

## ETIOLOGY OF SUPPURATIVE CORNEAL ULCERS IN RURAL POPULATION OF NORTHERN INDIA

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### ABSTRACT:

**Background:** A prospective, interventional, comparative study carried out in rural population of northern India in western UP. Fungal keratitis studies available in literature were also reviewed.

**Methods:** Patients presenting in eye OPD of UPRIMS & R Saifai, Etawah, UP with suspected microbial keratitis were recruited to the study. Corneal ulceration was defined as loss of corneal epithelium with clinical evidence of infection in form of corneal infiltrate with or without hypopyon. Microscopy and culture were performed on all corneal specimens.

**Results:** A total of 105 patients presenting with suppurative keratitis were enrolled in the study from Jan 2014 to April 2015. Fungi were identified as the dominant causative agent of infection (including mixed infections) in 52 patients (49.52%). Bacteria were isolated from 32 cases (30.47%). Mixed infection was present in 9 cases (8.57%). In each case of mixed infection a single bacterial species was associated with a single fungal species. In 21 cases (20%) had no definitive laboratory diagnosis. The principal causative micro-organisms noted in rural population were filamentous fungi (*Fusarium* and *Aspergillus* species) in 49.52 % cases. The most common bacterial pathogen was gram positive streptococci.

**Conclusion:** Infections of the cornea due to filamentous fungi are a frequent cause of corneal ulcer in rural population of north India and are very difficult treat. Corneal scrapping is very useful to assess etiology empirically and start anti-fungal drugs earliest. Knowledge of regional etiology will helpful in management of suppurative keratitis for private practitioner when microscopy cannot be performed.

**Keywords:** Corneal uclcer, Fungal corneal ulcer, Rural area, Suppurative keratitis.

### INTRODUCTION

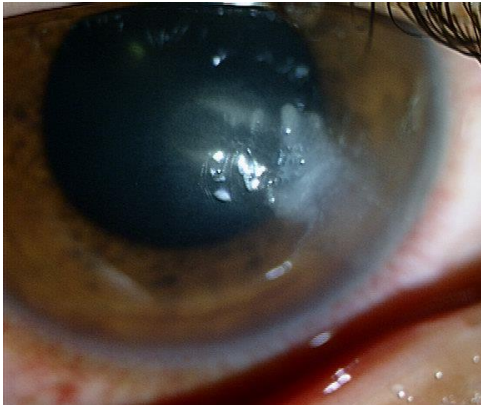
Scarring of the cornea as a result of suppurative keratitis is an important cause of preventable blindness. In developing countries like us, corneal infections are the second commonest cause of blindness after unoperated cataract.<sup>1-3</sup> Suppurative corneal ulcers may be caused by bacteria, fungi, and protozoa. However, within the tropics, and in India, as many as two thirds of ulcers may be due to filamentous fungi.<sup>1-3</sup> This type of ulceration is commonly associated with ocular trauma (vegetative matter most commonly).<sup>2-9</sup> Untreated, suppurative keratitis may lead to opacification and, if associated with corneal melting may lead to perforation of the cornea.<sup>1,3</sup> The associated morbidity is the result of difficulties in patient management because of a lack of diagnostic facilities and appropriate treatment in our country. Specific treatment requires prompt and accurate identification of the causative micro-organisms.<sup>9,10</sup> Within the setting of rural eye hospitals in the tropics laboratory facilities are rare and diagnosis is based on clinical characteristics. As a direct result of this, treatment is often empirical. The microbial causes of suppurative keratitis vary considerably between continents and countries and also within countries. It is essential to determine the "loco-regional etiology" within a given region when planning a corneal ulcer management

strategy. Several studies have investigated the epidemiology of corneal ulceration, causative micro-organisms, and effective treatments, particularly in the Indian subcontinent. However there is a paucity of information in the literature with regard to the experience in rural population of India.<sup>6-8,11,12</sup> The following study was conducted at rural tertiary care hospital of northern India. The aims of this study were to improve facilities for laboratory diagnosis, to determine the predominant causative micro-organisms, to identify the most suitable treatments, and encourage rapid referral of patients.

