Pathogenesis of ocular fungal Infections

Dr Lalitha Prajna. MD.
Department of Ocular Microbiology, Aravind Eye Hospital, Madurai. Tamil Nadu.
Introduction

- Fungal infections of the eye are rare
  (exception: keratitis)
- High morbidity and in some cases with mortality.
- The incidence is higher in a tropical country.

**Issues with fungal infections of the eye:**

- Difficulty in recognition
- In diagnosing fungal infections of the eye.
- Effective anti-fungal agents for treatment is limited.
Fungal infections of the eye.

blepharitis,
conjunctivitis,
adnexal
lacrimal gland infections.

The more serious infections with increased morbidity:
- corneal ulcers
- endophthalmitis
- uveitis.
- Orbital
• blepharitis,
• *Cryptococcus neoformans* after trauma
• *Rhinosporidium seeberi*
• *Candida* spp: antibiotics or immunosuppressive drugs

• The prevalence of fungal conjunctivitis is low,
  secondary to inflammation of the cornea, lacrimal sac and tear ducts
• *Rhinosporidium* .
• *Candida* spp., *Aspergillus* spp.
• *Sporotrichum* spp., *Blastomyces* spp.,
• *Coccidioides* spp.,
• *Malassezia* spp. and dermatophytes
Fungal infections of the Obit

- Orbital fungal infections are vision-threatening.
- Also associated with high mortality.
- Imperative to diagnose fungal disease at the earliest and initiate appropriate therapy.
- The most common orbital fungal infections are: *mucormycosis and aspergillosis.*
Fungal endophthalmitis

- **Exogenous:**
  intraocular surgery, keratitis, trauma

- **Endogenous:**
  associated with systemic fungal diseases
Fungal endophthalmitis

Clinical example: confirmation of the culture.

Aspergillus Flavus
Isolated both from Sputum and anterior chamber.
Incidence of fungal endophthalmitis

- Incidence varies with geographical regions:
  - Higher in tropical vs temperate  
    Gupta A, 2008; Lalwani GA, 2008

- Endogenous endophthalmitis accounts for 2–15%
- Endogenous endophthalmitis:
  50% Binder; 68.7% Leibovitch; 62% Schiedler

- Post-operative Fungal endophthalmitis accounts for 5–10%
- Postoperative endophthalmitis:
  21.8% Anand AR
- Aravind Data from 2010 to 2014: 39(8.9%)
# Causative organisms

<table>
<thead>
<tr>
<th>Post-operative endophthalmitis</th>
<th>Traumatic endophthalmitis</th>
<th>Endogenous endophthalmitis</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Aspergillus flavus</strong></td>
<td>Aspergillus fumigates</td>
<td><strong>Aspergillus flavus</strong> (40%)</td>
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<tr>
<td><strong>Aspergillus fumigatus</strong></td>
<td>Aspergillus niger</td>
<td><strong>Candida albicans</strong> (40%), <strong>Candida tropicalis</strong> (10%)</td>
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<tr>
<td><strong>Aspergillus terreus</strong></td>
<td>Curvularia</td>
<td><strong>Aspergillus fumigates</strong> (10%),</td>
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<tr>
<td>Fusarium</td>
<td>Dematiaceous fungi</td>
<td>Penicillium (10%).</td>
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<tr>
<td>hyaline fungus</td>
<td><strong>Aspergillus terreus</strong>, hyaline fungus Lasodiplopia</td>
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</table>
intravitreal triamcinolone injection /anti VGEF obtained from the same compounding pharmacy.

- Long duration of onset
- Poor outcome
- Vitreous tap inadequate/direct vitreous biopsy preferred for laboratory tests
- fungal infection was confirmed in 57% (8/14)
Risk factors: Endogenous endophthalmitis

- debilitating disease
- intravenous drug use,
- chemotherapy,
- corticosteroids therapy,
- alcoholism, diabetes.

*presenting sign of a systemic fungal infection.*

*Endophthalmitis within 10 days to 25 days of contaminated dextrose infusion*

_Candida endogenous endophthalmitis_

*Chakrabarti 2008; Narang 2001*
Challenges in fungal endophthalmitis

- Identification of risk factors
- Delay in presentation
- Clinical presentation
- Diagnosis
- Management

- Rare
- Suspect fungal aetiology at presentation
- Accurate diagnosis
- Laboratory confirmation
- Prompt therapy with antifungals
Fungal corneal ulcers

• Fungal ulcers of the cornea are a major cause of blindness.
• *Fusarium* and *Aspergillus*:
• *Topical anti-fungal eye drops*: Natamycin, Voriconazole.
• But nearly 50% of ulcers fail treatment.
### Microbiology data from Aravind Eye Hospital: 2013 to 2017

