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To Study the Comparison of Therapeutic Effect of Topical Intrastromal and Intracameral Voriconazole in Patients of Fungal Corneal Ulcer

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Introduction

Diseases of cornea are a major cause of blindness, second only to cataract resulting in more than 1.5 million new cases of vision loss annually. In India, it is estimated that there are approximately 6.8 million people who have vision less than 6/60 in atleast one eye due to corneal diseases; of these, about a million have bilateral involvement.(1,2)

The burden of corneal disease in our country is reflected by the fact that 90% of the global cases of ocular trauma and corneal ulceration leading to corneal blindness occur in developing countries. (5)

Treatable or avoidable blindness can be tackled by an effective and accessible health care service delivery network. In cases of corneal ulceration sight can be restored with timely and prompt management.

Fungal Keratitis

Fungal keratitis was described by Leber in 1879. It can be caused by several species of fungi and also accounts for nearly 50% of all cases of infectious keratitis in developing countries and tropical regions. (6,7)

Incidence of Fungal Keratitis

Prevalence varies by region and is highest in South India (36.7% of corneal ulcers) but is also common in West India (36.3%) and East India (26.4%), Northern India (7.3%).(8)

Classification of Fungi

1. Yeast: Candida species (albicans)
   Cryptococcus
2. Filamentous: Septate – Fusarium species (solani)
   - Aspergilus species (Fumigatus, flavus, niger)
   - Dematiaceous (alternaria, cladosporium, curvularia)
   - Non –septate – Mucor
   - Rhizopus
3. Diphasic:
   - Blastomyces
   - Coccidiodes
   - Histoplasma

Contributing Factors

Contributing factor for the development of fungal infection is trauma outdoor/or one which involves vegetative matter (including contact lens).

Another factor is use of topical medication like corticosteroids, anaesthetic drug abuse, topical broad spectrum antibiotic for long time. Chronic ocular surface disorders like dry eye, HSV, HZV, neurotropic ulcer, vernal / allergic keratoconjunctivitis also predispose to fungal infection. Systemic immunosuppression, Diabetes mellitus, malnutrition, alcoholism, chronically ill patients increase risk of development of fungal keratitis.

Corneal surgery (PK, refractive surgery) facilitates spread of fungal keratitis.

Causative Fungi

Filamentous Fungi: Filamentous fungal keratitis usually occurs in healthy young males engaged in agricultural or other outdoor work; these fungi do not penetrate an intact epithelium and invasion is secondary to trauma. Trauma is the key predisposing factor, occurring in 40-60% of patients others being previous corneal surgery, ocular surface ds, previous use of corticosteroids and contact lens use.

Traumatizing agents of plant or animal origin (even dust particles) either directly implant fungal conidia in the corneal stroma or abrade the epithelium, permitting fungal invasion.

Species of fusarium, aspergillus, curvularia, phaeohypomycetes, scedosporium apiospemum and paecilomyces are the principal cause of filamentous fungal keratitis.

Yeast Like and Related Fungi

Keratitis due to candida albicans and related fungi, one or more ocular (e.g. insufficient tear secretion, defective eyelid closure) or systemic (e.g. DM, immunosuppression) conditions predispose to the infection.

Symptoms

Symptoms are less compared to signs and slow in onset.

- Pain
- Redness
- Photophobia
- Discharge (mucoid, watery, mucopurulent or frankly purulent)
- Decreased visual acuity foreign body sensation

Signs

Patient examination on slit lamp biomicroscopy reveals

- Conjunctival and ciliary congestion
- Epithelial defect
- Stromal infiltrates
- Elevated areas, branching ulcers, irregular feathery margins
- Dry and rough texture
- Satellite lesions
- Wessley sterile immune ring
- Absent corneal vascularisation
- Endothelial plaques
- Non-sterile hypopyon
- Raised IOP
- Anterior uveitis

Laboratory Diagnosis

Diagnosis is based on

1. Gram and Giemsa stain
2. Potassium hydroxide (KOH) 10-20% wet mounts
3. Culture on saboraud's medium and blood agar
4. Anterior chamber tap to aspirate hypopyon and/or endothelial plaque.
5. PCR
6. Confocal microscopy

Smear

Direct microscopic evaluation is the most valuable and rapid diagnostic tool for the detection of fungal filaments in corneal scrapings. Giemsa stain and gram stain are equally sensitive in detecting fungal elements.

