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DEMOGRAPHIC PATTERN, RISK FACTORS, CLINICAL AND MICROBIOLOGICAL CHARACTERISTICS OF MICROBIAL KERATITIS AT A TERTIARY CARE HOSPITAL

1. **ASHOK KUMAR NARSANI**
FCPS
2. **MUHAMMAD MUNEER QURAISHY**
FRCOphth
3. **MAHESH KUMAR LOHANA**
DCP
4. **SHAFI MUHAMMAD JATOI**
FCPS
5. **MAHTAB ALAM KHANZADA**
FCPS

1. ***Associate Professor***
Department of Ophthalmology
LIAQUAT UNIVERSITY EYE HOSPITAL, HYDERABAD.
2. ***Associate Professor***
Department of Ophthalmology
DOW MEDICAL COLLEGE KARACHI
3. ***Clinical Pathologist***
Department of ophthalmology
LIAQUAT UNIVERSITY EYE HOSPITAL, HYDERABAD.
4. ***Chairman and Head***
Department of Ophthalmology
LIAQUAT UNIVERSITY EYE HOSPITAL, HYDERABAD.
5. ***Assistant Professor***
LIAQUAT UNIVERSITY EYE HOSPITAL, HYDERABAD.

Correspondence Address:
DR. ASHOK KUMAR NARSANI
FCPS
Associate Professor
Department of Ophthalmology
LIAQUAT UNIVERSITY EYE HOSPITAL HYDERABAD.
03023929327
amnarsani@hotmail.com

ABSTRACT

PURPOSE: *To analyze the demographic pattern, risk factors, clinical and microbiological characteristics of microbial keratitis in patients seen at tertiary care centre.*
DESIGN: *Quasi experimental*
SETTING: *Department of Ophthalmology Unit I, Eye Hospital, Liaquat University of Medical and Health Sciences Hyderabad Sindh from April 2006 to March 2009.*
MATERIAL AND METHODS: *Patients above 15 years of age with suspected microbial keratitis attending the outpatients department were included in this study. After detailed history, every patient underwent slit lamp bio-microscopical examination, for characteristics of corneal ulcer. Corneal scrapings were collected under aseptic conditions under magnification of slit lamp or operating microscope. The scraping material obtained from leading edge and base of each ulcer was spread onto labeled slides for staining. Another portion was inoculated on to the surface of solid and the liquid culture medias. The inoculated medias were incubated at appropriate temperature for appropriate time. The treatment started soon after taking material, which was further modified if needed.*
RESULT: *Three hundred and ninety seven eyes of 397 patients were enrolled, of these 67 patients were lost in follow up were excluded from study. Of the remaining 330 patients 139 (42.12%) were examined first time in the out patients department. The age of patients ranged from 16 years to 79 years. Of the 330 patients 194 patients were male and 136 females. Two hundred and twenty eight patients belongs rural and 102 from urban population. One hundred and eighty two patients were farmer by occupation, trauma with vegetative material was by far the most common risk factor; encountered in 161 patients. The diameter of the corneal ulceration was of 1-3 mm in 149 patients. Hypopyon was found in 138 patients. Other signs included feathery projection of ulcer, satellite lesions, anterior chamber reaction, endothelial plaques, and pigmentation of lesion, suppuration and stromal infiltration.*
Out of 330 patients, 161(48.80%) has positive culture for bacteria, 87 (26.36%) has positive culture for only fungi, in 12 (3.64%) were found positive culture for both bacteria and fungi, and 2 (0.61%) has the positive culture for Acanthamoeba.
The Staphylococcus aureus was the most common isolated bacteria, and Candida albicans was the most common isolated fungi.
Forty percent of patients had good visual out come with visual acuity same or better than the level at admission. Among the others 60% patient's final outcome was poor.
Secondary glaucoma was develop in 28 patients, other rare complications included, endophthalmitis, anterior staphyloma, cataract, corneal perforation, and descmetocele
CONCLUSION: *The suppurative keratitis, most often occurs after a vegetative or non vegetative trauma. Bacterial ulcers are more common than fungal ulcers. Staphylococcus aureus and canddida species' were the most common bacteria and fungus respectively. The regional variations in causative organisms are clinically important for initiating intensive ocular antimicrobial agents represent decisive steps in the management of such pathologies.*

KEY WORDS: Microbial keratitis, laboratory, staphylococcus aureus, canddida albicans, visual acuity.

