

**ORIGINAL RESEARCH**

# The comparative study of intrastromal voriconazole versus topical voriconazole therapy in fungal keratitis

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### ABSTRACT

**Introduction:** Mycotic Keratitis is one of the difficult forms of keratitis to diagnose and treat successfully. It is important ocular infection, especially in young male outdoor workers with history of vegetative trauma to eye. In this study patients of fungal keratitis were treated with voriconazole drug with topical eye drops versus intrastromal injections and the results were compared. **Aim:** To compare the efficacy of intrastromal voriconazole versus topical voriconazole in fungal keratitis. **Material and method:** This is a prospective study conducted on 100 cases of fungal corneal ulcers presented to out-patient department / in-patient department. Cases were diagnosed clinically and confirmed on KOH wet mount preparation, divided into two groups with 50 cases treated with intrastromal VCZ (group I) and 50 cases treated with topical VCZ (group II). The healing response results are compared in both the groups. **Results:** Out of 100 cases, 73% were males with maximum incidence (67%) in age group of 31-60 years. 69% cases were from rural background, agriculturist (73%) by occupation, majority of cases (87%) had history of vegetative trauma causing fungal ulcer. On treatment the healing response in intrastromal VCZ (group I) and topical VCZ (group II) was statistically significant ( $p < 0.05$ ) that is earlier healing was seen in intrastromal VCZ treated group. On comparison the best corrected visual outcome at 12 weeks, the difference between two groups was insignificant ( $p > 0.05$ ). Fungal ulcers were healed with corneal opacity. **Conclusion:** Our data suggests that the voriconazole drug was beneficial in treatment of fungal keratitis and early healing of ulcer was seen with intrastromal voriconazole treatment than topical VCZ treatment. **Abbreviations:** VCZ: voriconazole, KOH: potassium hydroxide, SDA media: sabourauds dextrose agar media

**Key words:** fungal keratitis, KOH wet mount, voriconazole

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### INTRODUCTION

Mycotic Keratitis is one of the difficult forms of keratitis to diagnose and treat successfully. It is characterized by severe inflammation, formation of corneal ulcer and hypopyon and with the presence of fungal hyphae within the corneal stroma. In most of the cases it is associated with trauma due to vegetative matter or objects contaminated with soil in both developing and developed countries. In India, the estimated incidence of fungal keratitis is 113 per 100,000, with *Aspergillus* being the most common etiology.<sup>1,2</sup>

Filamentous fungi such as *Fusarium* and *Aspergillus*, yeast like fungi such as *Candida*, are most commonly associated with mycotic keratitis. It is usually

diagnosed clinically and by inoculating corneal tissue or scrapings into KOH (10%) wet mount, Gram staining, Giemsa staining, lactophenol cotton blue, Grocotts methanamine silver, Acridine orange and Calcoflour white.<sup>3</sup> Culture can be done on sheep blood agar, chocolate blood agar, sabourauds dextrose agar (SDA media), thioglycollate broth, brain heart infusion broth.

Clinically dry looking elevated ulcers with extension beyond the edge of ulcer into surrounding corneal tissue, multifocal granular or feathery grey white satellite stromal infiltrate, an immune ring and thick hypopyon<sup>4</sup> are suggestive of mycotic keratitis.

Management includes treatment with broad spectrum antifungal agents (polyenes, azoles, pyrimidines,

allyllamines, echinocardins). Supportive treatment may be given in the form of cycloplegics, analgesics, antibiotics etc. Azoles (ketoconazole, fluconazole, voriconazole) target primarily the ergosterol synthesis pathway by inhibition of cytochrome P450 dependent C-14 $\alpha$  demethylase converted lanosterol to ergosterol, an essential component of fungal cell wall. Voriconazole (VCZ) is a newer generation triazole antifungal agent with broad spectrum activity and high intraocular penetration. VCZ eye drops are prepared with concentration of 10mg/ml (1%) and Intrastromal injection is prepared with the dose of 50mcg/0.1 ml. It has been proposed as good alternative to natamycin with minimal toxicity.<sup>5,6</sup>

