Mucormycosis in Asia: Where do we stand now?

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Very high incidence 0.14/1000 population

Uncontrolled diabetes MAJOR RISK FACTOR

India & China largest diabetics in world

Isolated renal mucormycosis

Many new flowers in the garden

Highlights of Asian epidemiology
Autopsy study from 3 corners

- 1983-2008 (26 years)
  - IFIs: 2.4% (365/15,040)
  - Mucormycosis: 0.6%

- 1969-1994 (26 years)
  - IFIs: 2.9% (17,064/5,94,263)
  - Mucormycosis: 0.1%

- 1989-2003 (15 years)
  - IFIs: 31% (314/1017)

Aspergillosis: 42%
Mucormycosis: 7%
Candidiasis: 5%
Cryptococcosis: 1%
Pneumocystosis: 22%
Others: 23%

Our Institute data
(Courtesy Prof. A Das)

Japan, National data
Yamazaki T et al. JCM 1999; 37: 1732

MD Anderson, Hematology malignancy
Chamilos G et al. Haematologica 2006; 91: 986
<table>
<thead>
<tr>
<th>Reference</th>
<th>Countries</th>
<th>Period</th>
<th>Cases No.</th>
<th>HM (%)</th>
<th>DM (%)</th>
<th>SOM/SOT</th>
<th>DFO (%)</th>
<th>HIV (%)</th>
<th>AI/CO</th>
<th>Trauma/ no</th>
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<tbody>
<tr>
<td>Roden, 2005</td>
<td>Global</td>
<td>1885-2004</td>
<td>929</td>
<td>21</td>
<td>36</td>
<td>7</td>
<td>6</td>
<td>2</td>
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<td>Bitar, 2009</td>
<td>France</td>
<td>1997-2006</td>
<td>63</td>
<td>17</td>
<td>16</td>
<td>7</td>
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<td>Pagano, 2009</td>
<td>Italy</td>
<td>2004-2007</td>
<td>60</td>
<td>62</td>
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<td>2</td>
<td>-</td>
<td>2</td>
<td>3</td>
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<tr>
<td>Saegeman, 2010</td>
<td>Belgium</td>
<td>2000-2009</td>
<td>31</td>
<td>77</td>
<td>6</td>
<td>13</td>
<td>-</td>
<td>3</td>
<td>-</td>
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<td>Global</td>
<td>2006-2009</td>
<td>41</td>
<td>63</td>
<td>17</td>
<td>10</td>
<td>-</td>
<td>-</td>
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<tr>
<td>Skiada, 2011</td>
<td>Europe</td>
<td>2005-2007</td>
<td>230</td>
<td>55</td>
<td>17</td>
<td>9</td>
<td>1</td>
<td>2</td>
<td>7</td>
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<td>Chakrabarti, 2006</td>
<td>India</td>
<td>2001-2005</td>
<td>178</td>
<td>1</td>
<td>74</td>
<td>1</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>19</td>
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<tr>
<td>Chakrabarti, 2009</td>
<td>India</td>
<td>2006-2007</td>
<td>75</td>
<td>9</td>
<td>44</td>
<td>5</td>
<td>-</td>
<td>1</td>
<td>29</td>
<td>14</td>
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<tr>
<td>Lanternier, 2012</td>
<td>France</td>
<td>2005-2007</td>
<td>101</td>
<td>50</td>
<td>23</td>
<td>3</td>
<td>-</td>
<td>-</td>
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<td>18</td>
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</tbody>
</table>

HM= Hematological malignancy, DM=Diabetes mellitus, DFO= Deferroxamine therapy, HIV= human immunodeficiency virus, AI/CO= Autoimmune/corticosteroid therapy, SOM/SOT=Solid organ malignancy/transplant
# Mucormycosis & diabetes in India