10% KOH wet mount is simple, cheap, rapid and easy to interpret even by an ophthalmic technician. It is an ideal method for practice in tropical and developing countries.

Fungal Culture

Fungus grows within 48-72 hr in blood agar and SDA kept at room temperature (27^0C). The rate of positive culture in microbial keratitis ranges from 52-68% but depends on the severity of the ulcer and the criteria established for positive culture.

Newer Diagnostic Tools

PCR and confocal microscopy are being used as new rapid diagnostic methods; they are not available in areas where fungal keratitis is highly prevalent.

PCR assay requires 4 hours to generate the results; which is significantly faster than 2 days - 2 weeks required by culture technique.

Confocal microscopy is a relatively new, non-invasive technique for imaging the cornea in normal and diseased states.

Management

Medical therapy- Prompt and appropriate antifungal therapy is the mainstay of the treatment of fungal keratitis. Antifungal therapy should only be instituted where corneal scraping reveals presence of fungal elements or cultures reveal the presence of fungal organisms at 36-48 hrs.

Since the corneal epithelium serves as a barrier to the penetration of most topical antifungal agents, debridement of the corneal epithelium is an essential component of the medical management of fungal keratitis.

Topical antifungals started hourly for 48 hrs and then tapered as signs permit.

- Systemic antifungals in severe forms, scleritis or endophthalmitis
- IOP lowering agents
- Broad spectrum antibiotics
- Cycloplegics

Voriconazole: Voriconazole is a newer generation triazole antifungal agent, only marketed in systemic formulation, with broad-spectrum activity and a high intraocular penetration. Voriconazole has demonstrated effectiveness against ocular mycosis. Voriconazole is a triazole having a structure similar to fluconazole with the addition of a methyl group to the propyl backbone and the replacement of a triazole moiety with a fluoropyrimidine group but with increased activity in vitro, an expanded spectrum, and poor aqueous solubility. [10]

Mechanism of Action of Voriconazole

The major effect of imidazoles and triazoles on fungi is inhibition of 14-sterol demethylase, a microsomal Cytochrome Peroxidase (CYP). Imidazoles and triazoles thus impair the biosynthesis of ergosterol for the cytoplasmic membrane and lead to the accumulation of 14-methylsterols. These methylsterols may disrupt the close packing of acyl chains of phospholipids, impairing the functions of certain membrane-bound enzyme systems, thus inhibiting the growth of the fungi.

Spectrum of Activity

Voriconazole is potent against a wide spectrum of fungi, namely, Candida albicans, Candida parapsilosis, Candida tropicalis, Aspergillus fumigatus, Aspergillus flavus, Fusarium solani, and other less common pathogens from the Paecilomyces, Histoplasma, Scedosporium, Curvularia, and Acremonium species. In a study by Marangon et al, in which the in vitro susceptibility of common pathogens to voriconazole was compared with that for amphotericin B, fluconazole, itraconazole, and ketoconazole, voriconazole demonstrated the lowest MIC90

Intraocular penetration of systemic voriconazole:

Three studies have investigated voriconazole penetration through the human cornea (non-keratitis) into the aqueous humor. Two of them investigated 1% voriconazole eye drops and one investigated 2% voriconazole eye drops. The sixth hourly and hourly dosing, voriconazole concentrations in the aqueous humor were studied which suggested that six-hourly dosing of 1% voriconazole eye drops may be ineffective. [16] Although voriconazole concentrations were detected in the aqueous humor after topical administration of voriconazole eye drops, this may not necessarily correlate with efficacy in the clinical setting of fungal keratitis.