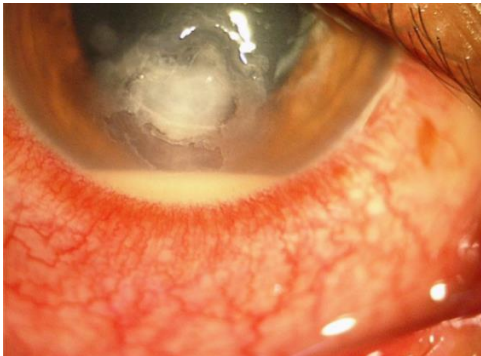
### MATERIALS AND METHODS

A prospective study of suppurative keratitis was conducted in UPRIMS & R, Saifai, Etawah a tertiary hospital in rural part of western UP from Jan 2014 to April 2015. Patients were recruited from the eye OPD. All patients presenting with suspected suppurative keratitis were included in the study. Corneal ulceration was defined as loss of corneal epithelium with underlying stromal infiltrate and suppuration associated with signs of inflammation, with or without hypopyon.<sup>1,6,7</sup> Patients with suspected or confirmed viral keratitis were excluded from the study. One eyed patients were excluded from study. Clinical examination and laboratory investigation was performed in all patients after well

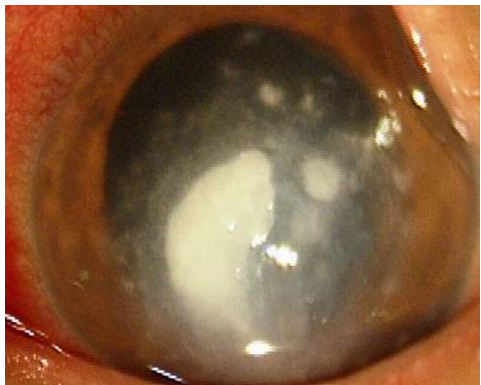
informed consent. Each patient was examined at the slit lamp clinical features were noted (*Fig. 1a,1b,1c,1d,1e,1f and 1g*) and a drawing made for patient records.



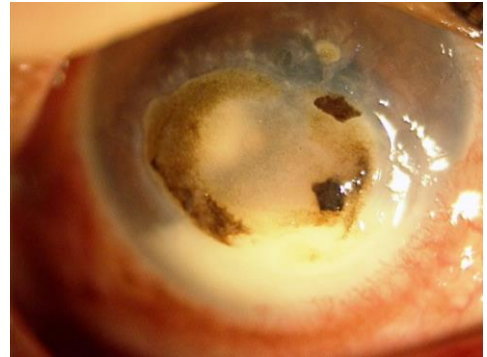
**Fig. 1a:** Shows Dry Raised Corneal Infiltrates



**Fig. 1(b):** shows epithelial defect with surrounding stromal infiltrate and hypopyon



**Fig. 1(c):** Shows Stellate Stromal Infiltrates and Endothelial Plaque



**Fig. 1(d), 1(e):** Shows Pigmented Corneal Ulcer with Dry Rough Looking Surface and Hypopyon



**Fig. 1(f):** Shows Dry Rough Raised Plaque of Corneal Infiltrates and Hypopyon



**Fig. 1(g):** Shows Immune Ring Shaped Corneal Infiltrates

A corneal scrape was performed by an ophthalmologist using a sterile 15 no blade after instillation of local anaesthetic lignocaine (lidocaine) 4%. If a patient was taking topical antibiotics at the time of presentation to the OPD then antibiotics stopped for 24 hours then sample for staining and cultures taken. Corneal material obtained from scraping the ulcer was smeared onto two slides which were stained with Gram stain and KOH mount for microscopic examination. Sterile swab for cultures also sent (swab were kept wet by 4% Lignocaine). Material was inoculated directly onto 5% sheep's blood agar and Sabouraud dextrose agar. Blood agar plates were incubated at 37°C. Sabouraud dextrose agar was incubated at 27°C. Bacteria was further identified using routine biochemical identification tests and selective media. Identification of fungi was carried out after correlating staining and culture characteristics on Sabouraud dextrose agar. Filamentous fungi were identified according to the macroscopic appearance of cultures on Sabouraud dextrose (fig1a,1b), and microscopic appearance of conidia and spore bearing structures. Colour production in culture or clinical appearance also used in identification. (Fig. 1 and 2)



**Fig. 2(a): Macroscopic view of culture plate with aspergillus species on sabouraud's dextrose agar media**



**Fig. 2(b): Macroscopic view of culture plate with fusarium species on sabouraud's dextrose agar media**

## RESULTS

A total of 105 patients presenting with suppurative keratitis were enrolled in the study. Fungi were identified as the dominant causative agent of infection (including mixed infections) in 52 patients (49.52%). Bacteria were isolated from 32 cases (30.47%). Mixed infection was present in 9 cases (8.57%). In each case of mixed infection a single bacterial species was associated with a single fungal species. In 21 cases (20%) had no definitive laboratory diagnosis. Streptococci (18.1%) and staphylococci (10.48%) were commonly associated with corneal infection (Table 2). In streptococci accounted for 59.38% of bacterial corneal ulcers followed by staphylococci (32.37%) and pseudomonas (3.1%). In our study 90% bacterial infection is caused by streptococci and staphylococci. (Table 1)