<table>
<thead>
<tr>
<th>Reference</th>
<th>Region</th>
<th>Population</th>
<th>Cohort</th>
<th>Diabetes</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mohapatra, 2010 IJMM</td>
<td>New Delhi</td>
<td>All mucormycosis</td>
<td>29</td>
<td>13</td>
<td>44.83</td>
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<tr>
<td>Chakrabarti, 2009 PMJ</td>
<td>Chandigarh</td>
<td>All mucormycosis</td>
<td>75</td>
<td>48</td>
<td>64.00</td>
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<tr>
<td>Chakrabarti, 2006 MM</td>
<td>Chandigarh</td>
<td>All mucormycosis</td>
<td>178</td>
<td>131</td>
<td>73.60</td>
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<tr>
<td>Mohindra, 2007 MM</td>
<td>Chandigarh</td>
<td>All mucormycosis</td>
<td>27</td>
<td>15</td>
<td>55.56</td>
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<tr>
<td>Nithyanandam, 2003 IJO</td>
<td>Bangalore</td>
<td>All mucormycosis</td>
<td>34</td>
<td>30</td>
<td>88.24</td>
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<tr>
<td>Chakrabarti, 2001 JI</td>
<td>Chandigarh</td>
<td>All mucormycosis</td>
<td>96</td>
<td>23</td>
<td>23.96</td>
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<tr>
<td>Sundaram, 2006 H</td>
<td>Hyderabad</td>
<td>Mucormycosis (CNS)</td>
<td>40</td>
<td>30</td>
<td>75.00</td>
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<tr>
<td>Sundaram, 2005 M</td>
<td>Hyderabad</td>
<td>Mucormycosis (CNS)</td>
<td>56</td>
<td>32</td>
<td>57.14</td>
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<tr>
<td>Sundaram, 2011 LI</td>
<td>Hyderabad</td>
<td>Mucormycosis (Pulmonary)</td>
<td>6</td>
<td>5</td>
<td>83.33</td>
</tr>
<tr>
<td>Chander, 2010 IJMR</td>
<td>Chandigarh</td>
<td>Mucormycosis (Nec fascitis)</td>
<td>9</td>
<td>1</td>
<td>11.11</td>
</tr>
<tr>
<td>Chakrabarti</td>
<td>Chandigarh</td>
<td>Mucormycosis (Nec fascitis)</td>
<td>18</td>
<td>1</td>
<td>5.56</td>
</tr>
</tbody>
</table>
## Countries with high diabetes burden

<table>
<thead>
<tr>
<th>COUNTRY/TERRITORY</th>
<th>2013 MILLIONS</th>
<th>COUNTRY/TERRITORY</th>
<th>2035 MILLIONS</th>
</tr>
</thead>
<tbody>
<tr>
<td>China</td>
<td>98.4</td>
<td>China</td>
<td>142.7</td>
</tr>
<tr>
<td>India</td>
<td>65.1</td>
<td>India</td>
<td>109.0</td>
</tr>
<tr>
<td>United States of America</td>
<td>24.4</td>
<td>United States of America</td>
<td>29.7</td>
</tr>
<tr>
<td>Brazil</td>
<td>11.9</td>
<td>Brazil</td>
<td>19.2</td>
</tr>
<tr>
<td>Russian Federation</td>
<td>10.9</td>
<td>Mexico</td>
<td>15.7</td>
</tr>
<tr>
<td>Mexico</td>
<td>8.7</td>
<td>Indonesia</td>
<td>14.1</td>
</tr>
<tr>
<td>Indonesia</td>
<td>8.5</td>
<td>Egypt</td>
<td>13.1</td>
</tr>
<tr>
<td>Germany</td>
<td>7.6</td>
<td>Pakistan</td>
<td>12.8</td>
</tr>
<tr>
<td>Egypt</td>
<td>7.5</td>
<td>Turkey</td>
<td>11.8</td>
</tr>
<tr>
<td>Japan</td>
<td>7.2</td>
<td>Russian Federation</td>
<td>11.2</td>
</tr>
</tbody>
</table>

IDF Diabetes Atlas, Sixth Ed, 2013
• 35 cases of consecutive 22,316 diabetics (1.6 cases/1000 diabetics); 48.6% diabetic ketoacidosis
• The mean informed duration of diabetes was 6.7±4.6 y before acquiring mucormycosis
• All patients treated with amphotericin B & 74% had extensive surgical debridement
• 68% survived
Prevalence of mucormycosis in diabetics in India

