Mucormycosis in Cairo, Egypt: review of 10 reported cases

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Abstract

We report on 10 cases of mucormycosis, as defined by The European Organization for Research and Treatment of Cancer and Mycoses Study Group (EORTC/MSG) standards of invasive fungal diseases, among patients with a recent history of neutropenia, prolonged use of corticosteroids and treatment with immunosuppressants. They were all observed at the Ain Shams University Specialized Hospital in Cairo, Egypt, during the year 2010. These cases were categorized as 50% proven and 50% probable, with none considered to be possible mucormycosis. The median age of the patients discussed in this report was 50 years (range 22–68 years), of which 80% were male and 20% were female. Uncontrolled diabetes with ketoacidosis was noted in 60% of cases, while 40% of the patients had undergone liver transplantations. Pulmonary mucormycosis was the predominant presentation as it was noted in 80% of cases, but there was only 20% sinus involvement. Members of the genus Lichtheimia were the most common etiologic agents (40% of all cases), whereas Rhizopus ssp. were recovered from 30% of cases, Syncephalastrum spp. in 20%, and 10% of patients were infected with Rhizomucor. Liposomal formulation of amphotericin B (LAMB) was successfully used to treat all the cases described in this report. We concluded that the incidence of mucormycosis was relatively high during the study period in this one-center study and that additional studies looking into the diagnosis and the control of mucormycosis in Egypt are required.

Key words: mucormycosis, epidemiology, ITS1-5.8s-ITS2, Egypt.

Introduction

Mucormycosis is considered the third most common invasive fungal disease after candidiasis and aspergillosis [1] and all such diseases are important causes of morbidity and mortality [2]. Risk factors for mucormycosis include corticosteroid and deferoxamine therapy, diabetic ketoacidosis, hematologic malignancy, solid organ transplant, penetrating trauma or burns, and complications of health care procedures [3]. The clinical presentations of mucormycosis fall in six major forms which include rhinocerebral, pulmonary, cutaneous, gastrointestinal, disseminated and uncommon or rare forms [1].

Members of the order Mucorales are the causative agents of mucormycosis worldwide and are composed of thermo-tolerant fungi which are ubiquitous in nature and generally found on decaying organic matter. Members of the genera...
Rhizopus, Lichtheimia and Mucor are most often recovered from clinical specimens while other Mucorales genera, such as Rhizomucor, Cunninghamella, Syncephalastrum, Saksenaea and Apophysomyces, are less common sources of infection [3,4].

The epidemiological pattern of mucormycosis in developed countries differs from that in developing countries. In the latter, the disease is rare and mostly seen in patients with hematologic malignancy and undergoing chemotherapy, bone marrow transplant recipients and patients receiving voriconazole therapy or prophylaxis. However, in developing countries, mucormycosis is more common, occurring mainly in patients with uncontrolled diabetes [5].

Uncontrolled diabetes mellitus is considered the main predisposing factor for invasive zygomycosis in developing and tropical countries [6]. Developing countries possess the largest number of diabetics among the diabetic global population and the majority of these cases are uncontrolled due to deficiency in health care facilities [6].

In this study, we have applied the European Organization for Research and Treatment of Cancer and Mycoses Study Group (EORTC/MSG) criteria for defining mucormycosis cases in order to understand the epidemiology of this fungal infection in Egypt.

Materials and methods

Study population

This study included patients hospitalized from January 2010 to December 2010 in Cairo at the Ain Shams Specialized Hospital. Those included had diabetic ketoacidosis or solid organ transplants with host factors such as recent history of neutropenia (l<500 neutrophils/mm3 for >10 days), prolonged use of corticosteroids in previous 90 days, and those treated with immunosuppressant in previous 30 days prior to the study period. The investigations described in this report adhered to the ethical principles for medical research of Helsinki Declaration. The microbiology and pathology laboratories records were reviewed daily. The corresponding medical records were reviewed and the clinical data analyzed included demographic characteristics such as the site of infection, host factors and the type of underlying disease at the time of diagnosis of infection.

