

Original article

A multicentre observational study on the epidemiology, risk factors, management and outcomes of mucormycosis in India

A. Patel^{1,2}, H. Kaur³, I. Xess⁴, J.S. Michael⁵, J. Savio⁶, S. Rudramurthy³, R. Singh⁷, P. Shastri⁸, P. Umabala⁹, R. Sardana¹⁰, A. Kindo¹¹, M.R. Capoor¹², S. Mohan¹³, V. Muthu¹⁴, R. Agarwal¹⁴, A. Chakrabarti^{3,*}

¹) Department of Infectious Diseases, Sterling Hospital, Ahmedabad, India

²) Department of Internal Medicine, University of South Florida, Tampa, FL, USA

³) Department of Microbiology, Postgraduate Institute of Medical Education and Research, Chandigarh, India

⁴) Department of Microbiology, All India Institute of Medical Sciences, New Delhi, India

⁵) Department of Clinical Microbiology, Christian Medical College, Vellore, India

⁶) St John's Medical College Hospital, Bangalore, India

⁷) Department of Microbiology, JIPMER, Pondicherry, India

⁸) Intensive Care Medicine, Sir Ganga Ram Hospital, New Delhi, India

⁹) Department of Microbiology, Nizam's Institute of Medical Sciences, Hyderabad, India

¹⁰) Department of Microbiology, Indraprastha Apollo Hospital, New Delhi, India

¹¹) Department of Microbiology, Sri Ramachandra Medical College, Chennai, India

¹²) Vardhman Mahaveer Medical College and Safdarjang Hospital, New Delhi, India

¹³) Department of Microbiology, Christian Medical College, Ludhiana, India

¹⁴) Department of Pulmonary Medicine, Postgraduate Institute of Medical Education and Research, Chandigarh, India

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ABSTRACT

Objectives: To describe the epidemiology, management and outcome of individuals with mucormycosis; and to evaluate the risk factors associated with mortality.

Methods: We conducted a prospective observational study involving consecutive individuals with proven mucormycosis across 12 centres from India. The demographic profile, microbiology, predisposing factors, management and 90-day mortality were recorded; risk factors for mortality were analysed.

Results: We included 465 patients. Rhino-orbital mucormycosis was the most common (315/465, 67.7%) presentation followed by pulmonary (62/465, 13.3%), cutaneous (49/465, 10.5%), and others. The predisposing factors included diabetes mellitus (342/465, 73.5%), malignancy (42/465, 9.0%), transplant (36/465, 7.7%), and others. *Rhizopus* species (231/290, 79.7%) were the most common followed by *Apophysomyces variabilis* (23/290, 7.9%), and several rare *Mucorales*. Surgical treatment was performed in 62.2% (289/465) of the participants. Amphotericin B was the primary therapy in 81.9% (381/465), and posaconazole was used as combination therapy in 53 (11.4%) individuals. Antifungal therapy was inappropriate in 7.6% (30/394) of the individuals. The 90-day mortality rate was 52% (242/465). On multivariate analysis, disseminated and rhino-orbital (with cerebral extension) mucormycosis, shorter duration of symptoms, shorter duration of antifungal therapy, and treatment with amphotericin B deoxycholate (versus liposomal) were independent risk factors of mortality. A combined medical and surgical management was associated with a better survival.

Conclusions: Diabetes mellitus was the dominant predisposing factor in all forms of mucormycosis. Combined surgical and medical management was associated with better outcomes. Several gaps surfaced in the management of mucormycosis. The rarer *Mucorales* identified in the study warrant further evaluation. **A. Patel, Clin Microbiol Infect 2020;26:944.e9–944.e15**

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* Corresponding author. A. Chakrabarti, Department of Medical Microbiology, Postgraduate Institute of Medical Education & Research, Chandigarh 160012, India.
E-mail address: arunalokey@hotmail.com (A. Chakrabarti).

Introduction

Mucormycosis is being increasingly diagnosed worldwide, particularly in India [1]. The rising trend is due to the increased awareness, advances in diagnostic techniques, and the increase in the prevalence of predisposing factors [2,3]. Mucormycosis mainly occurs in immunosuppressed hosts, including those with haematological malignancies, transplant recipients and in people with uncontrolled diabetes mellitus [4,5]. The *Mucorales* have a unique capability of angio-invasion causing vasculitis and thrombosis of vessels, resulting in large areas of infarction and necrosis [4,6,7]. Poor drug penetration in devitalized tissue mandates the need for surgical debridement.

In low- and middle-income countries including India, mucormycosis is associated with high mortality (45%–90%) [8–12]. The probable reasons include a delay in diagnosis and the high cost of managing mucormycosis. Many single-centre studies suggest that the epidemiology of mucormycosis is different in India compared with the developed world [3,8,12,13]. However, the existing data are from small studies, and there is a lack of a prospective, multi-centre data on mucormycosis from developing countries. Herein, we describe the epidemiology, predisposing factors, microbiology, management and outcome of patients with mucormycosis in India. We also evaluate whether combined surgical and medical treatment is associated with better outcomes in patients with mucormycosis.

