THE BURDEN OF FUNGAL KERATITIS IN INDIA

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KERATITIS
- Cornea is the outermost projecting part of eyeball
- Transparent
- Break in corneal epithelium
- Inflammation of the underlying corneal stroma

ENDOPHTHALMITIS
- Infection of posterior segment of eyeball
- Vitreous humour is infected
- Retina & choroid also infected

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Medical emergency

Patients suffer from significant pain & distress

Rapid initiation of aggressive treatment is needed to halt the disease process

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Multi-center, population-based cross-sectional study
8 months 2010; 168,673 individuals of all age groups participated.
Prevalence of past and active infectious keratitis = 0.19 %
Prevalence of viral, bacterial, & fungal keratitis = 0.11%, 0.075%, and 0.007%, respectively.
138 cases of infectious corneal blindness in at least one eye in the study population (prevalence of 0.082 %)
Risk factors identified for infectious corneal blindness (based on statistical analysis):
--- ocular trauma
--- alcoholic consumption
--- low socioeconomic levels
--- advanced age & poor education

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-- Preventable corneal disease, glaucoma, complications of cataract surgery & amblyopia caused 19% of overall blindness

In Andhra Pradesh  Dandona & Dandona, Br J Ophthalmol 2003; 87: 133
-- Corneal blindness if an eye had v/a < 20/200 due to a corneal disease
-- Corneal blindness in at least one eye in 86 of 10,293 (prevalence 0.66% [0.1% bilateral; 0.56% unilateral])
-- Major causes of keratitis during childhood (36.7%), trauma (28.6%) & keratitis during adulthood (17.7%)
-- Nearly 95% of all corneal blindness was avoidable
-- Prevalence significantly higher with ↓ socioeconomic status and ↑ age

-- Success of strategies to prevent 90% of preventable blindness due to corneal disease & glaucoma by 2020 would prevent 3.6 million blind persons in 2020 & 29 million blind person-years.

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Gonzales et al. Ophthalmic Epidemiol 1996; 3:159

- Retrospective, incidence, general community-based study in 1993 in Madurai district

1148 episodes of corneal ulceration noted in medical records = Incidence of 34 / 100,000 / year

Episodes of corneal ulceration seen but not recorded = Corrected incidence of 113 / 100,000 / year.

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- Corneal ulceration in the developing world: a silent epidemic
- More frequent in developing countries than previously recognised

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EPIDEMIC PROPORTIONS

<table>
<thead>
<tr>
<th>Region</th>
<th>New Cases / Year</th>
</tr>
</thead>
<tbody>
<tr>
<td>Madurai District</td>
<td>50,000</td>
</tr>
<tr>
<td>Whole of India</td>
<td>840,000</td>
</tr>
<tr>
<td>Developing world</td>
<td>&gt; 1,500,000</td>
</tr>
<tr>
<td>U.S.A.</td>
<td>27,000</td>
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</tbody>
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- Prevalence: unknown
- Incidence

Tuft and Tullo, Eye (Lond.) 2009; 23: 1308
-- 0.32 cases per million population/ year
-- 39 cases over 3 years

- Incidence of infectious keratitis (per 100,000 /year)

--- Hong Kong : 6.3 Lam et al., Eye 2002; 16 : 608
--- USA : 11.0 Erie et al., Arch Ophthalmol 1993; 111: 1165
--- Bhutan : 339.0 WHO, SEA Ophthal, 126 ,2004; 1
--- Myanmar : 710.0 WHO, SEA Ophthal, 126, 2004; 1

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Suppurative keratitis due to fungi as a proportion of total number of cases, by latitude.

