Original Article

Risk Factors and Treatment Outcomes of Fungal Keratitis : A Tertiary Eye Care Centre Experience

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Abstract

Purpose: *To study the epidemiological characteristics, microbiological diagnosis and treatment outcome of fungal keratitis in a tertiary eye care centre of Western India*

Materials and Methods: *A retrospective non-comparative observational review of the medical and microbiological records was done for all the patients with laboratory proven fungal keratitis on 10% KOH wet mount. Data was compiled in Microsoft Office Excel 2010 spreadsheet and analysis was done using Med-Calc software. Chi square test was used for categorical data.*

Results: *Between July 2016 to August 2018, 104/354 (29.3 %)corneal scrapings of microbial keratitis showed presence of fungal mycelia on 10% KOH wet mount. 72/104 patients were included in the study. Vegetative trauma (31/72 eyes, 43.06%) was the commonest risk factor. Aspergillus and Fusarium species were the major fungi isolated. 52/72 (72.2%) eyes healed with a scar, of which 21 (40.38%) healed with medical management alone, 29 (55.77%) needed additional tissue adhesive with bandage contact lens (BCL) and 2(3.85%) required intracameral Voriconazole. 334/52 eyes had a mean duration of presentation<=10 days (p<0.001) the need of keratoplasty (15/72) was seen in significantly larger number of patients presenting late (>10 days) (p<0.05).*

Conclusion: *History of late presentation, large extent of keratitis and presence of thinning/perforation at presentation were associated with the need for keratoplasty. Considering the recalcitrant course of fungal keratitis, the severity and time of presentation is highly predictive of the disease treatment and outcome.*

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Keywords: *Fungal, Koh, Trauma, Keratoplasty, Treatment Outcome*

Introduction

Fungal infections of cornea present as suppurative, usually ulcerative lesions which may be potentially vision threatening.[1-3] They can pose a major challenge to the ophthalmologist because of their tendency to mimic other types of stromal infections and because its management is restricted by the availability of effective antifungal agents and the extent to which they can penetrate into the corneal tissue. The microbiological and epidemiological pattern of fungal keratitis shows significant geographic variation.[4-10] Though there is enough literature regarding the risk factors and microbial etiology of suppurative keratitis from various parts of India, only a few preceding studies from Western India describe the microbiological and epidemiological profile of fungal keratitis in particular.[11-13] Besides, to the best of our knowledge, none of these studies report the outcome of different modalities of treatment of fungal keratitis in Western India.

The aim of our study was to elucidate the various predisposing factors, etiological agents and outcomes of different modalities of treatment in the management of patients with keratomycosis presenting to a tertiary care centre of Western India.

Methods

A retrospective analysis of the records of the patients presenting to the tertiary eye care centre in Western India, with laboratory proven KOH wet mount positive fungal keratitis, over a period of two years between July 2016 to August 2018, was done. The study was conducted in accordance with the ethical standards of the institutional committee and the Declaration of Helsinki 1975, as revised in 2013. Documentation of the patients included.

1. socio-demographic characteristics,
2. duration of symptoms,
3. predisposing factors,
4. treatment received prior to presentation to the centre
5. slit lamp bio microscopy findings,
6. associated ocular and systemic diseases,
7. treatment given at the study centre
8. response to the treatment and final clinical outcome during the follow-up period.

Inclusion Criteria

Clinically suspicious fungal keratitis with unilateral central and para-central corneal ulcers, with stromal infiltration with or without thinning or impending perforation, with or without hypopyon.

Exclusion Criteria

The patients with pre-existing ocular diseases (lid, conjunctival and corneal afflictions, dacryocystitis, glaucoma), patients on prior anti-fungal treatment from outside, history of ocular surgery (within six months), scleritis, endophthalmitis on presentation and clinically suspicious as well as microbiologically mixed infections.

