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Original Article

A prospective multicenter study on mucormycosis in India: Epidemiology, diagnosis, and treatment

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Abstract

Mucormycosis due to *Mucorales* is reported at large numbers in uncontrolled diabetics across India, but systematic multicenter epidemiological study has not been published yet. The present prospective study was conducted at four major tertiary care centers of India (two in north and two in south India) during 2013–2015 to compare the epidemiology, treatment strategies and outcome of mucormycosis between the two regions. Molecular techniques were employed to confirm the identity of the isolates or to identify the agent in biopsy samples. A total of 388 proven/probable mucormycosis cases were reported during the study period with overall mortality at 46.7%. Uncontrolled diabetes ($n = 172$, 56.8%) and trauma ($n = 31$, 10.2%) were the common risk factors. Overall, *Rhizopus arrhizus* ($n = 124$, 51.9%) was the predominant agent identified, followed by *Rhizopus microsporus* ($n = 30$, 12.6%), *Apophysomyces variabilis* ($n = 22$, 9.2%) and *Rhizopus homothallicus* ($n = 6$, 2.5%). On multivariate analysis, the mortality was significantly associated with gastrointestinal (OR: 18.70, $P = .005$) and pulmonary infections (OR: 3.03, $P = .015$). While comparing the two regions, majority (82.7%) cases were recorded from north India; uncontrolled diabetes ($n = 157$, $P = .0001$) and post-tubercular mucormycosis ($n = 21$, $P = .006$) were significantly associated with north Indian cases. No significant difference was noted among the species of *Mucorales* identified and treatment strategies between the two regions. The mortality rate was significantly higher in north Indian patients (50.5%) compared to 32.1% in south India ($P = .016$). The study highlights higher number of mucormycosis cases in uncontrolled diabetics of north India and emergence of *R. microsporus* and *R. homothallicus* across India causing the disease.

Key words: epidemiology, mucormycosis, diagnosis, *Rhizopus arrhizus*, *Rhizopus homothallicus*, therapy.

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Introduction

Mucormycosis is the third most common invasive fungal infection with high morbidity and mortality.[1,2] The disease is prevalent in uncontrolled diabetic patients of India,[3–6] in contrast to patients with hematological malignancies and transplant recipients of developed countries.[7,8] Over the years certain changes in epidemiology, diagnosis and management of mucormycosis have been noted.[9–11] An emerging clinical entity, the isolated renal mucormycosis in immunocompetent hosts has been recorded increasingly from north Indian patients.[10] Despite the rise in awareness of the disease, the early diagnosis of mucormycosis remains elusive due to difficulty in sample collection from deep tissues and absence of a biomarker.[2] In recent years polymerase chain reaction (PCR) for early diagnosis of mucormycosis has been evaluated with good results, but no standardized commercial kit is still available for routine use.[12–15]

The isolation of *Mucorales* fails in considerable number of cases due to the delicate nature of the hyphae. Occasionally, tissue materials are sent in formalin to histopathology laboratory only. The introduction of molecular technique has improved the identification of fungus even in paraffin-embedded tissue.[14,15] However, *Rhizopus arrhizus* is the predominant causative agent worldwide,[6,7] a geographical variation has been noted for other etiological agents.[7,16,17] *Apophysomyces variabilis* is the second most common agent in India.[17] Several new species are also recognized to cause mucormycosis in India including *Rhizopus homothallicus*, *Thamnostylum lucknowense* and *Mucor irregularis*.[18–20]

Isavuconazole, a new antifungal agent has been introduced in managing mucormycosis,[21] but the drug is not available in Indian market. Amphotericin B, oral posaconazole liquid suspension, and occasionally deferasirox are used to treat mucormycosis patients in this country.[4–6] Considering the rise in awareness and possible difference in epidemiology of mucormycosis across vast country like India, we conducted the present study at four major tertiary care centers (two each in north and south India) to compare the epidemiology, treatment strategies, and outcome between the two regions. As multiple series had been reported at time interval from one of the two centres in north India, the present data were also compared with earlier reports to find any change in epidemiology of the disease.