- **Computational model**
  - Reviewed all literature for past five decades (1960-2012)
  - **Prevalence - 0.14/1000 population** – ranges - 137,807 & 208,177, mean - 171,504 (SD: 12,365.6; 95% CI: 195,777-147,688) – 70 times to generally accepted rates
  - **Attributable mortality** - mean of 65,500/year (38.2%)  
    [Chakrabarti et al. Poster number 1044, 23rd ECCMID, Berlin, 2013].
**Uncontrolled diabetes & diabetic ketoacidosis**

<table>
<thead>
<tr>
<th>Condition</th>
<th>ROC</th>
<th>Pulmonary</th>
<th>Gastrointestinal</th>
</tr>
</thead>
<tbody>
<tr>
<td>Type 1 diabetes</td>
<td>n = 36 (%)</td>
<td>n = 13 (%)</td>
<td>n = 10 (%)</td>
</tr>
<tr>
<td>Type 2 diabetes</td>
<td>4 (11), (p = 0.47, OR = 4.8)</td>
<td>1 (8), (p = 0.9, OR = 1.21)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Diabetic ketoacidosis</td>
<td>14 (39), (p = 0.035, OR = 24)</td>
<td>0 (0)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Haematological malignancy</td>
<td>3 (8), (OR = 0.8)</td>
<td>3 (23), (p = 0.15, OR = 4.35)</td>
<td>1 (10), (OR = 1.1)</td>
</tr>
<tr>
<td>Steroids and ISAs</td>
<td>5 (14), (OR = 0.2)</td>
<td>6 (46), (p = 0.22, OR = 2.5)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Kidney transplant</td>
<td>0 (0)</td>
<td>2 (15), (p = 0.18, OR = 5.5)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Prematurity</td>
<td>1 (3), (OR = 0.13)</td>
<td>0 (0)</td>
<td>7 (70), (p&lt;0.001, OR = 149)</td>
</tr>
<tr>
<td>Alcoholic chronic liver disease</td>
<td>6 (17), (p = 0.6, OR = 1.8)</td>
<td>1 (8), (OR = 0.5)</td>
<td>1 (10), (OR = 0.7)</td>
</tr>
<tr>
<td>Breach of skin</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Bowel perforation</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>7 (70), (p&lt;0.001, OR = 149)</td>
</tr>
<tr>
<td>HIV infection</td>
<td>1 (3), (p = 0.45)</td>
<td>0 (0)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Graft versus host disease</td>
<td>1 (3), (p = 1, OR = 1.1)</td>
<td>0 (0)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Renal failure</td>
<td>11 (31), (OR = 0.63)</td>
<td>9 (69), (p = 0.015, OR = 5.5)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Metabolic acidosis</td>
<td>2 (6), (OR = 0.4)</td>
<td>2 (15), (p = 0.5, OR = 2.07)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Intensive care unit stay</td>
<td>1 (3), (OR = 0.25)</td>
<td>1 (8), (OR = 1.2)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Neutropenia</td>
<td>3 (8), (OR = 0.4)</td>
<td>3 (23), (p = 0.45, OR = 2.0)</td>
<td>3 (30), (p = 0.25, OR = 3)</td>
</tr>
<tr>
<td>Immunocompetent host</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>0 (0)</td>
</tr>
</tbody>
</table>

ISAs, immunosuppressive agents; n, number of cases; OR, odds ratio; ROC, rhino-orbito-cerebral.

Diabetes & mucormycosis

- Formidable risk factor in Asia


- Steroid induced diabetes in HM & transplants play role

- Not only rhino-cerebral type, but also in other types (except cutaneous & renal mucormycosis), diabetes significantly associated (Chakrabarti A et al. Postgrad Med J 2009; 85: 573)

- ?The incidence mucormycosis in diabetics is coming down in west (Kontoyiannis DP. CID 2007: 44: 1089)
Although many factors could influence the development of zygomycosis in diabetic patients, I would like to offer an explanation. I postulate that the widespread, continuous use of statins by patients with diabetes underlies such a trend. Statins induce an apoptosis-like cell death in *Mucor racemosus* [9]. In addition, we and others [10–12] recently revealed that lovastatin has in vitro activity against a range of Zygomycetes molds; lovastatin also had in vivo activity in a model of zygomycosis [12]. The antifungal effect of mevalonate synthesis inhibitors, such as statins, does not appear to be specific against *Zygomycetes* molds, because simvastatin and atorvastatin were recently shown to inhibit growth of both *Candida* species and *Aspergillus fumigatus* [13].
Diet Modification and Metformin Have a Beneficial Effect in a Fly Model of Obesity and Mucormycosis

