Journal of Population Therapeutics & Clinical Pharmacology

RESEARCH ARTICLE
DOI: 10.53555/jptcp.v31i7.6965

STUDY TO EVALUATE THE EFFICACY OF ANTIFUNGAL DRUGS AGAINST ASPERGILLUS KERATITIS

Dr. Pragya Prakash[1*]

[1*]Assistant Professor, Department Of Ophthalmology, Sri Aurobindo Medical College and PGI

***Corresponding author :**Dr. Pragya Prakash
*Email:- drpragyaeye@gmail.com

INTRODUCTION

Diseases affecting the cornea are the major cause of blindness in developing countries and infective keratitis is the main cause for this. Mycotic keratitis is more common in the tropical and developing countries which suggest warm climate and poor hygiene are important causative factors.Globally, the incidence of keratomycoses and systemic mycoses is rising.[1] The common cornea pathogenic fungi include *Aspergillus* spp., *Fusarium* spp. and *Candida* spp., with regional variations depending on geographical, occupational and host factors [2]. Corneal infections due to filamentous fungi are found to predominantly cause infection in tropical and subtropical regions, and yeasts more frequently in temperate climates [1-2]. The most common fungi isolated in tropical regions is *Aspergillus* spp. (31.1%), followed by *Fusarium* spp. (24.5%), *Alternaria* (10.5%), *Curvularia* (10.2%), [2]. The demonstration of fungal elements in microscopic examination and culture has been the gold standard for diagnosing fungal keratitis The use of antifungal medications is considered the main treatment for fungal keratitis. It is recommended to start antifungal therapy after confirmation of the clinical diagnosis with a smear or positive cultures. Topical application of antifungal medications is a mainstay for the treatment of fungal keratitis.

Current therapies are often ineffective. Ongoing research toward rapid diagnosis and specific drug therapy could minimize the morbidity caused by this preventable disease. [2] . high ocular morbidity and variations in drug effects prompted us to undertake this study for improving the outcome of fungal keratitis in our patients.

Materials and methods

This prospective study was conducted in department(s) of Cornea & Microbiology, Aravind eye hospital Coimbatore from july-2009 to jan-2011.The study was performed on 40 eyes of as many patients to determine and compare the efficacy of antifungal agents clinically (ulcer status)) against *Aspergillus* keratitis . Each patient with suspicion of fungal keratitis underwent a thorough clinical examination using a slit lamp biomicroscope to measure size and depth of ulceration following a detailed clinical and demographic history. Ulceration was defined as a loss of the corneal epithelium with underlying stromal infiltration and suppuration associated with signs of inflammation with or without hypopyon. A standardized form was filled for each patient documenting socio-demographic information as well as clinical information including duration of symptoms, previous treatment, predisposing ocular conditions and associated risk factors.

Inclusion riteria:

All patients with high index of clinical suspicion for fungal corneal ulcer were selected on the basis of thorough slit lamp examination & detailed history.

Exclusion criteria :

- Uncontrolled diabetic patient
- Immunocompromised patient
- Complicated ulcer like perforated ulcer /ulcer with impending perforations
- Large ulcers more than 7mm in largest diameter
- Small ulcer less than 2mm in smallest diameter
- Pediatric patients

CLINICAL PROCEDURES:

Each patient was examined by the slit lamp biomicroscope. The size of the epithelial defect after staining with fluorescein was measured with the variable slit on the biomicroscope and recorded in millimeters on a standardized form. In similar fashion, the size and depth of the stromal infiltrate was recorded. A sketch of each ulcer was also drawn on the form using standardized frontal and cross sectional diagrams, and the presence or absence of a hypopyon was recorded as shown in the clinical photographs (Fig 1a -1d). The height was measured in millimeters. Associated ocular conditions such as blepharitis, dacryocystitis, dry eyes, corneal anesthesia, or ocular leprosy were also noted.

After a detailed ocular examination corneal scrapings were performed under aseptic conditions using a Kimura spatula. Scrapings were performed under slit lamp biomicroscope after instillation of 4% lignocaine (lidocaine). Material obtained from scraping, the leading edge and the base of each ulcer was smeared on three separate glass slides: one each for Gram and Giemsa stain and the third for 10% potassium hydroxide (KOH) mount to identify fungus. All KOH smears were then sent to the laboratory for confirmation. Subsequent scrapings were plated onto blood agar, chocolate agar and potato dextrose agar (PDA). The patients with proved fungal keratitis (smear and culture positive for fungus) with an ulcer area of at least 2 mm^2 and not more than 7 mm^2 were included in the study. Fungal cultures inoculated onto PDA were incubated at 27ºC, examined daily, Fungi were identified by their colony characteristics on PDA and by their microscopic appearance in lactophenol cotton blue. Various *Aspergillus* species were identified.

Clinical photographs

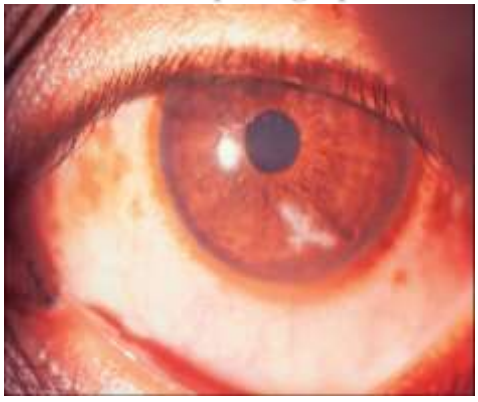


Fig1a: Very early fungal keratitis resembling showing dendritic ulcer typical feathery borders

