Original Article

A prospective study of mucormycosis in north India: Experience from a tertiary care hospital

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Abstract

Mucormycosis is an emerging angioinvasive infection caused by the ubiquitous filamentous fungi of the Order Mucorales and class of Mucormycetes. We conducted a prospective study of 38 patients who were diagnosed as having mucormycosis in a tertiary care hospital during January 2010 to June 2011. The cases were analyzed regarding the site of involvement, underlying disease and species of fungi isolated, antifungal susceptibility pattern of the isolates, and outcome of therapy. The mean age of the patients was 40.43 years, with 72% male. Rhino-orbital mucormycosis (61.5%) was the most common presentation followed by cutaneous manifestations (31%), gastrointestinal symptoms (5%), and pulmonary (2.5%). Diabetes mellitus (56%) was the significant risk factor in rhino-orbito-cerebral presentation (OR = 7.55, P = 0.001). Among 23 culture isolates, Rhizopus arrhizus (37.5%) was the most common, followed by Apophysomyces variabilis (29.2%), Lichtheimia ramosa (16.7%), Rhizopus microsporus (4.2%), Rhizomucor pusillus (4.2%), and Apophysomyces elegans (4.2%). Rhizopus arrhizus was most commonly isolated from rhino-orbito-cerebral mucormycosis and Apophysomyces species were generally obtained from cutaneous mucormycosis. In vitro antifungal susceptibility showed that 16 isolates were sensitive to amphotericin B (MIC less than 1 µg/ml), while in contrast, all isolates were found to be resistant to voriconazole (MIC- 0.25 to >8), fluconazole (MIC > 32), flucytosine (MIC > 32). Treatment regimens included antifungal therapy, reversal of underlying predisposing risk factors, and surgical debridement. Combination of surgery and medical treatment with amphotericin B was significantly better (OR = 0.2, P < 0.04) than amphotericin B alone (61.5% vs. 10.3% patient survival). The awareness of fungal diseases amongst clinicians is required to decrease the fatal outcome of disease.

Key words: Mucormycosis, Diabetes mellitus, Rhizopus, Apophysomyces, Amphotericin B.
**Introduction**

Mucormycosis, a serious angioinvasive infection caused by common filamentous fungi, that is, mucormycetes, constitutes the third most common invasive fungal infection following aspergillosis and candidiasis [1]. The disease can be transmitted by inhalation of spores or by direct inoculation of the spores into disrupted skin or mucosa. The etiologic agents can cause infections with high mortality in immunocompromised, mainly diabetic patients [2]. Mucormycetes are characterized by the presence of broad aseptate hyphae (coenocytic mycelia) and formation of zygosporangia. The order Mucorales includes several species involved in rhinocerebral, pulmonary, cutaneous, and gastrointestinal and other less frequent infections in immunocompetent and immunocompromised individuals, and all are characterized by tendency to disseminate. Members of the genus *Rhizopus* are by far the most common isolates recovered in a clinical setting with *Rhizopus arrhizus* occurring most frequently. Members of the genus *Mucor* are second to *Rhizopus* in order of frequency, whereas *Cunninghamella*, *Apophysomyces*, *Lichtheimia*, *Saksenaea*, *Rhizomucor* and *Cokeromyces*, *Syncephalastrum*, each constitute a significantly smaller percentage of clinical isolates [3–6].

Whatever the route of infection (inhalation of airborne spores, ingestion, or direct skin inoculation), the hyphae invade blood vessels, causing tissue infarction and necrosis [3]. Risk factors include prolonged neutropenia and use of corticosteroids, hematological malignancies (leukemia, lymphoma, and multiple myeloma) aplastic anaemia, myelodysplastic syndromes, solid organ or hematopoietic stem cell transplantation, human immunodeficiency virus (HIV) infection, diabetic and metabolic acidosis, intravenous drug abuse, prematurity, and advanced age [6,7]. The widespread use of voriconazole for prophylaxis or treatment of aspergillosis in patients with hematological malignancies has also been linked with rise in the numbers of cases of invasive mucormycosis. The patients with phagocytic dysfunctions caused by neutropenia or ketoacidosis, as well as with high iron serum concentrations, are at higher risk of developing mucormycosis. The rhinocerebral form of presentation is the most frequently reported localized symptom, followed by pulmonary, cutaneous, gastrointestinal, and disseminated infections [8,9].