INTRODUCTION:
Microbial keratitis is a serious ocular infectious disease that can lead to significant vision

loss and ophthalmic morbidity.[1] corneal infections are the second most common cause of monocular blindness after un-operated cataract in some developing countries in the tropics[2,3]. The severity of corneal infection depends on the underlying condition of the cornea and pathogenecity of the infecting organism.[4] It is rare in the absence of predisposing factors, and hence most commonly associated with ocular trauma or ocular surface disease[5]. The associated ocular morbidity is the result of several factors and patients management is directly affected by the lack of diagnostic facilities and initiation of appropriate antimicrobial therapy[6]. The spectrum of microbial keratitis can also be influenced by geographic and climatic factors. Many differences in keratitis profile have been noted between populations living in rural or in city areas in western, or in developing countries.[7,8] Gram positive bacterial species are more frequently recovered in temperate zones[9], and gram-negative species in tropic climates[10] and tropical countries. Humid atmosphere and outdoor occupations make the population more vulnerable to fungal infections[11]. Untreated, infective keratitis may lead to opacification and, ultimately, to perforation of the cornea. The purpose of this study was an effort to analyze the epidemiologic features, predisposing factors and the main causative organisms for microbial corneal ulcer in this part of Asia to provide a useful guide for the practicing ophthalmologists.

MATERIALS AND METHOD

This three years prospective study (from April 2006 to March 2009) of microbial keratitis was conducted at Department of Ophthalmology Liaquat University Eye hospital Hyderabad, a tertiary referral center in southern Pakistan. Microbial keratitis was defined as a loss of the corneal epithelium, with underlying stromal infiltration and suppuration associated with signs of inflammation with or without hypopyon.

Patient above 15 years of age were included after the initial clinical diagnosis of suppurative corneal ulcer was made. The ulcers excluded from this study were viral ulcers, marginal keratitis, mooren's ulcer, intersititial keratitis, neurotropic keratitis, non-healing ulcers. Patient's written consent was obtained and a standardized Proforma was used to record the data.

patients

Detailed history and examination of the patients was taken regarding demographic features, time of onset of symptoms, pre-disposing factors including corneal injuries

Table 1. Demographic data

Age (years)	Sex				Total (%)
	Male		Female		
	Rural	urban	Rural	Urban	
11-20	2	0	6	2	10 (3.03)
21-30	23	13	13	5	54 (16.36)
31-40	30	11	18	8	67 (20.30)
41-50	36	17	22	13	88 (26.70)
51-60	26	12	19	9	66 (20)
> 60	18	6	15	6	45 (13.64)
Total	**135**	**59**	**93**	**43**	**330 (100)**

Table No 2. Risk factors for microbial keratitis

Risks	Frequency	percentage (%)
Vegetative trauma	161	48.80
Non vegetative trauma	22	6.70
Ocular surgery	13	3.94
Foreign boy	21	6.40
Contact lens	07	2.12
Lid margin disease	23	07
Ocular surface disorder	18	5.50
No evident cause	65	19.70
Total	**330**	**100**

Table 3. Occupations

Occupation	Frequency	Percentage (%)
Farmer	182	55.15
Labourer	61	18.43
Student	31	09.40
Government employee	18	05.50
Old age	38	11.52
Total	**330**	**100**

Table 4. Size of lesions

Size (mm)	Frequency	percentage (%)
1-3	149	45.15
4-6	116	35.15
>6	65	19.70
Total	**330**	**100**