## MATERIALS AND METHODS

A prospective study of patients of fungal corneal ulcer, positive on KOH wet mount preparation, was done in the Outpatient / Inpatient department of Ophthalmology in association of Microbiology department of Government Medical College Amritsar. Out of the total 100 patients taken 50 were treated with intrastromal VCZ and 50 were treated with topical VCZ. This study was conducted after obtaining approval from Ethical and Thesis committee (Institutional Review Board) in adherence with the Declaration of Helsinki. The inclusion criteria was patients with suspected fungal corneal ulcer who came positive on KOH wet mount. Other features considered in making the diagnosis of fungal corneal ulcer were history of injury with vegetative matter or foreign body and clinical sign and symptom of fungal corneal ulcer i.e. firm, dry, elevated central ulcer with satellite lesion with immobile hypopyon and immune ring.

The exclusion criteria was already impending perforation, patient aggressively treated with antibiotic and antifungal drugs for longer duration, known case of drug allergy, uncooperative children for taking corneal scrapping, pregnancy, previous history of penetrating keratoplasty, any adverse reaction to VCZ, no perception of light in affected eye. The patients of group I were treated with

intrastromal VCZ (50mcg/0.1ml) injected at 5-6 sites circumferentially around the infiltrate in the form of star. The patients of group II were treated with topical application of 1% VCZ (freshly prepared), given ½ hourly for first 24 hours, 1 hourly for next 48 hours, 2 hourly till 28 days. The clinical response to both the groups was noted and compared on day 1, 3, 5, 7, 14, 28, 42. The patients were followed up to 12 weeks to see the improvement in visual acuity.

## Results

Out of 100 patients, 73 (73%) patients were males and 27 (27%) were females. The maximum incidence (67%) of fungal corneal ulcer was seen in age group of 31-60 years. The majority of cases (69%) were from rural background with 73% agriculturists (Farmers and farm workers). 64% of ulcers were superficial and 36% had involved deep stromal layer, with hypopyon present in 30% of cases only. Culture done on SDA media revealed *Aspergillus* (34% in group I and 26% in group II) most commonly grown followed by *Fusarium* (14% in group I and 6% in group II) and *Candida* (2% in group I and 4% in group II).

