted or negative. Rapid diagnosis and early initiation of treatment is fundamental to decrease morbidity and mortality in AIFRS cases. Histopathology and direct microscopy give a clue to presence of fungi and the culture confirms the etiological agent.

CONSENT

All authors declare that written informed consent was obtained from the patient for publication of this paper.

ETHICAL APPROVAL

Ethical Committee of the Faculty of Medicine, Mansoura University approved the study.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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