

Original Research Article

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Fungal Keratitis-Study at a Tertiary Eye Care Hospital in Hyderabad, India

B.M. Shanker Venkatesh^{1*}, S. Jayaprakash Rao² and V. Mallikarjun Rao³

¹Department of Microbiology, Sarojini Devi Eye Hospital Hyderabad, Telangana, India

²Department of Microbiology, Osmania Medical College, Hyderabad, Telangana, India

³Department of Microbiology, Shadan Medical College, Hyderabad, Telangana, India

*Corresponding author

ABSTRACT

Keratitis is the inflammation of the cornea. Fungi are the most common etiological agents which account for 30–40% of the cases whereas bacteria account for 13–48% of all cases of suppurative keratitis; this varies by geographical area¹. Fungal keratitis is an inflammation of the cornea caused by fungi. It is worldwide in distribution, but is more common in the tropics and subtropical regions. Fungal keratitis is an important cause of ocular morbidity and blindness worldwide and is a growing threat that has substantial morbidity and cost. In view of the rise in cases of fungal keratitis in recent years, this study has been undertaken to better understand the epidemiological pattern, microbiological profile, risk factors of fungal keratitis at a Tertiary Eye Care Referral Center - Sarojini Devi Eye Hospital - Hyderabad Telangana. This is a Retrospective study done, at Sarojini Devi Eye Hospital Hyderabad - a tertiary eye care teaching hospital from May 2017 to April 2018 (1 year) and included cases of fungal Keratitis (both OP and IP CASES). All patients with suspected fungal keratitis presenting to the OPD and IPD, at SD Eye hospital Hyderabad were evaluated. Socio-demographic data and information pertaining to the risk factors, clinical presentation was recorded. After provisionally diagnosing Keratitis clinically by the ophthalmologist, corneal scraping and cultures were taken. A presumptive diagnosis was based on clinical features and history, diagnosis was confirmed by KOH preparation and culture. During the study period, 173 patients with suspected fungal keratitis were evaluated. Cultures were positive in 147 cases, though while in 14 cases KOH was positive and culture was negative, they were also included in study. Thus the total cases that were studied were 161. Among these culture positive cases, 11 (6.83%) had mixed fungal and bacterial infections. The most common risk factor was ocular trauma as seen in 142 cases (82.08%), followed by co-existing ocular disorder in 12 cases (6.94%). The fungal pathogen that was most commonly isolated was *Aspergillus* spp with 83 (51.56%), followed by *Fusarium* spp in 56 (34.79%), *Curvularia* spp 12 (07.45), *Penicillium* spp. 06 (03.73%), and *Candida albicans* 04 (02.47%). The maximum incidence was seen in the age group of 41-60 years, and a majority of them were males. The typical clinical features at presentation in this culture-positive fungal keratitis were as follows: dry, thick and raised corneal surface, stromal infiltrates with feathery margins, typical satellite lesions, Hypopyon and corneal abscess. The prompt diagnosis of mycoses requires a high index of suspicion and an appreciation of specific risk factors that may predispose a patient to ocular fungal infections. Determination of the identity of the specific etiological agent of mycotic disease is very important for the therapeutic considerations. The clinical suspicion by an ophthalmologist is the most important factor in making a diagnosis of fungal infection of cornea. Fungal Keratitis most often occurs after a superficial trauma with vegetative or organic materials. *Aspergillus* spp and *Fusarium* spp were the most common fungi. These findings have important public health implications for the treatment and prevention of suppurative corneal ulceration. The overall knowledge of fungal keratitis with its clinical determinants and risk factors would aid in general awareness and prevention of complications associated with it.

Keywords

Areca nut, UHPLC,
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Introduction

Eye is one of the important organs of sensory perception. Normally it is kept free from microbial infections by the natural protective mechanisms. Breach of these protective barriers can predispose to ocular diseases leading to significant ocular morbidity. Keratitis is an inflammation of the cornea, due to infectious, physical or chemical causes, which can result in vision loss.