Methods

Study design

A prospective multicenter study was conducted during January 2013 through December 2015 at four major tertiary care centres in India: from north India, Postgraduate Institute of Medical Education and Research (PGIMER), Chandigarh (inpatient beds: 1948 beds with ∼85 000 admissions per year) and All India Institute of Medical Sciences (AIIMS), New Delhi (2362 beds with ∼23 0000 admissions per year); from south India, St. John's Medical College (SJMC), Bengaluru (1400 beds with ∼50 000 admissions per year) and Nizam's Institute of Medical Sciences (NIMS), Hyderabad (1300 beds with ∼42 000 admissions per year). All the four participating centers are well-known tertiary care centers of the country with similar healthcare access and delivery system. All the centers have super speciality units including hematology, transplant, pulmonary medicine, nephrology, neurology, endocrine care, and so forth. The medical mycology laboratories of all the four centers have routine diagnosis facilities including biomarker tests. The study was approved by the Institutional Ethics committee at the respective institutes. All consecutive cases of mucormycosis (caused by *Mucorales*) during the study period were enrolled in the study. The cases were classified as proven or probable mucormycosis as per European Organization for Research and Treatment of Cancer/Mycoses Study Group (EORTC/MSG) guidelines.[22] The modification was nonadherence to the host specific criteria of EORTC/MSG guidelines to define some of the probable cases, where uncontrolled diabetes was the major underlying disease.[23]

Study population

The clinical details of patients with proven and probable mucormycosis were noted prospectively during the study period. The clinical details included the anatomical site of involvement, underlying illness, mode of diagnosis, causative agents, treatment, and outcome of the disease. Based on the anatomical site affected clinical cases were categorized into: rhino-orbito-cerebral (ROCM) mucormycosis, pulmonary, gastrointestinal, cutaneous, isolated renal and disseminated diseases. Disseminated mucormycosis was defined as the noncontiguous involvement of more than one deep organ. Mucormycosis in post-pulmonary tuberculosis refers to invasion of *Mucorales* in the existing pulmonary cavities after anti-tuberculosis treatment. Clinical outcome was assessed as all-cause mortality at the time of death or discharge from hospital.

Histopathology and microbiology

The mucormycosis cases were diagnosed using histopathology and mycological techniques (calcofluor white/potassium hydroxide stained wet mount and isolation). The *Mucorales* were presumptively identified on the basis of their microscopic morphology and temperature tolerance. The identification was confirmed by sequencing of internal transcribed spacer (ITS) region of ribosomal DNA of the isolates.[17,24] In culture negative cases, the identification of the *Mucorales* in tissue specimens was attempted by extraction of the fungal DNA from tissue. The small ribosomal subunit (18S rDNA) region was sequenced, and BLAST analysis was performed.[14,15]

Table 1. Clinical spectrum of mucormycosis in north and south India.

S. No	Site of infection	Total cases N (%)	North India		South India	
			PGIMER N (%)	AIIMS N (%)	SJMC N (%)	NIMS N (%)
1	Rhino-orbito-cerebral	248 (63.9)	162 (41.8)	39 (10.1)	38 (9.8)	9 (2.3)
2	Renal	21 (5.4)	20 (5.2)	–	1 (0.3)	–
3	Gastrointestinal	25 (6.4)	22 (5.7)	1 (0.3)	1 (0.3)	1 (0.3)
4	Cutaneous	37 (9.5)	20 (5.2)	8 (2.1)	2 (0.5)	7 (1.8)
5	Pulmonary	50 (12.9)	39 (10.1)	5 (1.3)	2 (0.5)	4 (1.0)
6	Others$	7 (1.8)	4 (1.0)	1 (0.3)	–	2 (0.5)
	Total	388 (100)	267 (68.8)	54 (13.9)	44 (11.3)	23 (5.9)

$Included 4 cases of disseminated infection and one case each of sub-glottis, middle ear, and bone infection.
AIIMS, All India Institute of Medical Sciences; NIMS, Nizam's Institute of Medical Sciences; PGIMER, Postgraduate Institute of Medical Education and Research; SJMC, St. John's Medical College.