All the patients had received a thorough slit lamp examination by a single experienced ophthalmologist. The size of the epithelial defect after staining with a fluorescein strip was measured with a variable slit on the bio- microscope in the greatest dimension and along the axis

perpendicular to it and recorded in millimetres. The size and depth of the stromal infiltrate was also recorded clinically on the slit lamp biomicroscope. Serial slit lamp photographs with documentation were used to record the findings on each follow-up. The presence or absence of hypopyon was recorded and its height measured in millimetres. Posterior segment evaluation was done by ultrasonography in cases where the fundus could not be evaluated on ophthalmoscopy. A systemic work-up of all the patients including the complete blood count, blood sugar, liver and renal function tests was done.

After a detailed ocular examination and informed consent, the corneal scrapings were taken in all cases by a trained ophthalmologist under aseptic conditions using a sterile 15 number blade. The scraped material was obtained from the leading edge and base of each ulcer and was initially spread onto labelled slides in a thin even manner for 10% KOH wet mount and Gram's stain. Direct inoculation on Sabouraud Dextrose agar in C shaped streaks and on blood agar and chocolate agar was done with meticulous care. Sabouraud's dextrose agar was incubated at 27°C, examined daily and discarded in four weeks if no growth was seen. Blood agar and chocolate agar were incubated at 37°C examined daily and discarded at three weeks if there was no growth.

All laboratory methods followed a standard protocol. A definitive diagnosis of fungal keratitis was made if the corneal scrapings revealed fungal elements on 10% KOH wet mount with/ without,

1) confluent growth of fungus in any solid media or
2) fungal growth in more than one medium.

Treatment at the centre was started after the microbiological sample was taken depending on clinical suspicion and in most cases after getting an immediate positive smear result. Topical Natamycin (5%) eye drops were started as per the need and judgement of the clinician and tapered according to the clinical response in cases of resolving keratitis. Associated supportive treatment included topical cycloplegic Atropine (1%) eye drops with lubricating eye drops and intraocular pressure lowering agents as per need. Systemic Fluconazole (150 mg twice a day for initial three days followed by once a day for 18 days) was prescribed to patients with ulcer depth more than 50% of cornea, maximum linear diameter > 5mm, deep stromal infiltration and presence of hypopyon. Topical Voriconazole (1%) was added in cases which showed no improvement within seven to ten days of the start of initial anti- fungal therapy. Periodic therapeutic debridement of the ulcer bed was done unless corneal thinning prevented it. Additional procedures were performed at the discretion of the attending ophthalmologist for patients with extreme thinning or impending perforation at presentation or cases not responding to medical treatment and they included N butyl 2-cyanoacrylate glue with bandage contact lens (BCL) application, anterior chamber wash with intracameral injection of Voriconazole (50mcg / 0.1ml) and therapeutic/ tectonic penetrating keratoplasty.

Treatment was considered as a success if the keratitis healed with a visible scar on slit lamp bio-microscope. The final outcome measures included percentage of healed cases in each group of the different modalities of treatment and treatment failures like the requirement of keratoplasty or the eyes going into phthisis.

Statisical analysis

Data was compiled and entered in Microsoft Office Excel 2010 spreadsheet and analysis was done using Med-Calc software. Proportions, mean and standard deviation were used for quantitative data. Chi square test was used for categorical data. A p value of <0.05 was considered as statistically significant.

Results

During the study period, corneal scrapings were done in 354 patients clinically diagnosed as microbial keratitis. Of these, no organisms were isolated on smears in 105 clinical samples (29.7%), while 145 (41%) showed presence of bacteria with or without presence of fungal mycelia on Gram staining and KOH wet mount and 104 (29.3%) showed presence of fungal mycelia on KOH wet mount. Of the 104 KOH positive scrapings, 63 (60.58%) showed fungal growth on cultures. Aspergillus was the commonest fungus isolated (40/63) (63.5%).

