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Original article

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

A. Patel [1, 2], H. Kaur [3], I. Xess [4], J.S. Michael [5], J. Savio [6], S. Rudramurthy [3], R. Singh [7], P. Shastri [8], P. Umabala [9], R. Sardana [10], A. Kindo [11], M.R. Capoor [12], S. Mohan [13], V. Muthu [14], R. Agarwal [14], 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 2019;▪:1**

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

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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, multicentre 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-calcofluor 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′-TCCGTCAATTCCTTTAAGTTTC-3′) and ZM3 (5′-CAATCCAAGAATT TCACCTCTAG-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′-GGATTTGGTCGTATTGGG-3′; reverse primer: 5′-GGAAGATGGTG ATGGGATT-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

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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 co-morbid 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 21 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 co-morbid 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).

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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
Rhizopus[c]	11	114	0	2	32	65	7	231 (80.8)	0.28
Rhizopus arrhizus	8	88	0	2	20	54	4	176	
Rhizopus homothallicus	0	11	0	0	6	4	1	22	
Rhizopus microporus	3	15	0	0	6	6	2	32	
Rhizopus asexualis	0	0	0	0	0	1	0	1	
Rhizomucor spp.	1	2	0	0	1	0	0	4 (1.4)	
Apophysomyces variabilis	15	2	2	2	0	1	1	23 (7.9)	
Lichtheimia corymbifera	1	4	0	0	4	1	0	10 (3.5)	
Saksenaea vasiformis	1	1	0	0	0	0	0	2 (0.7)	
Mucor spp.	1	9	0	1	3	2	0	16 (5.5)	
Cunninghamella bertholletiae	1	1	0	0	1	0	0	3 (1.0)	
Syncephalastrum racemosum	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).

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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			
Rhizopus 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*

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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.

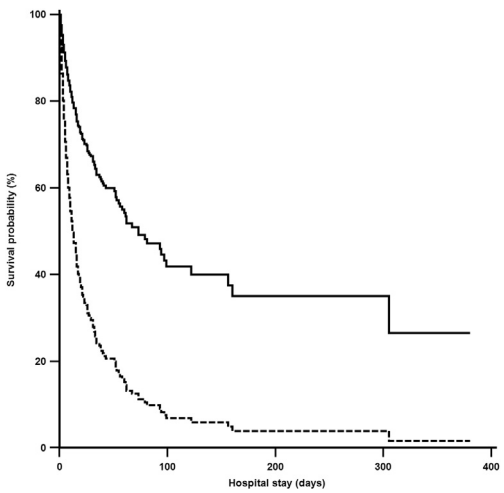

Fig. 1. Mortality (90-day) in participants with mucormycosis who underwent combined medical and surgical management (solid line) versus those managed medically (dotted line). The time to death was significantly less in those managed only medically.

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

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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.