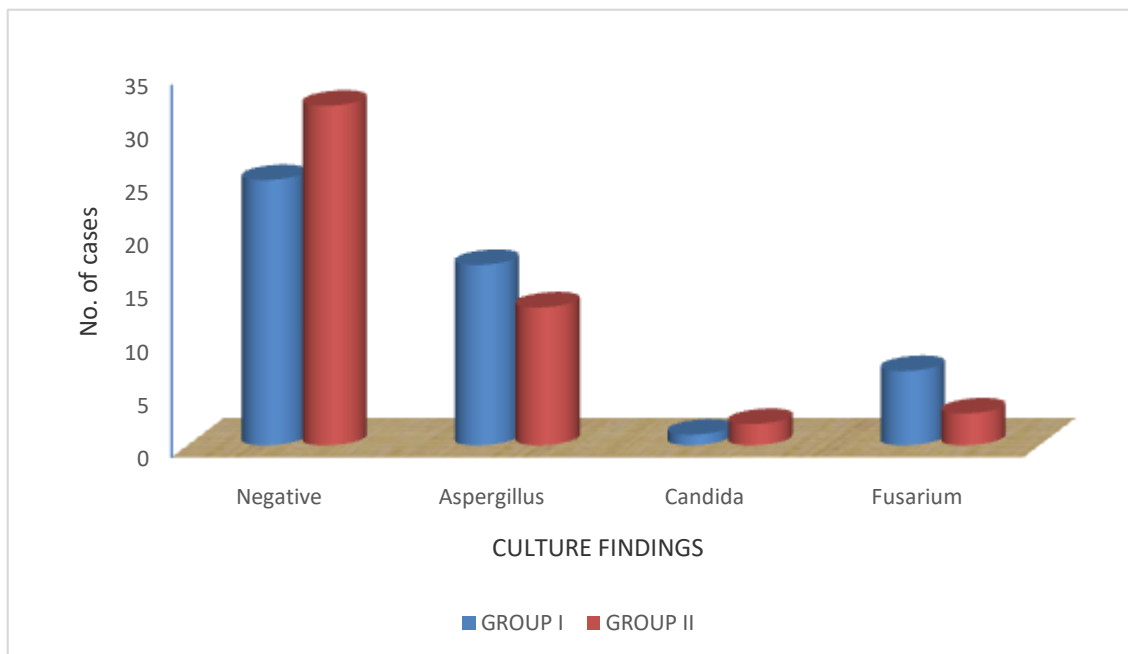
When the healing duration in both the groups was compared it showed that irrespective of the size and depth of corneal layers involved the difference between the group I and group II was statistically significant (p value < 0.005). The healing was earlier in grade I and grade II ulcers in group I (intrastromal VCZ) as compared to group II (topical VCZ). There was no comparative data present in grade III and Grade IV. The average duration of healing in grade III superficial ulcers was 14 days and 28 days in grade III deep ulcers. The average duration of healing response in grade IV deep ulcer was 42 days in group II. This showed an earlier healing response to intrastromal VCZ (group I) than topical VCZ (group II). On comparison of BCVA in both the groups, the difference was found to be statistically insignificant (p value >0.05%). There was no difference in final visual outcome in both the groups. The best corrected visual acuity was observed maximum in peripheral ulcer followed by paracentral and then central ulcer.

**Table 1: fungal culture**

Culture findings	Group i		Group ii	
	No.	%age	No.	%age
<b>Negative</b>	25	50.00	32	<b>64.00</b>
<b>Aspergillus</b>	17	34.00	13	<b>26.00</b>
<b>Candida</b>	1	2.00	2	<b>4.00</b>
<b>Fusarium</b>	7	14.00	3	<b>6.00</b>
<b>Total</b>	<b>50</b>	<b>100</b>	<b>50</b>	<b>100</b>

X<sup>2</sup>: 3.326; p=0.344

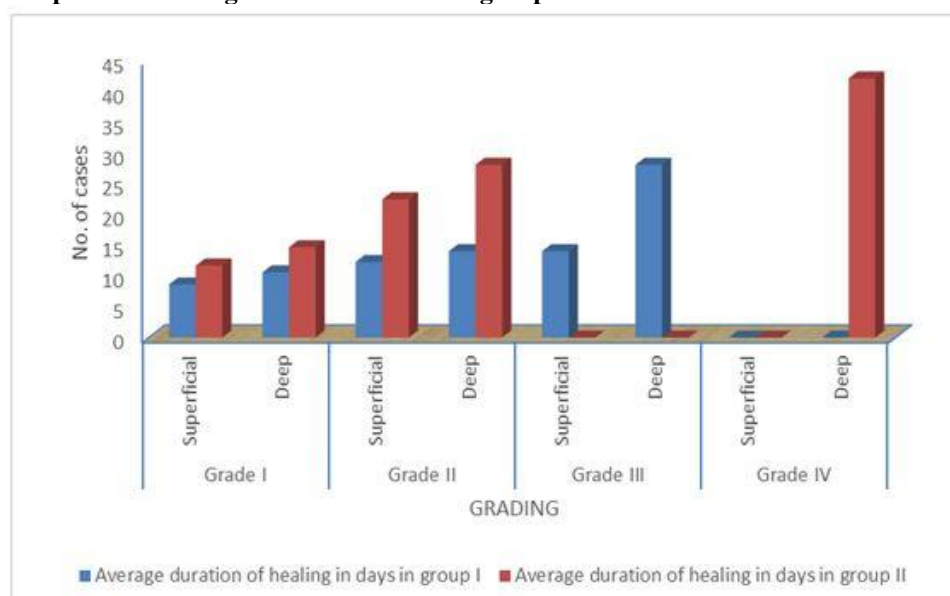
**Figure 1: fungal culture growth in both the groups**