Most of the studies done on this emerging disease in India, as well as investigations throughout the world, are retrospective. Due to increase in number of cases, diverse risk factors and inclusion of immunocompetent and immunocompromised patients, there is need of prospective study so that suspected cases can be diagnosed in a timely manner, various risk factors can be analyzed and accordingly patients appropriately treated, which should result in the increase of patient survival. The present study was carried out to understand the clinical behavior, natural history, and changes in incidence, epidemiology, clinical course, and outcome of the disease.

**Material and Methods**

**Study groups**

Both adults and children presented in various clinical departments with a high index of clinical suspicion of mucormycosis from January 1, 2010, to June 30, 2011, were included in the study. Enrolled patients comprised both immunocompromised and immunocompetent individuals.

**Defined Parameters**

The case histories of the patients with mucormycosis were analyzed prospectively regarding site of involvement, underlying disease, biochemical and haematological investigations, clinical course, mode of diagnosis, agents isolated, treatment instituted, and outcome of disease. The diagnosis of mucormycosis was confirmed when broad aseptate/ sparsely septate, ribbon like hyphae with right angled branching were demonstrated in tissue specimen or aseptically aspirated material from lesion with or without isolation of mucormycetes. Those patients with a clinical suspicion of mucormycosis but without mycology or histopathology confirmation were not included.

**Sample processing**

The biopsy material from individuals with clinically suspected mucormycosis was collected in two sterile containers, one with normal saline and the other 10% formalin and processed in the Departments of Microbiology and Pathology, respectively. Testing of samples in Department of Microbiology included direct KOH/Calcofluor white mount examination, fungal culture, and antifungal sensitivity testing of the isolates. The direct demonstration of fungal elements in the clinical sample is essential in establishing diagnosis. The microscopic examinations of specimens in KOH (10–20% potassium hydroxide) wet mount [10] were done to detect characteristic broad, sparsely septate, ribbon-like hyphae with wide-angle or right-angle branching at irregular intervals (Fig. 1a). The wet mounts were also examined with Calcofluor/blankophor white (Fig. 1b) under Nikon 90i fluorescent microscope with excitation filter (300–412 nm). The size, morphology, and quantity of any fungal elements were noted. For histopathological examination tissue sections were stained with...
Figure 1. (a) KOH wet mount preparation showing direct evidence of presence broad sparsely septate fungal hyphae suggestive of mucormycosis (400× magnification); (b) Calcofluor white staining of tissue sample showing fluorescent ribbon-shaped broad sparsely septate hyphae (1000× magnification).

Figure 2. (a) Haematoxylin and eosin (H&E) staining showing broad, sparsely septae hyphae suggestive of mucormycosis in tissue (1000× magnification); (b) Grocott methenamine silver (GMS) staining showing cut sections of broad sparsely septate fungal hyphae (black color) with right-angle branching (1000× magnification).

Hematoxylin and eosin (H&E) (Fig. 2a), Periodic acid Schiff stain (PAS), and Grocott methenamine silver stain (GMS) (Fig. 2b). These were evaluated and interpreted for presence or absence of specific fungi [11–13].

The tissue sample was cut into small pieces and inoculated without crushing in two tubes containing Sabouraud Dextrose Agar (SDA) with antibiotics (with chloramphenicol and gentamicin, without cycloheximide) and on two tubes without antibiotics, with one tube from each set incubated at 37°C and at 22°C. The sample was also inoculated in brain heart infusion broth (BHB) and blood agar and incubated at 37°C. Cultures were examined for growth daily for the first week and twice a week for the subsequent period. The fungal isolates were finally identified by conventional techniques like lactophenol cotton blue (LCB) mount.