Fusarium species and Aspergillus species were isolated from 73.08% of all fungal infections and comprised 73% of identified fungal isolates (Table 2). Although Fusarium species were the most prevalent fungal pathogens reported in India, followed by Aspergillus species. In our study 34.46 % of fungal isolate were Fusarium and 34.62% Aspergillus species. (Table 2)

**Table 1: Types of Bacteria isolated from corneal ulcer**

Bacteria	No of cases	% Bacterial cases	% of total cases
Gram Positive Cocci			
- Streptococci	19	59.38%	18.1%
- Staphylococci	11	32.37%	10.48%
Gram Positive Bacilli			
- Bacillus	-	-	-
Gram Negative Bacilli			
- Pseudomonas	1	3.1%	0.95%
- Enterobactor	-	-	-
- Klebsiella	1	3.1%	0.95%
<b>Total</b>	<b>32</b>	<b>100%</b>	<b>30.47%</b>

**Table 2: Types of Fungi isolated from corneal ulcer**

Fungi	No of cases	% of fungal cases	% of total cases
Fusarium	20	38.46%	19.04%
Aspergillus	18	34.62%	17.14%
Candida	2	3.84%	1.90%
Curvularia	-	-	-
Unidentified	12	23.07%	11.42%
<b>Total</b>	<b>52</b>	<b>100%</b>	<b>49.50%</b>

**Table 3: Comparison of incidence of fungal keratitis in different studies/ countries**

Place	Reference	Year	No. of cases	% Fungi	Organism1	Organism2
Europe						
London	Coster <sup>30</sup>	1981	67	3%	2 cases only	
London	Personal comm	2001	42	3%	Candida 58%	Aspergillus26%
Sweden	Neumann <sup>31</sup>	1993	78	0%		
North America						
Florida	Liesegang <sup>27</sup>	1980	663	20%	Fusarium spp62%	Candida spp7.5%
Florida	Rosa <sup>32</sup>	1994	125		Fusarium spp62%	Candida spp12.5%
Philadelphia	Tenure <sup>13</sup>	2000	24		C. albicans 46%	Fusarium spp25%
California	Ormanod <sup>14</sup>	1987	227	11%	C. albicans 4%	Penicillusspp 2%
Atlanta	Harris <sup>15</sup>	1988	108	32%	Candida spp 94%	
Northern USA	O' Day <sup>16</sup>	1987	33		Candida spp 42%	Aspergillus spp 30%
Southern USA			285		Fusarium spp55%	Candida spp 9.5%
South America						
Paraguay	Mino de Kasper <sup>28</sup>	1991	26	58%	Fusarium spp 42%	Aspergillus spp 19%
The Middle East						
Soudi Arabia	Khairallah <sup>21</sup>	1992	191	14%	Aspergillus spp 41%	Fusarium spp Candida albicans
Africa						
Ghana	Hagan <sup>6</sup>	1995	199	34%	Fusarium spp 52%	Aspergillus spp 15%
South Africa	Carmichael <sup>22</sup>	1985	274	25%	Curvulariaspp 33%	
Nigeria	Gugani <sup>11</sup>	1976	59		Fusarium spp 36%	Penicillusspp
Tanzania	Mselle <sup>23</sup>	1999	212	15%	Fusarium spp 75%	Aspergillus spp
South Africa	Ormarod <sup>24</sup>	1987	120	2.5%	3 different isolates	
Asia						
Nepal , Dharan	Khanal <sup>9</sup>	2001	86	44%	Aspergillus spp 60%	Fusarium 13%
India , New Delhi	Panda <sup>8</sup>	1997	211	10.8%	Aspergillus spp 40%	Fusarium spp 11%
India , New Delhi	Mahajan <sup>33</sup>	1985	674	19.7%	Aspergillus spp 37%	Fusarium spp 10%
India , Mumbai	Deshpande <sup>18</sup>	1999	367		Aspergillus spp 60%	Candida spp 10%
India , Hyderabad	Kunimoto <sup>12</sup>	2000	102	19%	Aspergillus spp 37%	Curvulariaspp 16%
India , Hyderabad	Garg <sup>16</sup>	2000	557		Fusarium spp 38%	Aspergillus spp 30%
Sri Lanka	Gonawardina <sup>35</sup>	1994	66	33%	Aspergillus spp 18%	Single isolate
Thailand	Imwidththaya <sup>36</sup>	1995	145	25%	Aspergillus spp 34%	Fusarium spp 26%
Singapore	Wong <sup>37</sup>	1997	29		Fusarium spp 52%	A. Flavus 17%
Hong Kong	Houang <sup>29</sup>	2001	223	2%	Fusarium 60%	