**Table 2: comparative healing duration in both the groups:**

Grading according to		Average duration of healing in days in group i	Average duration of healing in days in group ii	P-value
Size	Depth			
<b>Grade I</b>	Superficial	8.60	11.66	<b>0.001</b>
	Deep	10.55	14.63	<b>0.001</b>
<b>Grade II</b>	Superficial	12.25	22.40	<b>0.001</b>
	Deep	14.00	28.00	<b>0.001</b>
<b>Grade III</b>	Superficial	14.00	-	-
	Deep	28.00	-	-
<b>Grade IV</b>	Superficial	-	-	-
	<b>Deep</b>	-	<b>42.00</b>	-

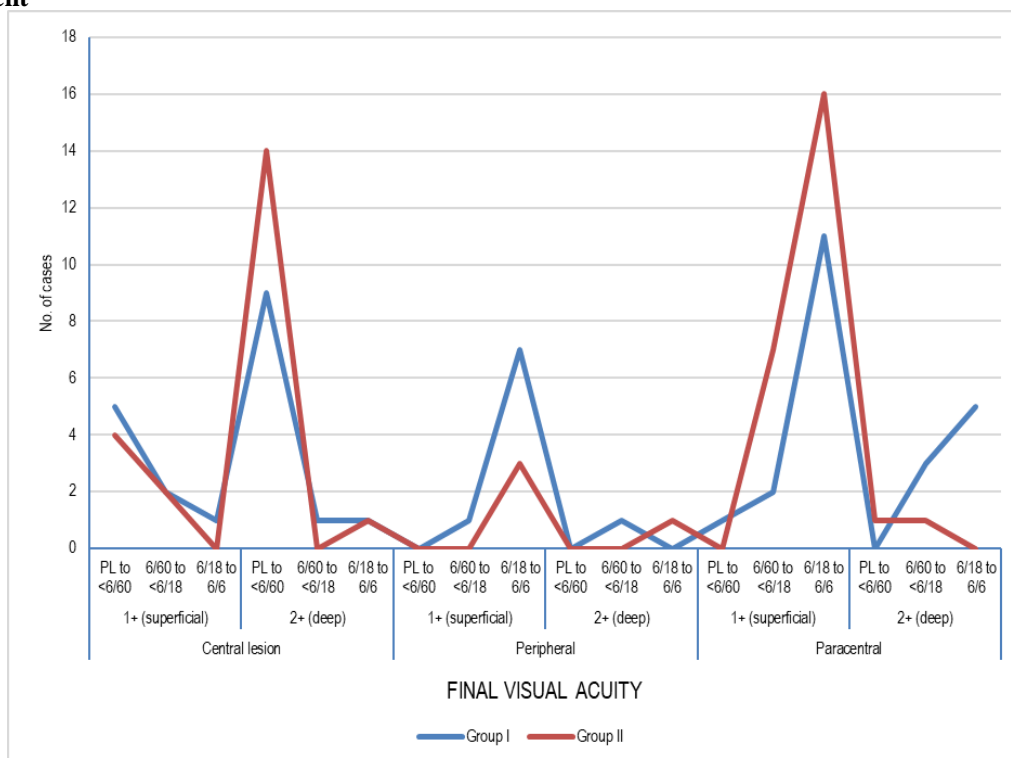
**Figure 2: comparative healing duration in both the groups**