Socio-demographic variables of the study population-

Of the 104 KOH positive cases, 72 eyes of 72 patients with unilateral corneal ulcer, with above mentioned inclusion and exclusion criteria were enrolled in the study. The socio-demographic characteristics of the patients is described in (Table 1). Mean age of the patients was 47.44 ± 16.63 years (range: 13 to 70 years). Vegetative trauma was the most

Table 1: Socio-demographic characteristics of the patients with mycotic keratitis

Characteristics	Patient Details	Number of patients (%)
Age	11-30	19 (26.39%)
	31-50	17 (23.61%)
	51-70	
	36 (50%)	
Gender	Males	45 (62.5%)
	Females	
	27 (37.5%)	
Residence	Urban	21 (29.17%)
	Rural	
	51 (70.83%)	
Visit	Primary	28 (38.89%)
	Referral	
	44 (61.11%)	
Occupation	Farmers	47 (65.28%)
	Labourers	09 (12.5%)
	House-wives	08 (11.11%)
	Office job	02 (2.78%)
	Unemployed	06 (8.33%)

common predisposing factor (Table 2). Mean duration of presentation was 12.3 ± 5.87 days (range: 3 days to 30 days) after the onset of symptoms and the mean follow-up of the patients was 6.19 ± 3.21 months.

Slit lamp and microbiological examination-

Thirty seven eyes presented with central keratitis while the rest 35 had para-central keratitis. The area of ulcers and stromal infiltration ranged from 3x3 sq mm to 11x9 sq mm. Most common findings on clinical examination were as per (Table 3). Out of the 72 eyes, cultures were positive in 36 (50%) cases. Of these, Aspergillus was the most common organism isolated followed by Fusarium (Table 4).

Treatment modalities and outcomes

All the patients were under medical management. 44 eyes had significant thinning within 7 to 10 days of presentation, which necessitated the application of N butyl 2 cyanoacrylate glue and Bandage Contact Lens (BCL). The mean duration of presentation in these eyes was 12.21 ± 3.61 days. 22 eyes needed tissue adhesive and BCL application at presentation itself due to thinning or impending perforation. 10 eyes required application of the tissue adhesive twice due to premature dislodgement of the glue. The need for application of glue and BCL was seen in larger number of patients (24 out of 34 eyes) with late presentation (>10 days), though this was not found to be statistically significant (p =.11).

Table 2 : Predisposing factors of fungal keratitis and their final outcomes

Predisposing factor	*Number*	*%*	*Final outcomes*		
1. Ocular			*Healed*	*Keratoplasty*	*Phthisis*
i.) Trauma					
• Plant/agricultural	31	43.06	21	10	-
• Stone injury	06	8.33	05	01	-
• Fall of dust	05	6.94	04	01	-
• Fingernail injury	02	2.78	02	-	-
Total	44	61.11	32	12	-
ii.) Prior topical antibiotic use	05	6.94	05	-	-
iii.) Prior topical steroid use	07	9.72	04	02	01
2. Systemic					
i.) Diabetes	05	6.94	02	-	03
ii.) Tuberculosis	01	1.39	-	-	01
3. No significant factor identified	10	13.9	09	01	-

Table 3 : Slit lamp examination findings at presentation in patients with fungal keratitis

Slit lamp examination signs	Number of eyes	%
Conjunctival injection	72	100%
Stromal infiltration	09	12.5%
• <50%		
• >50%	63	87.5%
Dry looking ulcer	48	66.67%
Feathery infiltrate	26	36.11%
Hypopyon	29	40.28%
Thinning / impending perforation	18	25%
Dry looking ulcer	48	66.67%
Feathery infiltrate	26	36.11%
Hypopyon	29	40.28%
Thinning / impending perforation	18	25%
C	04	5.56%
Ulcer size: maximum diameter	18	25%
• <5mm		
• >5mm	54	75%