Table 5. Signs of microbial keratitis

Signs	Frequency	percentage (%)
Hypopyon	138	41.82
Feathery projection	38	11.52
Satellite lesion	26	7.89
Anterior chamber reaction	149	45.15
Stromal infiltration	330	100
Suppuration	330	100
Endothelial plaque	18	5.50
Pigmentation	3	0.90

(agriculture, non agriculture, foreign bodies), contact lens wear, keratopathies (due to previous herpetic infection, corneal dystrophy or surgical procedure, as in bullous keratopathy), dry eye syndrome, eye lid abnormalities (blepharitis, entropion, ectropion, lagophthalmos, and others). Every patient underwent a comprehensive ophthalmic examination including slit lamp biomicroscopical examination, and characteristics of the ulcer such as the site, size and depth, thinning, satellite lesions, stromal infiltrate, margin, pigmentation and any impacted foreign body on the surface of ulcer were noted. Anterior chamber inflammation, hypopyon when present, was recorded. Best corrected visual acuity was taken using the Snellen's chart. After a detailed ocular examination corneal scrapings were collected under aseptic conditions after instillation of local anesthetic 0.5% proparacaine hydrochloride using a sterile Bard Parker blade (No 15). The procedure was performed under magnification of slit lamp or operating microscope. The scraping material obtained from leading edge and base of ulcer was spread onto labeled slides for 10% potassium hydroxide (KOH) wet mount, Gram staining and Giemsa staining. Another portion was inoculated on to the surface of solid media such as blood agar, chocolate agar, Mac Conkey agar, Sarboraud's agar respectively, in C – shaped streaks and also in the liquid media such as brain heart infusion broth. All inoculated media were incubated aerobically and meticulous care was taken in the collection of material and transferring it into the appropriate culture media aseptically. After growth of organisms on culture media's they were further examined by different chemical reaction for specific diagnosis of microorganism. These chemical reactions include indole, Simon citrate, urease, coagulase, catalase, and TSI (Triple Sugar Iron) tests

LABORATORY PROCEDURES.

The inoculated media – blood agar, chocolate agar, thioglycollate broth and brain heart infusion broth were incubated at 37°C and were evaluated daily for 72 hours. The inoculated fungal media- Sabouraud dextrose agar was inoculated at 27°C daily for 7 days. When KOH, smears were positive for amoebic cysts, a further corneal scraping was performed and material was inoculated on to non nutrient agar with Escherichia coli plates incubated at 37°C and were examined daily for 7 days. Microbial cultures were considered positive only if at least one of the following criteria were met.

a. The growth of the same organism was demonstrated on two or more solid media on the C –streak:

b. The same organism was grown from repeated scraping

c. Culture and smear result correlates.

d. Consistent with clinical signs

TREATMENT PROTOCOL

Two hundred and seven (62.80%) patients were treated as an out patients department

Table 6 Result of corneal scraping

Name of organism	Bacterial isolates	fungal isolates	Bacterial and isolates	acanthamoeba fungal isolates
Bacteria				
Gram positive cocci				
Staphylococcus aureus	78		04	
Staphylococcus epidermis	12		01	
Streptococcus pneumonia	14		02	
Streptococcus pyogenes	07		01	
Gram positive bacilli				
Bacillus	06			
Gram negative coco-bacilli				
Moraxella species	04			
Gram negative bacilli				
Pseudomonas aeruginosa	17		02	
Proteus species	09		01	
Klebsiella pneumonia	04			
Yersina species s	09		01	
Escherichia coli	01			
Fungi				
Candida albicans	67	08		
Aspergillus flavus	09	02		
Fusarium soloni	08	02		
Penicillium	02			
Aspergillus fumigatus	01			
Acanthamoeba				02

Table 7 Pre and post treatment visual status

Visual acuity	Frequency	percentage (%)
6/6 – 6/12	38	11.50
6/18 – 6/36	67	20.30
6/60 – 3/60	121	36.70
<3/60	104	31.50
Total	**330**	**100**

