

Demographic Pattern, Risk Factors, Clinical and Microbiological Characteristics of Fungal Keratitis

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ABSTRACT

PURPOSE: To document the clinico-epidemiological features and laboratory diagnosis of fungal corneal ulcer observed at a tertiary referral centre.

DESIGN: Prospective descriptive study.

SETTING: Department of Ophthalmology Unit III, Eye Hospital, Liaquat University of Medical and Health Sciences Hyderabad, Sindh-Pakistan from April 2007 to March 2010.

MATERIAL AND METHODS: Three hundred and fifteen patients of different ages having provisional diagnosis of suppurative corneal ulceration were registered for the study. Data were collected through history and slit lamp examination. Corneal scraping was performed. A portion of each scraping was examined by direct microscopy. Another portion was inoculated directly on to solid culture media.

RESULT: Among corneal scraping from 315 patients, the fungus grew in 116 (36.8%). The mean age of patients was 42.6 years (ranged between 9 and 82 years). Majority of patients (80; 68.97%) were between the 3rd and 5th decade of life. Males (72; 62.1%) were affected more often. Majority of the patients (80; 69%) was from rural areas. A majority of (52; 44.80%) patients was farmer by profession. Corneal trauma (70; 60.4%) was identified as the leading precipitating factor. Forty eight (41.4%) patients had corneal injury with vegetative material. The peak incidence observed in the months of October to December followed by March to June. The clinical features were dry, thick and raised grayish white corneal surface in 76 (65.51%) patients, stromal infiltrates with feathery margins in 64 (55.2%) patients, typical satellite lesions in 19 (16.38%) patients, hypopyon was present in 70 (60.3%) patients. *Candida albicans* was the most frequent organism which was isolated in eighty (69%) patients.

CONCLUSION: Fungi were found to be the frequent cause of suppurative corneal ulcer following agriculture trauma and *Candida albicans* being the most commonly isolated pathogen. For the diagnosis of fungal infection direct microscopic examination with potassium hydroxide 10% is a rapid, inexpensive, and reliable method.

KEY WORDS: Fungal keratitis, vegetative trauma, potassium hydroxide, *Candida albicans*, Corneal Trauma

INTRODUCTION

Microbial keratitis is a serious ocular infectious disease that can lead to significant vision loss and ophthalmic morbidity¹. Fungi are one of the most common infective organisms responsible for this morbidity, especially in the agricultural countries with temperate climates². It has been found to account for 6% to 50% of all the cases of ulcerative keratitis^{3,4}.

So far 70 genera of fungi have been isolated with fungal keratitis⁵. Among these in 70% of cases the pathogen belongs to *Fusarium* and *Aspergillus* species⁶. Fungi reside as commensals in 3% to 28% of conjunctival sac of healthy eyes⁷. Therefore in tropical countries, working outdoor in humid atmosphere makes one more vulnerable to fungal infection⁸. The severity of corneal infection depends on the underlying condition of the cornea and pathogenecity of the infecting organism. Also the increased number of vegetative

trauma, frequent use of broad spectrum antibiotics and steroids, and increasing use of corneal contact lenses⁹ are also responsible for blinding disorder.

Untreated, infective keratitis may lead to opacification and perforation of the cornea. The associated morbidity is the result of lack of diagnostic facilities and appropriate treatment. The specific treatment requires prompt and accurate identification of causative microorganisms¹⁰.

The purpose of this study is to document the epidemiological, clinical, and microbiological characteristics of infecting organism with emphasis on early treatment of fungal keratitis.

MATERIAL AND METHODS

This prospective, descriptive study was performed among patients with laboratory proven fungal corneal ulcer during period of three years, April 2007 to March 2010 at Liaquat University Eye hospital Hyderabad.

The 315 patients of different ages with the clinical diagnosis of suppurative corneal ulceration and having symptoms of pain, redness, watering, photophobia and decreased vision, with or without hypopyon were selected for the study. All the ulcers having typical characteristic of viral keratitis, marginal, neurotropic, and autoimmune association were excluded. Patient's written consent was obtained and a standardized proforma was used to record the data including, occupation of patient, duration of symptoms, predisposing factors, history of corneal injury, associated ocular and systemic disorders, previous treatment, visual acuity at the time of presentation and all other relevant clinical features.

