# Fungal keratitis:84 cases report in Southern Pakistan

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## Abstract

• AIM: To identify the predisposing factors, causative fungi and to improve the facilities for the laboratory diagnosis of fungal keratitis.

• METHODS: Two hundred and forty eyes of 240 patients of clinically suspected microbial corneal ulcer were included in the study. Data was collected through history and slit lamp examination. Using standard techniques, corneal scraping was performed. A portion of each scraping was examined by direct microscopy for the presence of fungi, bacteria and acan-thamoeba by using 100g/L potassium hydroxide and also by Gram and Giemsa stainings. Another portion was inoculated directly on the surface of solid media such as blood agar, Mac-Conkey agar, chocolate agar and Sabouraud agar in C-shaped streaks for culture.

• RESULTS: A total of 240 patients with suppurative corneal ulcer were enrolled in the study, out of which fungi were identified in 84 (35%) patients. Of these, 48 (57.1%) were males and 36 (42.9%) females. The age ranged between 22-80 years. Sixty patients (71.4%) belonged to rural population and twenty four (28.6%) to urban population. Trauma with vegetative material was the most frequent cause noted in eighteen (21.4%) patients. Peak incidence was in the months of October-November. Out of 84 eyes with fungal keratitis, fungi alone were the etiologic agents in 74 (80.10%) cases and bacteria with fungi were identified in 10 (11.90%). The most frequently isolated organism was Candida albicans which was found in 66 (78.6%) patients.

• CONCLUSION: Fungal keratitis is the leading cause of infective corneal ulcer and Candida albicans being the most commonly isolated pathogen in the patients belonging to Southern Pakistan. The direct microscopic examination with potassium hydroxide 100g/L method is a simple, rapid,

inexpensive and reliable method in the diagnosis of this infection.

·Clinical Research ·

• KEYWORDS: fungal keratitis; agriculture; candida

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### INTRODUCTION

**F** ungal keratitis is one of the most severe eye diseases world wide that may lead to blindness, especially in the agricultural countries with temperate climates <sup>[1]</sup>. It has been found to account for 6% to 50% of all the cases of ulcerative keratitis <sup>[2-6]</sup>. Factors that have been correlated with this increasing incidence include the growing number of trauma cases, widespread abuse of broad spectrum antibiotics and steroids and increasing use of corneal contact lenses<sup>[7]</sup>.

Fungi reside as commensals in flora of the conjunctival sac in 3% to 28% of healthy eyes <sup>[8-10]</sup>. Thus in the tropical countries, humid atmosphere and outdoor occupations make the population more vulnerable to fungal infections<sup>[11]</sup>.

Despite the advances in diagnosis and medical treatment of keratomycosis 15% to 27% of patients require surgical intervention such as keratoplasty, enucleation or evisceration because of either failed medical treatment or advanced disease at presentation<sup>[12]</sup>.

This study is a humble effort to analyze the epidemiologic features, predisposing factors and the main causative organisms for fungal keratitis in this part of Asia to provide a useful guide for the practicing ophthalmologists.

## MATERIALS AND METHODS

This quasi-experimental study of fungal corneal ulcer was conducted at Liaquat University Eye hospital Hyderabad Sindh Pakistan from April 2006 to March 2008.

Patients above 15 years of age presenting with suspected corneal ulceration and having symptoms of pain, redness, watering, photophobia and decreased vision were registered for the study. Patients written consent was obtained and a standardized profoma was used to record the data.

Detailed history of the patients was taken regarding demographic features, time of onset of symptoms, predisposing factors and any previous medical therapy. All the patients underwent slit lamp examination to locate the size, depth and the nature of the infiltrate; presence of pigmentation; endothelial exudates and anterior chamber reaction or hypopyon at the time of presentation.