Case definition

We applied the criteria of the (EORTC/MSG) for proven, probable or possible invasive fungal disease (IFD) [2]. As such, classifying a case as a proven IFD requires histopathologic, cytopathologic, or direct microscopic examination of a specimen obtained by needle aspiration or biopsy in which hyphae are seen accompanied by evidence of associated tissue damage. Alternatively, a proved case can be described upon recovery of a mold by culture of a specimen obtained by a sterile procedure from a normally sterile and clinically or radiological abnormal site consistent with an infectious disease process. Probable IFD cases require presence of a host factor, clinical features, and mycological evidence consistent with the IFD, whereas a possible invasive infection has the same features accept for the absence of mycological evidence [2].

Sampling, culturing and strain identification

The collected lung aspiration fluid and sinus biopsy tissue samples were directly cultured on Sabouraud dextrose agar (SDA) and potato dextrose agar (PDA). Part of the fluid and the biopsy tissue was sent to the pathology laboratory for the preparation of tissue slides stained with Hematoxylin-and-Eosin and periodic acid-Schiff procedures. Sputum samples were concentrated and cultured on SDA and PDA.

The obtained isolates were identified through examination of micro- and macro-morphologic features in accord with standard morphological criteria [3,4,7].

Molecular identification was used by comparing the ITS1.5.8S-ITS2 rDNA region sequence data of the isolated strains with reference strains data deposited in GenBank.

Extraction of DNA

Fungal isolates were grown on PDA and DNA extraction was conducted in accord with the instructions provided by Fermentas Genomic DNA Purification Kit #K0512 (Thermo Fischer Scientific, EU). Briefly, a sufficient inoculum was suspended in 200 µl TE buffer (10 mM Tris-HCl, pH 8.0, 1 mM EDTA) in a 2.2 ml Eppendorf tube, the tubes were boiled for 3 min and then placed in ice water for 10 min. Lysis solution (400 µl) was added, the tubes heated to 65 °C for 30 min and then 600 µl of chloroform were added and mixed carefully. The aqueous phase containing DNA was separated by centrifugation for 10 min at 12,000 rpm at 4°C and mixed with 800µl precipitation solution by several inversions at room temperature for 1 min each. The tubes are then centrifuged for 10 min at 12,000 rpm at 4°C. The DNA pellets were dissolized in 100 µl of 1.2 M NaCl solution by gentle vortexing. Ice-cold isopropanol (500 µl) was added to the solution, the tubes were incubated for 15 min at −20°C and then centrifuged for 10 min at 12,000 rpm at 4°C. The DNA pellet was washed with 1 ml ice cold 70% ethanol, dried and resuspended in sterile TE buffer.
Table 1. Characteristics of patients with proven and probable mucormycosis.

<table>
<thead>
<tr>
<th>No.</th>
<th>Age</th>
<th>Gender</th>
<th>Underlying disease</th>
<th>Host factor</th>
<th>Clinical presentation</th>
<th>Etiologic agent</th>
<th>Treatment</th>
<th>Outcome</th>
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<tbody>
<tr>
<td><strong>Proven cases</strong></td>
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<td></td>
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<tr>
<td>1</td>
<td>55</td>
<td>M</td>
<td>SOT</td>
<td>Neutropenia, immunosuppressant</td>
<td>Pulmonary</td>
<td>Syncephalastrum racemosum</td>
<td>LAMB</td>
<td>Death due to underlying condition</td>
</tr>
<tr>
<td>2</td>
<td>50</td>
<td>M</td>
<td>DM</td>
<td>Corticosteroids</td>
<td>Pulmonary</td>
<td>Rhizomucor pusillus</td>
<td>LAMB</td>
<td>Cured</td>
</tr>
<tr>
<td>3</td>
<td>68</td>
<td>F</td>
<td>DM</td>
<td>Corticosteroids</td>
<td>Pulmonary</td>
<td>Rhizopus oryzae</td>
<td>ITC</td>
<td>Cured</td>
</tr>
<tr>
<td>4</td>
<td>41</td>
<td>F</td>
<td>DM</td>
<td>Corticosteroids</td>
<td>Sinusitis</td>
<td>Rhizopus oryzae</td>
<td>LAMB</td>
<td>Cured</td>
</tr>
<tr>
<td>5</td>
<td>22</td>
<td>M</td>
<td>DM</td>
<td>Corticosteroids</td>
<td>Sinusitis</td>
<td>Rhizopus oryzae</td>
<td>LAMB</td>
<td>Cured</td>
</tr>
<tr>
<td><strong>Probable cases</strong></td>
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</tr>
<tr>
<td>6</td>
<td>55</td>
<td>M</td>
<td>SOT</td>
<td>Neutropenia, immunosuppressant</td>
<td>Pulmonary</td>
<td>Syncephalastrum racemosum</td>
<td>LAMB</td>
<td>Cured</td>
</tr>
<tr>
<td>7</td>
<td>55</td>
<td>M</td>
<td>SOT</td>
<td>Neutropenia, immunosuppressant</td>
<td>Pulmonary</td>
<td>Lichtheimia corymbfera</td>
<td>LAMB</td>
<td>Cured</td>
</tr>
<tr>
<td>8</td>
<td>55</td>
<td>M</td>
<td>SOT</td>
<td>Neutropenia, immunosuppressant</td>
<td>Pulmonary</td>
<td>Lichtheimia ramosa</td>
<td>LAMB</td>
<td>Death due to underlying condition</td>
</tr>
<tr>
<td>9</td>
<td>45</td>
<td>M</td>
<td>DM</td>
<td>Neutropenia, Corticosteroids</td>
<td>Pulmonary</td>
<td>Lichtheimia ramosa</td>
<td>ITC</td>
<td>Cured</td>
</tr>
<tr>
<td>10</td>
<td>55</td>
<td>M</td>
<td>DM</td>
<td>Neutropenia, Corticosteroids</td>
<td>Pulmonary</td>
<td>Lichtheimia ramosa</td>
<td>ITC</td>
<td>Cured</td>
</tr>
</tbody>
</table>