## DISCUSSION

In our prospective study Fungi were identified as the dominant causative agent of infection (including mixed infections) in 52 patients (49.52%). Bacteria were isolated from 32 cases (30.47%). Mixed infection was present in 9 cases (8.57%). In each case of mixed infection a single bacterial species was associated with a single fungal species. In 21 cases (20%) no definitive laboratory diagnosis was made. A review of the literature shows that there are distinct patterns of geographical variation in the aetiology of suppurative keratitis and considerable variation in the proportion of cases due to fungi has been documented. The proportion of

corneal ulcers caused by filamentous fungi increases towards tropical latitudes<sup>13-15</sup>. In more temperate climates, fungal ulcers are uncommon and are more frequently associated with Candida species than filamentous fungi.<sup>13-16</sup> Houang et al<sup>17</sup> reviewed (Table 3) the relation of fungal keratitis to the climate concluding that, although a higher incidence of fungal keratitis could be expected in countries with similar annual rainfall and temperature range, but this was not always so and was also dependent on the extent of urbanisation. Higher number of cases of fungal ulcers in rural area are due to higher incidence of vegetative matter trauma. Aspergillus and

Fusarium species are the most frequently reported fungal pathogens isolated from cases of fungal keratitis in the tropics and rural areas.<sup>5</sup> In our study also filamentous fungi Fusarium and Aspergillus accounted for 36.18% cases of suppurative corneal infection. Other studies in south India have reported Fusarium species to be more common than Aspergillus species. Fusarium species have also been found as the principal fungal pathogen in Florida, Paraguay, Nigeria, Tanzania, Hong Kong, and Singapore (Table 3). Aspergillus species predominate in northern India, Nepal, and Bangladesh.<sup>1,9,18-20</sup> This phenomenon may be explained on the basis of differences in climate and the natural environment. A similar pattern was also observed in our study in rural area of western UP. As observed by Khairallah<sup>21</sup> the higher proportion of corneal infections by Aspergillus species seen in drier climates may be due to the fact that spores of Aspergillus species can tolerate hot, dry weather conditions. Aspergillus species also predominate in more temperate latitudes. A significant increase in the number of reported cases of suppurative keratitis was observed during the harvest period and windy seasons<sup>21-23</sup>. However, the proportion of corneal ulcers due to fungi remained consistently high throughout the year. Other authors have made similar observations, noted an increase in cases of fungal keratitis during the dry, windy seasons compared with the wet, humid months of the year.<sup>4,27</sup> This trend is likely to be a direct consequence of increased agricultural activity before and immediately following the rains<sup>4,27</sup>. Some studies have reported an increase during the hot and humid months.<sup>8,25</sup> The majority of filamentous fungi associated with corneal ulceration in the tropics are thermophilic moulds that are found widely in this environment. These are ubiquitous in the soil and vegetation. Fusarium species are common plant pathogens, particularly of cereal crops or saprophytes of plant debris and are found in soil.<sup>26</sup> The Aspergilli are ubiquitous and have been found almost everywhere on every conceivable type of substrate, including soil and decaying organic debris<sup>26</sup>. Some of the less common isolates such as Bipolaris species and Exserohilum species are pathogens of grasses. Curvularia species mostly occur on dead plant material.<sup>26</sup> Although injury by vegetable matter is considered to be predictive for fungal keratitis, in this study fungal corneal ulcers were more often preceded by dust or mud particles in the eye. A shift in the predominant bacterial pathogens was observed when compared with earlier findings. Pseudomonas species were identified as the commonest bacterial isolate in a study of 142 cases of suppurative keratitis in Bangladesh in a study by Dunlop et al and also by Williams et al, who found that 40% of bacterial isolates were Pseudomonas species.<sup>4</sup> A predominance of Pseudomonas species has been reported in Hong