Table .8 complications

Complications	Frequency	percentage (%)
Glaucoma	28	8.50
Anterior staphyloma	18	5.45
Corneal perforation	10	3.03
Descemetocele	10	3.03
Cataract	10	3.03
Endophthalmitis	09	2.73

basis and 123 (37.20%) patients were hospitalized for treatment. The decision to admit patients and use of fortified antibiotics were influenced by the severity of the corneal ulcer and patient's compliance. The standard fortified therapy for bacterial corneal ulcer consisted of topical cefuraxime 50mg/ml and fortified tobramycin 9 mg/ml, or commercially available antibiotics used were topical fluroquinolone (moxifloxacin). For the fungal corneal ulcers natamycin drops were used and for the amoebic keratitis commercially available Propamidine isethionate 0.1% drops were used. The antimicrobial eye drops, administered alternately every fifteen minutes during the first 4 hours then every hour for the next 48 hours. Later, drops were progressively tapered according to the clinical response. Bottles of fortified antibiotics drops were freshly prepared and cefuraxime was changed every 72 hours. This standard treatment protocol was started for every patient and modified on patient's response and organism's susceptibility.

RESULT

Three hundred and ninety seven eyes of 397 patients were enrolled with a corneal infiltration that was clinically compatible with a diagnosis of microbial corneal ulcer during the three years study period. Of 397 patients 67 were lost in follow up, excluded from study. Of the remaining 330 patients 139 (42.12%) were examined first time in the out patients department, where as 191 (57.88%) were previously treated by general practitioners and ophthalmologists and were already being treated with topical antibiotics, corticosteroids and antifungal eye drops. The age of patients ranged from 16 years to 79 years (mean age 43 years). Majority of them were male 194 (58.79%) with male to female ratio of 2:1.4 (Table 1). Two hundred and twenty eight (69.09%) patients were belonging rural and 102 (30.91%) from urban population. One hundred and eighty two patients were farmer by occupation, 161 were labours, 31 were students, 18 were government employee and 38 patients were jobless due to old age (Table.2).

Trauma with vegetative material was by far the most common risk factor, encountered in 161 (48.80%) patients. Other risk factors were Lid margin disease in 23 (7%), Non vegetative trauma in 22 (6.7%), Foreign body in 21 (6.4%), Ocular surface disorder in 18 (5.50%), Ocular surgery in 13 (3.94%) and contact lens wear in 07 (2.12%) patients (Table.3). In sixty five (19.70%) patients no evident cause was found.

The diameter of the corneal ulceration was of 1-3 mm in 149 (45.15%), 4-6 mm in 116 (35.15%), >6 in 65 (19.70%) patients (Table.4). Hypopyon was found in 138 (141.82%) patients. Other signs included feathery projection of ulcer in 38 (11.52%) patients, satellite lesions in 26 (7.89%) patients, anterior chamber reaction in 149(45.15%), endothelial plaques in 18(5.50%) and pigmentation of ulceration were found in 3(0.9%) patients. While suppuration and stromal infiltration found in all patients (Table.5).

Out of 330 patients, 161 (48.80%) has positive culture for bacteria, 87 (26.36%) has positive culture for fungi, in 12 (3.64%) were found positive culture for both bacteria and fungi, and 2(0.61%) has the positive culture for Acanthamoeba. No organism were cultured in sixty eight patients.

Of the 173 bacterial isolates 119 (68.79%) had Gram positive Cocci, 6 (3.47%) had Gram positive bacilli, 4 (2.31%) had Gram negative Coco-bacilli, and 44 (25.43%) were found to be Gram negative bacilli. The most common isolated bacteria was Staphylococcus aureus 82 (47.40%) followed by Pseudomonas aeruginosa 19 (11%),

Fungal pathogens were isolated from 99 (30%) corneal ulcers. The commonest isolated organism was Candida albicans 75 (75.76%), followed by Aspergillus flavus 11 (11.11%), Fusarium soloni, Penicillium and Aspergillus fumigatus were least isolated organism (Table.6).