All the patients were examined by slit lamp biomicroscope. The size of the epithelial lesion after staining with 2% fluorescein was measured and the size and depth of the stromal infiltrate was recorded. The presence of pigmentation; endothelial exudates and presence or absence of a hypopyon was also noted. Associated surface ocular and eye lid and its margin conditions such as blepharitis, conjunctivitis, chronic dacryocystitis spheroidal corneal degeneration, dry eyes, bullous keratopathy, pre-existing viral keratitis, lid abnormalities, Bell's palsy, lagophthalmos, trichiasis, dystichiasis, suture infiltrates were noted. The use of contact lenses and of topical corticosteroids and other systemic combinations were also recorded. After all aseptic measures, a thorough ocular examination and corneal scraping was done. We used a sterile Bard-Parker blade (No 15) or bent 25 to obtain material from margin and base of ulcer. The procedure performed after instillation of topical anesthetic 4% lignocaine under magnification of slit lamp or operating microscope. We used inhalational general anesthesia only when patient was uncooperative or for very young patients. A portion of each scraping was spread onto labeled slides to examine microscopically for the presence of fungi, bacteria or acanthamoeba by using 10% potassium hydroxide wet mount (KOH), and Gram and Giemsa staining methods respectively. Another portion was inoculated directly on to solid media such as sheep's blood agar, chocolate agar, or Sabouraud's dextrose agar in C-shaped streaks. Deep inoculation in liquid media such as brain heart infusion broth was also done. Further corneal scrapings were performed and the material was inoculated onto non-nutrient agar in cases where Potassium Hydroxide smear was positive for amoebic cyst.

All inoculated culture media were incubated aerobically with different temperature. The Sabouraud's dextrose agar were incubated at 27°C and examined daily for maximum three weeks and then discarded in the absence of growth. The inoculated blood agar, choco-

late agar, and brain heart infusion broth were incubated at 37°C, examined daily, and discarded at 7 days in the absence of growth. To detect the Acanthamoeba species, the inoculated non-nutrient agar plates were incubated at 37°C after overlaying with thin film of Escherichia coli broth culture and were examined daily and discarded at 3 weeks in the absence of growth. A diagnosis of fungal corneal ulcer was confirmed in the presence of at least one of the following: 1) Smear of corneal scraping examination revealed fungi 2) growth of the same fungi in two culture media 3) and fungus grew largely at the inoculated site on a single solid medium.

The data were analyzed on SPSS version 10 for chi-square test.

RESULT

During the study period of three years, three hundred and fifteen eyes of 315 patients having the clinical diagnosis of suppurative corneal ulceration were examined at our department. One hundred sixteen (36.8%) of 315 patients grew fungus, 124 (39.37%) had bacterial, while in 75 (23.81%) cases we found either Acanthamoeba species, mixed bacterial and fungal growth and no growth. The mean age of patients in this series was 42.6 years (**Table I**) (ranged between 9 and 82 years). Majority of patients (80; 68.97%) were between the ages 21 to 50 years. Males (72; 62.1%) were affected more often than females. Majority of patients (80; 69%) was from rural areas, whereas 36 (31%) belonged to urban population ($p < 0.004$). More than 50% of all (52; 44.80%) our patients were farmer by profession ($p < 0.000$) while 28 (24.1%) laborer, 18 (15.5%) house wives, students and teachers each were 4 (3.4%), while 10 (8.6%) were jobless aged peoples (**Table II**).

