

Original Research Article

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A Study on Fungal Etiological Agents in Suppurative Keratitis

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ABSTRACT

Fungal keratitis is a major ophthalmic problem. In view of the importance in timely diagnosis and treatment of keratitis which would otherwise lead to serious complications like loss of vision, hypopyon formation, endophthalmitis, etc., this study was intended to bring into light the fungal etiological agents in suppurative keratitis. The specimen was collected from suppurative keratitis cases irrespective of prior antibiotic treatment during the period of one year from March 2012 to February 2013. Those cases with loss of corneal epithelium with signs of inflammation, and stromal infiltration were taken as suppurative keratitis cases. Five or more corneal scrapings are to be taken, based on the condition of the patient. The corneal scrapings are used for 10% KOH mount and Gram staining. Sabouraud's Dextrose Agar (SDA) is inoculated by touching the medium with the needle. Extreme care is taken to keep the entire process sterile. During the one-year period, out of the 100 patients with suppurative keratitis, there were 56 (82.36%) fungal isolates. *Aspergillus spp.* was the commonest, constituting 22 (39.29%) of the total fungal isolates. *Fusarium* is the second most common in this study 9(16.7%). Although corneal ulceration is a very rare cause of mortality, it is one of the important causes of morbidity and disability in South Kerala, especially among lower socio-economic groups. The present study gives an insight about the fungal aetiological agents of suppurative keratitis in this part of Kerala. The results of this study are similar to that in other parts of India. These findings have important public health implications for the treatment and prevention of corneal ulceration in the developing world.

Keywords

Suppurative keratitis,
Corneal scrapings,
SDA.

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Introduction

Corneal ulcers pose a challenging problem to the ophthalmologists, as their etiology is of varied origin and their course is unpredictable. Prognosis is uncertain. A specific therapy is still a confused issue, as effective specific antifungal broad-spectrum agents are not still available. Corneal ulcers occur more frequently in elderly debilitated, arteriosclerotic and anaemic persons. They occur among the lower classes exposed to injury and they are prone to neglect the infection. They occur specially during harvest

season and by the time the patient seeks medical aid, the ulcers are much advanced and they may recover without perforation but with opacities of varying density or finally end in perforation with blindness as its sequelae. Major causes of blindness in India are: - Cataract - 62.6%, Refraction Error - 19.7%, Glaucoma - 5.8%, corneal pathologies 0.9% and other causes- 11.0% (Arlappa). Wide geographical variation exists in the epidemiology of microbial keratitis (MK) based on economic and climate factors.

Fungal keratitis (FK) accounts for about 50% of all cases of culture-proven MK in some developing countries. The protracted course of the condition and the diversity of clinical presentation may contribute to unfavorable outcomes (Ibrahim et al., 2011).

Fungi gain access into the corneal stroma through a defect in the epithelium, then multiply and cause tissue necrosis and an inflammatory reaction. The epithelial defect usually results from trauma (e.g., contact lens wear, foreign material, prior corneal surgery). The organisms can penetrate an intact descemet membrane and gain access into the anterior chamber or the posterior segment. Mycotoxins and proteolytic enzymes augment the tissue damage (Kindo et al., 2009).

Incidental ocular trauma by plant leaves or vegetative materials is a facilitating factor in most patients, which can lead to fungal keratomycosis and corneal ulcer after the release of cytotoxins. The inflammatory reaction occurs after the release of mycotoxin and proteolytic enzymes, which are responsible for the catastrophic corneal melting in some patients (Tabatabaee et al., 2013).

A number of studies indicate that indiscriminate use of antibiotics and corticosteroids potent actual cause of the infestation by fungi. Antibiotics and corticosteroids have been variously implicated separately or in combination for this potentiation. Steroids either in combination with antibiotics or without them are freely prescribed not only by ophthalmologists but also by general practitioners and nonophthalmic specialists. The abuse of corticosteroids has done more harm than good as in herpetic keratitis and has hastened the progression of the superficial diseases of the cornea to perforation and made the opportunistic fungi like *Cephalosporium*,

Penicillium and *Fusarium* species to become pathogenic. Secondly the suppressive, anti-inflammatory effect of corticosteroids may delay the clinical diagnosis of a possible fungal infection which is partly made from the antibiotic resistant characters of fungal infections (Srinivasan, 1969).