After the instillation of local anesthetic 5g/L proparacaine hydrochloride, corneal scrappings were obtained aseptically with a sterile No. 15 surgical blade from the base and edges of each ulcer. A portion of each scrapping was examined microscopically for the presence of fungi, bacteria or acanthamoeba by using 100g/L potassium hydroxide, Calcoflour white and Gram and Giemsa staining methods. Another portion was inoculated on to Sarboraud's agar, brain heart infusion broth and chocolate agar respectively, in C-shaped streaks and cultured for the potential growth of fungi, bacteria or acanthamoeba. Sarboraud's agar slants were incubated at 28°C while others at 37°C. All media were cultured for a period of fourteen days and observed daily.

A diagnosis of fungal keratitis was made when at least one of the following was confirmed: (1) Corneal scraping examination revealed fungal presence in smears; (2) The same fungal pathogens grew in  $\geq$  two culture media; (3) Fungus grew confluently at the inoculated site on a single solid medium and (4) Histopathologic examination revealed fungal presence . **RESULTS** 

A total of two hundred and forty eyes of 240 patients with suppurative corneal ulcer were enrolled in the study. Of these fungal keratitis was identified in eighty four (35%) patients. Among these, forty eight (57.1%) patients were males and thirty six (42.9%) were females. The age ranged between 22 and 80 years. The mean age was 44.6 years. Sixty patients (71.4%) belong to rural population while twenty four patients (28.6%) belong to urban background. Of the eighty four patients with fungal keratitis, forty two (50%) were farmers, eighteen (21.4%) were labourers, six (7.14%) students, six (7.14%) teachers and twelve (14.28%) were jobless aged people.

Trauma with vegetative material was the main predisposing factor noted in eighteen (21.4%) patients. Six(7.1%) patients gave the history of corneal contact lens wear while history of ocular surgery and foreign body was also found in six (7.1%) patients each. Forty eight patients (57.1%) had no significant

prior history of ocular disorder.

The duration from the onset of symptoms to the presentation to our department ranged from 6 to 150 days. (mean 62 days). Regarding seasonal variation, the peak incidence occurred in the months of October to December followed by March to June. Before the initial visit to our institution seventy two patients (85.5%) received topical antibiotics, antivirals, antifungals and/or corticosteroids either alone or in combination whereas 14.5% had no history of medication. All patients with fungal corneal ulcer presented with the history of ocular pain photophobia, watering and decrease vision. On slit lamp examination the diameter of the ulcers ranged from 3.2-12 (mean 6.3-2.8)mm. Fifty four (64.28%) eyes had larger ulcers (diameter >6mm). The depth was from 1/3rd to almost full thickness of cornea. Epithelial defect, stromal infiltrates and corneal suppuration were present in all the eighty four eyes (100%). Forty eight eyes (57.1%) had feathery projections while anterior chamber reaction was also found in forty eight (57.1%) eyes. Thirty (35.7%) eyes had hypopyon and twenty four eyes (28.6%) had satellite lesions.

Corneal scrapings obtained from eighty four eyes of fungal corneal ulcer patients were examined by direct microscopy with potassium hydroxide 100g/L and inoculated for culture. Of these, fungi alone were the etiologic agent in seventy four (80.1%) and bacteria along with fungi were identified in ten (11.9%) cases.

Candida albicans was the most frequent organism which was isolated in sixty six (78.6%) patients. Other isolated fungi included aspergillus flavus in eight (9.5%) patients, fusarium soloni in eight (9.5%) patients and penicillium species two (2.4%) patients.

#### DISCUSSION

Mycotic keratitis is an important ophthalmic problem in all parts of the world and a major cause of visual loss especially in developing countries where a large number of population belongs to agriculture. Moreover, the climate is mild and humid, and malnutrition is common <sup>[7]</sup>. All these factors predispose people to the development of fungal corneal ulcer from minor eye trauma. In the various published reports, fungal corneal ulcer has been found to be 6% to 50% of all cases of corneal ulcer <sup>[2-6]</sup>.

Of the two hundred and forty patients with infective keratitis from April 2006 to March 2008 presenting at our department, fungal corneal ulcer was diagnosed in 84 (35%)

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eyes. Shokohi *et al* <sup>[12]</sup> and Parshant Garg *et al* <sup>[11]</sup> reported, respectively 37.5% and 30.4% incidence of fungal corneal ulcer which is nearly similar to our study. Javadi *et al* <sup>[13]</sup> and Mirshahi *et al* <sup>[14]</sup> reported 83% cases of fungal corneal ulcer which are quite higher than our study.