The incidence of microbial keratitis is about 11 per 100,000 persons/year in the United States, and the reported incidence of corneal ulceration in India is 1130 per million (Erie *et al.*, 1993; Upadhyay *et al.*, 2001; Srinivasan *et al.*, 1997). Eye is vulnerable to infections with fungal spp. mostly due to trauma and excessive steroid use with or without antibiotic. There have been increasing trends of infectious corneal blindness owing to higher incidences of direct ocular trauma added with personal and social factors like increased alcohol consumption, higher age, low socioeconomic status and lack of health education (Song *et al.*, 2014; Wang *et al.*, 2014). Fungal keratitis is seen more in tropical climates and is of lesser frequency in temperate areas. Its incidence is between 6 to 20% of all microbial keratitis cases depending on the geographic location. There is a great regional variation in the epidemiological pattern and causative agents for fungal keratitis (Collier *et al.*, 2014). Fungal Keratitis is associated with some predisposing or risk factors. Risk factors include trauma, ocular surface disease, systemic disease, inadvertent use of contact lens 1 and topical steroid use.

Ocular trauma is most common predisposing condition of infectious Keratitis in developing countries, whereas pre-existing ocular disease and contact lens are common risk factors in developed countries (Sharmeen Ahmed *et al.*, 2010; Gopinathan *et al.*, 2002).

Tropical climates show a preponderance of filamentous fungi, like *Fusarium* spp and *Aspergillus* spp. With a strong relationship to trauma. Temperate climates show higher percentages of yeast infections (Upadhyay *et al.*, 1991). In the USA, corneal infection by fungus was, more common in debilitated or immunocompromised patients and the causative organism being yeast, such as *Candida albicans*.

Filamentous fungi in these latitudes were rarely reported (Prajna *et al.*, 2013). *Fusarium* keratitis associated with a type of contact lens solution displaced yeasts as the most common fungal corneal infection in some areas.

Broad-spectrum treatment should be therefore administered once there is a strong probability of a mycotic infection without relying on the geographical distribution.

Indian scenario

Especially in rural India, where agriculture is the main occupation minor eye injuries sustained in agricultural farms often lead to infectious corneal ulceration and loss of vision (Srinivasan *et al.*, 1997; Sharma and Athmanathan, 2002). It is considered more a disease of rural areas and is frequently caused by trauma with a vegetative material. Its incidence in urban population is due to widespread use of contact lenses, especially bandage contact lenses and topical steroid usage.

Ocular fungal infections, or ophthalmic mycoses, are being increasingly recognized as an important cause of morbidity and blindness; certain types of ophthalmic mycoses may even be life-threatening (Sharma and Athmanathan, 2002). Fungal keratitis is one of the major causes of the ulcerative and sight-threatening infection of the cornea but its incidence is usually

underestimated in the community (Levin *et al.*, 1996; Yohai *et al.*, 1994). Even the trivial trauma of a dust particle falling on the cornea may disrupt the integrity of the corneal epithelium, predisposing to mycotic keratitis. The prompt diagnosis of mycoses requires a high index of suspicion and an appreciation of specific risk factors that may predispose a patient to ocular fungal infections.

Determination of the identity of the specific etiological agent of mycotic disease is very important for the therapeutic considerations. Classical diagnosis of fungal infections depends on direct microscopic examination or staining, and the isolation of the fungus in culture

The potassium hydroxide (KOH) wet mount and its modifications are widely used for the rapid detection of fungal hyphae in necrotic tissue samples from patients with infectious keratitis (Cao *et al.*, 2014).

Materials and Methods

All patients clinically provisionally suspected with fungal keratitis presenting to the OPD & IPD, at Sarojini Devi Eye hospital - Hyderabad Telangana from May 2017 to April 2018 were included in the study. A standardized form was filled up for each patient which included - socio-demographic information as well as clinical information including duration of symptoms, previous treatment, predisposing ocular conditions, history of trauma and associated risk factors and visual acuity.

Inclusion criteria

All those cases sent by the ophthalmologist with a provisional diagnosis as fungal keratitis were cultured and those found positive for corneal fungal growth and fungal isolates were included in this study.

Exclusion criteria

All those microbial keratitis cases with typical features of viral infection, interstitial keratitis, bacterial keratitis and not provisionally diagnosed as fungal keratitis were excluded.