The purpose of the study is to evaluate the current pathogens causing fungal keratitis at tertiary care centre.

Materials and Methods

Specimens were collected from both OP and IP patients with suppurative keratitis in a minor theatre and inoculations done immediately. After properly cleaning eye with normal saline, a drop of local anaesthetic (proparacaine 0.5%) was instilled, then eyes were focused on a slit lamp, and the corneal ulcer cleaned with sterile cotton swab soaked in sterile saline. Scrapings were taken from centre, edges, and base of the ulcer with a No. 26 G needle. Five or more scrapings were taken based on the condition of the patient. The corneal scrapings taken were used for 10% KOH mount. Another specimen was inoculated by making a well with a needle on Blood agar and MacConkey agar. Sabouraud's Dextrose Agar (SDA) was inoculated by touching the medium with the needle. Extreme care is taken to keep the entire process sterile. Study period was from February 2012 to March, 2013.

Results and Discussion

Out of 100 patients included in the study, 68 were males, and 32 were females. Age of patients included in the study ranged from 10-82 years, and keratitis cases were maximum in 41-50 year age group (29%), followed by 51-60 years (19%). Minimum incidence was found to be in extremes of age.

Occupational profile of the patients included in this study showed a maximum incidence among manual labourers, followed by housewives and students. 66% of patients included in this study were from rural areas, 22% from semi-urban areas, and 12% from urban areas. 82% of patients included in this study belonged to the low socio-economic group. Among the 56 fungal isolates, 39 cases occurred in the rainy season (May–July). Prior treatment was taken by 63% of patients included in this study. Topical antibiotics alone were taken by 55.5% of patients, and 57% of patients gave history of ocular trauma. 25.39% of patients took topical antibiotics with topical antifungals.

Corneal blindness is a major public health problem worldwide and infectious keratitis is one of the predominant causes. Corneal infection of fungal aetiology is very common and represents 30% to 40% of all cases of culture-positive infectious keratitis in South India. Fungal keratitis is a major ophthalmic problem. In view of the importance in timely diagnosis and treatment of keratitis which would otherwise lead to serious complications like loss of vision, hypopyon formation, endophthalmitis, etc, this study was intended to bring into light fungal etiological agents. In the present study, fungal isolates were 56 (82.36%) of the total 68 isolates.

Of the 100 patients included in the study 68 were males and 32 were females. Male: Female ratio is 2.125:1. This finding is in accordance with the results got in studies done by Punia *et al.*, (2014); Bharati *et al.*, (2014) and Saha *et al.*, (2006) in India. In this study, age of patients ranged from 10-82 years, and keratitis was maximum in 41-50 year age group (29%), followed by 51-60 years (19%), and 31-40 years (15%).

Minimum incidence was found to be in extremes of age groups. In the age group with

more than 70 years, it was found to be 4%, and in the less than 10 years age group, it was 1%. Similar results were obtained in studies done by Narayan (2013). In his study, done in Oman majority (64.36%) of them were over 60 years of age.

Majority of the patients included in this study were manual laborers which included construction workers, factory workers, and non-specific agriculture related workers. Farmers constituted less than 10% of the cases. This may be due to the decreasing areas of agricultural land, and a dwindling agricultural workforce in South Kerala. The other significant groups were housewives (12%), students (9%), and office-workers (4%). But, farmers were mostly affected in studies done in Vishakaptnam (Anuradha *et al.*, 2013) and Bhopal (Sharma *et al.*, 2013).