SOT, Solid organ transplant; DM, Diabetes mellitus; LAMB, Liposomal amphotericin B; ITC, Itraconazole.

Oligonucleotides

The oligonucleotide primers used for amplification and sequencing of the ITS regions were those described by White et al. [8]. This study used ITS5 (5′-GGAAGTAAAGTCGTAACAAGG-3′) and ITS4 (5′-TCCTCCGCTTATGC-3′) (Bioneer Corporation, South Korea).

PCR and DNA sequencing of ITS1-5.8S-ITS2 region rDNA of fungal species

Amplification reactions were performed in 20 µl reactions containing 2.5 µl of each primer (10 pm), 2.5 µl of genomic DNA (5 µg/ml), and one PCR-Gold Master-Mix bead (Bioron-Germany; buffers, dNTP, enzyme, stabilizers, Tris-HCl, KCl, and MgCl2). Amplification was performed with a PCR Thermal Cycler (Techne Genius - UK) using the initial denaturation at 96°C for 5 min, 35 cycles of denaturation at 94°C for 30 sec, annealing at 52°C for 30 sec, extension at 72°C for 80 sec, and a final extension at 72°C for 10 min. The PCR reaction products were sequenced directly using a Big-Dye terminator reagent kit including Taq polymerase and that protocol recommended by the manufacturer (Model 3100 automated DNA sequencer; PerkinElmer Inc./Applied Biosystems – Bioneer, South Korea).

Nucleotide accession numbers

The nucleotide sequence data reported in this study appear in the NCBI GenBank nucleotide sequence database. The accession numbers of the isolated strains are listed in Table 2.

Results

According to the (EORTC/MSG) criteria, we could identify 10 cases with mucormycosis among monitored patients with the appropriate host factors in Ain Shams University Specialized Hospital, Cairo, during the year 2010. The cases were categorized as 50% (five cases) proven and 50% (five cases) probable mucormycosis. No patients were classified as having possible mucormycosis. The median age of the patients with proven and probable mucormycosis was 50 years (range 22–68 years), of which 80% (8/10 cases) were male and 20% (2/10 cases) were female. Six patients (60%) had diabetes mellitus with ketoacidosis as underlying disease while the other four patients (40%) were liver transplant patients. Demographic characteristics, sites of infection, host factors and the types of underlying diseases are presented in Table 1.