Sample collection

Visual acuity was tested by an ophthalmologist at the time of presentation and was recorded. After a detailed ocular examination using standard techniques, the ophthalmologist took corneal scrapings under aseptic conditions. These samples were labeled with the patient's name and the date of collection.

Corneal scrapings were taken after installation of 4% preservative-free lignocaine by an ophthalmologist using the no. 15 Bard-Parker blade with strict asepsis under slit-lamp. The collected scraping material from the leading edge and base of the ulcer was inoculated into solid/liquid culture medias and then smeared on 2 different slides in a thin, even manner for Gram stain, Giemsa stain and 10% Potassium hydroxide (KOH) wet mount.

The use of KOH wet mount lies in dissolving the keratinized epithelial cells that possess a diagnostic difficulty owing to its similarity with fungal strands. The KOH wet mount is very useful for detecting fungi from the ocular specimens.

Culturing

Scrapings were inoculated directly on Blood agar, chocolate agar, thioglycolate broth, brain-heart infusion broth and Sabouraud's dextrose agar and incubated aerobically. When KOH smears were positive for amoebic cysts further, corneal scrapings were performed, and the materials were inoculated onto non-nutrient agar.

Inoculated Sabouraud's Dextrose Agar (SDA) was incubated and maintained at 27°C for 3 weeks. Blood agar and chocolate agar were incubated at 37°C for 7 days. These culture media were examined daily for any traces of growth. They were discarded if there were no growths.

The growth of fungus in the primary isolated medium was re-cultured in SDA after incubating for 15 days to demonstrate the sporulation.

Similarly, the temperature for inoculation of non-nutrient agar plates was maintained at 37°C after overlaying with *Escherichia coli* broth culture. It was examined regularly for the traces of *Acanthamoeba* species. These culture media were examined daily for any traces of growth. They were discarded if there were no growths.

Fungal identification

Fungi after the subsequent growth and isolation were identified by their macroscopic and microscopic features. Colony characteristics on SDA and by the morphological appearance of the spores in lactophenol cotton blue stain.

KOH mount microscopy if it reveals hyphae in corneal smear, but failed to grow in culture, the causative organism was reported as fungi.

The microbial cultures were considered as positive when it satisfies at least one of the following criteria (Levin *et al.*, 1996):

Growth and isolation of the same organism from more than one solid medium,

Confluent growth in one solid medium at an inoculated site with direct microscopy findings (staining and morphology with Gram-stain, KOH preparation)

Growth and isolation of the similar organism repeatedly from the same scrapings. All laboratory methods were performed following standard protocols (Levin *et al.*, 1996; Yohai *et al.*, 1994; Rao, 1989).

Results and Discussion

A total of 173 patients with clinically suspected fungal keratitis were examined of which 161 proved to be having the fungal infection while the rest of them were KOH and culture negative. Out of all the positive fungal cases, 150 had the only fungal infection while 11 had mixed infection with fungi and bacteria.

Of the total 173 patients included in the study, the ages ranged from 0 to 80 years among which males were 121 (69.94%), and females were 52 (30.06%). Out of the total 173 patients included in the study, 112 (64.74%) patients in the age group of 41 to 60 were the highest followed by the age group 21 to 40 with 46 (26.59%), age group 0 to 20 were 08(0.461%) and 61 to 80 age group being 07 (04.06%). The majority of the cases were from rural background (89.02%) farm laborers working in rice fields.

The most common risk factor was ocular trauma as seen in 142 cases (82.08%) and in which vegetative matter was seen in 108 cases (62.43) followed by co-existing ocular disorder in 12 cases (6.94%) Co-existing systemic disease in 04 cases (2.31%), wearing of refractive contact lenses is the presumed risk factor in 08 cases (4.62%) and inadvertent use of steroids in 07 cases (4.05%)

Aspergillus spp. (51.56%) was the single most fungi isolated and *Fusarium* spp. (34.79%) was the second most fungi isolated, and they together accounted for 139 (86.35%) cases. *Curvularia* spp. 12(7.45%), *Penicillium* spp 06(3.73%) and *Candida* spp. 04(2.47%) were

the other fungi that have been isolated. This is a one year study conducted at Sarojini Devi Eye Hospital, Hyderabad to analyze the profile of fungal keratitis cases with a sample size of 173 cases from May 2017 to April 2018.