References

[1] Prakash H, Chakrabarti A. Global epidemiology of mucormycosis. J Fungi (Basel) 2019;5.
[2] Chakrabarti A, Das A, Mandal J, Shivaprakash MR, George VK, Tarai B, et al. The rising trend of invasive zygomycosis in patients with uncontrolled diabetes mellitus. Med Mycol 2006;44:335–42.
[3] Prakash H, Ghosh AK, Rudramurthy SM, Singh P, Xess I, Savio J, et al. A prospective multicenter study on mucormycosis in India: epidemiology, diagnosis, and treatment. Med Mycol 2019;57:395–402.
[4] Farmakiotis D, Kontoyiannis DP. Mucormycoses Infect Dis Clin North Am 2016;30:143–63.
[5] Dioverti MV, Cawcutt KA, Abidi M, Sohail MR, Walker RC, Osmon DR. Gastrointestinal mucormycosis in immunocompromised hosts. Mycoses 2015;58:714–8.
[6] Ibrahim AS, Spellberg B, Walsh TJ, Kontoyiannis DP. Pathogenesis of mucormycosis. Clin Infect Dis 2012;54:S16–22.
[7] Rammaert B, Lanternier F, Poiree S, Kania R, Lortholary O. Diabetes and mucormycosis: a complex interplay. Diabetes Metab 2012;38:193–204.
[8] Chakrabarti A, Chatterjee SS, Das A, Panda N, Shivaprakash MR, Kaur A, et al. Invasive zygomycosis in India: experience in a tertiary care hospital. Postgrad Med J 2009;85:573–81.
[9] Roden MM, Zaoutis TE, Buchanan WL, Knudsen TA, Sarkisova TA, Schaufele RL, et al. Epidemiology and outcome of zygomycosis: a review of 929 reported cases. Clin Infect Dis 2005;41:634–53.
[10] Ruping MJ, Heinz WJ, Kindo AJ, Rickerts V, Lass-Florl C, Beisel C, et al. Forty-one recent cases of invasive zygomycosis from a global clinical registry. J Antimicrob Chemother 2010;65:296–302.
[11] Spellberg B, Ibrahim AS, Chin-Hong PV, Kontoyiannis DP, Morris MI, Perfect JR, et al. The deferasirox-ambisome therapy for mucormycosis (Defeat Mucor) study: a randomized, double-blinded, placebo-controlled trial. J Antimicrob Chemother 2012;67:715–22.
[12] Patel AK, Patel KK, Patel K, Gohel S, Chakrabarti A. Mucormycosis at a tertiary care centre in Gujarat, India. Mycoses 2017;60:407–11.
[13] Bala K, Chander J, Handa U, Punia RS, Attri AK. A prospective study of mucormycosis in north India: experience from a tertiary care hospital. Med Mycol 2015;53:248–57.
[14] Bialek R, Konrad F, Kern J, Aepinus C, Cecenas L, Gonzalez GM, et al. PCRr based identification and discrimination of agents of mucormycosis and aspergillosis in paraffin wax embedded tissue. J Clin Pathol 2005;58:1180–4.
[15] Zaman K, Rudramurthy SM, Das A, Panda N, Honnavar P, Kaur H, et al. Molecular diagnosis of rhino-orbito-cerebral mucormycosis from fresh tissue samples. J Med Microbiol 2017;66:1124–9.
[16] Lau A, Chen S, Sorrell T, Carter D, Malik R, Martin P, et al. Development and clinical application of a panfungal PCR assay to detect and identify fungal DNA in tissue specimens. J Clin Microbiol 2007;45:380–5.
[17] Chakrabarti A, Das A, Sharma A, Panda N, Das S, Gupta KL, et al. Ten years' experience in zygomycosis at a tertiary care centre in India. J Infect 2001;42:261–6.
[18] Jeong W, Keighley C, Wolfe R, Lee WL, Slavin MA, Kong DCM, et al. The epidemiology and clinical manifestations of mucormycosis: a systematic review and meta-analysis of case reports. Clin Microbiol Infect 2019;25:26–34.
[19] Corzo-Leon DE, Chora-Hernandez LD, Rodriguez-Zulueta AP, Walsh TJ. Diabetes mellitus as the major risk factor for mucormycosis in Mexico: epidemiology, diagnosis, and outcomes of reported cases. Med Mycol 2018;56:29–43.
[20] Petrikkos G, Skiada A, Drogari-Apiranthitou M. Epidemiology of mucormycosis in Europe. Clin Microbiol Infect 2014;20:67–73.
[21] Petrikkos G, Skiada A, Lortholary O, Roilides E, Walsh TJ, Kontoyiannis DP. Epidemiology and clinical manifestations of mucormycosis. Clin Infect Dis 2012;54:S23–34.
[22] Dolatabadi S, Ahmadi B, Rezaei-Matehkolaei A, Zarrinfar H, Skiada A, Mirhendi H, et al. Mucormycosis in Iran: a six-year retrospective experience. J Mycol Med 2018;28:269–73.
[23] Meis JF, Chakrabarti A. Changing epidemiology of an emerging infection: zygomycosis. Clin Microbiol Infect 2009;15:10–4.
[24] Prakash H, Ghosh AK, Rudramurthy SM, Paul RAa, Gupta S, Negi V, et al. The environmental source of emerging *Apophysomyces variabilis* infection in India. Med Mycol 2016;54:567–75.
[25] Pandey M, Singh G, Agarwal R, Dabas Y, Jyotsna VP, Kumar R, et al. Emerging *Rhizopus microsporus* infections in India. J Clin Microbiol 2018;56.
[26] Manesh A, Rupali P, Sullivan MO, Mohanraj P, Rupa V, George B, et al. Mucormycosis—a clinicoepidemiological review of cases over 10 years. Mycoses 2019;62:391–8.
[27] Cornely OA, Arikan-Akdagli S, Dannaoui E, Groll AH, Lagrou K, Chakrabarti A, et al. ESCMID and ECMM joint clinical guidelines for the diagnosis and management of mucormycosis 2013. Clin Microbiol Infect 2014;20:5–26.
[28] Skiada A, Lass-Floerl C, Klimko N, Ibrahim A, Roilides E, Petrikkos G. Challenges in the diagnosis and treatment of mucormycosis. Med Mycol 2018;56:93–101.