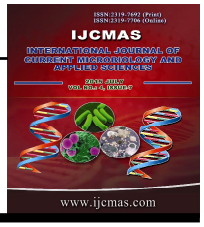
Int.J.Curr.Microbiol.App.Sci (2015) 4(8): 664-670

International Journal of Current Microbiology and Applied Sciences
ISSN: 2319-7706 **Volume 4 Number 8 (2015) pp. 664-670**
http://www.ijcmas.com



Original Research Article

A Study of Fungal Etiology of Infective Keratitis

Uma Sri Mallareddy[1], Parameswari katay[2] and Geethanjali Anke[3*]

[1]Regional Laboratory, Warangal, India
[2]Siddhartha Medical College, Vijayawada, India
[3]Govt. Medical College, Anantapur, India
**Corresponding author*

A B S T R A C T

Microbial keratitis is an important cause of ocular morbidity and avoidable visual impairment in all age groups, and is commonly encountered by ophthalmologists worldwide. Aims of the study are to isolate the fungi in infective keratitis; to determine its risk factors and other epidemiological characteristics. Corneal scrapings have collected from 100 patients with corneal ulceration for two years and fungal identification has done by direct microscopy using 10% Potassium Hydroxide (KOH), fungal culture and other standard Microbiological techniques. Fungal isolates were obtained in 36 out of 100 samples. The predominant fungal isolate was *Aspergillus* spp. (36.11%), followed by *Fusarium* spp. (25%). Corneal ulcers showed a higher prevalence in age group 31–70 years (83.3%). Male preponderance was seen, with 66.6% of the fungal. Agricultural laborers contributed to 58.33% of the corneal ulcers. Among the predisposing factors, History of corneal trauma (77.7%) was the most common predisposing factor noted. 10% KOH mount was positive in 32 out of 36 fungal ulcers- 88.8% sensitivity. 10% KOH mount is a rapid, reliable and inexpensive diagnostic modality which would facilitate the institution of early antifungal therapy. Help Ophthalmologists in treating corneal ulcers and in prevention of loss of vision.

Keywords

Keratitis, Fungi, 10% KOH, *Aspergillus, Fusarium*

Introduction

Microbial keratitis is a common potentially vision threatening ocular infection that may be caused by various pathogens like bacteria, fungi, viruses or parasites.

Microbial keratitis is an important cause of ocular morbidity and avoidable visual impairment in all age groups, and is commonly encountered by ophthalmologists worldwide (Schaefer *et al.,* 2001). Despite advances in treatment, infective keratitis remains clinically challenging and although the outcome can be favorable with appropriate management, there is potential for significant and permanent visual impairment in addition to social and healthcare costs (Schaefer *et al.,* 2001; Levey *et al.,* 1997).

Scarring of the cornea as a result of suppurative keratitis is an important cause of preventable blindness. In some developing

countries in the tropics, corneal infections are the second commonest cause of blindness after unoperated cataract (Upadhyay *et al.,* 1991; Gonzales *et al.,* 1996; Whitcher *et al.,* 2001). Suppurative corneal ulcers may be caused by bacteria, fungi, and protozoa. However, within the tropics, as many as two thirds of ulcers may be due to filamentous fungi. This type of ulceration is commonly associated with ocular trauma (Gonzales *et al.,* 1996; Whitcher *et al.,* 2001; Srinivasan *et al.,* 1997).

More than 70 species of fungi have been reported as pathogenic to human cornea; the most frequently isolated pathogen varies with the geographical area studied (Foster *et al.,* 1992; Kelly *et al.,* 1994). Across the world, the single most commonly reported fungus isolated from mycotic keratitis is *Aspergillus* spp (Levey *et al.,* 1997). Fungal keratitis is reported more frequently from regions with a warm, humid climate and/or with an agricultural economy (Foster *et al.,* 1992; Kelly *et al.,* 1994).