Statistical analysis

Statistical analysis was performed using SPSS v.22. A χ^2 test was used to analyze the qualitative variables. A two-sided P value of <.05 was considered significant. The statistically significant variables by univariate analysis ($P < .05$) were included in the multivariate model. For multivariate analysis, backward, stepwise multivariate, likelihood logistic regression approach was used. The data from the present case series of PGIMER (2013–2015) was compared with the reports published earlier from same centre (1990–2007).[4–6]

Results

During the study period 388 proven/probable cases of mucormycosis were recorded from four centers in India: PGIMER ($n = 267$, 68.8%), AIIMS ($n = 54$, 13.9%), SJMC ($n = 44$, 11.3%), and NIMS ($n = 23$, 5.9%). Of the 388 cases, 281 were proven cases diagnosed using histopathology of deep tissue ($n = 276$, 71.1%) and direct microscopy of samples collected from sterile sites ($n = 5$, 1.3%). A total of 107 cases (27.6%) were diagnosed as probable mucormycosis. The male to female ratio was 2.3:1 (271:117) and the median age of the patients was 45.5 years (range, 1 month to 85 years). Thirty-one patients were younger than 16 years, and 15 patients were ≤2 years old. The age distribution of the patients with the clinical spectrum of the disease is presented in Supplementary Table S1. Clinical details could be recorded in 303 patients and treatment and outcome details in 276 patients.

Site of infection

The clinical spectrum of the disease at different geographical locations is presented in Table 1. Based on the site of infection, the cases were categorised as ROCM type in 248 (63.9%), pulmonary 50 (12.9%), cutaneous 37 (9.5%), gastrointestinal 25 (6.4%), isolated renal 21 (5.4%), disseminated mucormycosis in four (1%) cases. Among other sites one patient each had involvement of bone, sub-glottis and middle ear. In ROCM cases ($n = 190$), the disease was restricted to sino-nasal area in 80 patients (42.1%), sino-orbital involvement in 84 (44.2%), and intracranial extension in 26 (13.6%) patients. In pulmonary mucormycosis ($n = 38$), 11 (28.9%) of patients had pleural involvement. In gastrointestinal mucormycosis ($n = 18$), the sites commonly affected were ileum and jejunum ($n = 12$, 66.7%), stomach ($n = 2$, 11.1%), colon ($n = 2$, 11.1%), caecum and appendix ($n = 2$, 11.1%).

Predisposing diseases/risk factors

The underlying diseases/risk factors associated with mucormycosis in north and south Indian population were depicted in Table 2. Diabetes mellitus ($n = 157$, $P < .0001$) and post-pulmonary tuberculosis ($n = 21$, $P = .006$) were the significant factors associated with mucormycosis in north Indian patients. The predisposing disease/risk factors were also analysed against different clinical categories of mucormycosis ($n = 303$) (Supplementary Table S2). Overall, uncontrolled diabetes mellitus was the major risk factor in 172 (56.8%) patients, and 31 (18%) had diabetic ketoacidosis. The majority of patients with ROC mucormycosis had uncontrolled diabetes ($n = 113$, 65.7%). Solid organ transplant and steroid therapy were significant risk factors for both ROC and pulmonary mucormycosis. Post-pulmonary tuberculosis (OR: 0.279, $P = .002$) was identified as risk factor for pulmonary mucormycosis patients. Penetrating trauma (OR: 0.16, $P = .0001$) and diabetes mellitus (OR: 2.02, $P = .04$) were significantly associated with cutaneous mucormycosis. Among patients with gastrointestinal mucormycosis 44% cases were in paediatric age group. A significant number of isolated renal mucormycosis were seen in apparently immunocompetent hosts (OR: 0.236, $P = .006$).

Diagnosis and etiological agent

Of 388 cases, 115 (29.6%) cases were diagnosed by histopathology alone, 112 (28.9%) by direct microscopic examination of

Table 2. Risk factors associated with mucormycosis in north and south India.