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1 Madras, S India (n = 150)
2 Madurai, S India (n = 434)
3 Ghana, W Africa (n = 199)
4 Sri Lanka (n = 66)
5 Trichy, S India (n = 774)
6 Thailand (n = 145)
7 Tanzania (n = 212)
8 Hyderabad, India (n = 102)
9 Bangladesh (n = 66)
10 Dharan, Nepal (n = 86)
11 South Florida (n = 663)
12 New Delhi, India (n = 674)
13 Hong Kong (n = 223)
14 Chandigarh, N India (n = 730)
15 Kathmandu, Nepal (n = 405)
16 Karnataka, S India (n = 295)
17 London, UK (n = 72)
18 Sweden (n = 48)
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Leck et al., Br J Ophthalmol 2002; 86: 1211

- 39 studies (1976 to 2001)
- 23 from Asia (India 12, Bangladesh 4, Nepal 3, Sri Lanka, Thailand, Hong Kong, Singapore)
  - 7 from North America (all USA)
  - 6 from Africa & Mid East (South Africa (2), Nigeria, Tanzania, Ghana, KSA)
  - 2 from Europe (London) 1 from South America (Paraguay)

- 2% to 58%
- Principal fungal isolates
  - Aspergillus spp.-- 17 ; Fusarium spp.-- 12 ; Candida spp. -- 5

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- Shah et al., Br J Ophthalmol 2011; 95: 762

Highest % of bacterial corneal ulcers -- from North America, Australia, Netherlands, Singapore

Highest % of fungal corneal ulcers – India, Nepal

- statistically significant coefficient correlations between gross national income and percentages of:
  -- bacterial K (0.85 [95% CI 0.68 to 0.91]) &
  -- FUNGAL KERATITIS (-0.81 [95% CI -0.9 to -0.66])

Mycotic cause in 1.2 % to 62 % of Inf. keratitis

  Ritterband et al., Cornea 2006; 25: 264
  Xie et al., Ophthalmology 2006; 113: 1943
  Nath et al., Indian J Ophthalmol 2011; 59:267

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- retrospective study of antifungal eye drops sales from only authorised pharmaceutical ophthalmologic laboratory in Brazil (6 years)
- 26,087 antifungal eye drop units sold (mean = 2.3 / patient).
- Significant variation in antifungal sales during the year.
- By linear regression a significant association between reduced relative humidity & antifungal drug sales (R2 = 0.17, p<0.01).

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Absolute numbers

-- ~ 340 cases/ year - single centre (southern India)

-- ~ 200 cases/ year - single centre (central China)

-- ~ 100 cases/ year - single centre
   Xie et al., Ophthalmology 2006; 113: 1943
   Nath et al., Indian J Ophthalmol 2011; 59:267
   Sengupta et al., Indian J Ophthalmol 2011; 59:291

-- ~ 100 cases/ year - 11 centres (USA)
   Keay et al. Ophthalmology 2011; 118; 920

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- >50% of oculomycoses Srinivasan et al., Acta Ophthalmol 1991; 69: 744

- 2 distinct forms due to:
  --filamentous fungi
  --yeasts & yeast-like fungi

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Aetiological Agents of Filamentous FUNGAL KERATITIS

Aspergillus  Curvularia  Scedosporium

Fusarium  Bipolaris  Lasiodiplodia

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Aetiological Agents of Filamentous FUNGAL KERATITIS

Auerswaldia lignicola

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Auerswaldia lignicola

Colletotrichum dematium

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Filamentous FUNGAL KERATITIS

- Increased frequency towards tropical latitudes (wind, temperature, rainfall)
  Leck et al., *Br J Ophthalmol* 2002; 86: 1211

- Increased *Curvularia* keratitis during hot moister summer months along Gulf of Mexico

- Tends to occur more frequently in adults & elderly than in children (<16 yr)
  Parmar et al., *Cornea* 2006; 25: 264

- Occupation-related
  Gopinathan et al., *Cornea* 2002; 21: 555

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Saad-Hussein et al., East Mediterr Health J 2011; 17: 468

- Statistically significant ↑ in relative frequency of FK (1997-2007) in greater Cairo area

- Rise correlated significantly with
  - ↑ minimum temperature
  - ↑ maximum atmospheric humidity
  - ↑ in CO₂ emissions & surface temperature

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Filamentous FUNGAL KERATITIS

Seasonal occurrence

- During paddy harvesting in Assam (Jan & Feb) Nath et al. 2011
- Fungi cultured significantly more frequently during summer months (Australia) Green et al. Cornea 2008; 27:33
- Incidence of FUNGAL KERATITIS higher between June & Sept (southern India). Bharathi et al. Ophthalmic Epidemiol 2007; 14:61
- Highest during harvest seasons, including summer and autumn (northern China). Xie et al. Ophthalmology 2006; 113: 1943