CLINICAL OUT COME

Visual acuity on presentation ranged from 6/6 to no light perception. Forty percent of patients had good visual out come with visual acuity same or better than the level at admission (Table.7). Among the others 60% patients final outcome was poor

Complications of bacterial corneal ulcer were noted in 85 (25.77%) patients. Among them, glaucoma in 28 patients, endophthalmitis in 9, anterior staphyloma in 18 patients and corneal perforation, descmetocele, cataract each develops in 10 patients (Table 8). Two hundred and forty five patients has no severe complication except scaring at the site of lesion.

DISCUSSION

Corneal infection is the leading cause of ocular morbidity and blindness worldwide. Corneal ulcer is rare in the absence of a predisposing factor. In this study most of the patients (74%) were agricultural worker and labour, an occupational profile similar to south Indian study[12,13] (66.8% and 79.3% respectively) presented with history of ocular trauma. One hundred and eighty three patients (55%) were associated with various types of ocular injuries. Vegetative trauma accounts 161 (48.80%) patients. Vajpayee et al[7] reported 77.5% of cases of bacterial corneal ulcer occurred by trauma in low income countries, where a large number of population are concerned to agriculture. Moreover, the climate is humid, and malnutrition is common[14]

Foreign body induced corneal ulcer was the second most common 21 (6.40%) predisposing factor in this study. Most (55%) of these patients had also history of foreign body removal by own or by other family members and followed by self-medications.

Ocular surface disorders such as dry eye syndrome and eye lid pathologies and Keratopathies accounting 13% of cases. Bourcier et al[4] reported 21% of cases of supurative corneal ulcer with ocular surface disorder.

Contact lenses remain the least common cause of bacterial corneal ulcer in our study. In contrast Radford CF et al[15] reported that contact lenses has greatly increased the risk of microbial keratitis which is estimated to be 10-15 times higher with the use of extended wear disposable contact lenses[16].

The incidence of microbial keratitis is more in males (59%) than females. Similar findings was reported by other authors[17,18].

The increased risk in males in our country is probably due to their more active involvement in out door activities, which subsequently increase their vulnerability to this blinding disease.

The older individuals are more frequently affected in this study, majority (61%) of patients were in fifth decades and above. Age profile in our patients is comparable to Schaefer[8] and Cohen[19] study.

The duration from the onset of symptoms to the presentation at our department ranged from 7 to 105 days (mean 41 days).This delay presentation to tertiary center may be due to the fact that the patient were already receiving therapy from their nearest Ophthalmologist or Doctor and were referred when the ulcers did not respond. Unlike west Bengal study (11.1%)[20], 139 (42.12%) patients presented during the first week of their illness. Same were reported in south India study where 60% of patients presented[12]. Lixin Xie[14] et al reported the first visit of 41.0% between 16 and 30 days

In this study, the most common signs on slit lamp examination were epithelial defect, stromal infiltrate and suppuration presents in every case, while anterior chamber reaction and hypopyon was observed in 45% of

patients. This is in accordance with European study[4].

In the published reports, Bacterial corneal ulcer has been found to be 13 % to 29.3% of all cases of ulcerative corneal ulcer[21] and fungal corneal ulcer has been found to be 6 % to 50 % of all cases of corneal ulceration [22-24].

The prevalence of various organisms responsible for microbial keratitis differs in different regions of world.[25,26.] The results of our study are similar to those of the studies conducted in Nepal 1991[2] and Bangladesh 1994[24], where 80% and 81.7% cultures were positive respectively. The studies conducted in Madurai Tamil Nadu in 1997[12] in Ghana in 1985[10] and India[13] in 2003 has smaller rate of isolation of organisms then our study. The isolation rate reported from Madurai, Tamil Nadu 68.4%, from Ghana 53.7% and India 69.59% of positive cultures. The higher isolation rate of organisms could probably be attributed to be more frequent use of topical corticosteroids than antibiotics.

