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Original Article

INTRASTROMAL INJECTION VERSUS TOPICAL DROPS OF VORICONAZOLE FOR RESISTANT FUNGAL CORNEAL ULCER

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Abstract

Aim: *To evaluate safety and efficacy of intrastromal injection of voriconazole in treating resistant fungal corneal ulcer.* ***Patients and methods:*** *A prospective, randomized study performed on patients with resistant fungal ulcer. Patients were randomly distributed into two groups: Group (A) included 10 eyes of patient treated with intrastromal injection(s) of voriconazole (50 µg/0.1 ml) and group (B) included 10 eyes treated with topical voriconazole eye drops 1%. Primary outcome measure was healing of the fungal ulcer. Secondary outcome measures included duration of healingin healed cases, any reported complication and the visual outcome.* ***Results:*** *Healing of fungal ulcer was higher in group A (80%) than in group B (30%) and the difference was statistically significant. The range of duration of healing in group A was less than that in group B. Ocular trauma (especially with plant matter) was considered as important risk factor.* ***Conclusion:*** *Voriconazole eye drops might be considered as effective treatment for resistant fungal ulcer. Delivering voriconazole by intrastromal injection could raise the healing rate and decreasing the resolution period with no significant complications.*

Keywords: *Intrastromal injection, Fungal ulcer, Topical, Voriconazole*

1. Introduction

Fungal ulcer accounts for 5-20% of all corneal infections. It results in significant ocular morbidity. Unlike bacteria, fungi are capable of penetrating the intact Descemet's membrane which is difficult to treat with antifungal agents as ocular penetration and bioavailability of many of the available topical antifungal preparations are poor [1]. Voriconazole is available commercially for systemic administration in the form of oral and intravenous formulations. It has an excellent broad spectrum antifungal activity. It is available in the form of oral and intravenous formulations. Topical voriconazole eye drops, used in an off-label manner, have also been prescribed for the treatment of keratitis with promising results [2]. The aim of this study is to evaluate and compare the safety and efficacy of intrastromal injection versus topical voriconazole for treatment of resistant fungal ulcer.

2. Patients and Methods

A prospective randomized study that included patients with resistant fungal ulcer. Patients were randomly distributed into two groups: group A included eyes of patients who received intrastromal injection of voriconazole and group B included eyes which received topical voriconazole eye drops.

2.1. *Inclusion criteria*

*) Proven fungal ulcer by direct smear and/or culture. *) Patient with fungal corneal ulcers with evidence of resistance to other antifungal therapy for at least 2 weeks.

2.2. *Exclusion criteria*

*) Perforated corneal ulcers or impending corneal perforation. *) Scleral involvement. *) Total corneal involvement or corneal melting. *) Endophthalmitis. *) Patients with history of previous surgery for corneal ulcer. An informed consent was obtained after detailed explanation about the condition and the methods of management for the patients. All patients were subjected to a history taking which included risk factors for fungal ulcer such as history of trauma, contact lens wear, and prolonged use of topical steroids. Duration of ulcer was also reported. Complete ophthalmic examination was performed especially the cornea and anterior segment. B-scan ultrasonography was done if needed. Corneal scraping from the ulcer was done to all patients. The material from scraping was subjected to direct smear and Sabouraud dextrose agar culture to form a film stained with Giemsa and Gram stains for visualization of the different types of fungi microscopically (direct film). Voriconazole (VFEND; Pfizer Inc, New York, USA) is available as 200 mg of powder in a glass vial. Voriconazole, intrastromal injection or eye drops, was started as soon as the diagnosis was confirmed.

2.3. *Preparation of intrastromal voriconazole and method of injection*

Voriconazole powder was divided in Epindorf tubes; each containing 5 mg of powder and was reconstituted with 10 ml of lactated Ringer solution to obtain a concentration of 500 μg/ml (50 μg/0.1 ml) according to the technique described by some studies as Solaiman, et. al. [3]. The reconstituted solution was loaded in one ml syringes. After topical anesthesia, local anesthesia in some cases, the voriconazole was injected into cornea under the operating microscope according to the technique described by Prakash, et. al [4]. The needle of syringe was inserted obliquely from the clear cornea to reach near the ulcer at the midstromal level. Then, the drug was injected inducing corneal hydration. Injections were given around the circumference of the ulcer (three to five injections to form a deposit of the drug around the circumference of the lesion). Intraoperative complications; if any, were reported. If no signs of clinical improvement were observed within one week (as decreased size of infiltrations and corneal abscess, diminished hypopyon level, starting vascularization and healing of epithelial defects) or there was worsening observed within three days (as increased size or depth of ulceration and/or infiltrations), another injection was given with three successive injections as a maximum (with interval ranging from 72 hour to 1 week). If no response occurred after 3 injections, the treatment was considered as a failure and no more injections were given.