Methods

We conducted a prospective observational study from 1 January 2016 to 30 September 2017 at 12 tertiary-care centres across India (see [Supplementary material, Table S1](#)). The study protocol was approved by the Institute Ethics Committee of all the individual participating centres, and a written informed consent was obtained from all the study subjects. The study is registered at the clinical trial registry of India (ctri.nic.in; CTRI/2016/02/006644).

Study objectives

The study objectives were to describe the epidemiology, risk factors, treatment practices and 90-day mortality of individuals with mucormycosis. This was an exploratory study in which we also evaluated the risk factors for mortality.

Study participants

All consecutive individuals with proven mucormycosis were enrolled in this study. We defined proven mucormycosis as those individuals with clinically compatible disease and the demonstration of fungi in the tissue (or body fluids) either by direct microscopy (broad ribbon like aseptate hyphae), culture or molecular methods. All participants received treatment at the discretion of the treating physician.

Study procedure

We collected the following information on a standardized case report form (see [Supplementary material, Appendix S1](#)): (a) demographic details; (b) clinical features; (c) predisposing factors (diabetes mellitus, glucocorticoid therapy, transplant, malignancy, immunosuppression, and others) (participants with multiple risk factors were graded in a hierarchical manner, for example, if the patient had undergone stem cell transplantation and also developed prednisolone-induced diabetes, then stem cell transplant was considered the primary risk factor, and not diabetes); (d) co-morbid

illnesses (ischaemic heart disease, chronic kidney disease, chronic liver disease, chronic respiratory illnesses, and others); (e) site of disease (pulmonary, rhino-orbital with or without cranial extension, cutaneous, renal, gastrointestinal and disseminated); (f) histopathological and microbiological findings; (g) details of treatment given (antifungal agent, dose and duration of antifungal agent, nature of surgical treatment); and (h) mortality at 90 days.

The exposure variable was chosen to be combined surgical and medical management with all other variables assumed as potential confounders. Participants who left the hospital against medical advice were assumed to be dead for the purpose of mortality analysis (worst-case scenario analysis). We also performed a sensitivity analysis by excluding these individuals.

Processing of sample

Tissue samples, such as nasal/sinus tissue biopsies and biopsies from ulcers, were subjected to conventional microscopy, culture, histopathological examination or molecular diagnostic techniques, as appropriate. Microscopy was performed using the KOH-calcocofluor mount method. The patient samples were also inoculated onto two sets of Sabouraud dextrose agar and one tube of brain–heart infusion agar. The positive cultures were identified by their macroscopic and microscopic characteristics, and through sequencing of the internal transcribed spacer (ITS) region of rDNA. The tissue samples submitted for histopathological examination were examined using haematoxylin & eosin, periodic acid Schiff or Gomori's methenamine silver stain.

The genomic DNA extraction was attempted from deparaffinized blocks [14], using phenol-chloroform-isoamyl extraction after tissue digestion with proteinase K and lysis buffer (100 mM Tris–HCl (pH 8.5), 0.5 M EDTA, 10% SDS and 5 M NaCl) [15]. The 18S region of rDNA was amplified using semi-nested PCR with the *Mucorales*-specific primers ZM1 (5'-ATTACCATGAGCAAATCAGA-3'), ZM2 (5'-TCCGTC AATCTCTTAAAGTTTC-3') and ZM3 (5'-CAATCCAAGAATTTCACCTCTAG-3'), as described by Bialek *et al.* [14], while the ITS2 was amplified using the panfungal primers ITS3 and ITS4 [16]. Amplification of the human GAPDH gene (forward primer: 5'-GGATTTGGTTCGTATTGGG-3'; reverse primer: 5'-GGAAGATGGTGATGGGATT-3') and Tris–EDTA (TE) buffer without template DNA acted as positive and negative controls, respectively. The amplicons were subjected to gel electrophoresis. The bands were excised and purified using a gel extraction kit (Qiagen, Hilden, Germany). The amplicons were sequenced using the BigDye terminator cycle sequencing ready reaction kit (version 3.1; Applied Biosystems, Foster City, CA, USA). The reaction products were analysed on an ABI Prism 3100 automated DNA analyser. Consensus sequences were obtained using BIONUMERICS software (version 7.5; Applied-Maths, Ghent, Belgium). The sequences were compared with the GenBank/International Society for Human and Animal Mycology (ISHAM) Barcode and Centraalbureau voor Schimmelcultures (CBS) databases to identify the agents.