S. No	Risk factor	No of patients# (N)	North India N (%)	South India N (%)	P value
1	Diabetes mellitus	172	157 (91.3)	15 (8.7)	<.0001*
2	Diabetic ketoacidosis	31	28 (90.3)	3 (9.7)	.108
3	Haematological malignancy	19	16 (84.2)	3 (15.8)	.775
4	Post-pulmonary tuberculosis	21	21 (100)	0	.006*
5	Chemotherapy	16	14	2	.537
6	Steroid therapy	30	27 (90)	3 (10)	.107
7	Solid organ transplant	19	16 (84.2)	3 (15.8)	.775
8	Penetrating trauma	31	21 (67.7)	10 (32.3)	.171
9	Dialysis	23	18 (78.3)	5 (21.7)	. . .
10	Chronic kidney disease	27	15 (55.6)	12 (44.4)	.006*
11	Neutropenia	18	16 (88.9)	2 (11.1)	.380
12	Immunocompetent	32	30 (93.8)	2 (6.3)	.023*

*$P < .05$ is considered statistically significant.

#A total of 303 (north India = 236; south India = 67) cases were analyzed.

calcofluor white/potassium hydroxide stained wet mount, and 161 (41.5%) by both histopathology and direct microscopy. The causative agents could be identified in 239 (61.6%) cases (Table 3). *Mucorales* were isolated in 183 (47.2%) cases. Additional 56 (14.4%) cases were identified by molecular techniques from paraffin embedded tissues. No geographic variation was observed among the *Mucorales* isolated from the north and south Indian population. Overall, *R. arrhizus* ($n = 124$, 51.9%) was the predominant agent, followed by *R. microsporus* ($n = 30$, 12.6%) and *A. variabilis* ($n = 22$, 9.2%). The species of *Rhizopus* could not be confirmed in 32 (13.4%) cases. *Rhizopus homothallicus* was isolated from six patients. In four ROCM cases mixed infection with *Aspergillus* was noted. The association of etiological agents with the clinical category and the predisposing disease/risk factor is presented in Supplementary Tables S3 and S4.

Treatment and outcome

The details of treatment and outcome were noted in 276 patients (Table 4, Supplementary Table S5). No significant difference in treatment strategies was noted between the two regions (Table 4). The overall mortality rate among 276 patients was 46.7%. Mortality rate was significantly higher in north India (50.5%) in comparison to 32.1% in south India ($P = .016$). The mortality rate was high when patients treated with surgery alone (60.9%) or amphotericin B alone (54.4%). No statistical difference was observed in the mortality rate between patients either treated by surgery alone or with only with amphotericin B therapy ($P = .63$). Patients who were managed with a combination of surgical debridement and amphotericin B therapy had significantly lower mortality (32.4%, $P < .0001$) (Table 4). Combination therapy with surgical debridement and amphotericin B was effective than surgery alone ($P = .011$) and amphotericin B alone ($P = .003$) therapy. Univariate analysis among survivors and nonsurvivors revealed that the mortality was higher in

patients with gastrointestinal (94.1%, OR: 9.58, $P = .0001$) and pulmonary (76.5%, OR: 2.44, $P = .0001$) mucormycosis (Supplementary Table S6). ROCM group of patients had better survival rate (64.4%, OR: 0.53, $P = .0001$). Among risk factors, steroid therapy (OR: 1.73, $P = .027$) and chronic kidney disease (OR: 1.91, $P = .018$) were the independent risk factors associated with high mortality (Supplementary Table S7). Among the different *Mucorales*, infections caused by *R. homothallicus* and *Mucor* species were associated with 100% mortality (Supplementary Table S8). On multivariate analysis, gastrointestinal (OR: 18.70, $P = .005$) and pulmonary infection (OR: 3.03, $P = .015$) were significantly associated with high mortality (Table 5).

Discussion

This is the first multicenter study at four major tertiary care centers of India that evaluated the epidemiology, risk factors, causative agents, treatment, and outcome of mucormycosis and compared the results of the two regions of the country. The cohort of 388 patients is the largest series on mucormycosis from any single country, and the number is alarming. Though the four tertiary care centers were similar in demography, patient groups and care, the majority (68.8%) of the cases were from PGIMER, Chandigarh. Awareness of fungal diseases among clinicians of that center is high due to regular medical autopsies and clinic-pathological conferences. In addition, the mycology laboratory at PGIMER, Chandigarh, is the reference center of advance research in medical mycology for the country. Similar high proportion (70%) of mucormycosis cases from that centre was noted in an earlier review of 461 cases from India. The authors of the review attributed the high incidence at that center to better awareness, expertise and competence in mycological diagnosis.[25] Three large series of mucormycosis cases were reported at different time intervals from the same center.[4–6] While comparing with the earlier series, a rise in incidence has been observed from

Table 3. Spectrum of *Mucorales* isolated from north and south India.