Table 4 - Fungal species isolated in culture from corneal ulcer patients

Type	Fungal species	Number	Percentage
Hyaline filamentous fungi			
1 Aspergillus.			
	Aspergillus niger	11	42.3
	Aspergillus flavus	7	26.9
	Aspergillus fumigatus	8	30.8
		26	72.22
2 Fusarium.	Fusarium solani	8	22.22
3.Scedosporium	Pseudallescheria boydii	01	2.78
Dematicious fungi	Curvularia	01	2.78
Total		36	100%

The final clinical outcome of healed scar was achieved in 52 (72.2%) patients with the mean healing time being 6.9 weeks. The healing was achieved within 4 weeks in 5 patients and within 6 weeks in 29 patients. The mean duration of topical antifungal therapy was 9.8±2.13 weeks and the topical therapy was continued 3 weeks after the healed scar was observed clinically. There was no statistically significant association between the age (p=.06) and gender and the number of healed eyes (p=.41) (Table 5). A majority of healed eyes (34 eyes) had a mean duration of presentation ≤10 days, which was found to be statistically significant. (p=.001)(Table-6).All the cases with a lesser extent of keratitis (maximum linear diameter<5 mm) healed. The final outcome of different modalities of treatment is described in (Table 5).

Table 5 : Outcomes of various modalities of treatment of fungal keratitis in relation to different parameters

Outcomes	Healed with medical management	Healed with tissue adhesive BCL and medical therapy	Healed with medical therapy + Intracameral Voriconazole	Total healed	Number of eyes gone into Phthisis	Number of eyes needing keratoplasty	Total
Parameters							
Gender							
males	14	18	02	34	03	08	45
females	07	11	-	18	02	07	27
Total	21	29	02	52	05	15	72
Age (years)							
11-30	06	06	01	13	-	05	18
31-50	06	04	-	10	02	07	19
51-70	09	19	01	29	03	03	35
Total	21	29	02	52	05	15	72
%	29.17%	40.28%	2.78%	-	6.94%	20.83%	-
Mean duration of presentation (days)	7.47± 2.94	12.14±3.57	15	10.36±4.05	23±7.58	15.66±5.61	-
Fungi isolated in culture	Aspergillus -niger (4 eyes) -fumigatus (2 eyes) -flavus (1 eye) Fusarium (2eyes) P boydii (1 eye) None (11 eyes)	Aspergillus -flavus (4 eyes) -fumigatus (4 eyes) -niger (3 eyes) Fusarium (4 eyes)	None isolated	-	None isolated	Aspergillus -niger (4 eyes) -fumigatus(2 eyes) -flavus (2 eyes) -Fusarium (2eyes) -Curvularia (1 eye) -None (5 eyes)	-
Size of the ulcer (maximum linear diameter) at presentation							
< 5 mm	15	03	-	18	-	-	18
>5 mm	06	26	02	34	05	15	54
Total	21	29	02	52	05	15	72

Of the twenty eyes which did not heal, 15 eyes needed keratoplasty, at a mean duration of 13.66± 6.99 days after presentation (range- 3 to 23 days), indications for which are given in (Figure 1). All these eyes had corneal ulceration with stromal infiltration involving more than 6 mm of cornea at presentation, were referrals from primary centres, and a majority (ten eyes) had needed tissue adhesive application at presentation itself. 10/15(66.67%) eyes needed a graft more than 8 mm in diameter due to the large extent of corneal melt. Postoperatively, topical antifungals, cycloplegics and analgesics were started and after that if no recurrence of the infection was found by 3 weeks, topical steroids were started in a guarded manner with gradual withdrawal of antifungal therapy over a period of 6 weeks. The final outcomes of keratoplasty at 6 months postoperatively are described in figure 2. A majority of the eyes needing keratoplasty had trauma as the risk factor (12/15 eyes), though it was not found statistically significant (p=.16) (Table 3).The need for keratoplasty was more when the patients had presented later than 10 days. (p=0.023)(Table 6)

Of the five eyes which went into phthisis, three patients had uncontrolled diabetes mellitus, one had active tuberculosis and was on anti-tuberculosis treatment, while the other had a poor compliance to the treatment advised. The mean duration of presentation was late for all the eyes (23 ± 7.58 days).