Proven cases

The median age of the five patients with proven mucormycosis was 47 years (range 22–68 years), of which three were male and two females. Diabetes mellitus with ketoacidosis was reported in four patients, while the fifth patient was a liver transplant recipient. Corticosteroids were given
to four patients (80%) in the month before onset of the infection. One patient (20%) received immunosuppressive agents in the 4 weeks preceding the diagnosis. The lungs were the site of infection in three cases (60%) while in the other two patients (40%) the sinuses were the infected sites. Pulmonary nodules appeared in the three pulmonary cases, while pleural effusion could be noticed only in two patients. In the two patients with sinus involvement, computed tomography (CT) scans showed air fluid level denoting acute infection. Histopathological examination of the lung aspiration and sinus biopsy tissue slides stained with periodic acid-Schiff procedure showed broad, aseptate, and ribbon-like hyphae indicating mucormycosis (Fig. 1a–e). Examination of PDA cultures inoculated with lung aspiration fluid and portions sinus biopsy samples from case 1 revealed fast growing, floccose white colonies which turned black with

<table>
<thead>
<tr>
<th>Histopathological findings</th>
<th>Description</th>
</tr>
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<tbody>
<tr>
<td>(a) Lung aspiration section showing ribbon-like aseptate hyphal elements and (sporangiohyphae, vesicles and merosporae) (Case 1).</td>
<td></td>
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<tr>
<td>(b) Lung aspiration section showing ribbon-like aseptate hyphal elements (arrows) (Case 2).</td>
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<tr>
<td>(c) Lung aspiration section showing ribbon-like aseptate hyphal elements (Case 3).</td>
<td></td>
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<tr>
<td>(d) Sinus biopsy tissue section showing ribbon-like aseptate hyphal elements (Case 4).</td>
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<tr>
<td>(e) Sinus biopsy tissue section showing ribbon-like aseptate hyphal elements (Case 5).</td>
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</tbody>
</table>

**Figure 1.** Histopathological findings in periodic acid-Schiff stained lung aspirations and biopsy tissue sections and their descriptions of the five proven cases. This Figure is reproduced in color in the online version of *Medical Mycology.*
Table 2. Mucorales species causing mucormycosis in 10 patients.

<table>
<thead>
<tr>
<th>Species</th>
<th>AUMC No.</th>
<th>GenBank Acc. No.</th>
<th>No. of patients (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rhizomucor pusillus</td>
<td>7966</td>
<td>KC117252</td>
<td>1 (10%)</td>
</tr>
<tr>
<td>Lichtheimia corymbifera</td>
<td>7960</td>
<td>KC117258</td>
<td>1 (10%)</td>
</tr>
<tr>
<td>Syncephalastrum racemosum</td>
<td>7964</td>
<td>KC117253</td>
<td>2 (20%)</td>
</tr>
<tr>
<td>Syncephalastrum racemosum</td>
<td>7965</td>
<td>KC117254</td>
<td></td>
</tr>
<tr>
<td>Rhizopus oryzae</td>
<td>7957</td>
<td>KC117255</td>
<td>3 (30%)</td>
</tr>
<tr>
<td>Rhizopus oryzae</td>
<td>7958</td>
<td>KC117256</td>
<td></td>
</tr>
<tr>
<td>Rhizopus oryzae</td>
<td>7959</td>
<td>KC117257</td>
<td></td>
</tr>
<tr>
<td>Lichtheimia ramosa</td>
<td>7961</td>
<td>KC117259</td>
<td>3 (30%)</td>
</tr>
<tr>
<td>Lichtheimia ramosa</td>
<td>7962</td>
<td>KC117260</td>
<td></td>
</tr>
<tr>
<td>Lichtheimia ramosa</td>
<td>7963</td>
<td>KC117261</td>
<td></td>
</tr>
</tbody>
</table>

AUMC, Assuit University Mycological Center, Assuit, Egypt.

Molecular identification

The presumptive morphologic identification of the Mucorales isolates showed three Lichtheimia ramosa, three Rhizopus oryzae, two Syncephalastrum racemosum, one Lichtheimia corymbifera and one Rhizomucor pusillus. All the isolates were deposited in the culture collection of Assuit University Mycological Center (AUMC), Assuit, Egypt, and their numbers are listed in Table 2. All 10 isolates yielded a unique PCR amplification. The sequences of the ITS1-5.8S-ITS2 rDNA region of the 10 isolates were from 549–777 bp. The NCBI GenBank was accessed to identify the isolated species through BLAST homology searches using the obtained ITS data. The ITS data of isolated Rhizomucor pusillus (590 bp), Syncephalastrum racemosum (549 bp), S. racemosum (567 bp), Rhizopus oryzae (583 bp), R. oryzae (651 bp), R. oryzae (584 bp), Lichtheimia corymbifera (682 bp), Lichtheimia ramosa (763 bp), L. ramosa (777 bp), and L. ramosa (772 bp). These were 99% identical with the ITS GenBank data of Rhizomucor pusillus (GenBank Acc. No. HM999962), Syncephalastrum...
racemosum (GenBank Acc. No. HM999979), S. racemosum (GenBank Acc. No. HM999979), Rhizopus oryzae (GenBank Acc. No. EU484274), R. oryzae (GenBank Acc. No. JX523619), R. oryzae (GenBank Acc. No. AY803930), Lichtheimia corymbifera (GenBank Acc. No. HM104216), Lichtheimia ramosa (GenBank Acc. No. HM104204). L. ramosa (GenBank Acc. No. GQ342868) and L. ramosa (GenBank Acc. No. HM104210).