Bacterial keratitis accounted more frequently in this study compared to those conducted by Kunimoto et al[27] and Clinch et al [28]. Of the 173 bacterial isolates 46.82% were Staphylococcus aureus. Similar results were reported by Cruz et al[29] and Omerod et al[30]. Study from India reported 41.85% of Streptococcus Pneumoniae[13]. Studies from Nepal[2] and Africa[31] also reported the Streptococcus Pneumonia, frequent bacterial isolates. Norina TJ et al[32] reported 58.6% of Pseudomonas responsible for bacterial corneal ulcer.

Other bacterial etiological agents isolated from corneal ulcers included Pseudomonas 11%, Streptococcus pneumonia 9.25%, Staphylococcus epidermis 7.51%, Proteus and Yersina each has 5.80%. The isolation of Gram positive bacilli from these cases were less when compared with result of the study by Kunimoto et al[2], but significantly more than the western series on microbial keratitis among children. [28,29]

Of the total fungal isolates 87 were pure fungal isolates and 12 were mixed fungal and bacterial infections. Candida albicans accounts 75.76% of total fungal isolates in this study. The results of our study are more or less similar to those of the study conducted temperate climate of Nepal and in china. The study conducted in Madurai in 1997[12], South Florida in 1980[33] and in Ghana in 1995[10], where Fusarium was the frequent, isolated fungus. Javadi et al[12] in1996 and Mirshahi[34] 1999 also reported Fusarium, the most frequent isolated fungi, while Shokohi[35] in1999 (33.3%) and Berenji[36] in 2001 reported (55%) Aspergillus the frequent isolated fungi.

Other fungal isolates were Aspergillus flavus 11.11%, Fusarium soloni 10.10%. Penicillium and Aspergillus fumigatus has the least contribution in microbial keratitis in this region.

The standard treatment of bacterial corneal ulcer in 73% of our patients consists of topical instillation of fortified antibiotics (cefuraxime 50mg/ml and fortified tobramycin 9mg/ml). This has been the "gold standard" for bacterial corneal ulcer[21] therapy. However the use of fortified antibiotics were associated with complain of ocular irritation or intense conjunctival reactions during drop instillation. This was due to the local corneal and conjunctival toxicity to the fortified drops. The same issue was noted by Cutarelli et al in 1991[37]. Remaining 27% patients of bacterial corneal ulcer were treated by fluoroquinolones (Moxifloxacin) antibiotics. This is a new fourth generation fluoroquinolone with a broad spectrum of activity against Gram positive (including methicillin resistant Staphylococus aureus and ciprofloxacin resistant Staphylococus aureus) and Gram-negative micro-organisms. In addition, it penetrates well in the anterior chamber and remains fairly stable for at least 12 hours[38]. This treatment was advised to those patients who did not stay at hospital and had small infiltration.

For the patients of fungal corneal ulcer the commercial natamycin eye drops with initial frequency of every one hourly and for the amoebic keratitis the commercially available propamidine isethionate o.1% eye drops with same frequency as antifungal were used. Further the dosing time was reduced on decreasing severity of the disease. Visual prognosis after microbial corneal ulcer depends on the size, location, and depth of the ulcers as well as on the risk factors, bacterial isolate, age and general health of patient [5]. In this study poor out come was seen in patients having chronic surface ocular disorder, large size of ulcer, involving more than 2/3 of depth of the cornea, poor visual acuity at presentation and associated complications. Only forty percent of patients had good visual out come with visual acuity better than the level at admission. Among the other 60% patient's final outcome was same or poor than time of presentation.