Statistical methods

The data were analysed using the commercial statistical package SPSS 21.0 for MS-Windows (IBM Inc., Chicago, IL). The descriptive statistics are presented as frequencies, mean with standard deviation (SD), or median and interquartile range (IQR), as appropriate. The categorical variables were compared using chi-square test (or Fischer's exact test) while the differences between continuous data were analysed using Mann–Whitney test or Kruskal–Wallis test, as appropriate. We also performed competing risk analysis to correct for the various variables that could be influenced by the time bias, namely mortality. A multivariate Cox regression analysis was

performed for identifying factors predicting mortality, by including variables that were significant ($p < 0.05$) on univariate analysis. Survival curves were constructed to study the effect of combined (surgical and medical) versus medical management on the time to mortality using Cox proportional hazard analysis. A p -value < 0.05 was considered as significant.

Results

A total of 485 individuals were diagnosed with mucormycosis during the study period, of whom 20 were excluded (incomplete case record forms). Among the 465 individuals enrolled, 438 (96.5%) were adults. The median (IQR) age of the study population (323/465, 69.5% men) was 48 (35–58.5) years (Table 1). Medical comorbid illnesses including chronic kidney disease (93/465, 20.0%) and cardiovascular diseases (67/465, 14.4%) were noted in 37.6% (175/465) of the participants (Table 1). The median (IQR) duration of symptoms before admission was 12 (7–30) days. Rhino-orbital mucormycosis (315/465, 67.7%) was the most common form followed by pulmonary (62/465, 13.3%), and cutaneous (49/465, 10.5%) mucormycosis (Table 1).

Predisposing factors

Most (410/465, 88.2%) of the participants had underlying risk factors (Table 2). Uncontrolled diabetes mellitus was the most common risk factor for all forms of mucormycosis, except cutaneous and renal (Table 2). Interestingly, in 44 (12.9%) of the 342 individuals with diabetes, their diabetes was diagnosed during the evaluation of mucormycosis. The median (IQR) duration of diabetes was 48 (3–120) months, and 81.6% had uncontrolled disease (median (IQR) HbA1c, 10.2 (8–12)); 14.6% (50/342) presented with diabetic ketoacidosis. Fifty per cent (7/14) of the participants with isolated renal mucormycosis did not have an identifiable risk factor, whereas trauma (26/49, 53.1%) was the most common predisposing factor in cutaneous mucormycosis (Table 2).

Table 1
Baseline characteristics of patients with mucormycosis

| | Total (n = 465) |
|-----------------------------|-----------------|
| Age*, in years | 48 (35–58.5) |
| Male sex | 323 (69.5) |
| Comorbid illnesses | |
| Any ^a | 175 (37.6) |
| Chronic kidney disease | 93 (20) |
| Cardiovascular | 67 (14.4) |
| Pulmonary | 30 (6.5) |
| Liver disease | 24 (5.2) |
| Neurological | 18 (3.9) |
| Others | 1 (0.2) |
| Duration of symptoms*, days | 12 (7–30) |
| Time to diagnosis*, days | 1 (1.4) |
| Clinical presentation | |
| Rhino-orbital | 315 (67.7) |
| with brain involvement | 103 |
| without brain involvement | 212 |
| Pulmonary | 62 (13.3) |
| Cutaneous | 49 (10.5) |
| Renal | 14 (3.0) |
| Gastrointestinal | 12 (2.6) |
| Disseminated | 13 (2.8) |

All values are represented as number (%) or as median (interquartile range) (indicated by *) unless otherwise stated. All the percentages are provided for the total number of participants in the study (n = 465).

^a A given participant may have had one or more co-morbid illnesses.

Diagnosis

The diagnosis of mucormycosis was made by direct microscopy in 406/465 (87.3%) participants. Histopathology demonstrated aseptate hyphae in 340/465 (73.1%) participants. Culture identified the aetiological agent in 290/465 (62.4%) cases (Table 2). Among the culture-positive (n = 290) participants, *Rhizopus* spp. were the most common (231; 79.7%). Of the *Rhizopus* spp., *Rhizopus arrhizus* was isolated from 174/231 (75.3%) participants followed by *Rhizopus microsporus* and *Rhizopus homothallicus* (Table 2). The other *Mucorales* isolated include *Apophysomyces variabilis* (23/290; 7.9%), *Mucor* spp. (16/290; 5.5%), *Lichtheimia corymbifera* (10/290; 3.4%), and others (Table 2).

Molecular detection was attempted in 68 histopathology blocks where the culture was negative. *Mucorales* were identified in 23 cases (*Rhizopus* spp. (n = 12), *Mucor* spp. (n = 5), *Lichtheimia* spp. (n = 3), *Apophysomyces variabilis* (n = 1)); nucleic acid could not be extracted in 47 cases. Non-mucorales species were identified in one of the histopathology blocks (*Aspergillus* spp.).