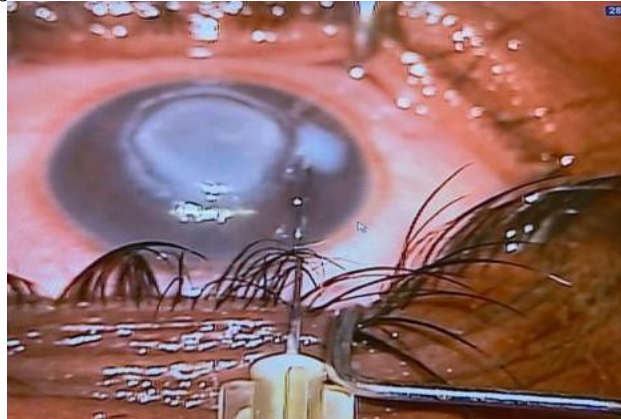


**Table 3: final best corrected visual acuity outcome at 12 weeks**

	Group i		Group ii		P-value
	No.	%age	No.	%age	
<b>Central lesion</b>					
<b>1+ (superficial)</b>	8	8%	6	6%	
<b>PL to &lt;6/60</b>	5	62.50	4	66.67	<b>X<sup>2</sup>: 0.843; p=0.656</b>
<b>6/60 to &lt;6/18</b>	2	25.00	2	33.33	
<b>6/18 to 6/6</b>	1	12.50	0	0.00	
<b>2+ (deep)</b>	11	11%	15	15%	
<b>PL to &lt;6/60</b>	9	81.82	14	93.33	<b>X<sup>2</sup>: 1.507; p=0.470</b>
<b>6/60 to &lt;6/18</b>	1	9.09	0	0.00	
<b>6/18 to 6/6</b>	1	9.09	1	6.67	
<b>Peripheral</b>					
<b>1+ (superficial)</b>	8	8%	3	3%	
<b>PL to &lt;6/60</b>	0	0.00	0	0.00	<b>X<sup>2</sup>: 0.413; p=0.813</b>
<b>6/60 to &lt;6/18</b>	1	12.50	0	0.00	
<b>6/18 to 6/6</b>	7	87.50	3	100.00	
<b>2+ (deep)</b>	1	1%	1	1%	
<b>PL to &lt;6/60</b>	0	0.00	0	0.00	<b>X<sup>2</sup>: 2.00; p=0.368</b>
<b>6/60 to &lt;6/18</b>	1	100.00	0	0.00	
<b>6/18 to 6/6</b>	0	0.00	1	100.00	
<b>Paracentral</b>					
<b>1+ (superficial)</b>	14	14%	23	23%	
<b>PL to &lt;6/60</b>	1	7.14	0	0.00	<b>X<sup>2</sup>: 2.673; p=0.262</b>
<b>6/60 to &lt;6/18</b>	2	14.29	7	30.43	
<b>6/18 to 6/6</b>	11	78.57	16	69.57	
<b>2+ (deep)</b>	8	8%	2	2%	
<b>PL to &lt;6/60</b>	0	0.00	1	50.00	<b>X<sup>2</sup>: 5.313; p=0.070</b>
<b>6/60 to &lt;6/18</b>	3	37.50	1	50.00	
<b>6/18 to 6/6</b>	5	62.50	0	0.00	

**Figure 3: the final best corrected visual outcome of healed fungal corneal ulcer at twelve weeks after treatment**



**Figure 4: intrastromal injection of voriconazole**

## DISCUSSION

Mycotic keratitis is an infection of cornea by fungus that causes ulceration and inflammation, usually following vegetative trauma. Due to increasing incidence and insignificant response to antifungal agents, fungal keratitis is one of the leading cause of visual loss and blindness worldwide.