<table>
<thead>
<tr>
<th>Particulars</th>
<th>2013</th>
<th>2014</th>
<th>2015</th>
<th>2016</th>
<th>2017</th>
<th>Total (%)</th>
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<tbody>
<tr>
<td><strong>corneal ulcer cases</strong></td>
<td></td>
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<td></td>
<td>2785</td>
<td>2988</td>
<td>2285</td>
<td>1982</td>
<td>950</td>
<td>10990</td>
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<td><strong>Culture Negative</strong></td>
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<tr>
<td></td>
<td>1058 (49.2%)</td>
<td>1214 (51.2%)</td>
<td>933 (48.5%)</td>
<td>707 (43.4%)</td>
<td>355 (45.4%)</td>
<td>4267 (48.2%)</td>
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<td><strong>Culture Positive</strong></td>
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<td></td>
<td>1092 (50.8%)</td>
<td>1156 (48.8%)</td>
<td>989 (51.5%)</td>
<td>922 (56.6%)</td>
<td>427 (54.6%)</td>
<td>4586 (51.8%)</td>
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<td><strong>Bacteria</strong></td>
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<tr>
<td></td>
<td>355 (32.5%)</td>
<td>409 (35.4%)</td>
<td>332 (33.6%)</td>
<td>242 (26.2%)</td>
<td>124 (29.0%)</td>
<td>1462 (31.9%)</td>
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<tr>
<td><strong>Fungus</strong></td>
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<tr>
<td></td>
<td>673 (61.6%)</td>
<td>657 (56.8%)</td>
<td>617 (62.4%)</td>
<td>648 (70.3%)</td>
<td>284 (66.5%)</td>
<td>2879 (62.8%)</td>
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<tr>
<td>Fungus</td>
<td>2013</td>
<td>2014</td>
<td>2015</td>
<td>2016</td>
<td>2013-2016</td>
<td>Total %</td>
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<tr>
<td><em>Fusarium sp</em></td>
<td>310</td>
<td>265</td>
<td>253</td>
<td>266</td>
<td>1094</td>
<td>39.8</td>
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<tr>
<td><em>Aspergillus flavus</em></td>
<td>133</td>
<td>119</td>
<td>135</td>
<td>155</td>
<td>542</td>
<td>19.7</td>
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<tr>
<td><em>Other hyaline spp</em></td>
<td>125</td>
<td>90</td>
<td>104</td>
<td>102</td>
<td>421</td>
<td>15.3</td>
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<tr>
<td><em>Other Aspergillus sp</em></td>
<td>48</td>
<td>42</td>
<td>36</td>
<td>36</td>
<td>162</td>
<td>5.9</td>
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<tr>
<td><em>Unidentified fungus</em></td>
<td>139</td>
<td>150</td>
<td>128</td>
<td>93</td>
<td>510</td>
<td>18.5</td>
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<tr>
<td><em>Candida sp</em></td>
<td>4</td>
<td>5</td>
<td>8</td>
<td>4</td>
<td>21</td>
<td>0.8</td>
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</table>
Trend in fungal keratitis in the world.

- Multistate outbreak of *Fusarium* keratitis associated with use of a contact lens solution
  
  *Khor WB et al 2006, Chang DC, et al, 2006*

- **Trend in fungal keratitis in USA 2001 to 2007**
  - *Fusarium*-related fungal keratitis cases among CLWs returned to baseline levels after the removal of ReNu with MoistureLoc from the market

- However, number of **non-Fusarium, culture-positive fungal keratitis** cases among both CLWs and non-CLWs had actually increased.
  
  *Gower et al 2010*
The outcome of fungal corneal ulcers is due to a combination of host factors and fungal virulent factors. As we understand the exact pathogenicity we might be able to have a more customized treatment options "personalized medicine"
Pathogenesis Fungal corneal ulcers

• To understand the pathogenesis underlying fungal keratitis:
  Comprehensive approach to examine the fungal virulence factors and the host response to the infection was undertaken.

• Aim to identify biomarkers that can be used for diagnosis/prognosis/treatment.
<table>
<thead>
<tr>
<th>Assembly and annotation</th>
<th>Genome sequencing and assembly</th>
<th>Global transcript analysis of clinical isolates</th>
<th>Proteome analysis of clinical isolates</th>
<th>Mycelial proteome</th>
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</thead>
<tbody>
<tr>
<td>Variability among clinical isolates</td>
<td>Fungal Pathogens</td>
<td>Comparative study of saprophytes and corneal isolates</td>
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<tr>
<td>Fungal Pathogens</td>
<td>Host susceptibility genes</td>
<td>Newer treatment options</td>
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<tr>
<td>Tear</td>
<td>Comparative quantitative proteomics of uninfected and keratitis patient tear</td>
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<tr>
<td>Corneal buttons</td>
<td>Human Host Response</td>
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<tr>
<td>Corneal scraping</td>
<td>Immune response to fungal antigens</td>
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<td>Serum</td>
<td>Host-pathogen interaction studies</td>
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<tr>
<td>Mice and Insect models</td>
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<tr>
<td>Ex vivo model</td>
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</tbody>
</table>
1. Tear from healthy individuals and fungal keratitis patients were collected (60-80µl).
2. Tear is collected using 10 µl-capillary tubes.
3. Tear samples are Centrifuge to remove cellular debris.
4. Frozen in liquid nitrogen until analysis.