To date, the penetration of topical voriconazole eye drops in patients with infective keratitis has been reported only twice, in the form of case reports.[18] In these studies good aqueous humor concentrations were achieved following hourly dosing of topical 1% voriconazole and provided

enough support for benefit of using voriconazole eye drops.

Side effects

Systemic side effects resulting from the topical administration of the voriconazole solution should be negligible . In the case of 2 % eye drops (the highest concentration that has been reported in humans), each drop contains only 0.001 mg of voriconazole which, when compared with the standard systemic doses of voriconazole, is unlikely to result in systemic concentration that is high enough to induce side effects. [25,26]

Topical voriconazole – 1% has to be prepared in pharmacy as it is only marketed in systemic formulation (voriconazole encapsulated with a beta-cyclodextrin derivative in the form of lyophilized powder of cyclodextrin –voriconazole complex). IV voriconazole is available as a glass vial that contains a white lyophilized powder containing 200 mg voriconazole and 3200mg sulfobutyl ether beta cyclodextrin sodium. Voriconazole (Vozole, aurolabs, Madurai, Tamilnadu, India) contains 30mg sterile lyophilized voriconazole powder.[27]

1% voriconazole e/d prepared in 19ml of sterile water for injection to produce a 20 ml solution with a concentration of 10mg / ml, were stable for at least 4 weeks when stored at 4^0C. Such long term stability data helps to minimize wastage and is pivotal to facilitate the use of the e/d in outpatient setting. Topical administration is convenient, simple and painless.[28,29]

Intrastromal Voriconazole therapy– Modality is advocated for non- healing fungal corneal ulcers in which injection is given in the vicinity of the stromal is This raises the local concentration of the antifungal agent enough to be effective in the eradication of the deep corneal infection.[30,31]

This approach proves effective with total elimination of the infection. The intrastromal injection can be repeated after a period of 48 to 72 hours.

Method of administration -After giving peribulbar anesthesia, under full aseptic conditions, the preloaded drug should be administered under operating microscope. With the bevel down, the needle is inserted obliquely from the uninvolved clear area to just reach the abscess at mid-stromal level (as the intended level for drug deposit). The drug then is injected and the amount of hydration of the cornea is used as a guide to assess the area covered. Once the desired amount of hydration is achieved, the plunger is withdrawn slightly to ensure discontinuation of the capillary column and thus prevent back-leakage of the drug. Five divided doses are given around the abscess to form a deposit of the drug around the circumference of the lesion. This is done in such a manner that a centripetally directed progressive wave of fluid appeared to encompass the abscess along each meridian. Circumferential injection will ensure the formation of a barrage of intrastromal voriconazole around the entire abscess. The total amount of drug injected intrastromally ranged from 0.05 ml to 0.1 ml.

Method of dilution for intracameral and intrastromal preparations.

Add 19 ml of distilled water	200 mg in 20 ml
Take 1 ml	10 mg in 1 ml
Add 9 ml distilled water	10 mg in 10 ml
Take 0.05 ml/0.1 ml	1 mg in 1 ml 50 microgram/100 microgram in 0.1 ml

Intracameral Voriconazole therapy

This modality ensures adequate drug delivery into the AC and may be especially useful to avoid surgical intervention in the acute stage of the disease. It should be performed under strict asepsis, injection can be repeated in case of inadequate response.

Patients with deep keratomycosis unexposed to conventional medical treatment are candidates for intracameral injection.

Method of administration- the usual dose is 50-100ug /0.1ml.

Intracameral voriconazole injection should be administered under aseptic conditions using an operating microscope. After instillation of topical proparacaine. A volume of 100 µg voriconazole in 0.1 mL is injected into the anterior chamber using

a 30-gauge needle attached to a 1.0-mL regular insulin syringe.