Treatment

Combined medical and surgical management was performed in 62.2% (289/465) of the participants. The surgical management rates ranged from 21% (13/62) for pulmonary mucormycosis to 79.6% (39/49) for cutaneous disease (Table 2). Radical surgery was feasible only in 107/289 (37%) participants, while the remainder underwent debulking. Ten (46/465) per cent of the participants left the hospital against medical advice before initiation of therapy, due to financial constraints. Amphotericin B was used in 381 (81.9%) of the 465 participants (liposomal: 62.4% (238/381); deoxycholate: 37.6% (143/381)). Amphotericin B was changed from liposomal to deoxycholate preparation in 4.2% of these individuals because of the high cost of therapy. A combination of antifungal agents was used in 22.1% (87/394) of the participants. The most common combination used was posaconazole (53/87, 60.9%) along with amphotericin B. Other agents combined with amphotericin B included caspofungin (n = 3), isavuconazole (n = 1), itraconazole (n = 8), voriconazole (n = 17), deferasirox (n = 6) and fluconazole (n = 5). The median (IQR) duration of the antifungal treatment was 15 (range 1–381) days.

Outcome

The 90-day mortality rate was 52.0% (242/465 participants). The duration of symptoms before hospitalization was significantly less in non-survivors (median, 10 versus 15 days). The presence of comorbid medical illnesses was associated with a significantly reduced survival (Table 3). A higher survival was observed in participants who received combined medical and surgical treatment (p 0.001), and patients receiving the liposomal compared with the deoxycholate preparation of amphotericin B (p 0.03). As the duration of antifungal therapy, duration of hospital stay and surgical treatment are affected by the immortal time bias, we performed competing risk analyses (competing risk: death; time factor: hospital stay). The subhazards ratio (SHR) for these factors (days of antifungal therapy: SHR 1.004, 95% CI 1.002–1.006, p 0.0001; combined surgical and medical management: SHR 2.2151, 95% CI 1.625–3.018, p 0.0001) remained statistically significant even after adjusting for the competing risk (mortality). On excluding the participants who left the hospital against medical advice (n = 112), the results were similar except that solid organ malignancy, immunosuppressant therapy was more frequent and the time to diagnosis was significantly longer in deceased subjects (see Supplementary material, Table S2).

Table 2

Risk factors, microbiology, diagnosis and treatment characteristics of patients with mucormycosis according to the site of involvement. ROM, rhino-orbital mucormycosis; ROCM, rhino-orbital mucormycosis with cerebral extension

