PROSPECTIVE STUDY OF EFFECTIVENESS OF INTRASTROMAL VORICONAZOLE INJECTION IN THE MANAGEMENT OF DEEP NON HEALING FUNGAL CORNEAL ULCER AS AN ADJUNCTIVE THERAPY
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ABSTRACT: INTRODUCTION: Fungal infections of the cornea usually are difficult to treat. Contemporary antifungal drugs in the treatment of mycotic keratitis are less effective than contemporary antibacterial drugs in the treatment of bacterial keratitis. Moreover, the penetration of many antifungal drugs into the cornea is suboptimal, which makes it difficult to treat cases of deep mycotic keratitis. In this present case study we report a series of thirty patients in which intrastromal voriconazole was used in conjunction with topical treatment to treat successfully deep seated recalcitrant fungal keratitis. AIM AND OBJECTIVE: To evaluate the role of intrastromal injection of voriconazole in the management of deep recalcitrant fungal keratitis as an adjunctive therapy. METHODS AND MATERIALS: In this interventional case series, thirty eyes of thirty patients with deep stromal recalcitrant fungal keratitis that were unresponsive to topical antifungal therapy underwent intrastromal injection of voriconazole 50µgms/0.1ml. Duration of study was 6 weeks per patient. RESULTS: Before the intracorneal injections, all thirty eyes had gradually worsening lesions on topical medications. After the intervention, a faster reduction in the size of corneal infiltration was documented and complete resolution of the ulcers was seen within 6 weeks of time. CONCLUSION: Targeted delivery of voriconazole by intrastromal injection may be a safe and effective way to treat cases of deep-seated recalcitrant fungal keratitis responding poorly to conventional treatment modalities. KEYWORDS: Recalcitrant fungal keratitis, Voriconazole, Intrastromal injection.

INTRODUCTION: Fungal infections of the cornea usually are difficult to treat. Contemporary antifungal drugs in the treatment of mycotic keratitis are less effective than contemporary antibacterial drugs in the treatment of bacterial keratitis. Moreover, the penetration of many antifungal drugs into the cornea is suboptimal, which makes it difficult to treat cases of deep mycotic keratitis. To overcome these problems, investigators have evaluated alternate routes such as intracameral and intrastromal injections of Amphotericin B to treat fungal keratitis.[1,2,3]

In this present case study we report a series of 30 patients in which intrastromal voriconazole was used in conjunction with topical treatment to treat successfully deep seated recalcitrant fungal keratitis.

Voriconazole is more recent azole antifungal that is available in the form of oral and I.V formulations. Oral voriconazole is highly bioavailable (96%) and has demonstrated good penetration into different parts of the eye, with sufficient concentration achieved to cover a wide range of keratitis-causative fungi.
However, oral voriconazole can be associated with side effects as well as significant drug interactions. Topical voriconazole eye drops, manufactured extemporaneously and used in an off-label manner, have also been prescribed for treatment of keratitis with promising result. With topical administration, voriconazole demonstrated good penetration through the cornea into aqueous humour, without compromising intraocular safety. Voriconazole acts by inhibiting the synthesis of ergosterol in the fungal membranes and, ultimately, the growth of the microorganism.

Voriconazole binds to the active site of P450-dependent enzyme lanosterol 14-demethylase (CYP51 or Erg11p) and ligates the iron heme cofactor via a nitrogen atom. This results in depletion of ergosterol and the accumulation of 14-methyl sterols such as lanosterol, affecting the integrity and function of the fungal membrane.

Voriconazole is ideal for use in the treatment of fungal keratitis, as it has a broad spectrum of activity with low minimum inhibitory concentrations (MIC), as well as high systemic intraocular penetration profile. Voriconazole is potent against a wide spectrum of keratitis-causative fungi, namely, the most common pathogens Candida albicans, C. parapsilosis, C.tropicalis, A. fumigatus, Aspergillus flavus, and Fusarium solani and other less common pathogens from the Paecilomyces, Histoplasma, Scedosporium, Curvularia, and Acremonium species.

In the study by Marangon, in vitro susceptibility of common pathogens to voriconazole was compared with that for amphotericin B, fluconazole, itraconazole, and ketoconazole; voriconazole demonstrated the lowest MIC. In addition, Voriconazole was only the antifungal agent that demonstrated 100% antifungal activity against 541 different fungal isolates comprising Candida, Aspergillus and Fusarium species.

Intrastromal voriconazole is useful for the successful treatment of deep-seated, recalcitrant, fungal keratitis. Voriconazole has a superior activity against fungi that are resistant to amphotericin B and itraconazole and also has a good safety profile.

Intrastromal injection of voriconazole facilitates the clearance of the nidus of infection from cornea and it may offer a less invasive, in-office alternative. Voriconazole 50µgms/0.1ml is used to inject circumferentially around the fungal abscess in the corneal stroma as an adjunctive to topical antifungal therapy.