Corneal trauma (70; 60.4%) was identified as the leading risk factor and the association between trauma and fungal keratitis was found significant ($p < 0.000$). Forty eight (41.4%) patients had corneal injury with vegetative material and this correlation was highly significant ($p < 0.000$). Twenty two (19%) patients reported foreign body and its removal traditionally. Contact lens, surface ocular disorder and ocular surgery each were 6 (5.2%) in number. Twenty eight (24.1%) patients had no prior history of ocular disorder (**Table III**). The duration of symptoms when presented at our department was found highly variable ranging from 6 to 153 days. (Mean 59.18 days). As far as seasonal variation is concerned we observed biphasic variation of fungal corneal ulcer, with first peak during October to December followed by March to June. All patients with fungal corneal ulcer presented with the history of ocular pain, photophobia, and watering and decrease vision. On slit lamp exami-

nation the diameter of the ulcers ranged from 3.2 mm to almost total corneal involvement. Seventy nine (68.1%) eyes had larger ulcers (diameter > 5 mm). The depth was from 1/3rd to almost full thickness of cornea.

The clinical features were as follows: dry, thick and raised grayish white epithelial lesion in 76 (65.51%), stromal infiltrates with feathery margins 64 (55.2%) patients, typical satellite lesions in 19 (16.38%) patients, Hypopyon was present in 70 (60.3%) patients, corneal perforation in 03 (2.59%) patients.

Corneal scrapings obtained from all patients, were examined by direct microscopy with potassium hydroxide 10%, and inoculated for culture. *Candida albicans* was the most frequent organism isolated in eighty (69%) patients, followed by *fusarium soloni* in 26 (22.4%), *Aspergillus flavus* in 8 (6.9%). *Pencilium* species were the least frequent in this study.

TABLE I: AGE

Age (years)	Frequency	Percentage
1-10	4	3.4
11-20	8	6.9
21-30	24	20.7
31-40	20	17.3
41-50	36	31
>50	24	20.7
Total	116	100

TABLE II: OCCUPATION

Occupation	Frequency	Percentage
Farmer	52	44.8
Labor	28	24.1
House wife	18	15.5
Student	4	3.5
Teacher	4	3.5
Nil	10	8.6
Total	116	100

TABLE III: RISK FACTORS

Risk factors	Frequency	Percentage
Agriculture trauma	48	41.3
Foreign body	22	19.0
Contact lens	06	5.2
Ocular surgery	06	5.2
Non healing ulcer	06	5.2
Nil	28	24.1
Total	116	100

DISCUSSION

Fungal corneal ulcer is not only the common disorder

but also a major cause of visual loss especially in those parts of world where a large number of populations belongs to agriculture⁹, malnutrition is common, and climate is hot and humid. All these factors predispose population to develop fungal corneal ulcer following ocular trauma.

Of the three hundred and fifteen patients with infective keratitis from April 2007 to March 2010 presenting at our department, fungal corneal ulcer was diagnosed in 116 (36.8%) eyes. Parshant Garg⁷ et al reported 30.4% while Shokohi et al⁸ also reported 37.5% incidence of fungal corneal ulcer which is in close agreement to our study. On the other hand a study conducted at University Hospital of Taiwan in 2004 reported an incidence of fungal keratitis in only 13.5% of 476 eyes with microbial corneal ulcer.¹¹ which is quite lower than our study. In contrast to our study Mirshahi et al¹² and Javadi et al¹³ reported 83% cases of fungal corneal ulcer which is much higher than our study. This variation in the incidence of fungal corneal ulcer confirms the regional difference of fungal corneal ulcer. Incidence of fungal corneal ulcer is nearly equal at the age of 21 to 50 years. Same was reported by Bharathi et al¹⁴ study. They reported younger people, aged 21-50 years, are more often affected by fungal keratitis compared to those above 50 years Male patients are predominant (62.1%) in our study; same was reported by Gopinathane et al¹⁵ and Kunimoto et al¹⁶. In our country increased risk in male highlights traditionally their more active involvement in outdoor activities which subsequently increase their exposure to the condition. This finding is identical to that reported by Bharathi et al¹⁴ where 61.28% fungal corneal ulcer were present in patients having the history of vegetative trauma. While Kunimoto et al¹⁶, Tenure¹⁷ reported much less frequency of 8.3% and 17.6% fungal corneal ulcer following vegetative trauma.