Discussion

This study presents the data regarding epidemiological, laboratory and clinical features and treatment outcomes of patients with mycotic keratitis from Western India. A

Table 6: Treatment outcome of patients with fungal keratitis in relation to duration of presentation (n=72)

Treatment outcome (No. of cases)	Duration of presentation		p value
	≤10 days No. (%)	≥10 days No. (%)	
Healed (n=52)	34 (65.38%)	18 (34.62%)	p <0.001
Penetrating keratoplasty (n=15)	04 (36.36%)	11 (63.64%)	p <0.05 (excluding 5 eyes gone into phthisis)
Phthisis (n=5)	-	05 (100%)	-

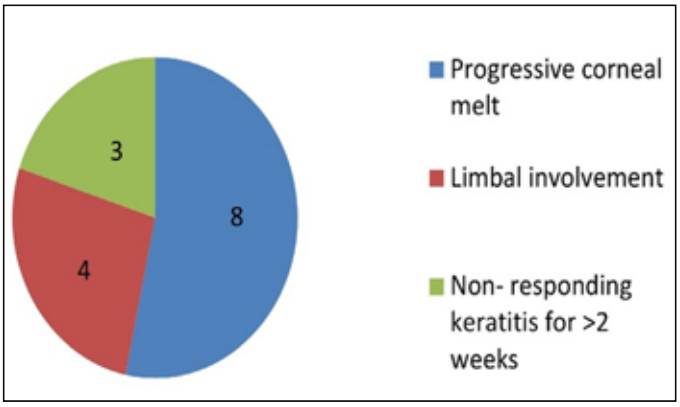

Figure 1: *Indications for Keratoplasty Number of eyes (n=15)*

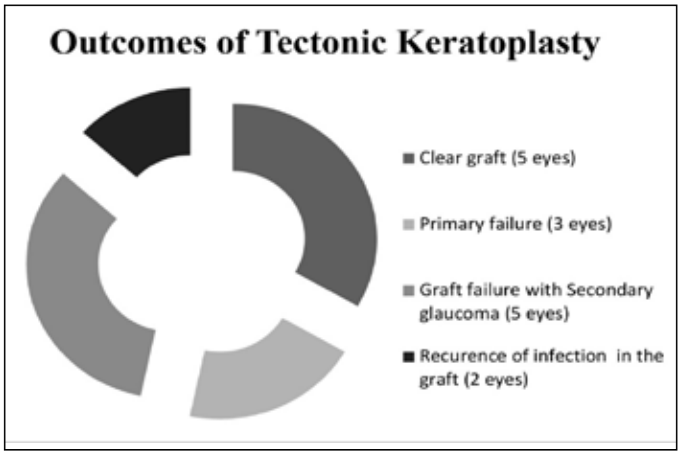

Figure 2: *Final outcomes of keratoplasty in 15 eyes at a 6 month follow-up period*

comparison of its results with a few recent studies reported from western as well as other parts of the country is shown in (Table 7). In the present study, fungal keratitis seems to be prevalent in males, the most common risk factor being corneal trauma, which is comparable with other studies.[14] Male patients may be more likely to be agricultural workers or involved in outdoor activities placing them at a higher risk of trauma or injury to cornea. Many studies report fungal keratitis to be more common in younger adults (<50 years) but in our study, 50% of the patients were in the age group of 51 to 70 years. Another study by Rautaraya et al had 63% of the cases in the age range of 50 to 60 years.