In majority of the studies, keratitis cases occurred commonly in low income groups, and a similar occurrence was noted in rural population. In this study, about 66% cases were rural population, 22% semi-urban, and 12% belonged to urban population. Similar finding was got in a study done in Bhopal. Rural residents have shown the higher incidence (83.1%) of keratitis in north Karnataka also 82% of patients who participated in the study belonged to low socioeconomic group, 16% to middle socioeconomic group and 2% to high socioeconomic group. In the study major predisposing factor for keratitis is found to be the history of corneal injury. This could be attributed to the fact that majority of patients were rural residents, belonged to low socioeconomic group and majority were manual labourers. Hence, their working and living environment exposed them to the threat of foreign body fall and the resulting corneal ulcer. Geographical location and climate are reported to influence the clinical presentation and outcome of fungal diseases, probably

through changes in the causative agent (Rautaraya et al., 2011). The incidence of fungal keratitis was higher in June, July and August. Maximum number of keratitis cases was seen in the months of July to October followed by November to February in north Karnataka. The peak incidence occurred in the winter months of June–August, during which there were 73 (38%) admissions in Australia (Butler et al., 2005).

63% of patients had taken treatment from outside before being referred. 37% of patients did not receive any treatment. This is a slightly higher percentage considering the fact that many of them had treatment from outside. This may be because 55.56% of patients had taken topical antibiotics alone and were not treated for fungus. Moreover, 25.4% of patients were on a combination treatment with topical antibiotics and antifungals, pointing to the awareness among the general practitioners about the increasing prevalence of fungal corneal ulcers. This demonstrates a very welcome trend, along with the fact that only 4 of the 100 cases were previously put on medications containing steroids. On the other hand, this may be due to the increasing availability of ophthalmologists in rural areas, and the increasing awareness regarding the seriousness of the corneal injury among the public. This can also be supported by the fact that majority of patients visited a medical practitioner in 2-3 days of occurrence of injury. Medical treatment was documented in 640 (93.2%) of the 687 patients before coming to the National Ophthalmology hospital in North Vietnam.

In this study, 57% gave history of ocular trauma prior to corneal ulceration, while 43% did not give any obvious history of injury. Corneal injury is the most important predisposing cause (and often the precipitating cause) of corneal ulceration,

especially in the developing countries where most of the population is daily wage manual labourers. The results obtained in this study are consistent with results obtained from different parts of India (Kumari et al., 2002). Corneal injury was caused by substances like sand dust, which contributed to 29%. Other particles which caused injury were wooden piece, building material, vegetative matter, insects, fingernail, and dirty water. None of the injuries reported in this study were caused by paddy husk which was one of the major causes in Central Kerala in the past, when rice cultivation was common.

In developed countries where agriculture and labor are largely mechanized, other predisposing factors become more important.

However, trauma still remains as one of the major predisposing factors in the development of corneal ulcers in certain developed countries like France, Switzerland, Australia, and USA (Schaefer et al., 2001; Gebauer et al., 1996). According to Elisabeth Karsten, *et al.*, trauma is quite commonly associated with infection by filamentous fungi such as *Fusarium solani*, *Aspergillus* spp and *Bipolaris* spp; especially when the trauma is caused by vegetative matter particularly in tropical climates.

In a retrospective study done on *Fusarium* keratitis in Taiwan, major predisposing factors were found to be corneal trauma with vegetable matter, or organic matter, usage of contact lens, and steroid use. Contact lens usage, although the commonest predisposing factor in developed countries (Bozorgmehr et al., 2011) was conspicuously absent in this study. This can be attributed to the virtual absence of contact lens usage among the low income group. It is also prudent to think that the contact lens users would prefer private hospitals for treatment.

Eight patients in the study were suffering from diabetes mellitus. Similar finding was obtained in a study done in Gujarat (Hoffart et al., 2012).

Out of the 56 culture positive cases, 48 cases yielded pure fungal isolates (Table 2). In 2 cases, there were mixed bacterial and fungal isolates, while 3 cases yielded two fungal isolates each. This study detected 56 fungal,

and 12 bacterial isolates from 100 cases of corneal ulcers. The fungal isolates constituted 82.35% of total isolates. While the prevalence recorded from the southern, western and north-eastern India is between 21-37%, it is only 7.3% from Chandigarh. In a larger study of 3528 microbial keratitis cases in Delhi (north India), the prevalence of fungal keratitis was reported to be 24.3% (Rautaraya et al., 2011).