The first case of fungal keratitis in the literature was reported by Leber *et al.* in 1879. Since then, despite numerous advances in the treatment of ocular infections, fungal keratitis is often a devastating event. Once considered rare, the last four decades have shown an increase in the percentage of microbial keratitis caused by fungal infections (Schaefer *et al.,* 2001).

Untreated, suppurative keratitis may lead to opacification and, ultimately, to perforation of the cornea. Specific treatment requires prompt and accurate identification of the causative micro-organisms (Foster *et al.,* 1992). Within the setting of rural eye hospitals in the tropics laboratory facilities are rare and diagnosis is based on clinical characteristics. As a direct result of this, treatment is often empirical.

The following study was Prospective conducted for two years at Government General Hospitals, Siddhartha Medical College in Andhra Pradesh to isolate the fungal agents in infective keratitis. The aim was to evaluate the importance of direct microscopy and isolation of etiological agents and institution of therapy.

Materials and Methods

Corneal scrapings has collected from 100 patients with corneal ulceration attending the ophthalmic O.P.D at Government General Hospital, Vijayawada and also from private ophthalmic clinics and 30 samples are collected from patients with refractive error who are considered as controls.

Corneal scrapings collection: Under aseptic measures, after instillation of anesthetic (4% lignocaine) drops into the eyes, under the magnification of slit lamp using Bard-Parker Blade (No.15), the material was scrapped from the base and margins of the ulcer.

Isolation of fungi: The Scrapings were subjected to direct microscopy by 10% KOH wet mount and also were inoculated on to Sabouraud Dextrose Agar. They were incubated in two test tubes at 25^0C and 37^0c for 8 weeks before declaring it as negative.
When growth appears, it has observed both on reverse and obverse side. Then Slide culture has kept using Corn meal Agar. Fungus exact morphology has identified by Slide culture and LPCB (Lacto Phenol Cotton Blue) stain.

Results and Discussion

Samples were collected from 100 patients (Cases) with corneal ulceration and 30 patients with refractive error (Controls).

In the present study out of 100 cases 36 fungal isolates (36%) has identified. Among 36 fungal isolates of corneal ulcers, *Aspergillus* spp. was the predominant organism about 36.11% followed by *Fusarium* spp (25%). The Fungal etiology of present study has depicted in table 1.

Out of 13 *Aspergillus* spp. isolates, 5 were *Aspergillus fumigatus*, 4 were *Aspergillus* flavus, 3 were *Aspergillus* terreus and 1 was *Aspergillus niger*.

Male preponderance was seen in Fungal Corneal ulcers about 66.66%. 37.5% of fungal ulcers in females.

Fungal Corneal ulcers showed higher prevalence in the age groups (31–70 years) representing a total of 83.33%. 2.7% and 8.3% of fungal corneal ulcers occurred in 11–20 yr and 21–30 yr age groups, 5.55% of fungal corneal ulcers occurred in 71 yrs & above age group

More number of fungal corneal ulcer cases has observed in rural areas about 75% and 25% of fungal corneal ulcers in urban areas. Agriculture laborers contributed to 58.33% of fungal corneal ulcers, followed by daily wage laborers of about 27.7% and household persons constituted about 13.88%. Peak incidence of corneal ulceration was seen during the monsoon season (July-Sep) representing 49.99% of fungal corneal ulcers. This was followed by high incidence during harvest season (Jan-March) representing a total of 36.1% of fungal corneal ulcers. Lower incidence was seen during summer (April-June) and during Oct-Dec about 8.32% and 5.55% respectively.

The most frequent predisposing factor was history of corneal trauma, representing 77.77% of fungal corneal ulcers, followed by post cataract ocular surgeries (8.33%), Chronic dacryocystitis (5.55%) and 2.77% of Fungal corneal ulcers observed in Hypertension, HIV, Topical Steroid usage each.

The Most frequent traumatic agent noted was history of injury with vegetative matter, contributing to 64.28% of bacterial corneal ulcers. This was followed by soil (21.42%), Animal horn (3.57%), Finger nail (7.14%) and unknown traumatic agent (3.57%).