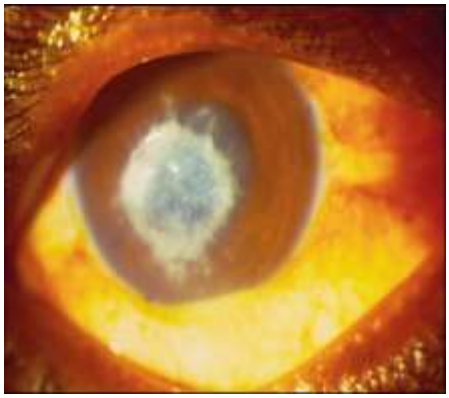


Fig1b: Fungal Keratitis Showing Dendritic Ulcer Base With Yellowish-White

Fig1c: Fungal ulcer with hypopyon

Fig1d: Ring infiltrate

All fungal ulcer patients [286 from july-2009 to feb-2010] were treated with standard regimen followed at our institution i.e. combination of antifungals Aurozole (eyedrops), Natamycin (eyedrops),Itraconazole (eye ointment) Homatropine (eyedrops), Diclofenac (tablet), ketoconazole (tablet) for 3days ,after KOH culture sensitivity results. After 1 month, among 286 fungal ulcer patients 40 *Aspergillus* culture positive cases were selected All patients were examined by slit lamp on daily basis.

Patients were discharged from the hospital at the end of the first week with instructions to instill the eye drops in the affected eye at intervals of 2 hours .Each patient was subsequently examined by the slit lamp for size and depth of the infiltrate at weekly intervals for up to 4 weeks. Details of signs including lid edema, congestion of the conjunctiva, and hypopyon were recorded for each subject. The presence or absence of hypopyon in the anterior chamber was noted, and quantified in millimeters.

Similar to Prajna et al [8], we also defined a healed corneal ulcer as a completely healed epithelial defect with no stain on fluorescein application, and non-progression of the stromal infiltration. A corneal ulcer was considered to be healing if the epithelial defect decreased in size by at least 20%, with non-progression or decrease in the size of the stromal infiltration by at least 20%. A corneal ulcer was considered to remain the same if the size and depth of the infiltrate remained same after initiating treatment.

These *Aspergillus* keratitis patients were monitored closely for the response of initial standard antifungal agent and change of any other antifungal agent if required in cases of non – healing ulcer following 7-antifungal drugs were studied:

1. Voriconazole

2. Natamycin
3. Ketoconazole
4. Amphotericin B
5. Itraconazole
6. Econazole
7. Clotrimazole

Observation &Results

Following are the results and observations of the prospective study.

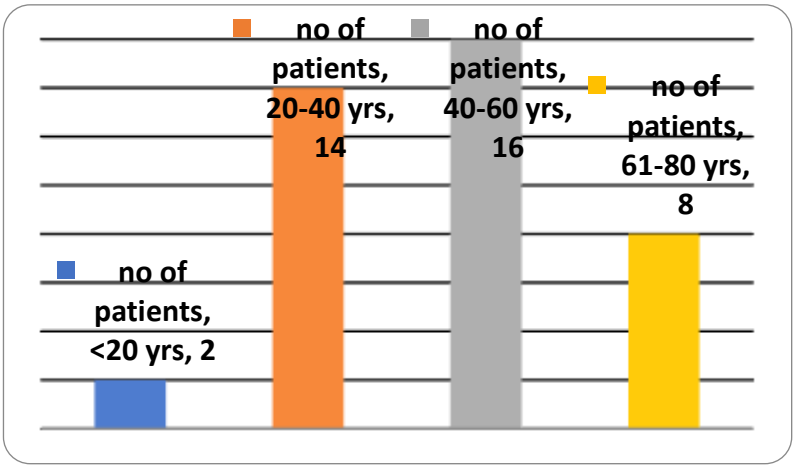
.

(1) AGE DISTRIBUTION:

Table-1: Age wise Distribution

S.no	Age Group	No. of patients (%)
1	< 20 yrs	2 (5%)
2	20-40 yrs	14 (35%)
3	40-60yrs	16 (40%)
4	61-80yrs	8 (20%)
total		**40**

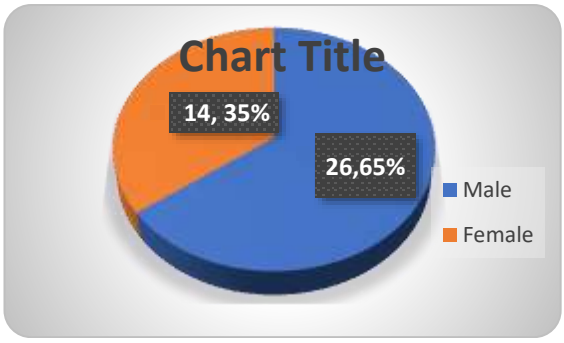
A total of 40 patients were included in the study. The youngest was 15 years of age and the oldest was of 73 years. According to age distribution, 2 (5%) patients had age less than 20 years and 14 (35%) were between 20-40 years. Majority i.e. 16 (40%) of patients belonged to 40-60 year age group. Rest 8 (20%) patients had age between 61-80 years (graph-1).



Graph 1: Age wise distribution

(2) SEX DISTRIBUTION:

Males constituted 26 (65%) cases while females constituted 14 (35 %) cases of the total 40 cases that were studied (graph-2)