S. No	Organism	North India					South India				
		ROCM N (%)	Pulmonary N (%)	Cutaneous N (%)	Others$ N (%)	Total[a] N (%)	ROCM N (%)	Pulmonary N (%)	Cutaneous N (%)	Others$ N (%)	Total[b] N (%)
1	*Rhizopus arrhizus*#	78 (42.4)	11 (6)	4 (2.2)	5 (2.7)	98 (53.3)	22 (40)	4 (7.3)	–	–	26 (47.3)
2	*Rhizopus microsporus*#	12 (6.5)	4 (2.2)	4 (2.2)	4 (2.2)	24 (13)	4 (7.3)	–	1 (1.8)	1 (1.8)	6 (10.9)
3	*Apophysomyces variabilis*#	10 (5.4)	–	5 (2.7)	3 (1.3)	18 (9.8)	–	–	4 (7.3)	–	4 (7.3)
4	*Rhizopus homothallicus*#	3 (1.6)	2 (1.1)	–	–	5 (2.7)	–	–	1 (1.8)	–	–
5	*Rhizopus* species§	12 (6.5)	2 (1.1)	1 (0.5)	2 (1.1)	17 (9.2)	12 (21.8)	1 (1.8)	1 (1.8)	1 (1.8)	15 (27.3)
6	*Rhizopus stolonifer*#	1 (0.5)	–	–	–	1 (0.5)	–	–	–	–	–
7	*Saksenaea vasiformis*#	–	–	1 (0.5)	–	1 (0.5)	–	–	1 (1.8)	–	–
8	*Lichtheimia* species§	4 (2.2)	3 (1.6)	1 (0.5)	1 (0.5)	9 (4.9)	1 (1.8)	–	–	–	1 (1.8)
9	*Cunninghamella* species§	4 (2.2)	–	1 (0.5)	–	5 (2.7)	1 (1.8)	–	–	–	1 (1.8)
10	*Mucor* species§	–	–	1 (0.5)	1 (0.5)	2 (1.1)	–	–	–	–	1 (1.8)
11	*Aspergillus* species§	4 (2.2)	–	–	–	4 (2.2)	–	–	–	–	–
	Total	128 (69.6)	22 (12)	18 (9.8)	16	184 (100)	40 (72.7)	5 (9.1)	8 (14.5)	2 (3.6)	55 (100)

ROCM, rhino-orbito-cerebral mucormycosis.

#Identification confirmed by DNA sequencing from culture and Formalin fixed paraffin embedded (FFPE) specimens: *R. arrhizus* (92 isolates & 32 FFPE), *A. variabilis* (15 isolates and 7 FFPE), *R. microsporus* (24 isolates and 6 FFPE), *R. homothallicus* (6 isolates), *S. vasiformis* (1 isolate), *Rhizopus stolonifer* (2 isolate), *Rhizopus* species (8), *Lichtheimia* species (28), *Cunninghamella* species (1), *Mucor* species (2), *Aspergillus* species (4).

§Isolates identified based on phenotypic characters: *Rhizopus* species (28), *Lichtheimia* species (8), *Cunninghamella* species (1), *Mucor* species (2), *Aspergillus* species (4).

$Others included the culture data for the clinical categories of renal, gastrointestinal, disseminated and middle ear infection.

The causative agents identified in other clinical categories from north India were: in renal infections the agents identified were *A. variabilis* (1 case), *R. arrhizus* (3), *R. microsporus* (1), *Rhizopus* species (1); in gastrointestinal infection, two cases each of *A. variabilis*, *R. arrhizus* and *R. microsporus* were identified and one case each of *R. microsporus* and *Lichtheimia* species were identified and *Rhizopus* species was isolated in middle ear infection.

From south India, *R. microsporus* (1) and *Rhizopus* species (1) were identified in disseminated mucormycosis.

Statistical analysis was performed to compare the significance of *Mucorales* isolated from north [a] and south India [b]. No statistical significance was observed between the groups analyzed.