Aims and Objectives

1. To study the comparison of therapeutic effect of topical, intrastromal and intracameral voriconazole in patients of fungal keratitis.
2. To study the complications associated with topical, intrastromal and intracameral voriconazole in patients of fungal keratitis.
3. To study the rate of recovery in patients of fungal corneal ulcer recieving topical or intrastromal or intracameral voriconazole.

Materials and Methods

This pre-designed prospective interventional study was conducted at the OPD of Upgraded Department of Ophthalmology at LLRM Medical College and associated S.V.B.P. Hospital, Meerut, India.

Patients of corneal ulcer were selected from the routine OPD over the period of June 2018 to June 2019 and diagnosed as a case of fungal keratitis on the basis of following inclusion criteria.

Inclusion Criteria

1. Patients with corneal scrapings positive for fungal filaments on Gram stain, Giemsa stain and KOH wet mounts obtained from corneal ulcer.
2. Patients with culture positive for fungal colonies after inoculation on SDA with chloramphenicol (50 □g/ml) and blood agar
3. Patients already diagnosed with fungal corneal ulcer not responding to other conventional antifungal therapy for at least 2 weeks

Exclusion Criteria

1. Patients with infectious keratitis negative for fungal filaments on Gram, Giemsa stain and KOH wet mounts.
2. Patients with Impending corneal perforation
3. Patients with Corneal perforation
4. Total corneal involvement .
5. Corneal melting
6. Patient with any known allergy to Azole group of drugs
7. No light perception in the affected eye.

Patient Selection

Patients were selected from the Eye OPD. 45 patients fitting in the inclusion criteria and taking view of exclusion criteria were selected for further evaluation and categorization after obtaining an informed consent.

Evaluation of Patients

A detailed history regarding trauma with organic matter, chronic use of topical corticosteroids, ocular surface disease – dry eye, HSV, HZV, VKC, systemic immunosuppresion- DM, malnutrition was elicited from the patients in the OPD. Followed which patients were undertaken for slit- lamp examination and looked for clinical signs suggestive of fungal keratitis – corneal epithelial defect, stromal infiltrates with feathery branching pattern, satellite lesions , ulcers with raised edges, thick yellow exudates , endothelial plaque, fixed hypopyon. After taking proper consent and without any financial interest a complete general examination was done, followed by obtaining adequate corneal scrapes from the edge and the base of corneal ulcer to be sent for urgent Gram stain, Giemsa stain, KOH wet mount and culture on blood agar and SDA.

A detailed Ocular examination was performed on patients with smear positive for fungal elements in the following sequence-

First visual acuity was recorded (BCVA) in an eye with ulcerative keratitis using Snellen's chart and logMAR chart .

External ocular examination was performed.

A careful slit lamp biomicroscopy of conjunctiva, cornea and anterior chamber was perfomed. Examination of cornea included the examination of corneal ulcer (location, measurement of size and depth of ulcer), dimensions of epithelial defect, infiltrate density and depth in the corneal stroma, endothelium and status of surrounding cornea and sclera

Grading of corneal ulcer*

Feature	Mild	Moderate	Severe
Size of ulcer (mm)	< 2	2-5	> 5
Depth of ulcer (%)	< 20	20-50	> 50
Infiltrate			
— Density	Dense	Dense	Dense
— Extent	Superficial	Extension upto mid-stroma	Deeper than mid stroma
Scleral involvement	Not involved	Not involved	May be involved

Anterior chamber was examined for hypopyon formation. Size of hypopyon was measured using the slit lamp micrometer. Mobility of hypopyon if present was tested by asking the pt. to lie supine for 10 minutes. IOP in all groups were recorded using NCT.