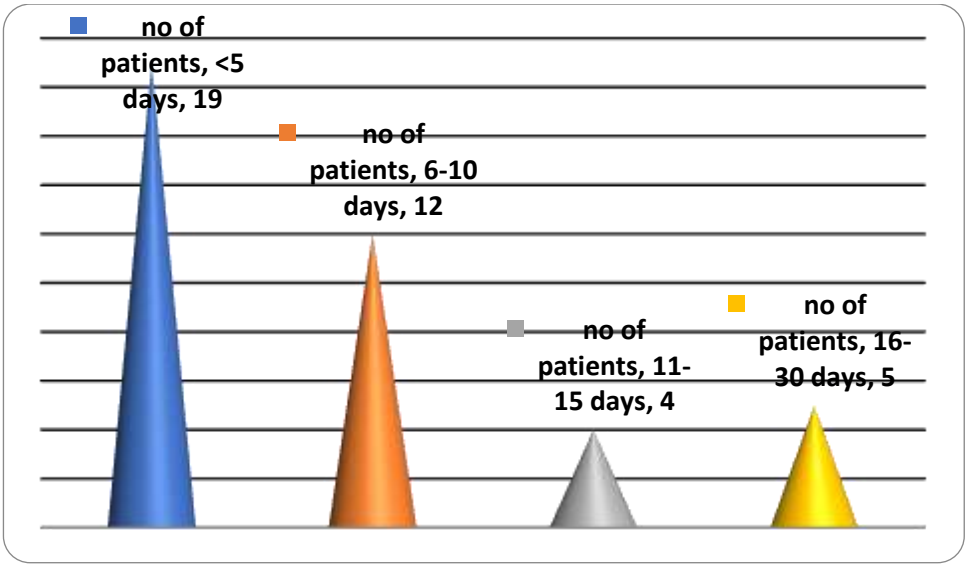
Graph -2: **Distribution of cases according to gender**

(3) DURATION OF SYMPTOMS:

Table-2: Distribution according to duration of symptoms

S.no	Duration of Symptoms	No. of patients (%)
1	< 5 days	19(47.5%)
2	6-10 days	12 (30%)
3	11-15 days	4 (10%)
4	16-30 days	5 (12.5%)
total		**40**

The earliest presentation in our study was within 1 day of starting of symptoms and the latest was 25 days after the symptoms appeared. Majority of patients i.e. 19 (47.5 %) presented within 5 days of appearance of symptoms.12 patients (30%) presented between 6-10 days and 4 (10%) patients presented between 11-15 days. A delayed presentation between 16-30 days was also observed in 5 (12.5%) patients (graph 3).



Graph 3: Distribution of cases according to duration of symptoms

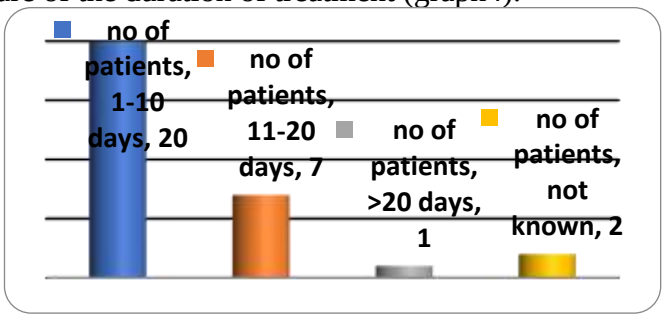
(4) DURATION OF PREVIOUS TREATMENT TAKEN:

Table-3: duration of previous treatment

Previous treatment taken (n=30)		**No previous treatment** (n=10)
Duration	**No. of patients (%)**	
1-10 days	20(50%)	-
11-20 days	7 (17.5%)	-

>20 days	1 (2.5%)	-
Not known	2 (5%)	-
Total	**30 (75%)**	**10 (25%)**

Out of total 40 patients, 30 (75%) patients had undergone some sort of treatment previously and 10(25%) patients had never taken any medication. Among these 30 patients, 20 (50%) had undergone treatment for 1-10 days. Another 7(17.5%) patients had taken treatment for 11-20 days and 1(2.5%) patient had undergone treatment for more than 20 days. 2 (5%) patients who took some sort of medication were not aware of the duration of treatment (graph4).



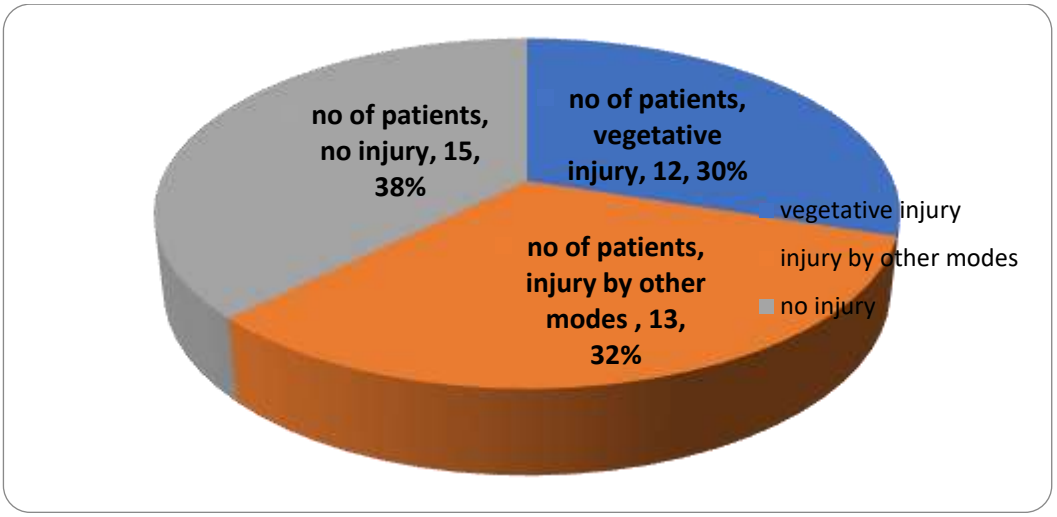
Graph 4: Distribution of cases according to duration of previous treatment

(5) MODE OF INJURY:

In Our study history of vegetative injury(by wooden stick etc.) was given by 12 (30%) patients while 13(33%) patients gave history of injury from other modality like cow's tail or stone injury.Rest 15 (37%) patients history was non-specific and no injury could be ascertained(graph 5).