Direct Smear examination was done by 10% KOH for fungi. Direct smear and Culture positivity correlation has depicted in table 2. Both Smear and Culture positivity was noted in 32 out of 36 fungal corneal ulcers.

19.44% of fungal corneal ulcers responded to treatment, 72.22% of fungal corneal ulcers deteriorated inspite of treatment with scarring of cornea and total corneal ulceration, and no follow up was present for 13.88% cases.

Controls: 30 Controls taken from healthy conjunctival sacs from patients with refractive errors shows 6.66% fungal isolates. Among which one was *Aspergillus fumigatus* and another was *Paecilomyces lilanicus*. These two samples showed male preponderance.

When study and control groups number of fungal isolates are compared, as per Chi square test with yates correction shows df=8.233, P=0.0041 which is Statistically significant.

Fungal isolates were obtained in 36% of cases in the present study. This coincides with Zhang *et al.*, (2002) - 43.8%, Bharathi *et al.* (2003) - 34.4%, Mohapatra *et al.* (2003), Shoja *et al.* (2004) reported a lower incidence of 18.73%, 6.25% respectively.

Table.1 No. of fungal isolates from corneal ulcers

S.No.	Organism	No of isolates	Percentage
1	*Aspergillus spp*	13	36.11%
2	*Fusarium spp*	9	25%
3	*Pseudallescheria boydii*	3	8.33%
4	*Pencillium spp*	2	5.55%
5	*Cladosporium spp*	2	5.55%
6	*Acremonium spp*	2	5.55%
7	*Curvularia lunata*	1	2.77%
8	*Bipolaris spp*	1	2.77%
9	*Paecilomyces lilanicus*	1	2.77%
10	*Alternaria alternata*	1	2.77%
11	Unidentified (hyaline fungi)	1	2.77%
Total		36	100%

Table.2 Co-relation of smear and culture positivity in corneal ulcers

Category	Fungal	
	Smear	Culture
Smear Positive & Culture Positive	32 (88.88%)	32 (88.88%)
Smear Negative & Culture Positive	-	4
Smear Positive & Culture negative	-	-

Table.3 Prevalence of fungi responsible for infective keratitis in various studies

S.No	Author	*Fusarium spp*	*Aspergillus spp*	*Cladosporium spp*	*Pencillium spp*	*Bipolaris spp*	*Alternaria alternata*	*Acremonium spp*	*Paecilomyces lilanicus*	*Curvularia lunata*	*Pseudallescheria boydii*	unidentified
1	Jyothi Padmaja (1990)	1.6	19.2	-	5.6	2.4	-	3.2	-	0.8	-	-
2	Panda *et al.* (1997)	10.7	39.5	-	7	-	10.2	-	-	7.4	-	-
3	Deshpande *et al.* (1999)	8.99	59.6	-	9.26	-	-	-	-	-	-	5.9
4	Kumari *et al.* (2002)	7.89	52.2	-	7.89	-	-	-	-	2.8	-	13.8
5	Gopinathan *et al.* (2002)	37.2	30.7	-	-	-	-	-	-	2.8	-	-
6	Mohapatra *et al.* (2003)	23	38.4	-	-	-	-	-	-	-	-	-
7	Bharathi *et al.* (2005)	42.8	26	4.82	0.36	2.36	1	-	-	2.64	-	10.5
8	Khanal *et al.* (2006)	22	38.4	-	-	-	-	-	-	-	-	-
9	Xie *et al.* (2006)	73.3	12.1	-	-	-	-	-	-	-	-	-
10	Present study (2008)	25	36.1	5.35	5.55	2.77	2.77	5.55	2.77	8.33	2.77	-

The incidence of mycotic keratitis ranges from 8.3% to 82.3% in North India and from 22.7% to 46.1% in South India as reported by various authors.

In this study *Aspergillus* spp was the predominant pathogen isolated, followed by *Fusarium* spp. Various studies by different authors observations are depicted in table 3 along with present study results.

These various incidences shown in the table 3 are due to different epidemiological pattern of fungi.