Antifungal Susceptibility Testing

The in vitro antifungal sensitivity was determined by microbroth dilution method according to the Clinical and Laboratory Standard Institute (CLSI) document (M38-A2) [14]. Inoculum suspensions were prepared from 7 days potato dextrose agar (PDA; Difco) cultures by adding sterile saline solution and slightly scraping the surface of mature colonies with a sterile cotton swab. The homogeneous conidial suspensions were then transferred to sterile tubes and the supernatants adjusted spectrophotometrically at 530 nm wavelength to an optical density (OD) that ranged from 0.15 to 0.17. The inoculum suspensions, which consisted primarily of nongerminated conidia, were diluted 1:50 in RPMI 1640 medium. Microdilution plates were incubated at 35°C and examined visually after 24 hr and 48 hr. Candida parapsilosis (ATCC 22019) was used
Table 1. Risk factors associated with various clinical presentations of mucormycosis.

<table>
<thead>
<tr>
<th>Risk factors</th>
<th>Total</th>
<th>Rhino-orbital</th>
<th>Cutaneous</th>
<th>Pulmonary</th>
<th>GIT</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(n, OR, P)</td>
<td>(n, OR, P)</td>
<td>(n, OR, P)</td>
<td>(n, OR, P)</td>
<td>(n, OR, P)</td>
</tr>
<tr>
<td>Diabetes mellitus</td>
<td>23</td>
<td>17</td>
<td>7.55</td>
<td>0.001</td>
<td>5</td>
</tr>
<tr>
<td>Injection</td>
<td>4</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>4</td>
</tr>
<tr>
<td>Breach of skin (I&amp;D, Trauma)</td>
<td>3</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>3</td>
</tr>
<tr>
<td>Deferoxamine</td>
<td>1</td>
<td>1</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Transplant</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Voriconazole therapy</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>None</td>
<td>7</td>
<td>5</td>
<td>-</td>
<td>-</td>
<td>0</td>
</tr>
</tbody>
</table>

Note. Diabetes mellitus has significant association with rhino-orbital-cerebral presentation (OR = 7.55, P = 0.001). However, diabetes mellitus is not significantly associated with pulmonary and cutaneous mucormycosis (P = 1, P = 0.37, respectively). Intramuscular injection and breach of skin has significant association with cutaneous mucormycosis (P = 0.0005, P = 0.004), respectively.

as quality control. The minimum inhibitory concentration (MIC) obtained with various antifungal drugs at 24 and 48 hr, respectively, were noted.

Statistical Methods
Chi square test and odds ratio (OR) were calculated to analyze the significance of association for clinical presentations, risk factors, treatment, and outcome of disease. Significance of our results: P value less than 0.05 was considered as statistical significant.

Results
A total of 38 cases of mucormycosis were diagnosed and identified on the basis of defined parameters. The proportion of male patients (n = 27) was higher than female patients (n = 11) and the mean age of all patients was 40.43 years. Most lived in Haryana state (43.6%), followed by Punjab state (41.1%). Rhino-orbital type (61.5%) was the most common presentation followed by cutaneous (31%), gastrointestinal (5%), and pulmonary (2.5%) mucormycosis. Various risk factors were associated with different clinical presentations (Table 1). Out of 38 patients, only 12 were immunocompetent, and 26 patients were immunocompromised due to underlying factors such as diabetes mellitus, diabetic ketoacidosis, chronic renal disease, operative procedures such as exploratory laparotomy, and trauma. None of our patients had undergone transplantation, chemotherapy, steroid use, severe burns, or HIV. On combining all the investigations, the mucormycosis cases were positive by KOH (84%), histopathology (58%), and culture (61%). Histopathological and microbiological (KOH & Culture) investigations were positive in 34.2% cases, and those processed by only microbiological investigations were positive in 23.7% cases. Direct examination with both modalities histopathology and KOH were positive in 23.7% cases. Only histopathology was positive in 7.9% cases. Out of 23 culture isolates, *Rhizopus arrhizus* (Fig. 3a) was the most commonly identified in nine cases (39.1%). *Apophysomyces variabilis* (Fig. 3b) was found in 30.4% cases followed by four cases (17.4%) of *Lichtheimia ramosa*. *Rhizopus microsporus* (4.3%) (Fig. 4a), *Rhizomucor pusillus* (4.3%) (Fig. 4b), *Apophysomyces elegans* (4.3%) was found as one each (Table 2). All isolates have been confirmed with two reference laboratories in Spain, namely, (i) Mycology Unit, Medical School, Rovira i Virgili University, Sant Florence 21, 43201, Reus and (ii) Mycology Reference Laboratory, Spanish National Centre for Microbiology, ISCIII, Madrid.