Based upon the detailed examination of the cornea the patients were divided into three groups :-

(a) Group A - included eyes with mild grade corneal ulcer (size of ulcer < 2 mm , depth of ulcer <20 % , superficial infiltrates with no sclera involvement)

(b) Group B - included eyes with moderate grade corneal ulcer (size 2-5 mm, depth 20-50 % , infiltrates extending upto mid stroma with no scleral involvement) and

Group C - Included eyes with severe grade corneal ulcer (size > 5mm , depth > 50 % , infiltrates extending deeper than mid stroma with / without scleral involvement . GROUP A: We started with medical management using topical 1% voriconazole hrly for 48 hrs and then tapered as sign permitted + cycloplegics + IOP lowering agents + broad spectrum antibiotics

Group B: Intrastromal injection of 50□g/0.1ml voriconazole in 5 divided doses around the abscess circumferentially.

The total amount of drug injected was 0.1ml around the ulcer + cycloplegics + IOP lowering agents + Broad spectrum antibiotics + topical 1 % Voriconazole

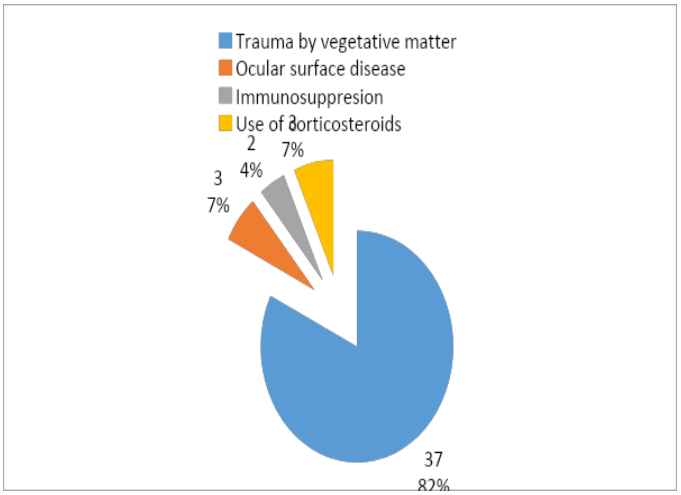
Group C: Intracameral injection of 50-100□g/ml voriconazole in AC using a 30-gauge needle attached to 1.0ml syringe + cycloplegics + IOP lowering agents + Broad spectrum antibiotics + topical 1% Voriconazole

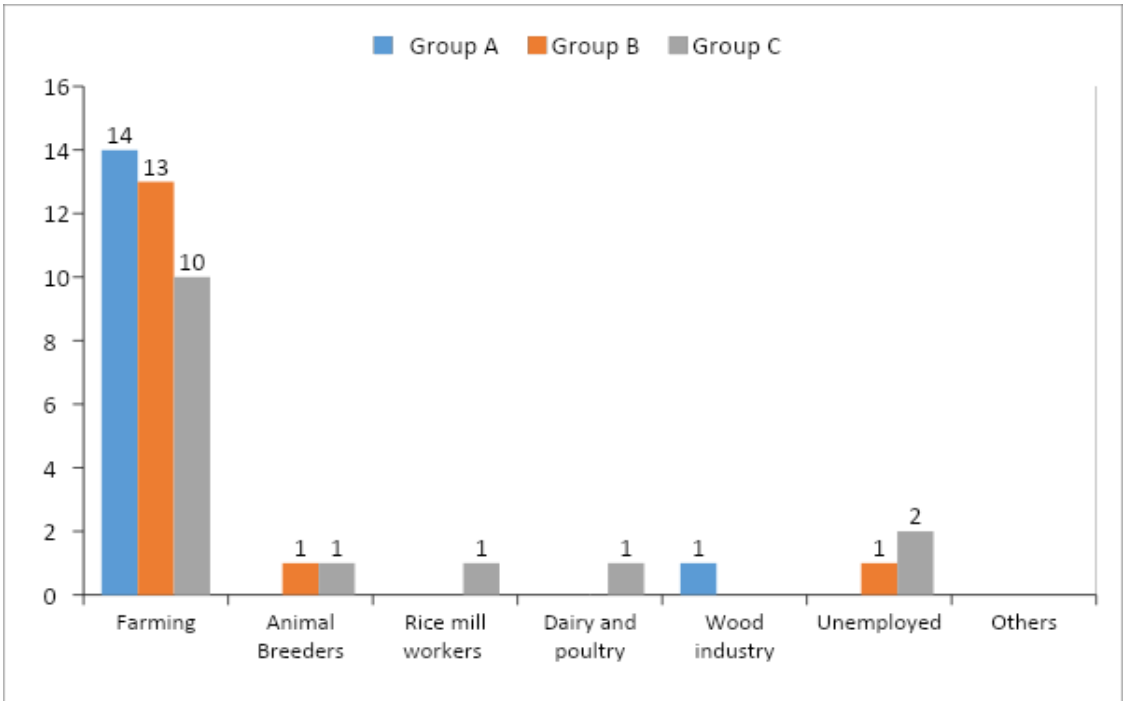
Total 45 patients were included in the study with 15 Spatients in each group. Oral voriconazole was not used in our patients included in the study.