Table.1 Direct smear positivity vs. culture positivity

Direct Smear Positivity (KOH)	No.	%	Fungal Culture Positivity	No.	%
	49	49%		56	56%

49% of cases were positive in direct smear (KOH), and 56% of cases gave growth in culture.

Table.2 Fungal pathogens isolated

Species	No. of isolates	%
<i>Aspergillus</i> spp	22	39.29
<i>Fusarium</i> spp.	9	16.07
<i>Penicillium</i> spp.	4	7.14
<i>Candida albicans</i>	2	3.57
<i>Candida tropicalis</i>	1	1.79
<i>Mucor</i> spp.	2	3.57
<i>Acremonium</i>	3	5.36
<i>Nigrospora</i>	2	3.57
<i>Curvularia</i> spp.	2	3.57
<i>Phoma</i> spp.	2	3.57
<i>Cladosporium</i>	1	1.79
<i>Dreschlera</i>	1	1.79
<i>Lasiodiplodia theobromae</i>	1	1.79
<i>Aureobasidium pullulans</i>	1	1.79
<i>Fonsecaea</i> spp.	1	1.79
Unidentified Hyphae	2	3.57
Total	56	

Among the 56 fungal isolates, 22 belonged to *Aspergillus* spp. Of this, *Aspergillus flavus* isolated (10 cases) was the most common isolate. Second most common was *Fusarium* spp.

Table.3 *Aspergillus* spp. isolated

<i>Aspergillus</i> spp.	No. of isolates	%
<i>Aspergillus flavus</i>	10	45.45
<i>Aspergillus fumigatus</i>	6	27.27
<i>Aspergillus niger</i>	6	27.27
Total	22	100%

Table.4 Corneal ulcers: other predisposing factors

Sl. no.	Predisposing factors	No
1	DM	8
2	History of fall and keratitis in the past	4
3	Facial Palsy	2
4	Dry eye	1
5	Lid abnormality	1
Total		16

Table.5 Ocular trauma: causes of injury

Sl. No.	Traumatic Agents	No
1	Particulate material (Sand, Dust)	29
2	Wooden Stick	9
3	Building Material	10
4	Insects	1
5	Fingernails	1
6	Dirty Water	1
7	Vegetative matter	5
Total		56

Table.6 Corneal ulcers: seasonal variation

Sl. No.	Months	Fungal isolation
1	Dec, Jan, Feb	12
2	Mar, Apr, May	4
3	Jun, Jul, Aug	32
4	Sep, Oct, Nov	8
Total		56

The possible reason for reduced bacterial corneal ulcers might be more successful treatment of bacterial corneal ulcers in the peripheral centers and/or by general practitioners. Since this study is conducted at tertiary referral eye care center, the large number of fungal ulcers reported may be due to their prolonged course and poor response to available topical medications. 49% of the cases included in the study were direct smear - 10% KOH positive, and 56% of cases yielded fungal isolates (Table 1). This shows KOH wet mount is a simple and sensitive method for diagnosis. Consequently, about 7 cases were missed on microscopic examination, which were positive in culture

for fungi. But, 3 cases gave a positive microscopy for fungi without yielding fungal growth in culture. These would have been either artifacts, or these fungi must have been suppressed due to administration of topical antifungals. Study done at L.V. Prasad Eye Institute concludes Gram staining is much inferior to 10% KOH mounts for visualization of fungal hyphae (Srinivasan et al., 1997).

Among the 56 fungal isolates, *Aspergillus spp.* was the commonest. *Aspergillus species* constituted 39.28% of the total fungal isolates. *Aspergillus* was speciated based on their macroscopic and microscopic morphological features. Accordingly 17.85%

was *Aspergillus flavus*, and 10.71% *Aspergillus fumigatus* and *Aspergillus niger* respectively. In 1993, Chander *et al.*, (1993) (Chandigarh) reported 35% of fungal isolates from corneal ulcers to be *Aspergillus*. In other studies Panda *et al.*, (1997) from New Delhi (1997), Dunlop *et al.*, (1994) from Bangladesh, Vemuganti *et al.*, from Hyderabad have all reported *Aspergillus* as the commonest isolate in corneal ulcer.