Antifungal Susceptibility Testing
*In vitro* antifungal susceptibility studies were conducted on 21 isolates out of 23, as two were not able to grow on RPMI 1640 medium. Out of the 21, 16 isolates were sensitive to amphotericin B having MIC less than 1 µg/ml (MIC range 0.03–0.25). One isolate of *Apophysomyces variabilis* was resistant to amphotericin B having MIC 2 µg/ml, three others had an MIC >4 µg/ml, as did one of *Lichtheimia ramosa*. Only one isolate, *Rhizomucor pusillus*, was found to be sensitive to itraconazole having MIC 0.03 µg/ml. The remaining 20 isolates were found to be resistant to itraconazole with MICs >0.125. All isolates were found to be resistant to voriconazole (MIC range 0.25 to >8), fluconazole (MIC >32), and flucytosine (MIC >32).

Treatment
During the medical management of the patients liposomal formulation of amphotericin B was given in dose of 5 mg/kg/day in 28 (74%) patients. Conventional amphotericin B was given only in 4 (11%) patients. All recovered patients received an additional 4 weeks to 8
weeks of amphotericin B depending on the underlying condition of patients. Continuous monitoring of renal function by serum urea and creatinine level was done. Of the nine patients who did not recovered, five did not get any medical treatment, two patients with rhino-orbital infections received amphotericin B for 3 days (TCD-750 mg) and 15 days (TCD-3750 mg), respectively, and two patients with cutaneous presentations received amphotericin B for 2 weeks (TCD-3500 mg) and 3 weeks (TCD-4500 mg), respectively. Despite treatment some patient died due to underlying complications such as cardiorespiratory collapse and septic shock. In six patients (15%) no treatment was given, as some patients were poor, not able to afford costly amphotericin B, or left against medical advice. A total of 31 patients were managed by surgical intervention, of which 11 patients were managed by functional endoscopic sinus surgery (FESS) and in one patient orbital exenteration was done. Two patients of oroantral fistula were managed by doing antrophy, and in 13 patients surgical debridement was performed. Gastrointestinal mucormycosis patients were managed by doing exploratory laparotomy. No surgical intervention was done in eight patients. Even there is difference between survival rate when comparison between liposomal amphotericin B and conventional amphotericin B was done (88% survival vs. 66% survival). Out of 38 patients, only 23 (61%) patients survived. Despite medical and surgical therapy, nine (24%) patients died due to mucormycosis. Six patient left against medical device despite explaining dire consequences of the same (Table 3).
Table 2. Distribution of various mucormycetes with various clinical presentations.