Categorization of keratitis tear samples

Based on Duration of symptoms

- <7 Days (Early stage)
- 7-14 Days (Intermediate stage)
- >14 Days (Late stage)
**Experimental Approach**

**Tear/Cornea Collection from Healthy/Cadaver Controls & Fungal keratitis patients**

Separation of Proteins by 1D/2D PAGE

Identification of differentially expressed protein spots by MALDI

LC-MS/MS

<table>
<thead>
<tr>
<th>Database/Accession no.</th>
<th>Mowse score(^a)</th>
<th>Mr/(pI) 2-D(^b)</th>
<th>Mr/(pI) database(^c)</th>
<th>Peptides Matched(^d)</th>
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</thead>
<tbody>
<tr>
<td>MSDB / UDHUP1</td>
<td>495 (36)</td>
<td>16/4.8</td>
<td>16.2/4.95</td>
<td>R.IIPGGVYDADLNDKWQR.A</td>
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<td>K.KQLCSFEIYEVWEDR.M</td>
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<td>55</td>
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</tbody>
</table>

\(^a\) Mowse score: Indicates the confidence of protein identification.

\(^b\) Mr/\(pI\) 2-D: Molecular weight and isoelectric point determined by 2D PAGE.

\(^c\) Mr/\(pI\) database: Reference database used for protein identification.

\(^d\) Peptides Matched: Peptides matched to the identified protein.

\(^e\) Sequence Coverage (%): Degree of sequence covered by identified peptides.

\(^f\) Function: Biological function of the identified protein.
Expression of Innate and Adaptive Immune mediators in Human Corneal Tissue Infected With Aspergillus or Fusarium

Rajapandian et al JID 2011:

- Gene expression studies of patients with corneal ulcers

  Corneal scrapping / Post transplant corneas

Expression of Dectin-1, Toll-like receptor 2 (TLR2), TLR4, TLR9, and NOD-like receptor protein were looked for.

- There is a common innate and adaptive immune response to these filamentous fungi, which includes the generation of T-helper 1 and T-helper 17 cell.

- Targeting mediators along with antifungal therapy, could restrict excessive cellular infiltration into infected corneas and minimize host- mediated tissue damage
**A. *flavus* corneal isolates are more virulent: Animal models**

**Corneal isolates produce more melanin**

**Corneal isolates in general are more virulent in Galleria larvae**

Saprophyte

Exoproteome differ between A. flavus strains
Clinical isolates secrete more proteins: Through non-classical pathways

Proteome depends on life style


- High resolution two-dimensional electrophoresis and mass spectrometry were used to generate A. flavus exoproteome reference map as well as to profile most of the exoproteins.

- Nearly 50% of the exoproteins possess catalytic activity

- *One of these, an alkaline serine protease (Alp1) is present in high abundance as well as multiple proteoforms.*

- Many proteins in the A. flavus exoproteome have been shown to be virulence factors in other pathogens indicating the probable role for these proteins in the corneal infection as well.

- Thus, this study provides a clue to the early strategies employed by the pathogen to establish an infection in an immunocompetent host.
Tear Proteomics to understand host response

- Multiple pathways are activated in response to fungal infection
- Complement system is a major mediator of inflammatory response
- An intricate balance exists between pro- and anti-inflammatory factors

Comprehensive approach to understand intra-species variation in A. flavus
Pathogenesis of fungal corneal ulcers


- In-depth identification and analysis of tear proteins revealed that *A. flavus* infection activates multiple pathways representing the host response, namely the complement and coagulation pathways along with the recruitment of neutrophils.

- All these pathways are activated only in patients and are favourable to the host in resolving the fungal infection.
Pathogenesis of fungal corneal ulcers


• However, the presence of a number of pro- and anti-inflammatory proteins in tear suggest that there is an intricate balance between these two groups of proteins and the outcome of the ulcer is dependent on which of these factors dominate.

• Further, studies with infected corneal tissue from keratitis patients also provide a direct characterization of the host response to pathogenic fungi in infected human tissues at early and later stages of disease
Fungal infection alters multiple pathways: Wound healing, inflammation and host defense

Conclusion

• These findings will allow us to identify potential targets for immune intervention.

Targeting immune mediators along with antifungal therapy, could restrict excessive cellular infiltration into infected corneas and minimize host-mediated tissue damage.

• The outcome of the study is expected to allow the development of better diagnostic methods in combination with effective treatment strategies.
Future work: Pathogenic *Aspergillus*: Interaction with innate immune cells

1. Analyzing the differential interactions of two *Aspergillus*, *A. fumigatus* and *A. flavus*, with their host in the context of their specific pathologies (*invasive vs superficial*)

2. Understanding the phagocytic responses toward *Aspergillus* morphotypes (dormant and germinating conidia)

3. Identifying and characterizing the fungal cell surface components which activate or repress the host immune response

4. Studying in depth the immunogenic function of the core cell wall fungal polysaccharides – uptake by phagocytes, surface receptor identification and degradation

5. Recognizing the components of the phagolysosome involved in the intracellular recognition of the fungus
“Intelligence & capabilities are not enough. There must be the joy of doing something beautiful.”

- Dr. V

Thank You!