Fusarium was the second commonest isolate, next to *Aspergillus* in this study. Similar findings were obtained in studies done in South India by Gar *et al.*, from the Prasad Eye Institute, Hyderabad (37.6%), and from Iran (Badiee, 2013; Thomas *et al.*, 2013; Zubair *et al.*, 2013).

Four corneal ulcers yielded *Penicillium* spp. in this study. This is 7.14% of the total fungal isolates. This is supported by Panda *et al.*, who reported 7% of fungal isolates to be *Penicillium* spp. in his study at New Delhi (Chander *et al.*, 1993).

Curvularia spp. is the commonest dematiaceous fungi in most of the studies conducted worldwide and in India. However in this study, there were 2 *Curvularia* isolates. This fungus is known to occur in the hot, humid, summer months Wilhelmus *et al.*, (Zubair *et al.*, 2013).

In a study in North India, *Curvularia* was the causative organism in 30.43% of cases followed by *Fusarium* (21.73%) (Sanjeevani *et al.*, 2010).

Acremonium spp. is one of the commonest hyalohyphomycete causing keratitis. Laspina *et al.*, (Paraguay) reported this organism as the commonest fungal isolate from corneal ulcers. Another study from Madras Venugopal *et al.*, (Sharma *et al.*, 2008) reports *Acremonium* as the second commonest fungal

isolate. *Acremonium* spp is also isolated in post-traumatic keratitis cases in Kancheepuram district of Tamil Nadu. In this study, there were 3 isolates of *Acremonium* spp (5.36%). In this study, *Candida* spp. constituted 5.36% of the total fungal isolates. *Candida* was further speciated into *Candida albicans*, and *Candida tropicalis*. *Candida albicans* was the predominant isolate in a study conducted in Hyderabad (Narsani *et al.*, 2012).

The other fungal isolates encountered in this study (*Mucor* spp., *Nigrospora*, *Phoma* spp. *Cladosporium*, *Dreschlera*, *Lasiodiplodia theobromae*, *Aureobasidium pullulans*, *Fonsecaea* spp.) though rare, are associated with corneal ulcers (Thomas *et al.*, 2013).

Though majority of cases healed well following aggressive treatment, few cases ended up in corneal opacification, vascularization and perforation.

The present study was conducted in Regional Institute of Ophthalmology, Thiruvananthapuram. This study was an attempt to reveal the fungal in suppurative keratitis. Hundred patients – both OP and IP patients, with signs and symptoms of suppurative keratitis were studied from March 2012 to February 2013.

The following conclusions were derived from the present study:-

The male to female ratio was 2.125:1, indicating male preponderance.

The majority of patients were in the age group 41 to 50 years.

Manual laborers, followed by housewives and students were the dominant group in our study.

The highest prevalence of suppurative keratitis was found in people living in rural areas.

Majority of patients belonged to low socio-economic background.

Majority of patients presented in monsoon months.

63% of patients included in this study had taken treatment before attending RIO. Majority had taken topical antibiotics alone.

57% of patients included in this study gave history of ocular trauma, and particulate material like sand and dust were the most common traumatic agents. Diabetes Mellitus was another important predisposing factor.

Out of 100 cases, 56 were culture positive for fungus

Direct smear positivity was found in 49% of patients, whereas culture positivity of fungus was found in 56% of patients.

Aspergillus spp. was the most common fungal isolate followed by *Fusarium* spp., and *Penicillium* spp.

Although corneal ulceration is a very rare cause of mortality, it is one of the important causes of morbidity and disability in South Kerala, especially among lower socio-economic groups. The present study gives an insight about the fungal and bacterial etiological agents of suppurative keratitis in this part of Kerala. The results of this study are similar to that in other parts of India. Few cases, especially *Fusarium* keratitis perforated within 1 to 2 weeks. So, early diagnosis and treatment with proper antifungal is of utmost importance. Microbiological culture, or a direct microscopy (KOH mount, or even a gram smear) is strongly advisable in this scenario. A complete microbiological investigation is mandatory and sight-saving in such a situation.

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