In conclusion the suppurative keratitis, most often occurs after a vegetative or non vegetative trauma. Bacterial ulcers are more common than fungal ulcers. Staphylococcus aureus and canddida species' were the most common bacteria and fungi respectively. This variation in causative organisms are clinically important for initiating intensive ocular antimicrobial agents and represent decisive steps in the management of such pathologies in this region.

REFERENCES

1. Stapleton F, Keay L J, Sanfilippo P G, Katiyar S, Edwards K P, Naduvilath T. Relation ship between climate, disease severity, and causative organism for contact lens-associated Microbial Keratitis in Australia. Am J Ophthalmol 2007;144:690-698.
2. Upadhyay MP, Karmacharya PC, Koirala S, Tuladhar NR, Bryan LE, Smolin G, et al. Epidemiologic characteritiscs, predisposing factors, and etiologic diagnosis of corneal ulceration in Nepal. Am J Ophthalmol 1991;111:92-99.
3. Gonzales CA, Sirinivasan M, Witcher JP, Samolin G. Incidente of corneal ulceration in Mandurari District, South India. Ophthalmic Epidemiol1996;3:159-66.
4. Bourcier T, Thomas F, Borderie V, Chaumeil C, Laroche L. Bacterial keratitis: predisposing factors, clinical and microbial review of 300 cases. Br J Ophthalmol.2003;87(7):834-838.
5. Miedziak AL, Miller MR, Rapuano CJ, et al. Risk factors in microbial keratitis leading to penetrating keratoplasty. Ophthalmology 1999;106:1166-1170.
6. Leck AK, Thomas PA, Hagan M, Kaliamurthy J, Ackuaku E, John M et al. Aetiology of Suppurative corneal ulcers in Ghana and South India and Epidemiology of Fungal keratitis. Br J Ophthalmol 2002;86:1211-15.
7. Vajpayee RB, Dada T, Saxena R, ET AL. Study of the first contact management profile of cases of infectious dermatitis: a hospital-based study. Cornea 2000;19:52-6
8. Schaefer F, Bruttin O, Zografos L, et al. Bacterial keratitis: a prospective clinical and microbiological study. Br J Ophthalmol 2001;85:842-7.
9. Beenett HG, Hay J, Devonshire P, et al. antimicrobial management of presumed microbial dermatitis: guideline for treatment central and peripheral ulcers. Br J Ophthalmol 1995;82:137-145.
10. Hagan M, Newman M, Wright E, et al. Causes of supparative keratitis in Ghana. Br J Ophthalmol 1995;79:1024-1028.
11. Garg P, Gopinathan U, Choudhary K, Rao GN. Keratomycosis: Clinical and Microbiologic Experience with Dematiaceous Fungi. Ophthalmology 2000;107:574-8.
12. Sirinivasan M, Gonzales CA, George C, Cevallos V, Mascarenhas JM, Asokan B, et al. Epidemiology and etiological diagnosis of corneal ulceration in Madurai, south India. Br J Ophthalmol1997;8:965-71.
13. Bharathi MJ, Ramkirshana R, Vasu S, et al. Epidemiological characteristics and laboratory diagnosis of fungal dermatitis: a three year study. Indian J Ophthalmol 2003;51:315-21.