| | Skin (n = 49) | ROM (n = 212) | Kidney (n = 14) | Gastrointestinal (n = 12) | Lung (n = 62) | ROCM (n = 103) | Disseminated (n = 13) | Total (n = 465) | p value |
|---|------------------|------------------|--------------------|------------------------------|------------------|-------------------|--------------------------|-----------------|---------|
| Risk factors | | | | | | | | | |
| No risk factor | 3 (6.1) | 22 (10.4) | 7 (50%) | 3 (25) | 5 (8.1) | 11 (10.7) | 4 (30.8) | 55 (11.8) | 0.0001 |
| One risk factor | 32 (65.3) | 148 (69.8) | 3 (21.4) | 7 (58.3) | 39 (62.9) | 82 (79.6) | 5 (39.5) | 316 (68) | |
| Two risk factors | 10 (20.4) | 31 (14.6) | 2 (14.3) | 2 (16.7) | 10 (16.1) | 6 (5.8) | 3 (23.1) | 64 (13.8) | |
| Three or more risk factors | 4 (8.2) | 11 (5.1) | 2 (14.3) | 0 | 8 (12.9) | 4 (3.9) | 1 (7.7) | 30 (6.4) | |
| Individual risk factors | | | | | | | | | |
| Diabetes mellitus | 19 (38.8) | 175 (82.5) | 5 (35.7) | 3 (25) | 44 (71) | 88 (85.4) | 8 (61.5) | 342 (73.5) | 0.0001 |
| Diabetes control | | | | | | | | | 0.49 |
| Uncontrolled | 14 | 140 | 5 | 3 | 35 | 76 | 6 | 279 | |
| Controlled | 4 | 33 | 0 | 0 | 7 | 9 | 2 | 55 | |
| Not known | 1 | 2 | 0 | 0 | 2 | 3 | 0 | 8 | |
| Diabetic ketoacidosis ^a | 2 (10.5) | 23 (13.1) | 0 | 1 (33.3) | 7 (15.9) | 16 (18.2) | 1 (12.5) | 50 (14.6) | 0.64 |
| Transplant | | | | | | | | | |
| Solid organ | 2 (4.1) | 11 (5.2) | 4 (28.6) | 0 | 11 (17.7) | 1 (1.0) | 1 (7.7) | 30 (6.5) | 0.0001 |
| Haematopoietic | 1 (2) | 4 (1.9) | 0 | 0 | 1 (1.6) | 0 | 0 | 6 (1.3) | 0.84 |
| Malignancy | | | | | | | | | |
| Haematological | 2 (4.1) | 18 (8.5) | 1 (7.1) | 1 (8.3) | 9 (14.5) | 3 (2.9) | 1 (7.7) | 35 (7.5) | 0.20 |
| Solid organ | 1 (2) | 2 (0.9) | 0 | 1 (8.3) | 1 (1.6) | 1 (1.0) | 1 (7.7) | 7 (1.5) | 0.23 |
| Steroids | 2 (4.1) | 5 (2.4) | 0 | 2 (16.7) | 4 (6.5) | 3 (2.9) | 1 (7.7) | 17 (3.7) | 0.15 |
| Immunosuppressants | 3 (6.1) | 12 (5.7) | 4 (28.6) | 0 | 11 (17.7) | 3 (2.9) | 1 (7.7) | 34 (7.3) | 0.0001 |
| Trauma | 26 (53.1) | 2 (0.9) | 0 | 1 (8.3) | 0 | 3 (2.9) | 0 | 32 (6.9) | 0.0001 |
| Burns | 2 (4.1) | 0 | 0 | 0 | 0 | 0 | 1 (7.7) | 3 (0.6) | 0.001 |
| Presence of co-morbid illnesses | 9 (18.4) | 81 (38.2) | 3 (21.4) | 5 (41.7) | 30 (48.4) | 41 (39.8) | 6 (46.2) | 175 (37.6) | 0.04 |
| Aseptate hyphae on smear | 39 (79.6) | 198 (93.4) | 5 (35.7) | 3 (25) | 54 (87.1) | 97 (94.2) | 10 (76.9) | 406 (87.3) | 0.0001 |
| Culture positivity | 30 (61.2) | 132 (62.3) | 2 (14.3) | 5 (41.7) | 40 (64.5) | 69 (67.0) | 8 (61.5) | 290 (62.4) | 0.001 |
| Histopathological diagnosis | 33 (67.3) | 165 (77.8) | 13 (92.9) | 9 (75.0) | 34 (54.8) | 73 (70.9) | 13 (100) | 340 (73.1) | 0.001 |