The peak of fungal corneal ulcer showed seasonal variation in our study is almost identical to that reported by Williamson et al⁹. He showed that the incidence of fungal keratitis is higher during the peak time of agriculture activity. The fungal corneal ulcer was most prevalent in our study during months of October to December followed by March to June. A hot, humid, windy climate and an agriculture-based occupation of a large population make fungal keratitis more frequent in tropical zones.¹⁵

The duration of symptoms at the time of presentation at our department was variable, ranged from 6 to 153 days. (Mean 59.18 days). This delayed presentation to tertiary care center may be due to the fact that the patient were already receiving therapy from their nearest physician and were referred when the ulcers did not respond. Before reporting to our department 85.7% patients had received some sort of topical

medication including antibiotics, and corticosteroids alone or in combination. Lixin Xie¹⁸ et al reported first visit of 41.0% patients between 16 and 30 days while in another study 46.66%¹⁴ patients reported within 7 days. We noticed that response to the antifungal therapy was better in the earlier presenters than those who reported late.

The clinical presentation of fungal keratitis is variable. It varies from case to case, depending upon the causative fungus, severity of the invading pathogen, liberation of toxin, resistance of host tissue and age and physical condition of the patient¹⁹. The biomicroscopic appearance is a dry, raised, grayish white lesion and/ or as stromal infiltration with a feathery finger projection seen in 76 (65.51%) and 64 (55.2%) respectively. Satellite lesions seen in 19 (16.38%) patients support the clinical diagnosis. Same were reported earlier²⁰. Other lesions that helped in diagnosis were cheesy hypopyon in 70 (60.3%) patients in this study while, Javadi et al¹³ noted hypopyon in 52% of eyes and Lixin Xie et al¹⁸ reported 46.3% incidence. Corneal perforation observed in 03 (2.59%) and endothelial plaque in 02 (1.72%) patients. For the detection of fungus the sensitivity of KOH wet mount preparation (86.93%) found higher than that of Giemsa-stained smear (64.13%) in this study. This high value lies in its ability to clear the scraping of cellular debris, which leads more easily detectable on microscopic examination.²¹ Xie¹⁸ et al and Panda³ et al reported 88.7% and 90% respectively. Singh and Choudhary²² reported a lower rate of 62% where as an incidence of 92.2% reported by Bharathi¹⁴ et al. Chander²³ et al, in their evaluation of Calcoflour white staining for diagnosis of fungal corneal ulcer confirmed the superiority of KOH+CFW in comparison with KOH and culture. In this study positive rate of culture was seen in 64.85% cases which are consistent with the previous studies²⁴.

Candida 80 (69%) was the predominant isolated species in this study. As reported in literature, this species is more common in developed countries²⁵. *Fusarium Soloni* 26 (22.4%) were the next most common species isolated. *Aspergillosis* 8 (6.9%) and *Pencillium* species were less often 2 (1.7%) observed in this study. In contrast the literature shows that the *Fusarium* is a commonly isolated species in North and South China and South India²⁵.

CONCLUSION

Fungi were found to be the frequent cause of suppurative corneal ulcer following agriculture trauma and *Candida albicans* being the most commonly isolated pathogen. For the diagnosis of fungal infection direct microscopic examination with potassium hydroxide 10% is a rapid, inexpensive and reliable method.