Table-4: Distribution as per mode of injury

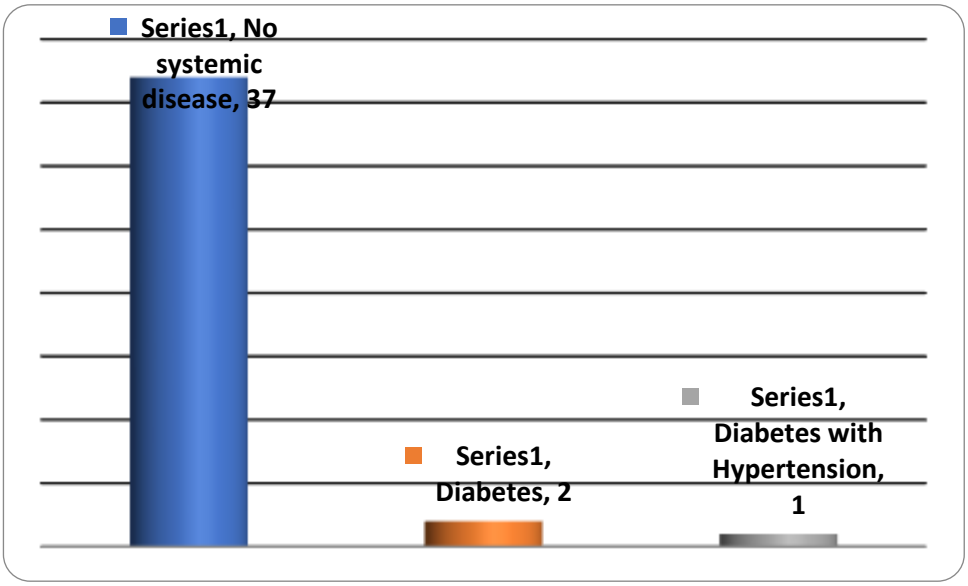
S.no	Age Group	No. of patients (%)
1	Vegetative injury	12 (30%)
2	Injury by other modes	13 (33%)
3	No injury	15 (37%)
total		**40**



Graph 5: Distribution of cases according to mode of injury

(6) ASSOCIATED SYSTEMIC DISORDERS:

In our study only, out of the total 40 patients only 2 patients (5 %) had Diabetes mellitus and 1 patient (2.5 %) had both Diabetes mellitus and hypertension. In remaining 37 patients (92.5%) no associated systemic disorder was found (graph 6)



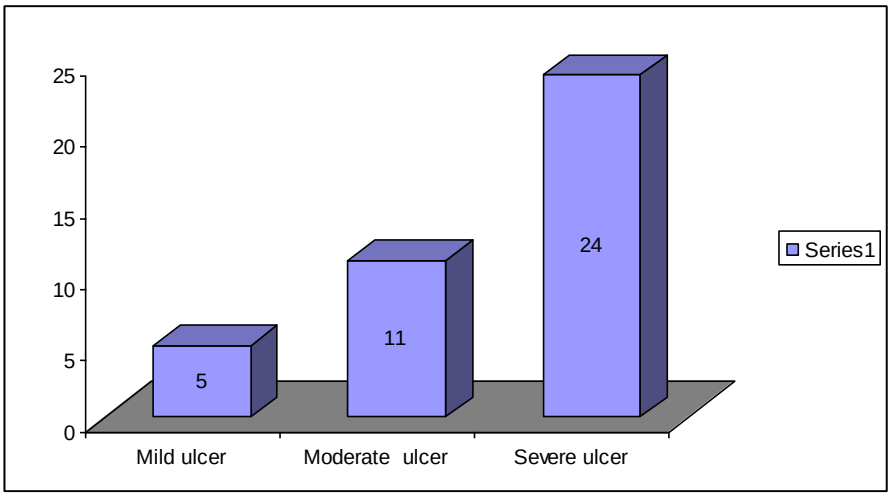
Graph 6: Distribution of cases as per associated systemic disorders

(7) ULCER SEVERITY:

Table-5: Distribution as per ulcer severity

S.no	Age Group	No. of patients (%)
1	mild	5 (12.5%)
2	moderate	11(27.5%)
3	severe	24 (60%)
total		40

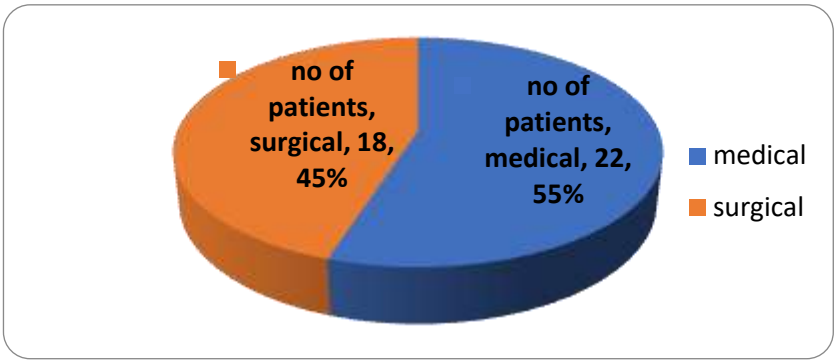
On assessment of the severity of corneal ulcers we found that out of 40 patients 5 (12.5%) and 11(27.5%) patients had mild and moderate ulcers respectively. Severe corneal ulcers were detected in 24 (60%) patients .



Graph 7: Distribution of cases as per Ulcer Severity

(8) MANAGEMENT:

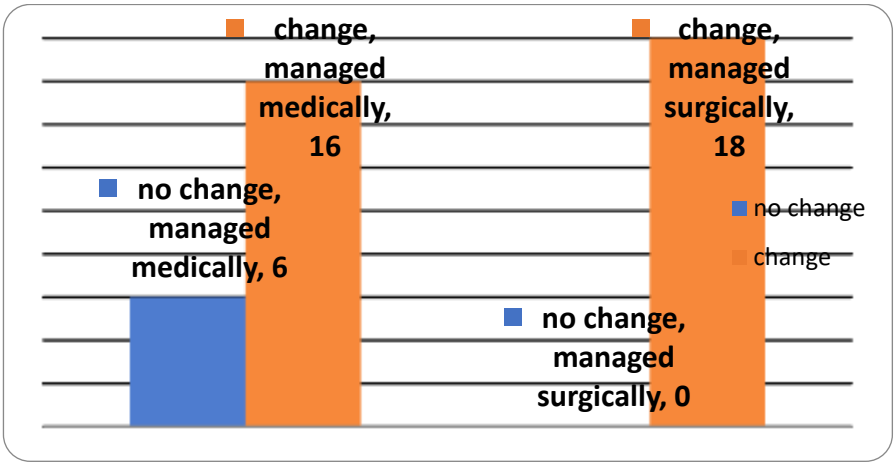
In this study, out of 40 patients, 22(55%) were managed successfully managed medically and 18 (45%) required surgical intervention