| Organism identified ^b | | | | | | | | | 0.16 |
| <i>Rhizopus</i> ^c | 11 | 114 | 0 | 2 | 32 | 65 | 7 | 231 (80.8) | 0.28 |
| <i>Rhizopus arrhizus</i> | 8 | 88 | 0 | 2 | 20 | 54 | 4 | 176 | |
| <i>Rhizopus homothallicus</i> | 0 | 11 | 0 | 0 | 6 | 4 | 1 | 22 | |
| <i>Rhizopus microsporus</i> | 3 | 15 | 0 | 0 | 6 | 6 | 2 | 32 | |
| <i>Rhizopus asexualis</i> | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 1 | |
| <i>Rhizomucor</i> spp. | 1 | 2 | 0 | 0 | 1 | 0 | 0 | 4 (1.4) | |
| <i>Apophysomyces variabilis</i> | 15 | 2 | 2 | 2 | 0 | 1 | 1 | 23 (7.9) | |
| <i>Lichtheimia corymbifera</i> | 1 | 4 | 0 | 0 | 4 | 1 | 0 | 10 (3.5) | |
| <i>Saksenaia vasiformis</i> | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 2 (0.7) | |
| <i>Mucor</i> spp. | 1 | 9 | 0 | 1 | 3 | 2 | 0 | 16 (5.5) | |
| <i>Cunninghamella bertholletiae</i> | 1 | 1 | 0 | 0 | 1 | 0 | 0 | 3 (1.0) | |
| <i>Syncephalastrum racemosum</i> | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 1 (0.4) | |
| Treatment | | | | | | | | | |
| Surgery | 39 (79.6) | 152 (71.7) | 9 (64.3) | 8 (66.7) | 13 (21.0) | 60 (58.3) | 8 (61.5) | 289 (62.2) | 0.0001 |
| Any antifungal | 38 (77.6) | 183 (86.3) | 12 (85.7) | 9 (75.0) | 54 (87.1) | 87 (84.5) | 11 (84.6) | 394 (84.7) | 0.74 |
| Amphotericin B | 35 (71.4) | 177 (83.5) | 12 (85.7) | 8 (66.7) | 51 (82.3) | 87 (84.5) | 11 (84.6) | 381 (81.9) | 0.37 |
| Liposomal | 15 | 114 | 10 | 4 | 32 | 55 | 8 | 238 | 0.04 |
| Deoxycholate | 20 | 63 | 2 | 4 | 19 | 32 | 3 | 143 | 0.30 |
| Posaconazole | 5 (10.2) | 29 (13.7) | 3 (21.4) | 2 (16.7) | 8 (12.9) | 6 (5.8) | 0 | 53 (11.4) | 0.37 |
| Voriconazole | 2 (4.1) | 9 (4.2) | 0 | 0 | 6 (9.7) | 0 | 0 | 17 (3.7) | 0.06 |
| Isavuconazole | 0 | 0 | 0 | 0 | 0 | 0 | 1 (7.7) | 1 (0.2) | 0.0001 |
| Itraconazole | 0 | 6 (2.8) | 0 | 0 | 0 | 2 (1.9) | 0 | 8 (1.7) | 0.65 |
| Fluconazole | 2 (4.1) | 0 | 0 | 0 | 1 (1.6) | 2 (1.9) | 0 | 5 (1.1) | 0.25 |
| Caspofungin | 0 | 2 (0.9) | 0 | 0 | 1 (1.6) | 0 | 0 | 3 (0.6) | 0.88 |
| Amphotericin and posaconazole combination | 5 (10.2) | 29 (13.7) | 3 (21.4) | 2 (16.7) | 8 (12.9) | 6 (5.8) | 0 | 53 (11.4) | 0.25 |
| Duration of symptoms, days | 14 (7–25) | 12.5 (7–30) | 15 (10–20) | 14 (6.3–37.5) | 15 (7–30) | 10 (7–20) | 15 (6.5–52.5) | 12 (7–30) | 0.74 |
| Time to diagnosis, days | 3 (1–6.5) | 1 (1–3) | 6.5 (2.8–16.5) | 7 (3–13) | 3 (1–9.3) | 1 (1–1) | 1 (1–1) | 1 (1–1) | 0.001 |
| Duration of hospital stay, days | 15 (6–23.5) | 17 (5.3–31) | 24 (7.5–46) | 24 (9.8–33.5) | 16 (8–35.5) | 11 (3–30) | 26 (11.5–51) | 16 (6–32) | 0.99 |
| 90-day mortality | 28 (57.1) | 82 (38.7) | 7 (50) | 8 (66.7) | 38 (61.3) | 71 (68.9) | 8 (61.5) | 242 (52) | 0.98 |