Kong, Florida, and Paraguay.<sup>27-29</sup> In reports from Nepal and south India, Gram positive cocci have been reported as the primary cause of bacterial keratitis.<sup>1, 7, 9</sup> In this study, Gram positive cocci accounted for the majority (74%) of bacterial isolates in rural India, as found previously by Thomas et al.<sup>12</sup> The proportion of bacterial ulcers caused by Streptococci species increased from 18.5% (1986) to 59.38% in this study. Similar diagnostic criteria were used in the previous study and, therefore, the trend may be attributable to a genuine change of bacterial flora within the geographical area, as influenced by climate and environment. It is imperative that the quality and quantity of specimen is optimal for accurate laboratory diagnosis. Self-administration of antibiotics by patients before seeking medical attention has been thought to affect the recovery of organisms in culture.<sup>4</sup> It is therefore not unreasonable to assume that this trend could be extrapolated. It is usually not possible to determine the significance of bacteria observed by microscopy alone<sup>3-6</sup>. Small numbers of Gram positive cocci may be contaminants from the lid margin. Conversely, the presence of fungal hyphae in corneal tissue is significant. In agreement with Dunlop et al, the sensitivity, specificity, and predictive value of Gram stain microscopy is much higher for fungal ulcers than those caused by bacteria.<sup>4</sup> In this study 95% of fungal infections could have been diagnosed based on the findings from microscopy and clinical characteristics alone. This is an important conclusion, since the majority of rural based clinics in areas where suppurative keratitis is a problem do not have culture facilities but may be able to perform simple microscopy.

A wet mount preparation, using Potassium Hydroxide (KOH) stain, was used as a supplementary stain in this study. Reports into the presence of fungi in the eyes of asymptomatic individuals have shown that a wide variety of fungi may be transient in the conjunctival sac. This may be the case in as many as 37% of healthy eyes, thereby discounting the use of single culture.<sup>11</sup> In conclusion, it is imperative to know the "loco-regional" etiology of keratitis in a particular region. The differences were observed within the same country. This is important information with regard to management, as many ophthalmologist in private practice do not have microscopy or culture facilities. Diagnosis is then dependent on clinical assessment and the treatment provided is empirical. It is clear that it is important to know the loco regional etiological organism when diagnosis is made by clinical examination alone.

Awareness of changes in etiology and antimicrobial resistance, when this information is available, are critical to managing keratitis case in any particular loco-regional area.

## CONCLUSION

Infections of the cornea due to filamentous fungi are a frequent cause of corneal ulcer in rural population of north India and are very difficult treat.

Corneal scraping is very useful to assess etiology empirically and start anti-fungal drugs earliest. Knowledge of loco- regional etiology will helpful in management of suppurative keratitis for private practitioner when microscopy cannot be performed for starting region selective empirical therapy