The patients in each group were assessed at Day 1 , 4 weeks and at the end of 3 months

The main outcome measures were BCVA, IOP and Rate of healing of ulcer in each groups.

Observations





Comparison of BCVA pre intervention and post intervention

BCVA (logMAR)	Group A	Group B	Group C
Pre-intervention	0.79±0.34	1.24±0.40	1.67±0.30
At 3 months	0.73±0.42	1.00±0.62	1.34±0.46

Comparison of IOP pre and post intervention

IOP (mmHg)	Group A	Group B	Group C
Pre-Intervention	15.6±1.55	19.06±2.98	23.93±2.37
At 3 months	16.2±1.37	17.13±1.88	19.40±2.94

Comparison of response to treatment

Response To Treatment	Group A	Group B	Group C	Mean
Healing	14(93 %)	12(80 %)	13 (86.67%)	13.00
Failure	1 (7 %)	3(20 %)	2(13.33%)	2.00
Total	**15 (100%)**	**15(100%)**	**15 (100%)**	

Rate of Healing of Ulcers

Duration of Healing (weeks)	Group A	Group B	Group C
Upto 2 weeks	1(6.67%)	0	0
2-4	10(66.67%)	4(26.66%)	0
>4-6	1(6.67%)	5(33.33%)	0
>6-8	2(13.33%)	2(13.33%)	9(60.00%)
>8-12	0	1(6.67%)	4(26.66%)

Results

The subjects in our study were more males 31 (68.89%) than females. Most were in the age group > 40 years (97 %). The most common probable cause of fungal keratitis was trauma by vegetative matter (82 %) followed by chronic use of corticosteroids (7%) and ocular surface disorders (7%) and then immunosuppression states (4 %) 37(82.22%) patients were Farmer by occupation.

Aspergillus species in 26 patients(57.78%) were isolated on Culture after 72 hours of incubation followed by Fusarium species in 9 patients (20.00%), Candida albicans in 5 patients (11.11%), Curvularia species in 4 patients (8.89%) and Penicillium in 1 (2.22%) patient.

All patients included in the study were non – respondent to conventional anti – fungal therapy for 2 weeks.

In group A topical 1 % voriconazole was given, in group B intrastromal voriconazole and in group C intracameral voriconazole was given. In group A topical Voriconazole was tapered according to the response to the treatment, in Group B intrastromal Voriconazole were repeated once or twice if the patient didn't respond to the first injection. Likewise in Group C intracameral injection were repeated once or twice if the patient didn't respond to the first injection.

In this study we found statistically significant improvement in BCVA (in terms of logmar units) at the end of 3 months in all the three groups . There was statistically significant decrease in IOP in groups B and C at the end of 3 months. IOP in group A was within normal range both pre intervention and post intervention. IOP in all groups were measured by NCT.

In 14 cases (93%) of fungal corneal ulcers healed with Topical 1 % Voriconazole and only one 1 case (7%) progressed towards non –healing corneal ulcer.