Table 4. Treatment and outcome of mucormycosis.

Mode of treatment# (N = 276)	Geographic location	Total no. of patients (N)	Death N (%)	Survival N (%)	P value
No therapy	Both regions	11	11 (100)	0	0.0001*
	North India	9	9	0	0.003*
	South India	2	2	0	0.099
Surgical intervention only	Both regions	23	14 (60.9)	9 (39.1)	0.192
	North India	16	13 (81.3)	3 (18.8)	0.017*
	South India	7	1 (5.6)	6 (85.7)	0.409
Amphotericin B therapy only	Both regions	68	37(54.4)	31(45.6)	0.163
	North India	61	34 (55.7)	27 (44.3)	0.368
	South India	7	3 (42.9)	4 (57.1)	0.669
Patients treated with surgery and amphotericin B only	Both regions	139	45 (32.4)	94 (67.6)	0.0001*
	North India	111	39 (35.1)	72 (64.9)	0.0001*
	South India	28	6 (21.4)	22 (78.6)	0.152

*P value < .05 was considered statistically significant.

#The other modes of treatment included:

a) Amphotericin B combination with other antifungals such as posaconazole (*n* = 3, death = 2), natamycin (*n* = 1, survived = 1), caspofungin (*n* = 1, death = 1), fluconazole (*n* = 3, death = 3).

b) Surgery plus amphotericin B therapy in combination with other antifungal agents such as posaconazole (*n* = 5, death = 2), fluconazole (*n* = 4, death = 3), itraconazole (*n* = 2, survived = 2), voriconazole (*n* = 3, death = 2), micafungin (*n* = 1, death = 1).

c) Treatment with other antifungals without amphotericin B; itraconazole (*n* = 2, survived = 2), fluconazole (*n* = 1, death = 1), voriconazole (*n* = 1, death = 1).

d) Patients treated with surgery plus other antifungals without amphotericin B; itraconazole (*n* = 2, survived = 2), posaconazole (*n* = 2, death = 2), fluconazole (*n* = 4, death = 4).

Table 5. Multivariate logistic regression analysis to predict factors associated with outcome of disease.

S. No	Factors	Odds Ratio	95% Confidence Interval		P value
			Lower Limit	Upper Limit	
1	Gastrointestinal mucormycosis	18.70	2.38	147.32	.005*
2	Pulmonary mucormycosis	3.03	1.236	7.447	.015*
3	Combination of surgery and amphotericin B therapy	0.399	0.234	0.681	.001*
4	Steroid therapy	2.344	0.925	5.943	.073

*P value < .05 was considered significant.

Significant factors (*P* < .05) by univariate analysis were included in regression analysis: Rhino-orbito-cerebral type, Gastrointestinal type, Pulmonary type, Chronic kidney disease, Steroid therapy, No treatment group and Combination of surgery and amphotericin B therapy.

24.7 to 89 cases/year (Supplementary Table S9). However, to establish a true rise one need to know the denominators of the cohorts. The only study with denominator was diabetic cohort at PGIMER, Chandigarh, which reported 1.6 cases per 1000 diabetics.[26]

The epidemiology of mucormycosis in India is different from European countries and the United States.[7–9] Uncontrolled diabetes mellitus is the predominant risk factor in India and overshadows other risk factors including hematological malignancy and organ transplantation which are major risk factors in developed countries.[7,8,10] The present study confirms the fact, as overall 56.8% of the patients had diabetes mellitus and majority of them presented with ROCM (65.7%). Uncontrolled diabetes as risk factor was significantly higher in north India (Table 2), though the prevalence of diabetes mellitus is more in the south Indian population compared to north India.[27] The reason of this contrast picture is not known and requires further study.