Fungal keratitis and its complications constitute important causes of ocular morbidity often leading to blindness if early management is not instituted. A proper clinical history coupled with detailed clinical examination would be beneficial to identify the predisposing factors for corneal perforation in FUNGAL keratitis.

In the present study, male patients were more affected than female patients which is in agreement with the study done by Tityal *et al.*, (2006). This pattern of gender variability maybe attributed to the increased outdoor activity of men especially in the working age group and also as per the prevailing socio-cultural structure, predominantly males go for the outdoor work and the same applies for the farming. In contrast, in the study done in China, women were more affected and most of them were over the age of 60 (Cao *et al.*, 2014). This could be due to higher employability of women particularly in the agricultural sector in China.

The majority of the patients 154 (89.02%) were from the rural background (farm workers), involved in agricultural activities. It is in agreement to other studies done by Sethi

et al., (2010), Keshav *et al.*, (2009), Amrutha and Venkatesha (2014) were in labourers/farmers contributed ranging from 54-70% and housewives/homemakers 11-13%.

In this study, the commonest age group affected were 41–60 years 112 (64.74), followed by 21-40 years 46 (26.59%), 0-20 years 8 (4.61%) and 61- 80 years 7 (4.062%) which is in agreement with the results of (Cameron *et al.*, 2006) in Sydney and (Das and Konar, 2013) in Kolkata, and Laltanpuia Chhangte *et al.*, in Uttarakhand (www.ijsrp.org). The most common age group who are affected (41-60 years) impact the socio-economic conditions as they usually support families financially. Similar age group was also most commonly affected in south India and western Orissa (Srinivasan *et al.*, 1997; Samir Mahapatra *et al.*, 2009). While in the eastern study and Bangladesh study, the commonest age group was 21-40 years (Sharmeen Ahmed *et al.*, 2010; Basak *et al.*, 2005).

In the present study, the most common risk factor was trauma to the eye 142 (82.08%) and the trauma in most cases 108 (62.43%) was due to vegetative matter, followed by the accidental piercing of eye with animal hair 12 (6.94%), foreign body, sand/stone related to material at construction sites 14 (8.09%) and sharp wooden structures 8 (4.62%), which is similar to study by (Sharma and Mehta, 2014) and (Katara *et al.*, 2013) (Table 1–4).

Table.1 Showing sex incidence (Total cases 173)

Sl. No.	SEX	NO. OF CASES	PERCENT
1	Male	121	69.94%
2	Female	52	30.06%

Table.2 Showing age incidence

Sl. No.	AGE	NO. OF CASES	PERCENT
1	0 -- 20	08	4.61%
2	21 -- 40	46	26.59%
3	41 -- 60	112	64.74%
4	61 -- 80	07	4.06%

Table.3 Showing habitat incidence

TYPE OF HABITAT	NO. OF CASES	PERCENT
Rural	154	89.02%
Urban	19	10.98%

Table.4 Showing risk factors incidence

RISK FACTORS	NO. OF CASES	PERCENT
TRAUMA	142	82.08
Vegetative trauma	108	62.43
Animal matter	12	06.94
Sand/stones related to material at construction sites	14	08.09
Wooden material	08	04.62
Co-existing ocular disorder	12	06.94
Co-existing systemic disease	04	02.31
Inadvertent use of steroids	07	04.05
Use of contact Lens	08	04.62

Table.5 Showing culture positive cases

Total cases	KOH and Culture Negative	KOH Positive and culture negative	KOH Positive and culture positive
173	12	14	147

Table.6 Showing type of fungus (species isolated)

Fungus Isolated	Number	Per cent
<i>Aspergillus spp.</i>	83	51.56
<i>Aspergillus flavus.</i>	39	
<i>Aspergillus fumigatus</i>	24	
<i>Aspergillus niger.</i>	20	
<i>Fusarium spp.</i>	56	34.79
<i>Curvularia spp</i>	12	07.45
<i>Penicillium spp.</i>	06	03.73
<i>Candida albicans</i>	04	02.47