<table>
<thead>
<tr>
<th>Isolates</th>
<th>Rhino-orbito cerebral</th>
<th>Cutaneous</th>
<th>Pulmonary</th>
<th>GIT</th>
<th>Total</th>
<th>Fatal (n, P, OR)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rhizopus arrhizus</td>
<td>6</td>
<td>2</td>
<td>1</td>
<td>-</td>
<td>9</td>
<td>3, 1 OR 0.75</td>
</tr>
<tr>
<td>Rhizopus microsporus</td>
<td>1</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>1</td>
<td>1, 0.38</td>
</tr>
<tr>
<td>Rhizomucor pusillus</td>
<td>1</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>1</td>
<td>0, 1</td>
</tr>
<tr>
<td>Apophysomyces variabilis</td>
<td>1</td>
<td>6</td>
<td>-</td>
<td>-</td>
<td>7</td>
<td>3, 1 OR 1.12</td>
</tr>
<tr>
<td>Apophysomyces elegans</td>
<td>-</td>
<td>1</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>1, 0.375</td>
</tr>
<tr>
<td>Lichtheimia ramosa</td>
<td>3</td>
<td>1</td>
<td>-</td>
<td>-</td>
<td>4</td>
<td>1, 0.63, OR 0.46</td>
</tr>
</tbody>
</table>

Note. Rhizopus arrhizus is most commonly isolated from rhino-orbito-cerebral mucormycosis. A. variabilis and A. elegans are most commonly isolated from cutaneous mucormycosis.

Table 3. Treatment and outcome of mucormycosis cases with respect to clinical presentations.

<table>
<thead>
<tr>
<th>Management</th>
<th>Outcome</th>
<th>ROC</th>
<th>Cutaneous</th>
<th>Pulmonary</th>
<th>GIT</th>
<th>Total (n, P, OR)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Amphotericin B alone</td>
<td>Survived</td>
<td>4</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td>Died</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>0, 0.5</td>
</tr>
<tr>
<td>Surgery alone</td>
<td>Survived</td>
<td>1</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>Died</td>
<td>-</td>
<td>1</td>
<td>-</td>
<td>-</td>
<td>2, 0.1, 7.4</td>
</tr>
<tr>
<td>Both</td>
<td>Survived</td>
<td>15</td>
<td>08</td>
<td>-</td>
<td>1</td>
<td>24</td>
</tr>
<tr>
<td></td>
<td>Died</td>
<td>2</td>
<td>02</td>
<td>-</td>
<td>-</td>
<td>4, 0.04,0.2</td>
</tr>
<tr>
<td>None</td>
<td>Survived</td>
<td>2</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>Died</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>1</td>
<td>1</td>
</tr>
</tbody>
</table>

Note. Combination of surgery and amphotericin B therapy was significantly better (OR = 0.2, P = 0.04) than amphotericin B alone (61.5% vs. 10.3% patient survival).

Discussion

Mucormycosis was first reported as a cause of human disease in 1885 [15]. During the last two decades, there has been a dramatic increase in the occurrence of invasive fungal infections observed worldwide largely as a result of the increase in the size of the population at risk. During this period of increased incidence, mucormycosis is not the exception. It is usually an acute necrotizing fungal infection with a fulminant course due to angioinvasion.

The rise in the number of patients with mucormycosis in developed countries has been particularly evident in hematopoietic stem cell transplant recipients and patients with hematological malignancies [16]. The rise in number of patients with invasive mucormycosis may be correlated with an increase in the population of diabetics in developing and tropical countries. Countries like India, China, United States, and Indonesia have the highest number of diabetic patients; for example, India alone probably has more than 30 million diabetic individuals. Therefore, mucormycetes can establish themselves on such patients with uncontrolled diabetes mellitus.