Fazal Shirazi\textsuperscript{1,9}, Dimitrios Farmakiotis\textsuperscript{1,9}, Yuanqing Yan\textsuperscript{2}, Nathaniel Albert\textsuperscript{1}, Kim-Anh Do\textsuperscript{2}, Dimitrios P. Kontoyiannis\textsuperscript{1*}

September 2014 | Volume 9 | Issue 9 | e108635
Phagocytic uptake of *Rhizopus oryzae* spores  
(*in vitro* assay)
• Not only nasal macrophages, but also peritoneal & alveolar macrophages are altered in diabetes

• Increased incidence of mucormycosis in the diabetic individuals may be due to a **poor recognition** of the fungi, **reduced uptake** and **low cytokine response** in diabetic patients towards the pathogen


• Further defects
  - Neutrophil dysfunction
  - Low serum pH decreases phagocytosis
  - Modifies transferrin system
  - Decreases serum inhibitory activity
Anatomical sites of involvement
Anatomic distribution of mucormycosis in India

- Pulmonary: 6-17%
- Renal: 5-14%
- GI: 5-13%
- ROC: 48-58%
- Cutaneous: 13-15%
- Disseminated: 5-12%

References:
- J Infect 2001; 42: 261
- Med Mycol 2006; 44: 335
- Mycoses 2007; 50: 271
- Postgrad Med J 2009; 85: 57
Low incidence of pulmonary mucormycosis in India

- Low level of suspicion
- Low diagnostic competence
- High no. of rhino-cerebral mucormycosis due to diabetes overshadow this type
- True difference in relative frequency between the two parts of the glove

<table>
<thead>
<tr>
<th></th>
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</tr>
</thead>
<tbody>
<tr>
<td>Cases</td>
<td>13</td>
<td>12</td>
<td>13</td>
</tr>
<tr>
<td>Antemortem</td>
<td>31%</td>
<td>50%</td>
<td>69%</td>
</tr>
<tr>
<td>Postmortem</td>
<td>69%</td>
<td>50%</td>
<td>31%</td>
</tr>
</tbody>
</table>
Diabetes also play role in pulmonary mucormycosis

- 17/m, type I diabetes, partially controlled over 10y
- Presented with low grade fever, occasional dry cough & progressive breathlessness
- Chest radiograph – collapse of lung
Diabetes also play role in pulmonary mucormycosis

47 year old male, type I diabetes, partially controlled over 10y

Presented with low grade fever, occasional dry cough & progressive breathlessness

Chest radiograph – collapse of lung

Bronchoscopy – mucosal plug

Flexible bronchoscopy could not remove the plug

Plug removed by rigid bronchoscopy under GA

PCR of the sample – *Rhizopus oryzae*

Lip AmB (3mg/Kg/d), surgery not possible as Carina involved

Patient on follow up
Another case

- 57/F, uncontrolled type II diabetes
- Cough, mucopurulent expectoration, progressive dyspnoea
- Bronchoscopy, bronchial biopsy – aseptate hyphae, PCR & sequencing – *Rhizopus oryzae*
- Patient died due to surgical complication
Gastrointestinal Mucormycosis - prematurity

<table>
<thead>
<tr>
<th>Presentation</th>
<th>No. of cases (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bowel gangrene</td>
<td>7 (70)</td>
</tr>
<tr>
<td><strong>Prematurity</strong></td>
<td><strong>7 (70)</strong></td>
</tr>
<tr>
<td>Septic shock</td>
<td>9 (90)</td>
</tr>
<tr>
<td>Anemia</td>
<td>2 (20)</td>
</tr>
<tr>
<td>Renal failure</td>
<td>1 (10)</td>
</tr>
<tr>
<td>Hematological malignancy</td>
<td>1 (10)</td>
</tr>
<tr>
<td>Steroid &amp; cytotoxic drug</td>
<td>1 (10)</td>
</tr>
<tr>
<td>Alcoholism</td>
<td>1 (10)</td>
</tr>
</tbody>
</table>