Another study conducted at University Hospital in Taiwan in 2004 registered the incidence of fungal keratitis in only 13.5% of 476 eyes with microbial corneal ulcer <sup>[15]</sup>, which is quite lower than our study. This variation in the incidence of fungal corneal ulcer confirms the regional difference of mycotic corneal ulcer.

In line with other studies <sup>[16-18]</sup>, males(57.1%) are predominant in our study also. The increased risk in males in our country is probably due to their more active involvement in out door activities, which consequently increases their vulnerability to this blinding disease.

Incidence of fungal corneal ulcer is almost equal in all age groups in this study, which differs from many studies which suggest that fungal keratitis is more common during 50 to 60 years<sup>[19]</sup>.

In the present study, 21.4% patients with fungal corneal ulcer had agriculture trauma, which is lower than described in literature <sup>[20]</sup>. In some other reports, a frequency of 8.3%<sup>[19]</sup> to 17.6% <sup>[18]</sup> has been described for mycotic keratitis in patients with corneal trauma with an organic material which may predispose to the fungal corneal infection.

As in previous study <sup>[7]</sup>, our research also shows higher incidence during the months of October till December followed by March till June. In an agricultural country, this seasonal variation of the incidence indicates a higher occurrence rate during the harvest season.

The duration from the onset of symptoms to the presentation at our department ranged from 6 to 150 days (mean 62 days). This delay presentation to our tertiary center may be due to the fact that the patients were already receiving therapy from their nearest ophthalmologists and were referred when the ulcers did not respond. Xie *et al* <sup>[7]</sup> reported the first visit of 41.0% between 16 and 30 days. We noted that the response to the antifungal therapy was better in the earlier presenters than those very late.

Before reporting to our department, 85.7% patients had received some sort of topical medication including antibiotics, antivirals, antifungals and corticosteroids alone or in combination.

In this study, the most common signs on slit lamp examina-

tion were epithelial defect, stromal infiltrate and suppuration found in 100% cases; feathery finger projection and anterior chamber reaction were present in (57.1%) cases, hypopyon in 35.7% patients and satellite lesion in 28.6% eyes. This is in accordance with another Asian study <sup>[12]</sup> which also showed the epithelial defects (57.1%) and suppuration (57.1%) as the most common signs. Javadi *et al*<sup>(13]</sup> noted hypopyon in 52% of eyes with fungal keratitis and Xie *et al*<sup>(7]</sup> reported 46.3% incidence.

Rosa *et al* <sup>[3]</sup> noted irregular feathery margins (62%) a dry texture (47%) and satellite lesion (47%) in their patients.

In our study 83.3% cases had a positive rate of microscopic examination of corneal scraps with potassium hydroxide 100g/L, which is nearly similar to the finding of Xie *et al*<sup>[7]</sup> (88.7%), and Panda *et al*<sup>[5]</sup> (90%). Singh and Choudhary <sup>[16]</sup> reported a lower rate of 62% whereas an incidence of 92.2% reported by Bharathi *et al*<sup>[21]</sup>. Chander *et al*<sup>[22]</sup>, in their evaluation of Calcofluor 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 67.15% cases which is consistent with the previous studies<sup>[23]</sup>.

In the current study candida (78.6%) was the predominant isolated species. As reported in literature, this species is more common in developed countries<sup>[24]</sup>. Aspergillus (95g/L) and Fusarium (95g/L) were the next most common species isolated. The study of the literature shows that the Fusarium is a commonly isolated species in North and South China<sup>[25]</sup> and South India<sup>[26]</sup>.

In conclusion, fungal keratitis is the leading cause of infective corneal ulcer and Candida albicans is the most commonly isolated pathogen responsible for fungal keratitis in Southern Pakistan. The direct microscopic examination with potassium hydroxide 100g/L method is a simple, rapid, inexpensive and reliable method in the diagnosis of fungal corneal ulcer.

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