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Risk factors for filamentous FUNGAL KERATITIS


- Injury caused by plants show a 3.8 fold greater chance of positive fungal culture. Cariello et al. Int Ophthalmol 2011; 31: 197

- Corticosteroids (Stern & Buttross, Ophthalmology 1991; 98:847)

- ‘allergic’ conjunctivitis

- ? traditional eye medicines

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Predisposing factors

TOTAL NO. OF CASES = 515.

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Risk factors for filamentous FUNGAL KERATITIS

- **Fungal**
  - Veg. Matter: 28
  - Mud: 47
- **Bacterial**
  - Veg. Matter: 28
  - Mud: 15

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Injury by needle

foreign body in the cornea

Welding spark

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Courtesy J. KALIAMURTHY
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Hydrophilic contact lenses
Liesegang, Cornea 1997; 16: 125, 265

Contact lens cleaning solution
(ReNu with MoistureLoc - Bausch & Lomb, Rochester, NY)
--- Singapore, Hong Kong, USA, West Indies

--- withdrawal controlled the outbreak
Khor et al., JAMA 2006; 295: 2867
Chang et al., JAMA 2006; 296: 953

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Keratitis due to yeasts and yeast like fungi

- *Candida albicans*

- No geographic localization, exogenous
  - Ocular (tears, eyelid closure)
  - Systemic (diabetes mellitus, immunosuppression)

- Pre-existing corneal lesions
  - Herpes keratitis, contact lens-associated keratitis

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- Tried to detect fungal hyphae in corneal scraping material by a cost-effective assembly of smartphone & pocket magnifier.
- A tissue sample was obtained by conventional corneal scraping from a clinically suspicious case of fungal keratitis.
- Smear stained by Gram stain; a 10% potassium hydroxide mount also prepared.
- Slides imaged by a smartphone coupled with a compact pocket magnifier & integrated light-emitting diode assembly at point-of-care.
- Photographs of multiple sections of slides were viewed using smartphone screen & pinch-to-zoom function. Same slides subsequently screened under a light microscope by experienced microbiologist.
- The scraping from the ulcer also cultured
- Smartphone-based digital imaging revealed gram-positive organisms with hyphae. Examination under a light microscope also yielded similar findings.
- Fusarium cultured from the corneal scraping, confirming the diagnosis of fungal keratitis.

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Laboratory Diagnosis of FUNGAL KERATITIS

- Corneal scrapes
  - Edges
  - Base
- Corneal biopsy
  - Lamellar
  - Formal
- Anterior chamber

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Laboratory Diagnosis: Direct Microscopy

- **Wet Mounts**
  - KOH, ink KOH, KOH-DMSO+AO  
    Gopinathan et al., Cornea 2002; 21: 555
  - LPCB  
    Thomas et al., Diagn Microbiol Infect Dis 1991; 14:219

- **Stained smears**
  - Gram  
    Gopinathan et al., Cornea 2002; 21: 555
  - Giemsa

- **Special stains**
  - GMS, PAS  
    Gopinathan et al., Cornea 2002; 21: 555
  - AO, CFW

- **Lectins**
  - FC, EC  
    Robin et al., Am J Ophthalmol 1986; 102: 797
    Garcia et al., Mol Vis 2002; 8: 10

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THE BURDEN OF FUNGAL KERATITIS IN INDIA: LABORATORY DIAGNOSIS

- Calcofluor white
- Lactophenol cotton blue
- Gram stain
- Methenamine silver stain

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THE BURDEN OF FUNGAL KERATITIS IN INDIA: CULTURE

Solid media

- Sheep blood agar
- Cystine tryptone agar
- Sabouraud agar
- Rose Bengal agar

Antibacterial added to media

Liquid media

- Brain heart infusion broth
- Thioglycollate

Incubation Temperature: 22°C, 30-35°C

Duration of Incubation

Controls

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DIAGNOSIS ESTABLISHED BY:

■ Fungal hyphae or yeast cells in direct microscopy of LPCB wet films, Gram- or CFW-stained smears

■ Growth of fungi in ‘C’ streaks of at least 2 solid culture media inoculated with corneal scrapes.