2.4. *Preparation of voriconazole eye drops*

The powder is reconstituted with 20 ml of sterile distilled water to produce a 20 ml aqueous voriconazole solution with a concentration of 10 mg/mL (1%). Reconstituted voriconazole was aseptically instilled in sterile droppers and it was given hourly after initiation of treatment, follow up was done on daily basis for one week and then twice weekly until either complete resolution or stoppage of treatment and failure.

3. Results

In this study, 20 eyes with resistant fungal ulcer, confirmed both clinically and by smear and/or culture, were randomly distributed into two groups: Group (A) included 10 eyes treated with intrastromal injection(s) of voriconazole and group (B) included 10 eyes treated with topical voriconazole eye drops. Patients' demographic data was shown that there is no statistically significant difference between both groups as regards age, sex and residency. Group A:included 6 males (60 %) and4 females (40 %), Their mean age was 53±11.83 years and eight (8) patients (80 %) were from rural areas while 2 patients (20 %) were from urban areas. While group B: included 7 males (70 %) and 3 females (30 %), their mean age was 49±12.24 years and six (6) patients (60 %) were from rural areas while 4 patients (40 %) were from urban areas. Table (1) shows the risk factors in all patients with no statistically significant differences between both groups of the study as regards all risk factors. It was noticed that the most common risk factor in both groups was ocular trauma. Results of growth on culture media among both groups, tab. (2) were Filamentous fungi (7 eyes in group A and 6 eyes in group B) and Yeast fungi (3 eyes in group A and 4 eyes in group B) with no statistically significant difference between both groups. The results of direct films and cultures, tab. (3) were Aspergillum species (5 eyes in group A and 4 eyes in group B), Fusarium species (2 eyes in group A and 2 eyes in group B) and Candida species (3 eyes in group A and 4 eyes in group B) with no statistically significant difference between both groups. This study showed that healing of fungal ulcer, fig. (1 & 2) was statistically significant higher in group A than in group B, tab. (4). In group A, 8 eyes (80%) responded to intrastromal injection of voriconazole with no complications related to injection were recorded. The numbers of intrastromal injections were two injections in 3 eyes (30%), three injections in 7 eyes (70%). In group B, 3 eyes (30%) responded adequately to 1% voriconazole eye drops with complete healing, while 7 eyes (70%) did not respond adequately to 1% voriconazole eye drops and they were considered as a failure. In group A; Among the 8 eyes who responded well to intrastromal injection of voriconazole with complete resolution, 5 eyes (62.5%) resolved in 3-4 weeks and 3 eyes (37.5 %) resolved in more than 4 weeks after 3 injections one week apart. While in group B; of the 3 cases who responded adequately to voriconazole eye drops, complete recovery was reported in one (33.3%) of them in 4 weeks, while others 2 cases after more than 4 weeks (66.6%) as been shown in tab. (5) with no statistically significant difference between both groups of the study. The mean duration of follow up after starting voriconazole treatment was 4.88± 2.11 weeks in group A and was 6.85±2.73 weeks in group B. The changes in the best corrected visual acuity (BCVA) on presentation and after treatment in both groups of the study are illustrated in tab. (6).

Table (1) Risk factors in patients of both groups of the study

	Group A		Group B		P
	No	%	No	%	
Plant trauma	5	50	5	50	
Blunt trauma	3	30	4	40	**0.9**
Contact lenses wear	**2**	**20**	**1**	**10**	

Table (2) Results of growth on culture media among both groups of the study.

	Group A		Group B		P
	No	%	No	%	
Filamentous	7	70	6	60	**0.64**
Yeast	**3**	**30**	**4**	**40**	

Table (3) Results of direct films among study groups.

	Group A		Group B		P
	No	%	No	%	
Aspergillums sp.	5	50	4	40	**0.88**
Fusarium sp.	2	20	2	20	
Candida sp.	**3**	**30**	**4**	**40**	

Table (4) Outcome of treatment among study groups.