Solid organ transplantation (*n* = 19, 6.3%), hematological malignancy (*n* = 19, 6.3%) and long term steroid therapy (*n* = 30, 9.9%) have emerged as other risk factors in the present series. Comparing present data of the reference center with the previous reported series showed that (Supplementary Table S9), solid organ transplantation (*P* = .036), steroid therapy (*P* = .036), post-tuberculosis (*P* <.0001), and chronic alcoholism (*P* = .025) as emerging new risk factors. The proportion of pulmonary mucormycosis cases has increased from 10% to 14.6% in the present series (Supplementary Table S9). Solid organ transplantation (OR: 0.25, *P* = .0001), steroid therapy (OR: 0.27, *P* = .001), and pulmonary tuberculosis (OR: 0.279, *P* = .002) were significant risk factors for pulmonary mucormycosis (Supplementary Table S2). The occurrence of isolated renal mucormycosis cases in healthy individuals in India and China remains an enigma.[10] In the present study, 33% of the renal mucormycosis infections were seen in

immunocompetent hosts. However, the pathogenesis of this entity is still unclear.

The introduction of molecular technique has improved the identification of the fungus. *Mucorales* could be identified from paraffin embedded tissues in 56 (14.4%) cases in the present series. Although the performance of molecular techniques is better in fresh tissues, they can also be performed in paraffin embedded tissues.[14,15,28] The sensitivity of detection of the fungus is lower in formalin fixed tissues due to denaturation of DNA in the presence of formalin. We could identify 50.9% of the *Mucorales* in paraffin embedded tissues in the present series. The accurate identification of the species of *Mucorales* helps in optimal management, as variability of antifungal susceptibility among the different species has been noted.[17,29]

Confirming previous studies,[3,6] *Rhizopus arrhizus* and *Apophysomyces variabilis* were the common etiological agents identified in the present series, and no significant variation in spectrum of agents was noted between two regions of the country. The emergence of *Rhizopus homothallicus* (2.5%) is an important finding in the present study, which was recently reported in Indian patients.[18,30] Recently, a fatal pulmonary mucormycosis case due to *R. homothallicus* has been reported from the western world.[31] *R. homothallicus* infection may be missed when molecular identification of *Mucorales* is not routinely performed. The higher rate of isolation of *Rhizopus microsporus* in the present series (14.5%, $P = .005$) compared to earlier series (1.5%) requires attention (Supplementary Table S9), as the agent is comparatively more resistant to amphotericin B compared to other *Mucorales*.[29,32] By phylogenetic analysis of sequences of large ribosomal subunit (rDNA), internal transcribed spacer region (ITS), actin and elongation factor-1, five species under genus *Rhizopus* have been found to be pathogenic to humans.[33] *R. microsporus* is the second most common species under *Rhizopus* causing human infection and this agent is abundantly present in the environment.[24]

In the present series, the overall mortality rate was 46.7%. The mortality was significantly higher in north Indian patients compared to south India ($P = .016$). The reason for the difference could not be ascertained in the present observational study. However, this may be due to inequalities in health care access in India, mainly due to socioeconomic status, geography, and gender.[34] Overall mortality was high in patients with gastrointestinal (94.1%) and pulmonary (76.5%) infection, similar to our earlier series[4–6] (Supplementary Table S9). Dioverti et al. reported mortality rate of 57% in the immunosuppressed patients with gastrointestinal mucormycosis.[35] The percentage of survival varied from 10% to 52% in patients with pulmonary infection.[36] Patients with ROCM had better survival rate (65%) possibly due to early diagnosis and ease of debridement, similar to our earlier series[4–6] (Supplementary Table S9).

Combined surgical debridement and amphotericin B therapy play major role in management of the disease.[37] In the present study, the mortality rate was high in patients treated with surgical intervention alone (39.1%) or amphotericin B therapy alone (43.4%), and the findings are similar to our earlier series[4–6] (Supplementary Table S9). Better survival rate (75.2%) was noted when the patients were managed with a combination of surgical debridement and amphotericin B therapy confirming the findings of other studies.[4–7,11] A multicenter prospective clinical trial is essential to optimize the therapy in mucormycosis.

In conclusion, this study provides current insights on mucormycosis in India. It highlights that mucormycosis remains a major fungal infection in diabetic population of this country especially in north India. The study also revealed the emergence *R. microsporus* and *R. homothallicus* as causative agents and improvement of identification using molecular technique. The mortality of mucormycosis especially in north India is very high, which stresses the need of a molecular tool for early diagnosis of the disease.

Supplementary material

Supplementary data are available at *MMYCOL* online.

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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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