Graph 8: Distribution of Patients as Per Management

(9) CHANGE IN MANAGEMENT AFTER INITIAL TREATMENT:

In 34 (85%) patients a change in management was required after correlation of clinical and laboratory findings including culture and sensitivity analysis. 16 (40%) patients required change in medical management and 18 (45%) patients required surgical intervention. Only 6 (15%) patients were managed without any alteration in initial management strategy.



Graph 9: Change in management after initial treatment

(10) CHANGES IN INITIAL MANAGEMENT ON BASIS OF RESPONSE:

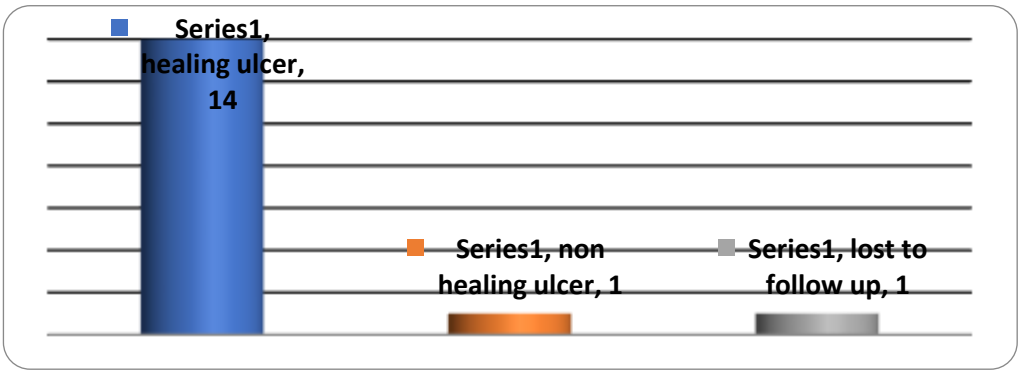
As mentioned previously, only 6 (15%) patients were managed without any alteration in initial management. In the rest 34(85%) patients a change in management either in the form of addition of antifungal agent or surgical intervention was required. Among these 34 patients, 16 were successfully treated medically with addition of Voriconazole, Amphotericin B, Natamycin and combined therapy in 9 (22.5%),4(10%),1(2.5%) and 2(5%) patients respectively. Remaining 18 (45%) patients required surgical intervention.

Table7: Distribution on the basis of change in treatment

S.No	**Change in treatment** (n=34)		**No change in treatment** (n=6)
	Intervention	**No .of patients**	
1	Add Voriconazole	9	-
2	Add Amphoterecin B	4	-
3	Combined	2	-
4	Add Natamycin	1	-
5	Surgery	18	-
	Total	34	6

(11) ULCER STATUS AFTER CHANGE IN MEDICAL THERAPY:

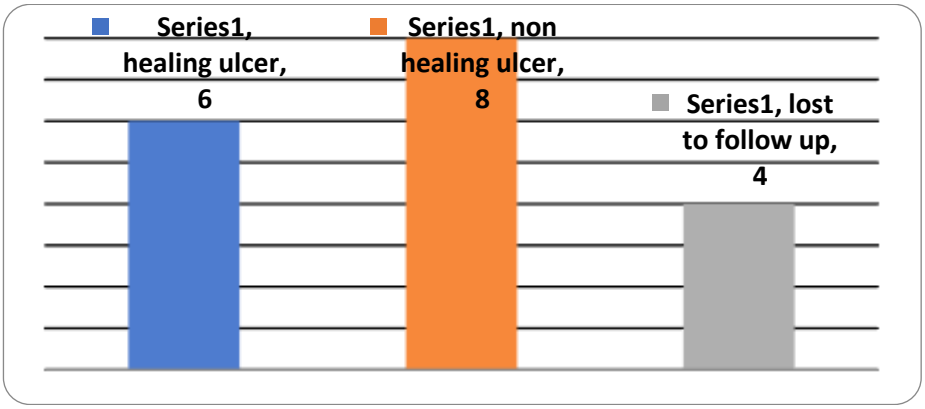
On correlating clinical and laboratory findings including culture and sensitivity 16(40%) patients required change in management and were managed successfully managed medically. Out of them, 14(35%) patients showed significant improvement in the form of ulcer healing. Non healing ulcer was detected in 1 (2.5%) patient and 1 (2.5%) lost to follow up.



Graph10: Distribution as per Ulcer status after change in medical therapy

(12) STATUS OF ULCERS AFTER SURGICAL INTERVENTION:

Similarly, after clinical and laboratory correlation, 18 patients (45%) required surgical intervention as change in management. Out of them, 6(15%) patients showed significant improvement in the form of ulcer healing. Unlike patients managed medically, here non-healing ulcers were detected in 8 (20%) patient and 4 (10%) lost to follow up (graph12).