Prevalence of genera and species

Lichtheimia was the predominant genus seen in four (40%) of the 10 cases followed by Rhizopus noted in three (30%) cases. Syncephalastrum was isolated from two (20%) specimens of two patients, while Rhizomucor was recovered from one (10%) case. Lichtheimia was the only genus represented by two species, i.e., L. corymbifera isolated from one patient with probable mucormycosis and L. ramosa recovered from three cases of probable mucormycosis. On the other hand, Rhizopus, Syncephalastrum and Rhizomucor were each the predominant species, in that each of them were recovered from three cases (30%), followed by S. racemosum obtained from samples of two (20%) cases. L. corymbifera and Rhizomucor pusillus were each the etiologic agents in one (10%) case (Table 2).

Treatment and outcome

All the patients diagnosed with proven or probable mucormycosis in this study received specific therapy. While eight patients survived, two died due to their underlying conditions. The five patients who received liposomal amphotericin B (LAMB) treatment from the start were cured. Three patients were initially treated with itraconazole (ITC), which was later replaced by LAMB, and were also cured. The median daily dose of LAMB was 5 mg/kg (range, 3–10 mg/kg) and the median duration of treatment was 41 days (range, 35–49 days).

Discussion

Mucormycosis is an opportunistic infection caused by fungi of the order Mucorales with an angio-invasive pathology in diabetics and other immunocompromised patients [9,10]. The incidence of mucormycosis has increased significantly over the past two decades due to the increase in the population at risk in parallel with medical technological advances [11–13]. Although it remains a relatively infrequent disease compared to candidiasis and aspergillosis, attention is being paid to mucormycosis because it often leads to an aggressive clinical course with high mortality rate even with appropriate medical management [14].

This study, to our knowledge, represents the first epidemiological report of mucormycosis in Egypt. During this one-year study, 10 cases of mucormycosis were recorded from one center, Ain Shams University Specialized Hospital, Cairo. Five cases matched EORTC/MSG criteria for defining proven cases, while the remaining five cases were defined as probable mucormycosis. The latest revision of EORTC/MSG criteria in 2008 [2] constitute an important diagnostic tool for epidemiological and clinical research [9,15].

Diabetes mellitus was considered the most common underlying disease for mucormycosis infection as noted in the largest English-language literature review of 929 cases of the diseases described between 1885 and 2004 [16]. However, recent reports from different developed countries in Europe indicate that the percentage of cases in which diabetes was an underlying disease contributing to mucormycosis was considerably low when compared with other underlying conditions [17]. On the other hand, uncontrolled diabetes was the most common underlying disease in developing and tropical countries such as India, in which the largest series of cases (n = 178) at one center were described in 3 years [6]. The results obtained in this study revealed that uncontrolled diabetes with ketoacidosis was common in 60% of cases of mucormycosis and liver transplantation was the second most common predisposing factor as the infection was noted in 40% of cases. The high percentage of diabetes as underlying disease in our study is consistent with the results obtained from India by Chakraborti et al. [6] and this can be attributed to the similarity between Egypt and India in that both have high populations of uncontrolled diabetic patients.

Among the 10 recorded cases, pulmonary mucormycosis was the predominant presentation representing 80% of cases, while sinus involvement constituted only 20%. Pulmonary involvement (40%) is also the primary clinical manifestation, followed by sinus infection (20%) noted in the diabetic population in this investigation. These results are in contrast with those found in the study of Roden et al. [16], which revealed that sinus involvement was the major manifestation in diabetic patients. All the liver transplant recipients developed pulmonary mucormycosis.