In this study the most common age group affected was 31–70 years representing 83.25% of the fungal isolates. Bharathi *et al.* (2003), Kumari *et al.* (2002), Gopinathan *et al.* (2002) and Deshpande *et al.* (1999) were observed that more incidence of fungal keratitis in 21–50 years. Chowdary *et al.* (2005) and Jyothi padmaja *et al.* (1990) has observed in 31–40 years. The higher incidence in the middle age group may be explained by the fact that they are more involved in outdoor activity and hence having a greater chance of injury and exposure to infection.

Male preponderance was seen in this study about 66.6% which in line with other studies such as Gopinathan *et al.* (2002)-71.5%, Bharathi *et al.* (2003)-65.08%, Jyothi padmaja *et al.* (1990)-68.51%, Chowdary *et al.* (2005)-68%.

Fungal ulcers occurred among agricultural laborers in 58.33% cases in present study and 27.7% from daily wage laborers. This coincides with Bharathi *et al.* (2003) - 64.75%, Deshpande *et al.* (1999) - 88%, Kumari *et al.* (2002) - 82%.

In the present study fungal ulcers most common in Monsoon season about 49.99%, followed by during harvesting season (36.10%). This coincides with Bharathi *et al.* (2003), Gopinathan *et al.* (2002) and Kumari *et al.* (2002). This may be due to cool, humid atmosphere, ideal climate for growth of microbes.

On assessing with predisposing factors in this study most of fungal corneal ulcers occurs in patients with history of corneal trauma (77.7%) followed by Post operative ocular surgeries (8.33%), Systemic diseases (5.54%). other studies also observed higher incidence in corneal trauma such as Bharathi *et al.* (2003)-92.15%, Kumari *et al.* (2002) - 81.58%, Deshpande *et al.* (1999) - 89.92%, Gopinathan *et al.* (2202) - 54.4%, Pande *et al.* (1997) - 55.3%. As per various studies corneal trauma is main risk factor fungal keratitis in India. Other countries shows higher incidence in both corneal trauma and steroid usage (Bhartiya *et al.*, 2007; Rondeau *et al.*, 2002).

Trauma due to vegetative matter contributing 64.28% of fungal corneal ulcers in the present study [Bharathi *et al.*, 2003 (92.15%), Panda *et al.*, 1997 (60.5%), Xie *et al.*, 2006 (25.7%)].

Sensitivity of 10% KOH mount in detecting fungal elements was 8.88% in the present study. This coincides with Poonam *et al.* (2003)-88%, Kumari *et al.* (2002)-100%, Bharathi *et al.* (2003)-99.2%. The value of 10% KOH wet mount preparation in the diagnosis of fungal keratitis lies in its ability to clear the scraping off cellular debris, thereby rendering hyphal fragments more refractile on microscopic examination.

10% KOH mount is a rapid, reliable and inexpensive diagnostic modality which would facilitate the institution of early antifungal therapy. Help Ophthalmologists in treating corneal ulcers and in prevention of loss of vision.

Acknowledgements

The authors are grateful to the entire staff of the department of Microbiology at Siddhartha Medical College for their support and cooperation in the study.

References

Bharathi, M.J., Rama Krishnan, R., Vasu, S., Meenakshi, R., Shiv Kumar, C., Palaniappan, R. 2003. Epidemiology of Bacterial keratitis in a referral centre in South India. *IJMM,* 21(4): 239–245.

Chowdary, A., Singh, K. 2005.Spectrum of fungal keratitis in North Inida. *Cornea,* 24(1): 8–15.

Deshpande, S.D., Koppikar, G.V. 1999. A study of mycotic keratitis in Mumbai. *IJMM,* 42(1): 81–87.

Foster, C.S. 1992. Fungal keratitis. *Infect. Dis. Clin. N Am.,* 6: 851–857.

Gonzales, C.A., Srinivasan, M., Whitcher, J.P. *et al.* 1996.Incidence of corneal ulceration in Madurai District, south India. *Ophthalmic Epidemiol.,* 3: 159–166.