	Group A		Group B		P
	No	%	No	%	
Healing	8	80	3	30	**0.024**
Failure	**2**	**20**	**7**	**70**	

P <0.05 = *Significant*

Table (5) Duration of healing after treatment among study groups.

Duration of healingin weeks	**Group A**		**Group B**		**P**
	No	**%**	**No**	**%**	
3-4	5	62.5	1	33.3	**0.38**
>4	**3**	**37.5**	**2**	**66.6**	

Table (6) BCVA on presentation and after treatment in study groups

	Group A				**Group B**			
	BCVA on presentation		**BCVA after treatment**		**BCVA on presentation**		**BCVA after treatment**	
	No.	**%**	**No.**	**%**	**No.**	**%**	**No.**	**%**
HMGP	**3**	**30**	**3**	**30**	**2**	**20**	**3**	**30**
CF up to less than 6/60	**5**	**50**	**4**	**40**	**6**	**60**	**4**	**40**
6/60 or better	**1**	**10**	**3**	**30**	**2**	**20**	**3**	**30**

HMGP: *Hand Movement with Good Projection*, ***CF***: *Counting Fingers*

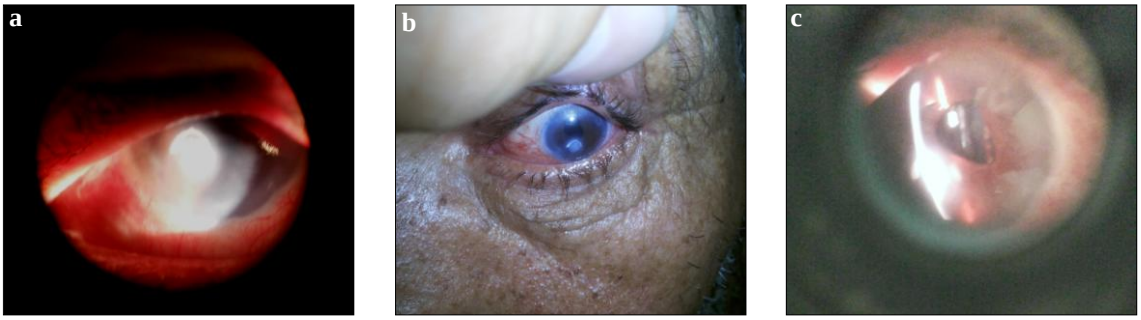

Figure (1) **a.** A case of fungal ulcer in an agricultural worker after plant trauma and BCVA of 3/60 with diffuse stromal infiltrations and epithelial defects, **b. & c.** four weeks after three intrastromal injections of voriconazole one week apart with resolution of stromal infiltrates and healing of epithelial defects with BCVA of 5/60.

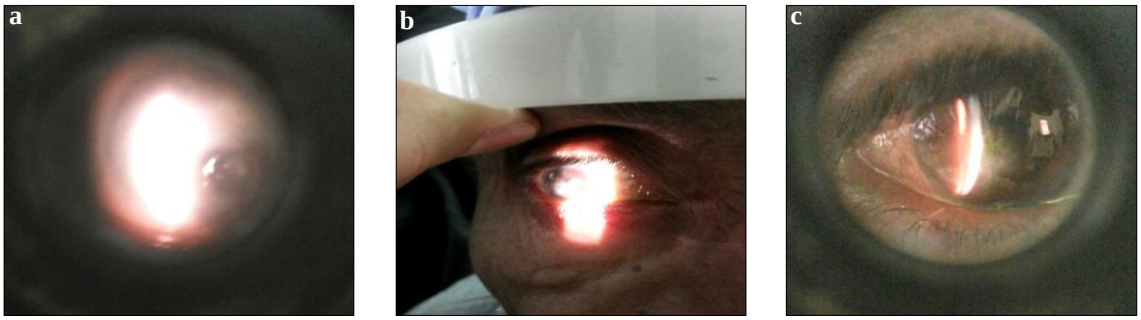

Figure (2) **a.** Resistant fungal ulcer and BCVA of 5/60, **b. & c.** complete healing of epithelial defects 5 weeks after administration of topical voriconazole eye drops 1 % with BCVA of 6/24.