In this study, positive culture results were obtained in 100% of the cases, with the etiologic agents identified by mycological methods and molecular techniques targeting the ITS region of the rDNA. In agreement with the results in other reports, ITS sequencing is considered a first-line method of identification of Mucorales as compared to other target genes in the ribosomal DNA [17–21].

Our data showed that members of genus Lichtheimia were the predominant species as they were seen in 40% of cases, while Rhizopus species was found in 30% of cases.
These results are in sharp contrast to the information in the literature in which \textit{Rhizopus} was the predominant genus in almost all cases [6,9,16,22–25]. Of note, we recorded two cases of infection caused by \textit{Syncephalastrum} (20%) and one case due to \textit{Rhizomucor} (10%), both of which are considered less common etiologies of mucormycosis according to Gomes et al. [4]. \textit{L. corymbifera}, \textit{L. ramosa} and \textit{L. ornata} are the clinically relevant members of the genus \textit{Lichtheimia} [26]. Based on molecular, biological and morphological characteristics, both \textit{L. corymbifera} and \textit{L. ramosa} were differentiated into two species [17,26]. A considerable proportion of infection reported to be caused by \textit{L. corymbifera} was found, after revision, to be caused by \textit{L. ramosa} [27]. These two species were found in our investigation with \textit{L. ramosa} recovered from three cases (30%) and of \textit{Lichtheimia corymbifera} from one patient (10%). \textit{Rhizopus oryzae} was obtained in 30% of our cases and was the only member of the genus recovered from clinical isolates. It should be noted that according to Roden et al. [16], \textit{R. oryzae} is considered the most commonly isolated \textit{Rhizopus} species.

Chronic and acute infection after trauma has been considered to be the most like mode of transmission of \textit{S. racemosum} [4]. However, there are only two recently reported cases of rhino-orbital infection caused by this mold [4,28]. Interestingly, we found two cases of pulmonary mucormycosis due to \textit{S. racemosum} in neutropenic liver transplant recipients. One of these was a proven case in which the fungus was recovered in cultures from a lung aspirate and typical ‘merosporas and sporangiophores’ were detected in tissue sections (Fig. 1a). The other was a probable case in which radiologic evidences demonstrated the presence of bilateral pleural effusion with pulmonary nodule and repeated inoculation of culture media with sputum specimens were positive. It is noteworthy to mention that these are the first proven and probable cases of pulmonary mucormycosis caused by \textit{S. racemosum}.

Only one proven case of pulmonary infection caused by \textit{Rhizomucor pusillus} was recorded in this study, which is the least frequent infection and this result is consistent with previous review that considered \textit{R. pusillus} a rare finding [4]. \textit{R. pusillus} represented 4% of the cases reported in Roden et al. study [16] and 11.6% of the unusual cases of mucormycosis reviewed by Gomes et al. [4]. Moreover, Gomes et al. reported that the majority of the cases infected by \textit{R. pusillus} were pulmonary infections [4].

Liposomal amphotericin B (LAMB) was the drug of choice for the treatment of mucormycosis in previous reports [9,29,30]. LAMB has been successfully used to treat all the cases we reported, although Itraconazole (ITC) was given empirically to three cases only.

Conclusion

This is the first report discussing the epidemiology of mucormycosis in Egypt. Despite the short study period, the incidence of mucormycosis was found to be relatively high in one center. The fact that the number of patients with uncontrolled diabetes mellitus is on the rise as the Egyptian Ministry of Health declared in 2012 [http://www.mohp.gov.eg], together with the poor healthcare procedures may cause the expansion of population at risk for mucormycosis. Moreover, lack of surveillance and low awareness among physicians about fungal diseases and absence of cooperation between clinicians, microbiologists and pathologists in medical centers may hinder determining an accurate figure of the incidence of mucormycosis in Egypt. More efforts must be paid to improve diagnosis and control of fungal diseases in Egypt.

Acknowledgments

The authors thank Dr Sahar Farouk, Ain Shams University Specialized Hospital, Cairo, and Dr Ahmed Mokhtar, Wadi El Nil Hospital, Cairo, for their assistance in defining the cases.

Declaration of interest

The authors report no conflicts of interest. The authors alone are responsible for the content and the writing of the paper.

References