Graph 11: Distribution as per ulcer status after surgical intervention

(13) FUNGAL STRAINS ISOLATED BY CULTURE:

Table 8

S.no	Fungi	**No. Of patients**	**%**
1	*Fusarium*	125	43.71
2	*Aspergillus spp*	40	13.99
3	*Cuvularia spp*	17	5.94
4	*Penecillium spp*	3	1.05
5	*Scedosporium spp*	3	1.05
6	*Cladosporidium spp*	7	2.45
7	*Exerohilum spp*	7	2.45
8	*Bipolaris*	4	1.4
9	*Candida*	18	6.29

10	Unidentified hyphae	36	12.59
11	Unidentified Dermatecious	26	9.09
TOTAL		**286**	**100**

In our institute *Fusarium* is the most common cause of fungal keratitis 125 cases (43.71%) followed by *Aspergillus* which contribute to 40 (13.99%) of the cases while other fungal species like *Candidia*(6.29%), *Culvularia(*5.94%) *Cladosporidium* etc. are less common

(14) FOLLOW UP OF PATIENTS:

All patients were followed up regularly. Majority of patients i.e.17 (42.5%) patients followed up to 14 days while 5 (12.5%) patients belonged to 15-30 days and 61-90 days group each. 4 (10%) patients followed up to 31-60 days and 6(15%) patients lost to follow up

Table-9: Distribution as per follow up

S.no	Age Group	No. of patients (%)
1	Upto 14 days	17(42.5%)
2	15-30 days	5 (12.5%)
3	31-60 days	4 (10%)
4	61-90 days	5 (12.5%)
5	>90 days	3 (7.5%)
6	Lost to follow up	6 (15%)

DISCUSSION

Fungal corneal ulcer is not only the common disorder but also a major cause of visual loss especially in those parts of world where a large number of populations belongs to agriculture malnutrition is common, and climate is hot and humid. All these factors predispose population to develop fungal corneal ulcer following ocular trauma.

Bharathi et al[3] reported that approximately, 65 % of the patients are in the age group 21 to 50 years, although it has been reported in extremes of age also.K.V. Raju et al[4] also reported that the peak age group was 41-50 years age group. [4] However, in study conducted by Vijaya S. Rajmane and collagues maximum number of corneal ulcer cases were observed between 51 to 70 years (44.59%) followed by 31 to 50 years (36.48%).[5]Similarly Srinivasan et al also documented the predominance of corneal ulceration in the middle years with sixth decade of life being most susceptible.[6]

In present study a total of 40 patients were included. The youngest was 15 years of age and the oldest was of 73 years. According to age distribution, 2 (5%) patients had age less than 20 years and 14 (35%) were between 20-40 years. Majority i.e. 16 (40%) of patients belonged to 40-60 year age group. Rest 8 (20%) patients had age between 61-80 years. In our study average age of presentation is between 41- 60 yr of age which is in correlation with other studies. This could be due to the fact that they are physically active and working outdoors and prone to injury.

S.No	Author	Age Group Most commonly affected
1	Bharathi et al [3]	21 to 50 years
2	K.V. Raju et al [4]	41-50 years
3	Vijaya et al [5]	51 to 70 years
4	Srinivasan et al [6]	sixth decade
5	Present study	41- 60 yr

Sharma N[2] et al has reported that fungal keratitis occurs more commonly in males than females with the ratio varying from 1.5:1 to 4.5:1. Bharathi et al reported that male patients were commonly affected (65%).[3] Males again predominated in the study by Vijaya et al accounting for 35(66.21%)

cases in contrast to females i.e.25(33.78%) cases. The male to female ratio was found to be1.96:1. [5]Similarly, in study conducted by Srinivasan et al amongst total 434 patients 266 (61.3%) were males and 168 (38.7%) were females.[6]
Similar to previous studies our study also shows that males are affected more than females. Males constituted 26 (65%) cases while females constituted 14 (35 %) cases of the total 40 cases .The male to female ratio was found to be 1.86:1.

S.No	Author	M:F ratio	% of males
1	Bharathi et al [3]	1.86:1	65%
2	Sharma et al [2]	1.5:1	-
3	Vijaya et al [5]	1.96:1	66.21%
4	Srinivasan et al [6]	1.41:1	61%
5	K.V. Raju et al [4]	3.28:1	77 %
6	Present study	1.86:1.	65%

Injury to the cornea is the leading cause of microbial keratitis, particularly fungal keratitis. It most often occurs outdoors and involves plant matter such as leaves and paddy grains. It may also occur after injury with mud or sand and even injury from animal origin such as due to cow dung and cow tail and may occur even with metal pieces. History of corneal trauma was given by 92% patients in Bharathi et al. , 72.6 % in K V Raju et al and in 65.4% by Srinivasan et al. [3,4,6] Among them corneal trauma with vegetable matter or organic matter was reported in 61% by Bharathi et al ,43.3 % by K V Raju et al. and 52.8 % by Srinivasan et al . [3,4,6]
In Our study history of corneal trauma was given by 25(63%) patients. History of injury by vegetative matter (by wooden stick etc.) was given by 12 (30%) patients while 13(33%) patients gave history of injury from other modality like cow's tail or stone injury. Rest 15 (37%) patients history was non-specific and no injury could be ascertained. As evident from all the studies fungal keratitis is more common in with patients with corneal trauma more so with vegetative matter.

S.No	Author	History of trauma	% of vegetative injury
1	Bharathi et al [3]	92%	61%
2	Srinivasan et al [6]	65.4%	52.8 %
3	K.V. Raju et al [4]	72.6 %	43.3 %
4	Present study	63%	30%

Filamentous fungi form the major etiologic agents of fungal keratitis. In study conducted by Srinivasan *Fusarium* species (37–62%) and *Aspergillus* species (24–30%) have been implicated as main pathogens. Dematiaceous fungi are the cause of 8 to16.7% of cases of fungal keratitis. [6] In another study by KV Raju et al out of the 30 cases studied the most common fungi isolated was *fusarium* species in 13 cases (42.9 %). This was followed by *Aspergillus* species in 6 cases (19.8 %) and *Curvularia* and *Pencillium* in 6.66% and 3.33% each respectively. [4] In one more study published by Srinivasan M and coworkers of 155 total isolates, 73 were *Fusarium* spp (47.1%), 25 were *Aspergillus* spp (16.1%), and six were *Botryodiplodia theobromae* (3.9%). [6] Vijaya and colleagues found 25 fungal culture positive cases; *Aspergillus* was more prevalent (18.91%). *Fusarium solani* was isolated in 5.4% and *Curvularia s*pecies in 2.7% of the cases. [5] Leck et al published their study where *Fusarium* was isolated in 39.9%,*Aspergillus* spp in 21.5% and *curvularia i*n 9.6% cases. [7] Prajna L et al in their study on 90 isolates detected 41 *Aspergillus* species,38 *Fusarium s*pecies, and 11 others including 4 *Curvularia* and 3 *Bipolaris* spp respectively. [8]
In our institute *Fusarium* is the most common cause of fungal keratitis 125 cases (43.71%) followed by *Aspergillus* which contribute to 40 (13.99%) of the cases while other fungal species like *Candida* (6.29%), *Culvularia(*5.94%) *Cladosporidium* etc. are less common which is in conjunction with other studies from this region.