The present study was conducted on 100 patients of fungal corneal ulcer which were clinically diagnosed and confirmed microscopically with KOH wet mount preparation. This study was done to compare the efficacy of intrastromal voriconazole therapy (50mcg/0.1ml) versus topical voriconazole therapy (1%) in fungal keratitis. In our study the maximum incidence (67%) was in age group of 31-60 years followed by 19% above the age of 60 and 14% at age of 20-30 years. This is in concordance with study done by Gupta et al<sup>7</sup> in 2015 who found the highest incidence in age group of 20-50 years. In our study the incidence of fungal corneal ulcer in males and females was 73% and 27% respectively which is similar to study by Chander J et al<sup>8</sup> who found the incidence of fungal corneal ulcer in males and females was 80.52% and 19.48% respectively. From the above analysis, it is revealed that the incidence of fungal corneal ulcer is more in young and middle aged adult males because they are manual workers in outfields as compared to housewives, children and old people who are less exposed to field work.

In our study the trauma was the major predisposing factor in 87% of cases out of which different causative factor were from vegetative matter like grass, paddy, sugarcane, twig, wheat straw, wooden dust, algae, animal fodder and in rest 13% cases cause was unknown. This was also in concordance with a study done by Gupta et al<sup>7</sup> which showed the vegetative trauma (73.94%) was the major predisposing factor for fungal corneal ulcer.

In our study out of 100 cases of fungal corneal ulcer 69% were from rural background and 31 % were from urban area. Similarly Chander J et al<sup>8</sup> also showed the incidence in rural population was 76.62% and in urban was 23.38%. In our study, 73% were agriculturists, 16% housewives, 5% carpenters and

6% others (Student, driver, dairy workers and rikhshaw pullers). Bharti MJ et al<sup>9</sup> which also reported the more incidence (64.75%) in agriculture workers.

In our study of all KOH positive cases, when inoculated on SDA culture media revealed 43 % growth of different types of fungus. Out of 43 positive culture cases 30 cases (69.76%) showed *Aspergillus* growth, 10 cases (23.25%) *Fusarium*, and 3 cases (6.97%) showed *Candida* growth. Reddy et al<sup>10</sup> also showed increased incidence of 50% of *Aspergillus* in culture.

In our study the ulcers which were superficial, healed faster than deep ulcer. The ulcers treated with group I (intrastromal voriconazole treatment) were healed earlier than the group II (topical voriconazole treatment).

The average duration of ulcer healing in group I was 14.57 days and 23.74 days in group II. The healing response was also checked according to grade and depth of ulcer in which average duration of all grades of superficial ulcers in group I was 11.62 days and 17.03 days in group II whereas in all grades of deep ulcers it was 17.52 days in group I and 28.21 days in group II. It showed that irrespective of the size of ulcer and depth of the corneal layers involved the difference between group I and group II was statistically significant ( $p < 0.005$ ), showing healing was earlier in group I as compared to group II (Intrastromal Group). Solaiman et al<sup>11</sup> showed healing response in fungal corneal ulcer was higher in group treated with topical and intrastromal voriconazole (85%) together than the group treated with topical voriconazole alone (55%). Sharma et al<sup>12</sup> also demonstrated the efficacy of intrastromal voriconazole in cases which were not responding to topical antifungal medication.

In terms of Best Spectacle Corrected Visual acuity, the p value was statistically insignificant in both the groups as there was no difference in final visual outcome in both the groups. Although the best corrected visual acuity was observed maximum in peripheral ulcer followed by paracentral and then central ulcer. Solaiman et al<sup>11</sup> also did a study which

showed that BCVA was statistically insignificant in group A (intrastromal with topical VCZ) versus Group B (topical voriconazole alone) with p value > 0.005.

### CONCLUSION

The present study concluded that the use of topical and intrastromal voriconazole were beneficial for healing of fungal corneal ulcer. The healing response of intrastromal voriconazole (50 µg/0.1ml) was early as compared to topical voriconazole (1%). Intrastromal voriconazole had significantly hastened the healing rate and resolution period without significant complication of the injection.

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