In group B, 12 cases (80%) healed and 3 cases (20%) progressed towards non – healing corneal ulcer. In group C, 13 cases (86.67%) healed and only 2 case (13.33 %) progressed towards non – healing corneal ulcer.

Among 45 eyes, 39 (86.67%) corneal ulcers healed while 6 went into non-healing stage and were excluded from the study to be considered for therapeutic keratoplasty. 2 % of ulcers healed within 2 weeks , 35 % of ulcers healed within 4 weeks , 15 % of ulcers healed within a period of 6 weeks while 28 % of ulcers healed within a duration of 8 weeks and 11 % ulcers healed within 3 months duration among 45 cases .

Regarding complications ocular burning sensation was experienced by 1(2.2%) out of 15 patient in Group A , Periocular erythema was observed in 1(2.2%) out of 15 patients in Group A .Descemetocoele formation was observed in 2(4.4%) out of 15 patients in Group B .Difuse stromal haze was observed in 2(4.4%) patients in Group B .

No significant complication was observed in Group C after intracameral injection of Voriconazole.

Discussion

This study represents data of 45 patients of fungal corneal ulcer, visiting our OPD who conformed to the inclusion criteria as laid down earlier .

First line of treatment of fungal keratitis was discussed in an article by Zubair Ansari which states that natamycin was the first antifungal agent approved for fungal keratitis and continues to be the first line agent. Natamycin is considered as the most effective medication against Fusarium . Though Natamycin tends to be the first line treatment, it is limited by its inability to cover other fungal organisms such as Candida.

Voriconazole has been proposed as a good alternative to Natamycin with minimal toxicity and it is not only active against filamentous fungi but also against Candida.

Voriconazole has a wider therapeutic window. Heidar Siatiri et al conducted a prospective study to evaluate the course and outcome in 3 patients with recalcitrant fungal keratitis treated with intrastromal and topical voriconazole application. A dramatic therapeutic response was seen in 2 patients (66%).

In my study also rate of response to treatment was seen in 39 eyes out of 45 (86.67 %).

Sharma N et al carried out a randomized clinical trial in 40 eyes to compare the efficacy of topical Voriconazole and topical natamycin with that of intrastromal Voriconazole and topical natamycin. They observed that topical Voriconazole seemed to be a useful adjunct to natamycin in fungal keratitis not responding to topical natamycin alone also intrastromal injections did not offer any beneficial effect over topical therapy. The mean BCVA after treatment was 1.295±0.5 logMAR units in the topical group and 1.692±0.29 logMAR units in the intrastromal group. In my study the mean BCVA after treatment in topical group was

0.73±0.42, 1.00±0.62 in intrastromal group and 1.34±0.46 in intracameral group. There was statistically significant improvement in mean BCVA in all the three groups.

K Lekhanont et al reported the role of intrastromal and intracameral voriconazole injection in the management of fungal keratitis and concluded that intracameral and intrastromal voriconazole injections may offer safe and effective treatment options.

Conclusion

Fungal corneal ulcer is prevalent in tropical countries and its treatment is yet a challenge.

Identifying the causative fungus and planning the treatment plays a key role in the treatment of fungal keratitis.

Various antifungal drugs are available till date. Voriconazole has emerged as a broad spectrum antifungal agent in the treatment of recalcitrant fungal keratitis.

In our study Topical 1 % Voriconazole, Intrastromal and intracameral voriconazole were found safe and equally efficient in their respective groups.

All three modalities were safe with concern to IOP. Fusarium species were isolated in the non-healing groups.

Treatment failure rates were higher in intrastromal group as compared to other groups. Numerically there was significant improvement in BCVA after intervention in all the three groups and it was statistically significant so it can be concluded that administration of voriconazole improves BCVA in patients of fungal corneal ulcer.

Hence our study suggests that Voriconazole given by any of the three routes i.e. Topical, intrastromal and intracameral is almost equally effective in treating fungal corneal ulcer without any associated systemic side effects.

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