- **Prematurity** – GI mucormycosis in 20% of enterocolitis cases
- Suspect mucormycosis in neonate having intestinal perforation

Health-care related mucormycosis

- Cases of necrotizing fasciitis due to *Mucorales* is not unusual

- This is due to contaminated i.m. injection [(Chakrabarti & Singh. Mycoses 2014; 57 (Suppl. 3) 1-6)]

- Nosocomial mucormycosis in 9% (Chakrabarti *et al.* Postgrad Med J 2009; 85: 57)

- Infection at the site of ECG leads or adhesive tapes or from contaminated i.m. injection or from air in hospital
Renal mucormycosis

Pathology:
- Infarction
- Hilar vessel thrombosis
- Vasculitis
- Cortical & medullary necrosis
- Microabscess & granuloma

Renal mucormycosis

• 50 cases in last 2 decades at our center
• 42 cases reviewed
• 34 diagnosed antemortem
• 75% no risk factor reported
• 50% mortality
• Route of entry not known

Marak RS et al. Med Mycol 2010; 48: 1088
Primary renal zygomycosis due to *Rhizopus oryzae*

Jin YU & Ruo Yu Li
Peking University First Hospital, Beijing, PR China

- 46 cases reviewed
- Male : Female = 10:1
- Age – 3m-77y (5 children)
- Clinical presentation – similar to India (fever, flank pain, hematuria, anuria)
- Underlying illness
  - No – 30% (all children)
  - Yes – 70% (i.v. drug abuser, diabetes, kidney transplant, steroid therapy etc.)
Human pathogenic *Mucorales* in different series

- **India**: Chakrabarti et al., Mohapatra et al., India, Europe, France, Italy

- **Europe**: Skiada et al.

- **France**: Lanternier et al.

- **Italy**: Pagano et al.

- **Other species**: Other species, Cunninghamamella, Mucor, Rhizomucor, Lichtheimia, Apophysomyces, Rhizopus
### Recent study from Patel Chest, Delhi

<table>
<thead>
<tr>
<th>Organism</th>
<th>Rhino-cerebral</th>
<th>Pulmonary</th>
<th>Disseminated</th>
<th>Cutaneous</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Rhizopus arrhizus var. delemar</em></td>
<td>2</td>
<td>14</td>
<td>2</td>
<td>-</td>
</tr>
<tr>
<td><em>R. Arrhizus var. arrhizus</em></td>
<td>-</td>
<td>15</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td><em>Rhizopus microsporus</em></td>
<td>7</td>
<td>6</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td><em>Rhizopus stolonifer</em></td>
<td>1</td>
<td>-</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td><em>Syncephalastrum racemosum</em></td>
<td>5</td>
<td>-</td>
<td>-</td>
<td>6</td>
</tr>
<tr>
<td><em>Lictheimia ramosa</em></td>
<td>-</td>
<td>2</td>
<td>-</td>
<td>1</td>
</tr>
<tr>
<td><em>Mucor circinelloides</em></td>
<td>-</td>
<td>2</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td><em>Apophysomyces variabilis</em></td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>2</td>
</tr>
<tr>
<td><em>Apophysomyces elegans</em></td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>2</td>
</tr>
<tr>
<td><strong>Total cases (n=61)</strong></td>
<td><strong>15</strong></td>
<td><strong>39</strong></td>
<td><strong>4</strong></td>
<td><strong>13</strong></td>
</tr>
</tbody>
</table>

Chowdhary et al. Mycoses 2014; 57 (Suppl. 3) Epub
Indian garden has many new flowers!