Various risk factors are associated with the development of mucormycosis. In uncontrolled diabetes, ketoacidosis is considered the key factor for predisposition to mucormycetes infection, as low serum pH diminishes the phagocytic effect of macrophages, chemotactic and oxidative burst of neutrophils. Macrophages and neutrophils are the main host defenses against invasion of mucormycetes. Moreover, other serum components like the transferrin system is less active at acidic pH, allowing unbound iron to circulate in blood and the free iron is then utilized by mucormycetes [18]. In a review of 179 cases of paranasal sinus mucormycosis, 70% of patients had diabetic ketoacidosis [19]. In our study, diabetes mellitus (56%) remains the most common risk factor followed by intramuscular injection (10%), incision and drainage (5%), deferoxamine therapy (2%), and trauma (2%), and in 24% cases no risk factor was identified. In this study uncontrolled diabetes mellitus was considered when fasting blood glucose level was >140 mg/dl, and it was found in 56% patients as compared to 50% of patients who had uncontrolled diabetes mellitus [6]. Diabetic ketoacidosis was considered when blood sugar level was more than 200 mg/dl, low bicarbonate level <10 mmol/L, low serum pH ≤ 7.2. In this investigation, 30.7% patients with type 2 diabetes had ketoacidosis, which was consistent with other studies; in other words, Chakrabarti et al. in 2006 and 2009 reported diabetic ketoacidosis in 27.3% and 20% patients, respectively [19,20].
In a study conducted by Roden et al. of 929 patients diagnosed with mucormycosis, the mean age was 38.8 years, and disease was more common in males (65%) as compared to females (35%). In the present study the mean age was 40.43 years and the most common in males (72%) as compared to females (28%).

Mucormycosis, formerly thought to be always community acquired, is now recognized as also being a nosocomial infection. It has been associated with various procedures or devices used in hospitals, including antifungal prophylaxis, bandages or medication patches, intravenous catheters, and even tongue depressors. In one study 9% of patients had acquired nosocomial infection either at the site of the ECG leads or the adhesive tapes or from contaminated intramuscular injections or from air in the hospital environment [17]. In our study 15.4% patients acquired nosocomial infection from intramuscular injections or during the incision and drainage procedure of abscess. All of these patients presented with cutaneous mucormycosis and isolates obtained from five patients were Apophysomyces variabilis and Apophysomyces elegans from one patient.

Rhinocerebral mucormycosis is the commonest clinical presentation in most of the published series [16,18,20], as well as in the present study (61.5%). This clinical entity has a strong association with poorly controlled diabetes mellitus. The study done by Chakrabarti et al. reported that nearly 50% patients had uncontrolled diabetes mellitus and the association was significant (OR = 9.3; \( P \leq 0.001 \)). In our study, out of 61.5% cases of rhino-orbito cerebral presentation, 74% had diabetes mellitus and the association was significant (OR = 7.6, \( P = 0.001 \)); one patient was on dialysis and on deferoxamine therapy and developed rhino-orbital mucormycosis. However, this disease occurred in 21.7% of our patients who were otherwise healthy as compared to 23.5% patient with no underlying risk factors in a study done by Chakrabarti et al. [16]. This finding deserves serious consideration, though cases have previously been reported in healthy hosts.

Cutaneous involvement was second (29%) in frequency in present study as compared to other studies [16,20]. Cutaneous mucormycosis occurs in patients with disrupted cutaneous barrier, as a result of burn, trauma, maceration or injection [1,17]. In our study, cutaneous presentation, 38.4% patients were having diabetes. Fifty-four percent of our patient had breach of skin as the predisposing factor, and the association was significant (\( P < 0.004 \)). There was history of intramuscular injection in 31% of patients (\( P < 0.0005 \)), and 15.4% of patients had undergone incision and drainage for abscess and 7% of patient had a road traffic accident. Cutaneous mucormycosis spread locally and aggressively causing necrotizing fasciitis and causing the fatal outcome.

Gastrointestinal mucormycosis is the third most common (5%) type in the present study though it is considered as very rare [21]. This disease presents as necrotizing enterocolitis in premature neonates. Prematurity was the significant (\( OR = 1.49, P < 0.001 \)) underlying factor among patients with the gastrointestinal mucormycosis. Ninety percent of the gastrointestinal mucormycosis were diagnosed postmortem [22]. In our study two patients were diagnosed as gastrointestinal mucormycosis. One patient was 3 year old and presented with symptoms of intestinal obstruction. Exploratory laparotomy was done on emergency basis. The patient survived due to timely diagnosis and treatment with liposomal amphotericin B. The other patient was 28 year-old male who was also diagnosed as perforation peritonitis. Exploratory laparotomy was performed, and the excised biopsy sample was sent for histopathological examination. But the patient died due to septic shock before making the establishment of final diagnosis. In our study, the affected child patient was not premature, and no other obvious risk factor such as ingestion of contaminated food was observed.