## REFERENCES

- Upadhyay MP, Karmacharya PC, Koirala S, et al. Epidemiologic characteristics, predisposing factors, and etiologic diagnosis of corneal ulceration in Nepal. *Am J Ophthalmol* 1991;111:92-9.
- Gonzales CA, Srinivasan M, Whitcher JP, et al. Incidence of corneal ulceration in Madurai District, south India. *Ophthalmic Epidemiol* 1996;3:159-66.
- A K Leck, P A Thomas et al Aetiology of suppurative corneal ulcers in Ghana and south India and epidemiology of fungal keratitis. *B.J.O* 2002;86:1211-15.
- Dunlop AAS, Wright ED, Howlader SA, et al. Suppurative corneal ulceration in Bangladesh. *Aus NZ J Ophthalmol* 1994;22:105-10.
- Thomas PA. Mycotic keratitis—an underestimated mycosis. *J Med Vet Mycol* 1994;32:235-56.
- Hagan M, Wright E, Newman M, et al. Causes of suppurative keratitis in Ghana. *Br J Ophthalmol* 1995;79:1024-8.
- Srinivasan M, Gonzales CA, George C, et al. Epidemiology and etiological diagnoses of corneal ulceration in Madurai, south India. *Br J Ophthalmol* 1997;81:965-71.
- Panda A, Sharma N, Das G, et al. Mycotic keratitis in children: epidemiologic and microbiologic evaluation. *Cornea* 1997;16:295-9.
- Khanal B, Kaini KR, Deb M et al. Microbial keratitis in eastern Nepal. *Tropical Doctor* 2001;31:168-9.
- Foster CS. Fungal keratitis. *Infect Dis Clin N Am* 1992;6:851-7.
- Gugani HC, Talwar RS, Njoku-Obi AN, et al. Mycotic keratitis in Nigeria. A study of 21 cases. *Br J Ophthalmol* 1976;60:607-13.
- Garg P, Gopinathan U, Choudhary K, et al. Keratomycosis: clinical and microbiologic experience with dematiaceous fungi. *Ophthalmology* 2000 107:574-80.
- Tanure MA, Cohen EJ, Sudesh S, et al. Spectrum of fungal keratitis at Wills Eye Hospital Philadelphia, Pennsylvania. *Cornea* 2000;19:307-12.
- Ormerod LD, Hertzmark E, Gomez DS, et al. Epidemiology of microbial keratitis in southern California. A multivariate analysis. *Ophthalmology* 1987;94:1322-33.
- Harris DJ Jr, Stulting RD, Waring GO 3rd, et al. Late bacterial and fungal keratitis after corneal transplantation. Spectrum of pathogens, graft survival, and visual prognosis. *Ophthalmology* 1988;95:1450-7.
- O'Day DM. Selection of appropriate antifungal therapy. *Cornea* 1987;6:238-45.
- Houang E, Lam D, Fan D, et al. Microbial keratitis in Hong Kong: relationship to climate, environment and contact-lens disinfection. *Trans R Soc Trop Med Hyg* 2001;95:361-7.
- Deshpande SD, Koppikar GV. A study of mycotic keratitis in Mumbai. *Indian J Pathol Microbiol* 1999;42:81-7.
- Williams G, McClellan K, Billson F. Suppurative keratitis in rural Bangladesh: the value of gram stain in planning management. *Int Ophthalmol* 1991;15:131-5.
- Chander J, Sharma A. Prevalence of fungal corneal ulcers in northern India. *Infection* 1994;22:207-9.
- Khairallah SH, Byrne KA, Tabbara KF. Fungal keratitis in Saudi Arabia. *Doc Ophthalmol* 1992;79:269-76.
- Carmichael TR, Wolpert M, Koornhof HJ. Corneal ulceration at an urban African hospital. *Br J Ophthalmol* 1985;69:920-6.
- Mselle J. Fungal keratitis as an indicator of HIV infection in Africa. *Trop Doct* 1999;29:133-5.
- Ormerod D. Causation and management of microbial keratitis in subtropical Africa. *Ophthalmology* 1987;94:1662-8.
- Mahajan VM. Ulcerative keratitis: an analysis of laboratory data in 674 cases. *J Ocul Ther Surg* 1985;4:138-41.
- De Hoog GS, Guarro J, Gené J, et al. Atlas of clinical fungi. 2nd ed. 2000:30-5.
- Liesegang TJ, Forster RK. Spectrum of microbial keratitis in south Florida. *Am J Ophthalmol* 1980;90:38-47.
- Miño de Kaspar H, Zoulek G, Paredes ME, et al. Mycotic keratitis in Paraguay. *Mycoses* 1991;34:251-4.
- Houang E, Lam D, Fan D, et al. Microbial keratitis in Hong Kong: relationship to climate, environment and contact-lens disinfection. *Trans R Soc Trop Med Hyg* 2001;95:361-7.
- Coster DJ, Wilhelmus J, Peacock J, et al. Suppurative keratitis in London. The cornea in health and disease. Vth Congress of the European Society of Ophthalmology 1981;40:395-8.
- Neumann M, Sjostrand J. Central microbial keratitis in a Swedish city population. A three-year prospective study in Gothenburg. *Acta Ophthalmol (Copenh)* 1993;71:160-4.
- Rosa RH, Miller D, Alfonso EC. The changing spectrum of fungal keratitis in south Florida. *Ophthalmology* 1994;101:1005-13.
- Mahajan VM. Ulcerative keratitis: an analysis of laboratory data in 674 cases. *J Ocul Ther Surg* 1985;4:138-41.
- Kunimoto DY, Sharma S, Garg P, et al. Corneal ulceration in the elderly in Hyderabad, south India. *Br J Ophthalmol* 2000;84:54-9.
- Gonawardena SA, Ranasinghe KP, Arsecularatne SN, et al. Survey of mycotic and bacterial keratitis in Sri Lanka. *Mycopathologia* 1994;127:77-81.
- Imwidthaya P. Mycotic keratitis in Thailand. *J Med Vet Mycol* 1995;33:81-2.
- Wong TY, Fong KS, Tan DTH. Clinical and microbial spectrum of fungal keratitis in Singapore: a 5-year retrospective study. *Int Ophthalmol* 1997;21:127-30.