The incidence of the mycotic keratitis was found to be higher during the monsoon from July to September when the agricultural activity is greater, with higher chances of vegetative trauma and a greater propensity for fungal growth in a favourable humid environment, in contrast to the findings of another study done in Gujarat, which noted it to be common from September to December.

The clinical presentation of fungal keratitis is varied and largely depends on the type of fungus, severity of the invading pathogen, liberation of toxin, resistance of the host tissue and the age of the patient. The typical slit lamp bio microscopic presentation of a dry looking ulcer was seen in 66.67% and the feathery infiltration in 36.11%, as against 75.43% and 71.78% respectively observed in the study by Bharathi et al.

In almost all the studies, the diagnosis of fungal keratitis is remarkably efficiently done using simple methods such as KOH wet mount and Gram stain. Our study reported 104 (29.3%) of the total samples from corneal scrapings to be fungal on KOH wet mount, of which 72 were included in the study. Since the clinical acumen in the diagnosis of fungal keratitis varies according to the level of training and experience, it is essential to have a minimum facility of KOH wet mount in the clinic as a screening tool to diagnose it. In our study, the commonest causative organism isolated was Aspergillus niger and the second most common fungus was Fusarium, which is comparative to another study reported from north India. However, Saha et al reported Candida (19%) to be the second most common fungus isolated.

The overall success rate with only medical management in our study was 31.9%, which is lesser than that reported by the researchers (74.5%) using a similar protocol of medical management in their study.[17] 40.3% eyes requiring tissue adhesive application healed with scar formation, which was lesser in comparison to another study.[18]

Our study shows that treatment outcome in fungal keratitis remains less than satisfactory if the patient presents late, similar to the study in eastern India, where a large number of patients with poor outcome had presented later than 10 days of start of symptoms. The need for tissue adhesive application and penetrating keratoplasty was seen in significantly more number of patients who presented late which was similar to our study. However in our study, penetrating keratoplasty was performed after a mean duration of 14 days of presentation to the centre unlike in the study by Xie L et al, where it was done within 2 days of presentation in 82.7% cases.[19]

Limitations

The study is retrospective with a relatively small study sample and a short follow-up carried out at a referral centre, with a majority of the patients included, presenting late. Smear negative cases with positive fungal cultures were not included in the study. The surgical intervention in the form of keratoplasty was done relatively late due to late referrals at the tertiary centre and unavailability of donor corneas at that particular time.

Table 7:Comparison of microbiological and clinical data on mycotic keratitis from studies from various parts of India