AIMS AND OBJECTIVES: To evaluate the role of intrastromal injection of voriconazole in the management of deep recalcitrant fungal keratitis as an adjunctive therapy

MATERIALS AND METHODS: In this interventional case series, 30 cases of mycotic keratitis involving deep corneal stroma that were unresponsive to topical antifungal therapy underwent intrastromal injection of voriconazole 50µgms/0.1ml and were observed for a duration of 6 weeks each. Confirmed fungal corneal ulcer cases not responding to routine antifungal therapy were included in the study. Patients with Perforated mycotic corneal ulcer, anesthetic cornea, Lagophthalmos were excluded from the study. Each of the participating patients gave informed written consent for participation in the study and for the surgical and medical management.
The diagnosis of the fungal infection was made on the basis of clinical evaluation, positive smear, and culture of the fungus. At initial presentation, each patient underwent a detailed clinical evaluation that included recording medical history, Snellen’s visual acuity testing, and slit-lamp biomicroscopy. Corneal scrapings were obtained under topical anesthesia and were sent for microbiological investigation including potassium hydroxide(10% KOH) wet mount preparation, Gram smear and cultures on blood agar, chocolate agar and Sabarround agar.

Topical antifungal therapy was started as soon as fungus was identified by KOH wet-mount preparation and Gram smear. The initial topical therapy included 5% natamycin every two hours, drops of 1% cyclopentolate hydrochloride thrice a day and tablet itraconazole 100mg bd. If no objectively demonstrable response to therapy was seen in 7 to 14 days or if infection shows the signs of worsening, voriconazole was injected intrastromally around the fungal abscess.

Injection voriconazole is available as 200mg of white lyophilized powder in a glass vial. The powder was reconstituted with 20ml of lactated Ringer solution (LR) to obtain 20ml of clear concentrate containing 10mg/ml of voriconazole. A 1-ml aliquot of this solution was further diluted with 20ml of LR to a concentration of 0.5mg/ml (50µg/0.1ml). The reconstituted solution was loaded in a 1-ml tuberculin syringe with a 30-gauge needle. After administration of peribulbar anesthesia, the patient was shifted to the operating table. Under full aseptic conditions, the preloaded drug was administered under operating microscope.

With the bevel down, the needle was inserted obliquely from the uninvolved, clear area to reach just flush to the abscess at mid-stromal level (as the intended level of the drug deposit) in each case. The drug then was injected and the amount of the hydration of the cornea was used as a guide to assist the area covered. Once the desired amount of hydration was achieved, the plunger was withdrawn slightly to ensure discontinuation of the capillary column and thus prevent back leakage of the drug.

Five divided doses were given around the abscess to form a deposit of the drug around the circumference of the lesion. This was done in such a manner that a centripetally directed progressive wave of fluid appeared to encompass the abscess along each meridian. Circumferential injection ensured the formation of a barrage of intrastromal voriconazole around the entire abscess. The total amount of the drug injected intrastromally ranged from 0.05ml to 0.1ml.

Post-intrastromal injection, all patients were continued 5% natamycin eye drops hourly, tablet itraconazole 100mg bd and 1% cyclopentolate hydrochloride eye drops tid. Patients were examined every third day and response to the therapy was recorded, including best corrected visual acuity (BCVA) and measurement of size of abscess on slit-lamp biomicroscopy.

The infection was considered resolved when there was complete healing of the epithelial defect with resolution of corneal abscess and scar formation. The patients were continued to topical antifungal therapy for atleast one week after the complete resolution of the infection.
RESULTS AND OBSERVATIONS:

Keratomycosis is more common in males (56.7%) than in females (43.3%).

| Sl. No. | Gender | No. of Cases |
|---------|--------|--------------|
| 1.      | Males  | 17(56.7%)    |
| 2.      | Females| 13(43.3%)    |

Table 1: Distribution (%) of Patients according to Gender

The prevalence of keratomycosis was higher among 51-60% in males than 41-50% in females.

| Sl. No. | Age group | Males | Females | Total |
|---------|-----------|-------|---------|-------|
| 1.      | 10-20     | 2(11.8%) | 3(23.1%) | 5(16.7%) |
| 2.      | 21-30     | 2(11.8%) | 3(23.1%) | 5(16.7%) |
| 3.      | 31-40     | 2(11.8%) | 0       | 2(6.6%) |
| 4.      | 41-50     | 3(17.6%) | 6(46.1%) | 9(30%) |
| 5.      | 51-60     | 5(29.4%) | 1(7.7%)  | 6(20%) |
| 6.      | 61-70     | 3(17.6%) | 0       | 3(10%) |
| **Total** | **17(100%)** | **13(100%)** | **30(100%)** |

Table 2: Distribution (%) of patients according to age group and gender

This data shows that keratomycosis is seen mostly in agriculturists and agriculture labour. This could be linked to their nature of work.

| Sl. No. | Occupation       | Male | Percentage | Female | Percentage |
|---------|------------------|------|------------|--------|------------|
| 1.      | Agriculturists(farmers) | 11   | 64.7       | 3      | 23.1       |
| 2.      | Agriculture labour | 4    | 23.5       | 5      | 38.5       |
| 3.      | House wives      | 0    | 0          | 3      | 23.1       |
| 4.      | Students         | 0    | 0          | 2      | 15.3       |
| 5.      | Industrial workers | 2    | 11.8      | 0      | 0          |

Table 3: Distribution (%) of patients according to their occupation and Gender
In majority of the cases the cause of injury was vegetative matter (40%) followed by foreign body (23.4%).