All values are represented as number (%) or median (interquartile range) unless otherwise stated.

^a Percentages are those among diabetic participants.^b Percentages are those among culture-positive cases. The remaining percentages are for the total number of participants having a particular site of involvement, mentioned in the first row of the table.^c Total species identified: 218; 13 species not identified for logistical reasons.

On a multivariate analysis, the duration of symptoms before hospitalization, the site of involvement (rhino-orbital mucormycosis with cranial extension and disseminated disease), and treatment with deoxycholate amphotericin B preparation were associated with increased mortality. On the other hand, combined surgical and medical management and the duration of

antifungal therapy were independently associated with better survival (Table 4). The median duration of hospital stay was 16 (6–32) days. The median (IQR) time to death was 32 (23–41) days; this was significantly longer (median 58 days versus 12 days) in those with combined medical and surgical management (Fig. 1).

Table 3
Characteristics of survivors and non-survivors with mucormycosis

| | Survivors (n = 223) | Non-survivors (n = 242) | p value |
|---|---------------------|-------------------------|---------------|
| Age, in years | 45 (34–58) | 48.5 (36–60) | 0.20 |
| Male sex | 161 (72.2) | 162 (66.9) | 0.22 |
| Adult patients | 210 (94.2) | 228 (94.2) | 0.98 |
| Duration of symptoms ^a , days | 15 (7–30) | 10 (7–20) | 0.0001 |
| Predisposing factors | | | |
| None | 31 (13.9) | 24 (9.9) | 0.21 |
| One | 151 (67.8) | 165 (68.2) | |
| Two | 32 (14.3) | 32 (13.2) | |
| Three or more | 9 (4.0) | 21 (8.7) | |
| Individual predisposing factors | | | |
| Diabetes mellitus | 162 (72.6) | 180 (74.4) | 0.67 |
| Uncontrolled diabetes | 131/162 (80.9) | 148/180 (82.2) | 0.66 |
| Diabetic ketoacidosis | 20/162 (12.3) | 30/180 (16.7) | 0.26 |
| Transplant | | | |
| Solid organ | 12 (5.4) | 18 (7.4) | 0.37 |
| Haematopoietic | 3 (1.3) | 3 (1.2) | 1.00 |
| Malignancy | | | |
| Haematological malignancy | 15 (6.7) | 20 (8.3) | 0.53 |
| Solid organ malignancy | 0 | 7 (2.9) | 0.02 |
| Steroids | 11 (4.9) | 6 (2.5) | 0.16 |
| Immunosuppressants | 12 (5.4) | 22 (9.1) | 0.13 |
| Trauma | 14 (6.3) | 18 (7.4) | 0.62 |
| Burns | 1 (0.4) | 2 (0.8) | 1.00 |
| Co-morbid illnesses ^b | 63 (28.3) | 112 (46.3) | 0.0001 |
| Chronic kidney disease | 31 (13.9) | 62 (25.6) | |
| Cardiovascular | 25 (11.2) | 42 (17.4) | |
| Pulmonary | 10 (4.5) | 20 (8.3) | |
| Liver disease | 5 (2.2) | 19 (7.9) | |
| Neurological | 5 (2.2) | 13 (5.4) | |
| Others | 0 | 1 (0.4) | |
| Site of mucormycosis | | | 0.0001 |
| Rhino-orbital | | | |
| with brain involvement | 32 (14.3) | 71 (29.3) | |
| without brain involvement | 130 (58.3) | 82 (33.9) | |
| Pulmonary | 24 (10.8) | 38 (15.7) | |
| Cutaneous | 21 (9.4) | 28 (11.6) | |
| Renal | 7 (3.1) | 7 (2.9) | |
| Gastrointestinal | 4 (1.8) | 8 (3.3) | |
| Disseminated | 5 (2.2) | 8 (3.3) | |
| Microbiology | | | |
| <i>Rhizopus</i> spp. | 96 (43.0) | 135 (55.8) | 0.16 |
| Other species | 25 (11.2) | 30 (12.4) | |
| ^c Time to diagnosis, days | 1 (1–4) | 1 (1–4.3) | 0.61 |
| Treatment | | | |
| Amphotericin B | | | 0.0001 |
| Deoxycholate | 57 (25.6) | 70 (28.9) | |
| Liposomal | 135 (60.5) | 103 (42.6) | |
| Amphotericin and posaconazole combination | 32 (14.3) | 21 (8.7) | 0.89 |
| Duration of antifungal therapy, days | 21 (13–41) | 6 (2–16) | 0.0001 |
| Combined surgical and medical management | 171 (76.7) | 73 (30.2) | 0.0001 |
| Hospital stay, days | 24 (15–42) | 8 (3–20.3) | 0.0001 |

All values are represented as number (%) or median (interquartile range) unless otherwise stated. The percentages are for the survivors (n = 223) or non-survivors (n = 242), as applicable unless otherwise stated.

^a Duration of symptoms represent the time between onset of symptom to admission.

^b Many patients had more than one co-morbid illness.

^c The time to diagnosis indicates the time since admission till achieving the diagnosis of mucormycosis.

Discussion

We report the largest prospective multicentre study describing the epidemiology, predisposing factors, diagnosis, management practices and outcome of mucormycosis in India. Diabetes mellitus (73.5%) was the predominant risk factor. We observed a high mortality rate and identified several risk factors associated with mortality, including disseminated or rhino-orbital mucormycosis with cranial extension, shorter duration of symptoms, shorter duration of antifungal therapy, and the use of amphotericin B deoxycholate. A combined surgical and medical management was associated with better survival.

The prevalence of mucormycosis has been variably reported from different centres [1], partly because of the divergent risk factors prevalent in different settings [3,8,17,18]. Data from a global fungal infection registry reports haematological malignancy (63%) to be the most important underlying condition for mucormycosis [10]. In contrast, uncontrolled diabetes was the main predisposing factor in the current study. The situation might also be similar in other low- and middle-income countries, where diabetes is prevalent [19]. Interestingly, 11.8% of the cases of mucormycosis had no apparent risk factors, especially in those with isolated renal mucormycosis. The most common pathogen in our study was *R. arrhizus* (similar to other studies) [20–22]. *Apophysomyces*

Table 4
Cox regression analysis of factors predicting mortality in patients with mucormycosis

| | Adjusted HR (95% CI) | p value |
|--|----------------------|---------------|
| Duration of symptoms, days | 0.99 (0.98–0.99) | 0.009 |
| Malignancy | | |
| None ^a | - | |
| Solid | 3.01 (0.65–13.98) | 0.16 |
| Haematological | 1.33 (0.74–2.39) | 0.34 |
| Presence of co-morbid illnesses | 1.52 (1.15–2.02) | 0.06 |
| Number of risk factors | | |
| None ^a | - | |
| One | 1.67 (0.93–2.99) | 0.08 |
| Two | 0.67 (0.31–1.45) | 0.31 |
| Three or more | 0.87 (0.38–1.98) | 0.75 |
| Site of involvement | | |
| Rhino-orbital without cranial involvement ^a | - | |
| Cutaneous | 1.38 (0.79–2.42) | 0.25 |
| Solid organ (lung, kidney, gastrointestinal) | 1.12 (0.73–1.71) | 0.60 |
| Rhino-orbital with cranial involvement | 1.91 (1.30–2.79) | 0.001 |
| Disseminated | 2.81 (1.23–6.41) | 0.014 |
| Duration of antifungal therapy, days | 0.96 (0.95–0.97) | 0.0001 |
| Amphotericin B therapy | | |
| None ^a | - | |
| Deoxycholate preparation | 2.21 (1.12–4.38) | 0.023 |
| Liposomal | 1.25 (0.65–2.42) | 0.51 |
| Management | | |
| Medical management alone ^a | - | |
| Combined surgical and medical management | 0.52 (0.38–0.73) | 0.0001 |

Abbreviation: HR, hazard ratio.