Pulmonary mucormycosis may have a wide variety of disease manifestations including solitary nodular lesion, lobar involvement and cavitary or disseminated form [1,20]. Infiltrates or mass lesions without any specific lobar predilections are common findings. Pulmonary consolidation and cavitations are seen less frequently [1]. In the series reported from India, uncontrolled DM was the most common risk factor for most types of mucormycosis including pulmonary form [23]. In this series large numbers (44–100%) of pulmonary mucormycosis cases are diagnosed postmortem due to a lack of specific symptoms and signs [17]. Fever and cough are the most common findings [23]. Increased awareness can improve the proportion of cases with an antemortem diagnosis. In our study, an adult female patient who was diabetic presented with sign and symptoms of pneumonia and consolidation was the finding on chest X-ray, BAL sample was sent and pulmonary mucormycosis was diagnosed. But the patient died due to lack of timely management. Unfortunately, though sputum or bronchoalveolar lavage analysis is frequently employed, this process rarely leads to confirmation of diagnosis. Therefore, open lung biopsy, surgical extirpation, and transthoracic needle aspiration provide better results. High resolution chest computed tomography (CT) scan is the best method of determining the extent of pulmonary mucormycosis. Pulmonary mucormycosis can be suspected when patients have a reverse halo sign on CT of the chest, along with the right clinical findings.

In various series, renal and disseminated presentations of this disease were reported [5,17,20]. Renal mucormycosis was reported in immunocompetent young adults. The
exact route of entry of the pathogen is not known. Patients present with unilateral or bilateral flank pain, fever with chills, pyuria, or anuria. When diagnosed antemortem, surgery accompanied with amphotericin B treatment may save the life of patients [17]. In our study, there was no report of renal and disseminated form of mucormycosis.

For diagnosis of mucormycosis, CT scan and magnetic resonance imaging (MRI) may help especially when multiple infarcts are visible. These may also help in delineating the extension of the lesion. The imaging techniques also help to collect samples from the site of the lesion in deep tissue for establishing diagnosis. In our study, CT scan was done in 24 cases of rhino-orbito-cerebral cases, and it helped in delineating the extent of lesion. Fourteen cases of sinusitis and six cases of rhino-orbital and four cases of rhino-orbito-cerebral mucormycosis were identified with the help of these imaging techniques.

Among mucormycetes, members of the genera Rhizopus, Mucor, Lichtheimia, Rhizomucor, and Apophysomyces are commonly implicated in causing human infection, and overall Rhizopus species are the most commonly isolated agents from patients with mucormycosis [1]. In the present study, R. arrhizus was most commonly isolated from six cases of ROC mucormycosis, two cases of cutaneous mucormycosis, and from one case of pulmonary mucormycosis. Our findings are consistent with other studies. Rhizopus microsporus produces primarily cutaneous and gastrointestinal mucormycosis [1]. But in this study R. microsporus was isolated from one case of ROC mucormycosis. Rhizomucor pusillus was also isolated from one case of rhino-orbito-cerebral mucormycosis. A. elegans is rarely isolated and is known to cause cutaneous mucormycosis [1,20]. A study from India had reported the emergence of A. elegans infections and recorded infections at sites other than skin as well. In this series, A. elegans was the second most common agent (19%) isolated, and it also produces ROC, renal, and disseminated mucormycosis, besides cutaneous mucormycosis [15,24]. In another study we also reported Apophysomyces elegans from four cases of primary cutaneous mucormycosis and Saksenaea vasiformis and Absidia corymbifera from one each case of cutaneous mucormycosis [25]. In the present study also Apophysomyces variabilis is the second most common agent isolated from 29.2% of culture positive cases, and it is isolated from six cases of cutaneous mucormycosis and one case of ROC mucormycosis. A. elegans is isolated from one case of cutaneous mucormycosis. Injection abscess was the risk factor among 31% patients of cutaneous mucormycosis. Lichtheimia ramosa (Absidia ramosa) was isolated from 16.7% cases, and it is isolated from three cases of ROC mucormycosis and from one case of cutaneous mucormycosis. Apophysomyces elegans and Saksenaea vasiformis are occasionally responsible for infections in immunocompromised individuals; most cases are encountered in immunocompetent individuals as a result of trauma, leading to soft tissue infections with relatively low mortality rates [26].