Aspergillus spp and *Fusarium* spp are the most frequently reported fungal pathogens isolated from cases of fungal keratitis in the tropics .Studies in south India have reported *Fusarium* spp tend to be more common than *Aspergillus* species. *Aspergillus* species predominate in northern India, Nepal, and Bangladesh. This phenomenon may be explained by differences in climate and the natural environment.

S. No	Author	Total cases	Fusarium	Aspergillus	Others
1	Srinivasan [6]		37–62%	24–30%	Dematiaceous fungi
2	Vijaya [5]	25	5.4%	18.91%	Curvularia 2.7%
3	Srinivasan M et al[6]	155	47.1%	16.1%	*Botryodiplodia* 3.9%
4	K.V. Raju et al [4]	30	42.9 %	19.8 %	Curvularia 6.66% Pencillium 3.33%
5	Leck et al [7	353	39.9%,	21.5%	curvularia in 9.6%
6	Prajna L et al [8]	90	42.22%	45.55%	Curvularia 4.44% Bipolaris 3.33%
7	Present study	286	43.71	13.99	Candida (6.29%), Culvularia(5.94%)

In study conducted by Leck et al various *Aspergillus* species isolated were *A. flavus* in16.7%, *A fumigatus* in 4.2% and *A.niger* in 0.3% cases.[7] study by Vijaya et al *Aspergillus niger* (6.75%), *Aspergillus nidulans* (5.4%), *Aspergillus flavus* (4.05) & *Aspergillus tereus* (2.7%) were identified.In another study from South India, Prajna L et al detected 41 *Aspergillus* species of which *A. flavus* in 33.33%, *A. fumigatus* in 5.5 %, *A.teres* in 4.44% and *A.niger* in 0.3% cases were reported.[8]
In our institution, 40(13.99%) cases were caused by Aspergillus spp. Out of them 35 cases(12.24%) were due to Aspergillus flavus,2 cases(0.7%) were caused byA. Fumigatus and A.teres respectively and 1(0.35%) case was caused by A.niger.

Aspergillus spp	Leck et al[8]	Vijaya et al[5]	Prajna et al[9]	Present study
A.flavus	16.7%,	4.05 %	33.33%	**12.24%**
A.fumigatus	4.2%	-	5.55%	**0.7%**
A.teres	-	2.7%	4.44%	**0.7%**
A.niger	0.3%	6.75	2.22%	**0.35%**
A.nidulans	-	5.4%	-	-
total	21.5%	18.9%	45.55%	**13.99%(40/286)**

In our study only, out of the total 40 patients only 2 patients (5 %) had Diabetes mellitus and 1 patient (2.5 %) had both Diabetes mellitus and hypertension. In remaining 37 patients (92.5%) no associated systemic disorder was found (graph 6)
On assessment of the severity of corneal ulcers we found that out of 40 patients 5 (12.5%) and 11(27.5%) patients had mild and moderate ulcers respectively. Severe corneal ulcers were detected in 24 (60%) patients .
Srinivasan M et al reported in their study from South India that over 60% of the patients presented for examination during the first week of their illness. [6] The relatively short duration of symptoms before examination is an interesting contrast with findings in Nepal as published by Upadhyay et al where only 43.7% of patients were seen in the first week of their illness and 19.3% took longer than a month to reach to the hospital. [9] In our study, the earliest presentation was within 1 day of starting of symptoms and the latest was 25 days after the symptoms appeared. Majority of patients i.e. 19 (47.5 %) presented within 5 days of appearance of symptoms and 12 patients (30%) presented

between 6-10 days. This can be attributed to the fact that transportation is excellent in southern India and patients travel great distances The majority of patients in south India appear to have access, even if somewhat limited, to relatively sophisticated eye care.On correlating duration of symptoms with ulcer status we found that the duration of presentation also affects the prognosis of ulcer as reported by a previous study.

It is evident from the data that majority of the patients whose ulcers have healed well presented early in the disease while those presenting late had poor outcome (non-healing ulcer). The analysis shows that early presentation significantly improves the outcome in fungal keratitis patients. This again emphasizes the role of early diagnosis and prompt treatment for the patients of fungal keratitis in improving the final outcome .statistical analysis showed that 12.5% of the patients had mild ulcer, 27.5% of them had moderate ulcer and 60% of them suffered from severe ulceration.

Among the various available antifungal drugs, Natamycin was given to 52.5% patients as first line drug. The chi-square analysis showed the p-value 0.041, which is less than the level of significance 0.05. Hence H_1 hypothesis accepted. We therefore inferred that ulcer severity has significant association with the initial treatment given at Aravind eye hospital.Our study showed that we have changed the intial medical (anti-fungal) therapy in 16 patients out of 40 i.e. in 40% patients. Out of these 16 patients 8 patients presented with severe ulcer, 7patients with moderately severe ulcer & 1patient had mild corneal ulcer.Out of the the 8 patients suffering from severe corneal ulcer, the antifungal therapy was changed from Natamycin to Voriconazole in 7 patients and 1 patient was started on combined regimen of Voriconazole and Amphotericin B.In moderately severe ulcer patients out of 7 patients, 4 patients were shifted to Amphotericin B, 2 patients were shifted to Voriconazole and in 1 patient combined therapy was administered.In the only patient who had mild ulceration natamycin was changed from antibacterial medication after culture results..