4. Discussion

In fungal infections of the cornea; Response to topical natamycin 5% suspension and oral itraconazole is limited due to poor penetration of anti-fungal drugs. Oral fluconazole has been reported to provide higher intracorneal and intracameral drug concentration [1]. Newer drugs, as voriconazole, with wider spectrum and better bioavailability in the ocular tissues have been recently introduced [2]. Voriconazole (Vfend) is available as 200 mg of white powder in a glass vial. In this study the powder was divided and used as previously mentioned and this new method of reconstitution is valuable to preserve the fresh powder for further reconstitution of either injection or eye drops as in some other studies [3]. Though**,** Prakash et al [4] and Sharma et. al. [5] used another method of reconstitution that was different from this study. They prepared the injections where the powder was reconstituted with 19 ml of lactated ringer solution to obtain 20 ml of solution with concentration of 10 mg/ml of voriconazole. A 1 ml of this solution was further diluted with 20 ml of lactated Ringer to form a concentration of 0.5 mg/ml (50 µg/0.1 ml) for injection. In this study, 19 ml sterile distilled water was used for dilution of 200 mg dry lyophilized powder of voriconazole to produce a 20 ml aqueous voriconazole solution with a concentration of 10 mg/mL (1%)**.** However, in the study of Sharma et. al. [5], they used lactated ringer for reconstitution of voriconazole eye drops 1%. Also, they stored the topical 1% voriconazole solution under aseptic conditions at temperature of 2-8 °C under refrigeration for 1 week. However, in this study voriconazole eye drops were stored in complete aseptic conditions under refrigeration for 1 month. In this study, we followed the same method of preparation and storage of voriconazole eye drops described by Dupuis et. al. [6]. In this study, the used topical voriconazole eye drops were in a concentration of 1 %.The study by Al-Badriyeh et. al. [7], found that the concentration of voriconazole in the aqueous humor resulting from the 2% voriconazole eye drops was not significantly different from that reported for the 1% solution, suggesting that the penetration of voriconazole is not concentration-dependent, at least for the concentration range studied. It was also noticed that trauma of plant origin was the commonest significant risk factor for fungal ulcer (50 % in group A and 50 % in group B), tab. (1). In an Indian studies, 60% of fungal ulcers followed plant trauma (Gopinathan et. al.; Bharathi et. al.) [8,9]. In this study, analysis of laboratory studies, tab$_s$. (2 & 3) showed increased prevalence of filamentous fungal ulcer (70% in group A and 60 % in group B), 2 cases (20%) of Fusarium species were reported in group A and other filamentous fungi were of Aspergillus species. While yeasts constituted 30 % in group A and 40 % in group B. In the study of Sharma et. al. [5] all the 12 cases of the study were filamentous (8 cases were of Aspergillus species, 3 cases were of Fusarium species and 1 was of Culvuralia species). In the study of Prakash et. al. [4], they reported that all cases were filamentous (2 were Fusarium and 1 was Aspergillus). Leck et. al. [10] had reported that Fusariumand Aspergillus species were the most common organisms responsible for fungal ulcer in tropical regions. No complications of intrastromal voriconazole were recorded during injection. Repeated intrastromal injections of voriconazole (50 µg/0.1 ml) were tolerated with no long-term ocular toxicity noted. This study has 10 patients with fungal ulcer treated with intrastromal injection of voriconazole with a success rate of 80% (8 of 10 eyes) in comparison to 30% success of voriconazole eye drops alone (3 of 10 eyes), tab. (4). On comparison of the duration of healing in both groups, tab. (5), Response was more rapid in group A than that in group B. This proves that intrastromal injection shortens the resolution period for treatment of fungal ulcer. In this study, no side effects were reported after usage of voriconazole eye drops. None of the patients in the clinical

study by Al-Badriyeh et. al. [7] reported any side effects with the 2% voriconazole eye drops. In the clinical study by Vemulakonda et. al. [12], only two patients reported a mild transient stinging sensation on instillation of the 1% voriconazole eye drops. Of the 10 patients in the study by Lau et. al., four patients reported one or two instances of mild stinging and one patient reported sneezing after the initial dose [13].

5. Conclusion

In conclusion, Voriconazole may be a promising therapy for fungal ulcer that is resistant to standard antifungal agents. Voriconazole, either intrastromal injection or eye drops, is a very effective, recent and broad spectrum antifungal therapy. Targeted delivery of voriconazole by intrastromal injection is more effective as it delivers the drug at the site of infection, achieving a high intracorneal concentration, which may not be possible with topical and systemic antifungal therapy. Intrastromal injection hastens the resolution period for treatment of fungal ulcer without recorded significant complications related to injection.

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