Various other studies from India also reported about the emergence of Apophysomyces infections including various sites other than skin [15,20,25]. This fungus is thought to be nonsporulating on ordinary media but it behaved differently as it sporulated easily within 48–72 hrs without need of any specialized method for sporulation. On the basis of molecular diversity there is proposal for three new species of Apophysomyces, in other words, A. variabilis, A. osisiformis, and A. trapeziformis [27]. In our earlier report four cases of Apophysomyces variabilis have been identified from cutaneous mucormycosis [28]. In the present study also Apophysomyces variabilis have been isolated from seven cases and Apophysomyces elegans from one case. There is an increase in incidence of A. variabilis causing human infections; molecular studies must be conducted to better understand the epidemiology and distribution of different Apophysomyces species.

The best management of mucormycosis is presumed to be aggressive surgical debridement combined with medical treatment and control of predisposing factors [1,4,8,29,30]. Amphotericin B is the first line drug of choice in most cases. In recent years posaconazole as a substitute for amphotericin B, especially as salvage therapy, has gained popularity [31]. The ischemic necrosis as a result of mucormycetes mediated angioinvasion is likely important mechanism by which the fungus survives therapy [4,32]. Surgery is considered necessary due to the massive amount of tissue necrosis occurring during disease process that may prevent entry of antifungal agents in adequate concentrations. Additionally surgery is supposed to minimize the fungal load in the tissue [1,4].

The present study is important as outcome of treatment could be evaluated prospectively in a considerable number of cases (38 patients) over a short period of time (18 months) from a single tertiary care center. Combination of surgery and medical treatment with amphotericin B was significantly better (OR = 0.2, P < 0.04) than amphotericin B alone (61.5% vs. 10.3% patient survival). These findings are consistent with other studies [4]. The patients who do not undergo surgery have major differences in severity of illness or co-morbidities. In the present study, comorbidity conditions such as poor general health, hypertension, chronic renal failure, and immunosuppression prevailed in the group in which surgery could not be done. Even there is difference between survival rate when comparison between liposomal amphotericin B and conventional amphotericin B was done (88% survival vs. 66% survival). The overall mortality rate was 23% of these 38 patients treated and
62% patients survived after therapy, but there is no information about 15% patients as they left against medical advice. The overall mortality rate was 45% of 53 patients treated in study carried by Chakrabarti et al. [21], and it was observed 46% in the largest meta-analysis done by Roden et al. [4]. The survival rate in mucormycosis varies with the site of involvement. Earlier studies reflect a better survival rate of 66.5% among 18 patients with rhino-orbito-cerebral involvement, compared with survival rates of <3% among patients with gastrointestinal and disseminated disease [33]. The higher survival rate in the present study may be because most (92%) of the patients included for outcome analysis were either in rhino-orbito-cerebral or cutaneous category, which were diagnosed easily and treated quickly. This study also showed a higher survival rate of 70.8% and 100% in rhino-orbito-cerebral and cutaneous mucormycosis, respectively [20].

The present study therefore emphasizes the need of further awareness of the disease and aggressive measures for early diagnosis and management.

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

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