	Eastern India	South India	West Bengal	North India	Western India			
Parameters	**Rautaraya et al 2016**	**Gopinathan et al 2002**	**Saha et al 2009**	**Chaudhary et al 2005**	**Deorukhkar et al. (Western India, Maharashtra) 2012**	**Tewari et al (Ahmedabad) 2012**	**Kumar A et al (Gujarat) 2011**	**Present study**
A. General								
Type of study	*Retrospective*	*Retrospective*	*Retrospective*	*Prospective*	*Retrospective*	*Prospective*	*Prospective*	*Retrospective*
Period of study with duration	*July 2006-December 2009 (3.5 years)*	*January 1991-December 2000 (10 years*	*January 2008 – December 2008 (1 year)*	*January 1999-June 2001 (2.5 years)*	*December 2004 to December 2009 (5 years)*	*July 2007-June 2008 (11 months)*	*September 2003-June 2005 (20 months)*	*July 2016-August 2018 (2 years)*
Sample size (total number of patients)	*997*	*3399*	*289*	*485*	*852*	*150*	*200*	*354*
Number of patients with proven fungal keratitis	*264 (26.4%)*	*1352 (39.8%)*	*110 (38.06%)*	*191 (39%)*	*311/537 (57.91%)*	*31/89 (34.9%)*	*22.5%*	*104 (29.3%) 72 included in the study*
B. Epidemiological factors								
Commonly affected Age range	*50-60 years (63%)*	*6-49 years (64.4%)*	*>50 years (60.81%)*	*31-40 years (37%)*	*21-30 years males 31-40 years females*	*21-40 years (52.6%)*	*<50 years*	*51-70 years (50%)*
Commonly affected Gender	*Males (70%)*	*Males (71.2%)*	*Males (65%)*	*Males (68%)*	*Males (68.31%)*	*Males (68%)*	*Males (61%)*	*Males (62.5%)*
Residence Urban Rural	*21% 79.5%*	*NA(Data not available)*	*NA*					*29.17% 70.83%*
Commonest risk factor	*Ocular trauma (40%)*	*Ocular trauma (54.4%) Vegetative trauma (13.9%)*	*Ocular trauma (47.29%) Vegetative trauma (22.97%)*	*Ocular trauma 42% Vegetative trauma (52.3%)*	*Ocular trauma (93.48%)*	*Ocular trauma (90%)*	*Ocular trauma (78.5%)*	*Ocular trauma (61.11%) Vegetative trauma (70.45%)*
Commonest months of presentation		*October to January & June to September*	*NA*	*September & October (37%)*	*NA*	*NA*	*September to December*	*July to September*
C. Microbiology								
Positive direct microscopy 10% KOH wet mount	*186 (70.5%)*	*1277*	*41/74 (55%)*	*119 (62%)*	*NA*	*21*	*68.4%*	*72/104 included in the study*
¬¬Gram stain positive	*199/252 (78.9%)*	*1181(88.2%)*	*NA*	*114 (60%)*	*NA*	*NA*	*NA*	*60/104(57.69%), 45 included in study*
Culture positive for fungus	*215*	*1352*	*74 (67.27%)*	*191*	*283*	*31*	*57 (40.9%)*	*36 (50%)*
Most common fungus isolated	*Aspergillus (27.9%)*	*Fusarium (37.2%)*	*Aspergillus (55.4%) Followed by Candida18.91%*	*Aspergillus (40.8%) Aspergillus niger (34%)*	*Fusarium (35.04%)*	*Aspergillus (35.4%)*	*Fusarium (29.82%)*	*Aspergillus (72.22%) Aspergillus niger (42.3%)*
D. Treatment given with outcome		*NA*			*NA*	*NA*	*NA*	
Healed scar with medical management	*35.6%*	-	*40.55%*	*NA*	-	-	-	*21 (29.16%)*
Tissue adhesive and BCL application	*18.9%*	-	*NA*	*NA*	-	-	-	*29 (40.28%)*
Tectonic/therapeutic keratoplasty	*19.7%*	-	*59.45% Aspergillus in 39.02%*	*18.8%*	-	-	-	*20.8% Aspergillus flavus in 4 eyes*
Evisceration/ phthisis	*3.4% eviscerated*		*NA*	*8 eyes eviscerated*				*5 eyes(6.94%) phthisis*
E. Follow up	*43±115 days*	*NA*	*NA*	*5.5 months*	*NA*	*NA*	*NA*	*6.19±3.21 months*

(NA- Data not available)

Conclusion

The regional information of common etiological agents and predisposing factors from our study can be helpful in starting an empirical therapy based on high degree of clinical suspicion and formulating preventive measures in a population at risk of fungal keratitis.

Our study also emphasizes that direct microscopy with KOH wet mount preparation can be relied upon as an easily accessible screening test for rapid diagnosis of mycotic keratitis. Cyanoacrylate glue, previously thought to be a temporary measure is effective and can be a definitive treatment in the absence of surgical quality donor tissue for

therapeutic or tectonic keratoplasty, thereby decreasing the need of early surgical intervention in severe cases of fungal keratitis. History of late presentation, large extent of keratitis and presence of thinning/perforation at presentation were associated with the need for keratoplasty.

Future prospective studies can be done to include more number of patients with detailed analysis of visual outcomes of different modalities of treatment in the same as well as different zone of India.

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