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Fungal Keratitis: Direct microscopic examination

Advantages

• Rapid, relatively inexpensive, relatively easy
• Good sensitivity and specificity for some methods

Disadvantages

Can detect fungi but very difficult to identify the genus and species involved
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Culture

Advantages: a) More sensitive than direct microscopy

b) May sometimes exceed molecular techniques in sensitivity and specificity

c) Organism can be identified & susceptibility testing can be done.

Disadvantage: a) Takes time

b) There may be no growth in culture due to

c) Some amount of expertise needed for proper identification


• Identified Fusarium isolates from ocular infections by morphological methods and by PCR analysis of ITS regions (ITS 1, 5.8 S & ITS2)

• At species level, morphologic classification correlated with genotypic classification in 25% (general ocular microbiology laboratory) and 97% (reference mycology laboratory)

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Fungal rRNA complex
• Coding regions 18S, 5.8S & 28S nuclear rRNA genes evolved slowly
  -- are relatively conserved among fungi
  -- provide molecular basis of establishing phylogenetic relationships

• Non-coding regions ITS1 and ITS2 evolved more rapidly
  – responsible for sequence variability among fungal genera & species

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Identification of pathogens

Single strand conformation polymorphism (SSCP) analysis after PCR

- Amplified products of target sequence denatured into single-stranded DNA fragments
- Single-stranded fragments subjected to non-denaturing PAGE
- PCR-SSCP can detect > 90% of single-base substitutions in a 200 bp fragment
- Used to diagnose FUNGAL KERATITIS identify the fungal species involved

Kumar and Shukla J Clin Microbiol 2005; 43: 662-668
Kumar and Shukla Diagn Microbiol Infect Dis 2006; 56: 45-51

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Identification of pathogens

Nucleic acid sequence analysis after PCR

- Provides most information since size, nucleotide composition and order of nucleotides are considered
- Best method for identification of pathogens since suspicion of aetiological agent is not necessary
- Used to identify the fungus causing MYCOTIC ENDOPHTHALMITIS & THE BURDEN OF FUNGAL KERATITIS IN INDIA


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1. Suspected ocular fungal infection where fungal species identification is not immediately required

Broad range PCR using universal fungal primers to confirm a fungal aetiology

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2. Suspected ocular fungal infection where fungal species identification is a crucial need

a) Single step or nested PCR amplification of 18S rRNA or 28S rRNA genes
   ITSs- 5.8S rRNA gene
   ITS2

b) Identification of the amplified product:
   nested PCR, DNA sequencing or SSCP

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3. Identification of fungal isolate and determination of genetic relatedness to other fungal isolates

Multilocus sequence typing (MLST)
- Used to determine relatedness between isolates causing disease in different patients, hospitals and countries
- Epidemiological association of Fusarium keratitis in 2006 performed by this method
  
  Chang et al. JAMA 2006; 296: 953-963.

Arbitrarily-primed PCR (AP-PCR)
- useful for determining whether two isolates of the same species are epidemiologically related.
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- Medical emergency
- Frequently occurs as an acute presentation
- Patients in a state of distress due to pain & loss of vision
- Index of suspicion based on demographics and clinical presentation of great importance
  - Fungi may cause 1.2 to > 60 % of infectious keratitis
  - Two important types:
    -- due to filamentous fungi
    -- due to yeast and yeast-like fungi
  - Aspergillus and Fusarium ; Candida albicans

- Laboratory diagnosis (conventional and new modalities) required for confirmation

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Prompt diagnosis & identification of the fungal pathogen is of utmost importance.

Direct microscopy is rapid and sensitive but species identification is difficult.

Culture allows species identification and susceptibility testing but is time-consuming.

The polymerase chain reaction permits rapid detection of fungal nucleic acid in corneal scrape material.

The polymerase chain reaction permits accurate species identification—helps treatment.

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