From the study is inferred that Voriconazole is better antifungal agent in severe grade ulcers and Amphoterecin B showed better results in moderately severe ulcers. On correlating in vitro and in vivo response we detected that out of 9 patients with severe keratitis in whom Voriconazole was been given 8 patients responded very well to the therapy (88.9%) . Similarly, all 4 patients who received Amphoterecin B either as single agent or in combination with Voriconazole responded well to treatment in form of healing of the ulcer.

Similarly, Srinivasan M. et al showed that Polyene antifungal antibiotics, the first-line therapy in fungal keratitis, are not effective in severe keratomycosis. Imidazole derivatives such as voriconazole may be the better choice in the future. [6]Hijab Mehta et al also proposed Voriconazole for the treatment of refractory Aspergillus fumigatus keratitis. With broad spectrum of coverage, good tolerability, and excellent bioavailability with oral administration, voriconazole may be a good alternative against fungi-resistant to standard antifungal agents; however, the expenditure involved in voriconazole treatment will pose a constraint in its more frequent usage. [10] Raoul Erbrecht et al in a multicenter study concluded that in patients with invasive aspergillosis, initial therapy with voriconazole led to better responses and improved survival and resulted in fewer severe side effects than the standard approach of initial herapy with amphotericin B. [11]Suganthini Krishnan et al also confirmed that voriconazole shows excellent fungicidal activity against A. fumigatus hyphae as determined by kill-curve experiments and a fungal cell viability test. This fungicidal activity is superior as compared with that of amphotericin B. [12] Suganthini Krishnan and co workers also addressed the intriguing question is why voriconazole is highly effective against *Aspergillus* hyphae as a fungicidal agent as compared to amphotericin B. Their recent investigation showing the effect of voriconazole on the gene expression profile of actively growing *A. fumigatus* mycelia by partial microarray experiments suggested several possible candidates, including hyp1 and chsE genes, whose functions are known to be important for the vegetative and reproductive growth phases of Aspergillus species. [12

Conclusion

The incidence of *Aspergillus* keratitis in our institution is 13.99% of all fungal corneal ulcers (40 cases out of total 286 cases examined) Most common age group affected is 41-60 years with male predominance (65%).Male to female ratio is 1.86:1. Corneal injury is the most important risk factor for developing fungal keratitis seen in 63% patients in our study. Injury vegetative matter which is more specific for fungal keratitis was observed in 30% of the patients.

Early presentation to the hospital i.e. short duration of symptoms significantly improve the outcome for fungal keratitis. Early diagnosis and prompt treatment is therefore imperative for improving the final outcome. Voriconazole is better antifungal agent in severe grade ulcers and Amphoterecin B showed better results in moderately severe ulcers than the existing Natamycin therapy. Correlating both in vivo and in vitro analysis authors recommend Voriconazole as drug of choice for severe corneal ulcers and Amphoterecin B as the drug of choice for moderately severe corneal ulcers caused by Aspergillus species. Natamycin can be used as initial treatment for mild and early presenting cases.

REFERENCES

1. Whitcher JP, Srinivasan M. Corneal ulceration in the developing world – a silent epidemic. Br J Ophthalmol. 1997;81:622–623.
2. Namrata Sharma , Prakash C. Agarwal , Rajesh Sinha et al. Fungal Keratitis.DOS Times 2009; 14(10);49-58
3. Bharathi M.J., Ramakrishnan R., Meenakshi R., Padmavathy S., Shivakumar C., Srinivasan M. Microbial keratitis in South India: Influence of risk factors, climate, and geographical variation. *Ophthalmic Epidemiol.* 2007;14:61–69.
4. K.V. Raju , M.S. Vijayalakshmi, Lakshmi J. Clinical Study of Fungal Corneal Ulcer. Kerala Journal of Ophthalmology 2008; 20(2):148-50.
5. Vijaya S. Rajmane, Mangala P. Ghatole and Sarita N. Kothadia. Prevalence of Oculomycosis in a Tertiary Care Centre. Al Ameen J Med Sci 2011; 4(4):334-38.
6. Srinivasan M, Christine A Gonzales, Celine George et al .Epidemiologyand aetiological diagnosis of corneal ulceration in Madurai, south India. Br J Ophthalmol 1997; 81: 965-71.
7. A K Leck, P A Thomas, M Hagan et al. Aetiology of suppurative corneal ulcers in Ghana and south India, and epidemiology of fungal keratitis. Br J Ophthalmol 2002; 86: 1211–15. 63.
8. Prajna Lalitha, Brett L. Shapiro, Muthiah Srinivasan et al.Antimicrobial Susceptibility of Fusarium, Aspergillus and Other Filamentous Fungi Isolated From Keratitis. Arch Ophthalmol 2007;125:789-93.
9. Upadhyay MP, Karmacharya PC, Koirala S, et al. Epidemiologic characteristics, predisposing factors, and etiologic diagnosis of corneal ulceration in Nepal. Am J Ophthalmol 1991; 111:92–964.
10. Hijab Mehta, Hitendra B Mehta, Prashant Garg et al. Voriconazole for the treatment of refractory Aspergillus fumigatus keratitis. Indian J Ophthalmol 2008; 56 (3); 243-4564
11. Raoul H Erbrecht, David w. Denning, Thomas F. Patterson et al. voriconazole versus amphotericin b for primary therapy of invasive aspergillosis.N Engl J Med 2002; 347, No. 6; 408-15.
12. Suganthini Krishnan, Elias K. Manavathu Pranatharthi H et al.Comparative study of fungicidal activities of voriconazole and amphotericin B against hyphae of Aspergillus fumigatus .Journal of Antimicrobial Chemotherapy 2005; 55: 914–20.