Gopinathan, U., Garg, P., Fernandes, M., Sharma, S., Athmanathan, S., Rao, G.N. 2002. The epidemiological features and laboratory results of fungal keratitis. A 10 year review at a referral eye care center in South India. *Cornea,* 21(6): 555–559.

Jyothi Padmaja, I., Saroja Venugopal, M.A. 1990. Study of keratomycosis. *Indian Pract. XLIII,* 1: 19–22.

Kelly, L.D., Pavan-Langston, D., Baker, A.S. 1994. Keratomycosis in a New England referral center: spectrum of pathogenic organisms and predisposing factors. In: Bialasiewics A.A., Schaal, K.P., (Eds). Infectious diseases of the eye. Butterworth-Heinemann, Boston. Pp. 184–190.

Khanaal, B., Deb, M., Panda, A., Sethi, HS. 2005. Laboratory diagnosis in ulcerative keratitis. *Ophthalmic Res.,* 37(3): 123–127.

Kumari, N., Xess, A., Shahi, S.K. 2002. A study of keratomycosis: one experience. *Indian J. Pathol. Microbiol.,* 45(3): 299–302.

Leber, T.H. 1879. Keratomycosis aspergillina als ursache von hypopyonkeratitis. *Graefes Arch Ophthalmol,* 25: 285–301.

Levey, S.B., Katz, H.R., Abrams, D.A. *et al.,* 1997. The role of cultures in the management of ulcerative keratitis. *Cornea,* 16: 383–6.

Mohapatra, D., Mallick, B., Panda, PL., Sarangi, G., Chayani, N., Panda, B., Patra, G.C. 2003. A study of infective keratitis with special reference to mycotic etiology. *IJMM,* 85

Panda, A., Sharma, N., Das, G., Kumar, N., Satpathy, G. 1997. Mycotic Keratitis in children: Epidemiologic and microbiologic evaluation. *Cornea,* 16(3): 295–299.

Poonam, S., Loomba., Mehfa, D.K., Mishra, B., Mandal, A. 2003. Fungal Keratitis in and around Delhi. XXVII National conference of Indian Association of Medical Microbiologists, Souvenir. 84 Pp.

Rondeau, N., Bourcier, T. *et al.* 2002. Fungal keratitis at the centre Hospitalies National d Ophthamologie des Quinze - Vingts: retrospective study of 19 cases. *J. France Ophthalmol.,* 25(9): 890–896.

Schaefer, F., Bruttin, O., Zografos, L. *et al.,* 2001. Bacterial keratitis: a prospective clinical and microbiological study. *Br. J. Ophthalmol.,* 85: 842–847.

Shoja, M.R., Manaviat, M. 2004.

Epidemiology and outcome of corneal ulcer in Yazd Shahid Sadoughi hospital. *Acta Medica Iranica,* 42(2): 136–141.

Srinivasan, M., Gonzales, C.A., George, C. *et al.,* 1997. Epidemiology and etiological diagnoses of corneal ulceration in Madurai, south India. *Br. J. Ophthalmol.,* 81: 965–971.

Upadhyay, M.P., Karmacharya, P.C., Koirala, S. *et al.* 1991. Epidemiologic characteristics, predisposing factors, and etiologic diagnosis of corneal ulceration in Nepal. *Am. J. Ophthalmol.,* 111: 92–99.

Whitcher, J.P., Srinivasan, M., Upadhyay, M.P. 2001. Corneal blindness: a global perspective. *Bull. World Health Organ.,* 79: 214–221.

Xie, L., Zhong, W., Shi, W., Sun, S. 2006. Spectrum of fungal keratitis in North China. *Ophthalmology,* 113(11): 1943–1948.

Zhang,W., Pan, Z., Wang, Z., Jin, X., Luo, S., Zou, Y., Wu, Y., Li, R. 2002. The variance of pathogenic organisms of purulent ulcerative keratitis. *Zhonghua Yan Ke Za Zhi,* 38(1): 8–12.