REFERENCES

1. Stapleton F, Keay L J, Sanfilippo P G, Katiyar S, Edwards K P, Naduvilath T. Relationship between climate, disease severity, and causative organism for contact lens-associated Microbial Keratitis in Australia. *Am J Ophthalmol* 2007;144:690-698.
2. Xie L, Hu J, Shi W. Treatment failure after lamellar keratoplasty for Fungal Keratitis. *Ophthalmology* 2008;115:33-6.
3. Panda A, Sharma N, Das G, et al. Mycotic keratitis in children: Epidemiological and microbiologic evaluation. *Cornea* 1997;16:295-9.
4. Dunlop AA, Wright ED, Howlader SA, et al. suppurative 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.
5. Agarwal PK, Roy P, Das A, Banerjee A, Maity PK, Banerjee AR. Efficacy of topical and systemic itraconazole as a broad-spectrum antifungal agents in mycotic corneal ulcer: A preliminary study. *Indian J Ophthalmol* 2001;49:173-76.
6. O'Day DM. Fungal keratitis. In: Pepose JS, Holland GN, Wilhelmus KR, editors. *Ocular infections and immunity*. St.Louis: Mosby;1997. 263-64.
7. Garg P, Gopinathan U, Choudhary K, Rao GN. Keratomycosis: Clinical and Microbiologic Experience with Dematiaceous Fungi. *Ophthalmology* 2000;107:574-8.
8. Shokohi T, Dailami KN, Haghghi TM. Fungal Keratitis In Patients With Corneal Ulcer In Sari, Northern Iran. *Arch Iranian Med* 2006;9:222-27.
9. Williamson J, Gordon AM, Wood R, et al. Fungal flora of the conjunctival sac in health and disease: influence of topical and systemic steroids. *Br J Ophthalmol* 1968;52:127-37.
10. Khanal B, Kaini KR, Deb M, et al. Microbial keratitis in eastern Nepal. *Tropical Doctor* 2001;31:168-9
11. Fong CF, Tseng CH, Hu FR, et al. Clinical characteristics of microbial keratitis in a University Hospital in Taiwan. *AM J Ophthalmol* 2004;137:329-36.
12. Mirshahi A, Ojaghi H, Aghashahi D, Jabarvand M. fungal keratitis in patients at Farabi Hospital, Tehran. *Bina* 1999;5; 135-43.
13. Javadi MA, Hemati R, Muhammadi MM, Farsi A, Karimian F, Einolahi B, et al. causes of fungal Keratitis and its management. Review of 23 cases from Labafinejad Medical Center (LMC). *Bina* 1996;2:38-54.
14. Bharathi MJ, Ramkirshana R, Vasu S, et al. Epidemiological characteristics and laboratory diag-

- nosis of fungal keratitis: a three year study. Indian J Ophthalmol 2003;51:315-21.
15. 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.
 16. Kunimoto DY, Sharma S, Garg P, et al. Corneal ulceration in the elderly in Hyderabad, south India. Br J Ophthalmol 2000;84:54-9.
 17. Tenure MA, Cohen EJ, Sudesh S, et al. spectrum of fungal keratitis at Wills Eye Hospital, Philadelphia, Pennsylvania. Cornea 2000;19:307-12.
 18. Xie L, Zhong W, Shi W, Sun S. Spectrum of Fungal keratitis in North China. Ophthalmology 2006;113:1943-48.
 19. Forster RK. Fungal keratitis and conjunctivitis: Clinical disease. In: Somalin G, Thoft RA, editors. The Cornea. New York: Little, Brown and Company;1994. 239-252.
 20. Agarwal V, Biswas J, Madhavan HN, Mangat G, Reddy MK, Saini JS, et al . Current perspectives in infectious keratitis. Indian J Ophthalmol 1994;42:171-91.
 21. Wilson LA, Sexton RR. Laboratory diagnosis in fungal keratitis. Am J Ophthalmol 1968;66:646-53
 22. Chowdhary A, Singh K. Spectrum of fungal keratitis in north India. Cornea 2005;24:8-15.
 23. Chander J, Chrabarti A, Sharma A, Saini JS, Panigrahi D. Evaluation of Calcoflour staining in the diagnosis of fungal corneal ulcer. Mycoses.1993;36:243-245.
 24. Sharma S. Silverberg M, Mehta, P, Gopinathan U, Agarwal V, Naduvilath TJ. Early diagnosis of mycotic keratitis: predictive value of potassium hydroxide preparation. Indian J Ophthalmol. 1998;46:31-35.
 25. Leck AK, Thomas PA, Hangan M, et al. Etiology of suppurative corneal ulcers in Ghana and South India, and epidemiology of fungal keratitis. Br J Ophthalmol 2002;86:1211-5.



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