*Saksenaea vasiformis*

*Apophysomyces elegans*

*Rhizopus homothallicus*

*Thamnostylum lucknowense*
Apophysomyces elegans infection

- Of ~100 cases published in literature, a major portion (~60%) of cases was reported from India
- Majority of patients had no underlying disease
- It usually causes cutaneous & subcutaneous infection
- Local wound contamination with soil after accident represents the single most common host risk factor
- The fungus has also been reported from rhinocerebral, pulmonary, renal, and disseminated mucormycosis
Cavitary Pulmonary Zygomycosis Caused by *Rhizopus homothallicus*

Arunaloke Chakrabarti,¹* Rungmei S. K. Marak,⁴ M. R. Shivaprakash,¹ Sunita Gupta,¹ Rajiv Garg,⁵ V. Sakhua,² Sanjay Singhal,⁵ Abhishek Baghela,¹ Ajai Dixit,⁴ M. K. Garg,² and Arvind A. Padhye⁶

We report the first two proven cases of cavitary pulmonary zygomycosis caused by *Rhizopus homothallicus*. The diagnosis in each case was based on histology, culture of the causal agent, and the nucleotide sequence of the D1/D2 region of the 28S ribosomal DNA.
Thamnostylum lucknowense

- Referred form AIIMS, New Delhi
- A 57-yr-old female diabetic patient presented with rhinorbitocerebral zygomycosis.
- Identified on typical morphological features and sequencing of ITS and 28S region.

- Earlier isolated form Indian soil.

Ecology of *Mucorales*

Types of soils:

- **Haryana** *(Calcireous sierozenic, pH>8, strongly alkaline)*
- **Punjab** *(Clay loamy, pH 7-7.5, neutral to slightly alkaline)*
- **Tamilnadu** *(Red Loamy, pH 6.8-7.2, neutral)*
- **Himachal Pradesh** *(Slity loamy, pH< 6, acidic)*
Spectrum of *Mucorales* in Indian soils

Punjab

- Apophysomyces variabilis: 5%
- Cunninghamamella sp.: 6%
- Lichtheimia sp.: 28%
- Rhizopus sp.: 3%
- Rhizomucor sp.: 2%
- Rhizopus homothallicus: 15%
- Mucor sp.: 21%
- Other Mucorales: 19%

Haryana

- Apophysomyces variabilis: 6%
- Cunninghamamella sp.: 24%
- Lichtheimia sp.: 29%
- Rhizopus sp.: 1%
- Rhizomucor sp.: 3%
- Rhizopus homothallicus: 2%
- Mucor sp.: 11%
- Other Mucorales: 6%

Tamilnadu

- Apophysomyces variabilis: 1%
- Cunninghamamella sp.: 6%
- Lichtheimia sp.: 29%
- Rhizopus sp.: 24%
- Rhizomucor sp.: 1%
- Rhizopus homothallicus: 0%
- Mucor sp.: 18%
- Other Mucorales: 20%

Himachal Pradesh

- Apophysomyces variabilis: 4%
- Cunninghamamella sp.: 2%
- Lichtheimia sp.: 31%
- Rhizopus sp.: 3%
- Rhizomucor sp.: 4%
- Rhizopus homothallicus: 18%
- Mucor sp.: 17%
- Other Mucorales: 21%
Soil properties and *Apophysomyces* spp. isolation

- Alkaline soils - higher *Apophysomyces* isolation
- No seasonal variation in isolation of *Apophysomyces* spp.
- The mean temperature – 34.5±7.8
- Bivariate analysis - nitrogen, phosphorous, zinc, copper was significantly associated with *Apophysomyces* isolation.
- Multivariate analysis - low nitrogen content, alkaline pH of soil were significantly associated.
Diagnosis
# Diagnostic techniques for mucormycosis

## Direct microscopy
- Wet mount, Calcofluor White, PAS, GMS
- Fluorescent in situ hybridization (FISH)
- Immunohistochemistry

## Culture

## Serology
- ELIspot

## Molecular techniques
- Conventional PCR, RFLP
- DNA sequencing
- Realtime PCR

## Fungal Identification
- Culture: Macro- and Micro-morphology
- DNA sequencing
- Conventional PCR, RFLP
- Realtime PCR
- MALDI-TOF
Molecular method for identification of Mucorales in paraffin-embedded or frozen tissues