14. Lixin Xie, Wenxian Zhong, Weiyun Shi, Shiying Sun. Spectrum of Fungal keratitis in North China. Ophthalmol 2006;113:1943-1948.
15. Radford CF, Minassian DC, Dart JKD. Disposable contact lens use as a risk factor for microbial keratitis. Br J Ophthalmol 1998;82:1272-5
16. Matthew TD, Frazer DD, Minassian DC, et al. Risks of keratitis and patterns of use with disposable contact lenses. Arch Ophthalmol 1992;110:1559-62.
17. Chowdhary A, Singh K. Spectrum of fungal keratitis in north India. Cornea 2005;24:8-15.
18. Gopinathan U, Garg P, Fernandes M, et al. The Epidemiological features and laboratory results of fungal keratitis : a ten year review at a referral eye care centre in South India. Cornea 2002;21:555-9.
19. Cohen EJ, Fluton JC, Hoffman CJ, et al. Trends in contact lens-associated corneal ulcers. Cornea 1996;15:566-70.
20. Basak SK, Basak S, Mohanta A, Bhowmick A. Epidemiological Diagnosis of Suppurative keratitis in Gangetic West Bengal, Eastern India. Indian J Ophthalmol 2005;53:17-22.
21. Chaudhuri PR, Godfrey B. Treatment of bacterial corneal ulcers with concentrated antibiotics eye drops. Trans Ophthalmol Soc UK 1982;102:11-14
22. Rosa RH jr, Miller D, Alfanso EC. The changing spectrum of fungal dermatitis in south Florida. Ophthalmology 1994:101:1005-13.
23. Panda A, Sharma N, Das G, et al. Mycotic keratitis in children: epidemiologic and microbiologic evalution. Cornea 1997;16:295-9.
24. Dunlop AA, Wright ED, Howlder SA, et al. Supparative corneal ulceration in Bangladesh. A study of 142 cases examining the microbiological diagnosis, clinical and epidemiological features of bacterial and fungal keratitis. Aust N Z J Ophthalmol 1994;22:105-10.
25. Kunimoto DY, Sharma S, Garg P, Gopinathan U, Miller D, Rao GN. Corneal ulceration in the elderly in Hyderabad, South India. Br J Ophthalmol 2000;84:54-9.
26. wahl JC, Katz HR, Abrams DA. Infectious keratitis in Baltimore. An Ophthalmol 1991;23:234-7.
27. Kunimoto DY, Sharma S, Reddy MK, Gopinathan U, Jyothi J, Miller D et al. Microbial in children. Ophthalmol 1998;105:252-7.
28. Clinch TE, Palmon FE, Robinson MJ, Cohen EJ, Barron BA, Laibson PR. Microbial keratitis in children. Am J Ophthalmol1994;117:65-71.
29. Cruz OA, Sabir SM, Capo H, Alfonso EC. Microbial keratitis in childhood. Ophthalmol1993;100:192-6.
30. Ormerod LD, Murphree AL, Gomez DS, Schanzlin DJ, Smith RE. Microbial Keratitis in children. Ophthalmol 1986;93:449-55.
31. Carmichael TR, Wolpert M, Koornhob HJ. Corneal ulceration in an urban African hospital. Br J Ophthalmol1985;69:920-926.
32. Norina TJ, Raihan S, Bakiah S, Ezanee M, Liza-Sharmini A T, Wan Hazzabah W H. Microbial Keratitis:aetiological diagnosis and clinical features in patients admitted to Hospital Universiti Sains Malaysia. Singapore Med J 2008;49(1):67-71..
33. Liesegang Tj, Foster RK. Spectrum of microbial keratitis in south florida. AM J Ophthalmol 1980;90:38-47.
34. Mirshahi A, Ojaghi H, Aghashahi D, Jabarvand M. fungal dermatitis in patients at Farabi Hospital, Tehran.{in Persian} Bina 1999;5;135-43.
35. Shokohi T, Dailami KN, Haghighi TM. Fungal Keratitis In Patients With Corneal Ulcer In Sari, Northern Iran. Arch Iranian Med 2006;9:222-27 .
36. Berenji F, Elahi SR, Fata AM, Khakshour H, Darakshan A. Fungal Keratitis in patients at Imam Raza Hospital Mashhad (9182-2001). Med J Mashhad Univ Med Sci. 2003;45:49-54.
37. Cutarelli PE, Lass HJ, et al. Topical fluroquinolones: antimicribial activity and in vitro corneal epithelial toxicity. Curr Eye Res. 1991;10:557-63.
38. Levey SB, Katz HR, Abrams DA. The role of cultures in the management of ulcerative keratitis. Cornea. 1997;16:283-86.