Use of contact Lens as a risk factor was seen among 08 (4.62%) patients and this may be attributed to increasing awareness and inclination towards improved cosmetic appearance. The overall incidence of contact lens-induced keratitis is increasing in the general population, and it could be due to improper handling of contact lenses along with lack of awareness of maintaining adequate hygiene as per study by (Rishi Mehta *et al.*, 2016)

In our study fungal keratitis due to inadvertent use of steroids was seen in 7(4.05%) cases. In India, most eye medications are sold over the counter without a prescription and the use of medications especially steroid eye drops lowers the immune system thereby reducing the host defense mechanism (Rishi Mehta *et al.*, 2016).

Moreover, due to illiteracy, patients keep on using these eye drops continuously for longer periods, many times even without prescription. Steroids are usually prescribed for various ocular diseases like blepharitis, iridocyclitis, choroiditis, and even allergic conjunctivitis. The use of steroids for a spectrum of ocular disorders, if unmonitored, leads to fungal keratitis.

Fungal keratitis secondary to co-existing systemic disease like Diabetes mellitus was found in 4 (2.31%) patients.

This is in similarity to the studies by Sharma and Mehta (2014), Katara *et al.*, (2013) and Kumar *et al.*, (2011).

In our study most commonly isolated fungal pathogen was *Aspergillus* spp.83 (51.56%), followed by *Fusarium* spp 56 (34.79%) both of which together constituted 139 (86.35%). This is in accordance with the study by Rishi Mehta *et al.*,²⁹ In similar studies done at Mumbai, parts of South India, North

India, Nepal and Bangladesh. (Kumar *et al.*, 2011; Bharathi *et al.*, 2003; Williams *et al.*, 1987; Despande and Koppikar, 1999; Garg *et al.*, 2000; Sunbaram *et al.*, 1989; Venugopal *et al.*, 1989) It was found that *Aspergillus* species was predominant. While other studies in South India reported *Fusarium* spp to be more common than *Aspergillus* spp (Leck *et al.*, 2002; Sharma and Athmanathan, 2002; Sunbaram *et al.*, 1989) *Fusarium* spp have also been found to be the principal fungal pathogen in Florida, Paraguay, Nigeria, Tanzania, Hong Kong and Singapore. (Dunlop *et al.*, 1994; Liesegang and Forstor, 1980; Tanure *et al.*, 2000) This phenomenon may be explained by differences in climate and the natural environment. Other fungi that have been isolated were, *Curvularia* spp 12 (7.45%) *Penicillium* spp 6 (3.73%) and from among the patients using contact lens *Candida albicans* 4 (2.47%) and *Fusarium* spp 4 were isolated (Tanure *et al.*, 2000; Gopinathan *et al.*, 2009).

In our study 11 (6.83%) cases had mixed fungal and bacterial infections. Mixed infections both by bacteria and fungi were similar to study by (Bharathi *et al.*, 2003) (2.4%) report from South India, and (Srinivasan *et al.*, 1997) (5.1%) (Leck *et al.*, 2002) (5.5%) (Table 5 and 6).

Fungal keratitis is the leading cause of ocular morbidity and continues to be a cause for concern among the ophthalmologists as it is an avoidable vision-threatening disease.

As the majority of the cases are from rural background (89.02%) i.e., farm laborers with predominance of agricultural activity and the traumatic vegetative eye injury being the most common risk factor and since agriculture sectors is the major contributor to the economy of our country, and fungal Keratitis is common among our agricultural farm laborers, improvement in our eye care

systems at the rural PHC with the knowledge of clinical cases with common causes, associated risk factors and presentations with complications would help in the early diagnosis of fungal keratitis and better clinical outcome and would be crucial in preventing blindness in our country.

In conclusion, meticulous microbiological examination, along with research in ophthalmic mycoses and the latest review of all clinical and laboratory aspects regarding fungal keratitis and development of new antifungal compounds is important in order to formulate better diagnosis and treatment strategies and to improve patients' outcome.

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