Statistically significant values are highlighted in bold type.

^a Reference category.

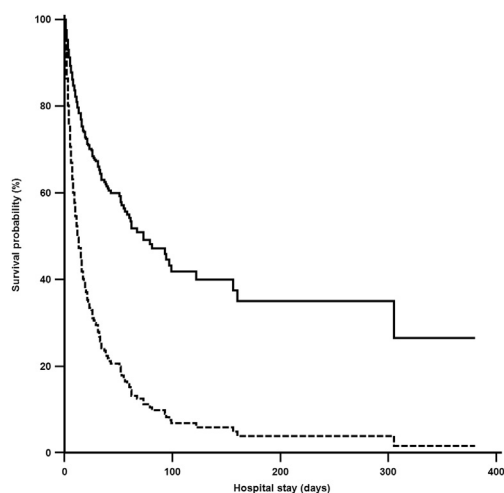


Fig. 1. Mortality (90-day) in participants with mucormycosis who underwent combined medical and surgical management (solid line) versus those managed medically (dotted line). The time to death was significantly less in those managed only medically.

variabilis was the most common agent responsible for cutaneous mucormycosis, consistent with previous knowledge [23]. We also isolated a larger proportion of *R. microsporus*, *A. variabilis* and *R. homothallicus*, which are abundantly present in soil samples from India [24] and are emerging pathogens in this country [24–26].

We identified several challenges in managing mucormycosis in our study including a delay in seeking health care, the lack of knowledge among physicians, and financial constraints. In fact, cutaneous mucormycosis was diagnosed after considerable delay, despite being easily amenable to diagnostic sampling, and possibly explains the high (57.1%) mortality observed. A combination of antifungal agents was prescribed in 22.1% of patients, despite the lack of any recommendation for this practice [27]. Further, the use of improper antifungals (voriconazole, itraconazole, fluconazole)

for management seen in a sizeable proportion of our participants, highlights the lacunae in knowledge among physicians. A significant proportion of our participants were unable to afford treatment or had to be switched from liposomal amphotericin to the deoxycholate preparation because of financial constraints.

Participants who underwent combined medical and surgical management had a significantly better outcome, similar to previous experience [28]. Surgical debridement of the necrosed tissue probably enables better penetration of antifungal agents, thereby improving outcomes. The surgical rate was highest in those with rhino-orbital mucormycosis. Unfortunately, even in rhino-orbital disease, radical surgery was not feasible in all individuals. Mortality was significantly high in patients with intracranial extension, where most were inoperable. Despite appropriate antifungal therapy, mortality was high among participants who were inoperable, suggesting a need for early diagnosis and better therapeutic strategies.

Finally, our study is not without limitations. Although an epidemiological study, we were unable to assess the exact incidence or prevalence of mucormycosis in different risk groups. Although we have described the predisposing factors, we were not able to assess the strength of association of these risk factors, because of the absence of a control group. However, the study provides a rough estimate of proven mucormycosis cases (about 40 cases on average over a 21-month period from each of the participating centres) in India, which is much higher compared with world literature [10,21]. We have reported the treatment outcome of mucormycosis from a heterogeneous population (various risk factors and different sites of involvement), so drawing conclusions for individual clinical presentations is difficult. Similarly, the study results may not be generalizable to centres where haematological malignancy and transplantation are the dominant risk factors. Though the financial toxicity seems apparent, we have not performed a formal health economic analysis. The prospective study design, the detailed description of the microbiology and management practices, and challenges faced in a low- and

middle-income country setting are the major strengths of the current study. Further, being a multicentre study from different regions of India, the results may be widely applicable.

In conclusion, mucormycosis is a serious problem in India with a high mortality. Uncontrolled diabetes mellitus was the major predisposing factor. A combined surgical and medical management was associated with better outcomes. The rarer *Mucorales* identified in our study warrant further evaluation. The gaps in knowledge identified in the study need to be addressed urgently and effectively.

Transparency declaration

The authors have submitted completed ICMJE forms to the corresponding author. On behalf of all authors, the corresponding author declares that they received funds from the Fungal Infection Study Forum to conduct the study. There were no other conflicts of interest. Mylan Pharmaceuticals provided an educational grant to the Fungal Infection Study Forum (a non-profit educational trust dedicated to the study of fungal infection in India). The Fungal Infection Study Forum supported the study from the same fund.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.cmi.2019.11.021>.

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