<table>
<thead>
<tr>
<th>Reference</th>
<th>Tissue</th>
<th>Method</th>
<th>Target</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dannaoui et al, 2010</td>
<td>Paraffin-embedded</td>
<td>PCR+ sequencing</td>
<td>ITS1</td>
</tr>
<tr>
<td>Hata et al, 2008</td>
<td>Frozen/fresh/paraffin-embedded tissue</td>
<td>Real-time PCR</td>
<td>Cyt b</td>
</tr>
<tr>
<td>Schwarz et al, 2006</td>
<td>Frozen/fresh tissue</td>
<td>PCR+sequencing</td>
<td>ITS</td>
</tr>
<tr>
<td>Machouart et al, 2006</td>
<td>Frozen/fresh tissue</td>
<td>PCR+RFLP</td>
<td>18S</td>
</tr>
<tr>
<td>Bialek et al, 2005</td>
<td>Paraffin-embedded</td>
<td>Semi-nested PCR</td>
<td>18S</td>
</tr>
<tr>
<td>Kobayashi et al, 2004</td>
<td>Frozen/fresh tissue</td>
<td>PCR+sequencing</td>
<td>28S</td>
</tr>
<tr>
<td>Hayden et al, 2002</td>
<td>Paraffin-embedded</td>
<td>In situ hybridization</td>
<td>18S</td>
</tr>
</tbody>
</table>

- Fresh or frozen tissue much better success
- Paraffin-embedded tissue – 70-90% success
Rapid screening for human-pathogenic Mucorales using rolling circle amplification

S. Dolatabadi,¹ ² M. J. Najafzadeh³ and G. S. de Hoog¹ ² ⁴ ⁵ ⁶ ⁷

Mycoses, 2014, 57 (Suppl. 3), 1–6
doi:10.1111/myc.12245

Even closely related varieties could be differentiated
## Serological tests

<table>
<thead>
<tr>
<th>Study</th>
<th>Target structure</th>
<th>Method</th>
<th>Specimen</th>
<th>Sensitivity/specificity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wyson DR, <em>et al.</em>, 1987</td>
<td><em>R. oryzae</em> Ag homogenate</td>
<td>Western blotting</td>
<td>Sera of 5 patients</td>
<td>n.a.</td>
</tr>
<tr>
<td>Potenza L, <em>et al.</em>, 2011</td>
<td>Mucorales specific IFN-γ producing T cells</td>
<td>ELISA (ELiSpot) or immuno-cyto-fluorimetric assay</td>
<td>Blood from 80 patients</td>
<td>Picked up all 3 cases with mucormycosis</td>
</tr>
</tbody>
</table>
Mucorales-specific T cells emerge in the course of invasive mucormycosis and may be used as a surrogate diagnostic marker in high-risk patients

*Leonardo Potenza,1 *Daniela Vallerini,1 *Patrizia Barozzi,1 *Giovanni Riva,1 *Fabio Forghieri,1 Eleonora Zanetti,1 Chiara Quadrelli,1 Anna Candoni,2 Johan Maertens,3 Giulio Rossi,4 Monica Morselli,1 Mauro Codeluppi,5 Ambra Paolini,1 Monica Maccaferri,1 Cinzia Del Giovane,1 Roberto D’Amico,1 Fabio Rumpianesi,6 Monica Pecorari,6 Francesca Cavalleri,7 Roberto Marasca,1 Franco Narni,1 and Mario Luppi1

(Blood. 2011;118(20):5416-5419)

• 3 of 28 patients developed mucormycosis
• 17 had other infections
• Mucorales specific T cells could be detected in 3 IM patients only
3 quantitative PCR assays using hydrolysis probes targeting *Rhizopus/Mucor, Lichtheimia, Rhizomucor*

10 patients with proven mucormycosis (2-9 serum samples)

No cross reaction with opportunistic fungi

Detection limit 3.7-15 fentogram/10µl

9/10 patients – DNA detection positive

DNA detected 69 & 3 days before mucormycosis diagnosis
Summary

- Mucormycosis is a serious problem – alarming rise
- Disease burden, risk factors
- Isolated renal mucormycosis – how it occurs?
- Spectrum of agents broad – many new flowers
- Antifungal susceptibility
- Real challenge is prompt diagnosis
- Solution - Bio-markers, serology, nucleic acid detection
- Therapy? – Dr. Kontoyiannis
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- Anup Ghosh
- Ritesh Agarwal

Thank you