**Table 4: Distribution (%) of patients according to size of ulcer**

| Sl. No. | Size of lesion       | No. of cases |
|---------|----------------------|--------------|
| 1.      | 1/4<sup>th</sup> to 1/2 of cornea | 21(70%)      |
| 2.      | 1/2 to 3/4<sup>th</sup> of cornea  | 8(28.7%)     |
| 3.      | > 3/4<sup>th</sup> of cornea     | 1(3.3%)      |

In majority of the cases (70%) the ulcer size was 1/4<sup>th</sup> to 1/2 of cornea.

**Table 5: Distribution (%) of patients with keratomycosis & type of fungi**

| Sl. No. | Types of fungus       | Males          | Females        |
|---------|-----------------------|----------------|----------------|
| 1.      | Fusarium species      | 7(41.2%)       | 8(61.5%)       |
| 2.      | Aspergillus species   | 7(41.2%)       | 5(38.5%)       |
| 3.      | Candida species       | 3(17.6%)       | 0              |

In majority of keratomycosis the Fusarium species was observed followed by Aspergillus species.

**Table 6: Distribution (%) of patients according to the time of (weeks) healing of ulcer after intrastromal voriconazole injection**

| Sl. No. | Weeks                                           | No. of cases |
|---------|-------------------------------------------------|--------------|
| 1.      | 4 weeks                                         | 10(33.3%)    |
| 2.      | 5 weeks                                         | 11(36.7%)    |
| 3.      | 6 weeks                                         | 5(16.7%)     |
| 4.      | Perforation of ulcer after 2 weeks of treatment. Under went TKP | 3(10%)       |
| 5.      | Did not come to follow up after one week treatment in hospital. | 1(3.3%)      |
Most of the cases started improving from 1st, 2nd week onwards and healed within 4-5 weeks after intrastromal voriconazole.

| Sl. No. | Residual Manifestations | Males    | Females   |
|---------|-------------------------|----------|-----------|
| 1.      | Nebula                  | 3(20%)   | 1(9.1%)   |
| 2.      | Macula                  | 8(53.3%) | 9(81.8%)  |
| 3.      | Leucoma                 | 3(20%)   | 1(9.1%)   |
| 4.      | Adherent leucoma        | 1(6.7%)  | 0         |

Table 7: Distribution (%) of patients with Residual manifestations

Since most of the cases of keratomycosis involve the stroma a gross opacity in the form of macula was observed in most of the cases.

| Sl. No. | Visual acuity | Intrastromal voriconazole |
|---------|---------------|--------------------------|
|         | Before No. of cases | After No. of cases |
| 1.      | PL+, PR+ to CF-1mt   | 27(90%)                  | 2(7.7%)             |
| 2.      | CF-2mts to CF-4mts  | 1(3.3%)                  | 10(33.3%)           |
| 3.      | 6/60 to 6/24        | 2(7.7%)                  | 9(30%)              |
| 4.      | 6/18 to 6/9         | 0                        | 5(16.7%)            |
| 5.      | Under went TKP      | 0                        | 3(10%)              |
| 6.      | Drop out            | 0                        | 1(3.3%)             |
| 7.      | P value < 0.001     |                          |                       |

Table 8: Distribution (%) of patients (keratomycosis) with treatment and improvement in Visual acuity (BCVA)

The above table shows visual acuity is improved in 10 cases of PL+, PR+ to CF-1mt group to CF-2mts to CF-4mts group and another 9 cases of same group improved to 6/60 to 6/24 group and 1 case to 6/18 to 6/9 group. This was the most significant advantage of Intrastromal Voriconazole injection.

| Sl. No. | 1st week | 2nd week | 3rd week | 4th week | 5th week | 6th week |
|---------|----------|----------|----------|----------|----------|----------|
|         | Reduction of inflammation | 0    | 10    | 14    | 2    | 0    | -       |
|         | Absorption of hypopyon    | 2    | 5    | 7    | 2    | 0    | -       |

Table 9: Distribution (%) of patients with Clinical improvement of ulcer

Most of the inflammatory signs and hypopyon disappeared in 2nd and 3rd weeks.
It was observed that the improvement of ulcer was associated with age of the patient, younger the patient earlier the ulcer healing.

The intervention shows significant impact (P value <0.001) on the improvement of keratomycosis. Each of the thirty patients was referred to us for management by peripheral ophthalmic clinics with a history of recalcitrant microbial keratitis and corneal abscesses involving up to posterior stroma. Patients had already received topical fluoroquinolone drops and antifungal agents for two to four weeks.

History of vegetative trauma in 12 patients, foreign body or dust in 7 patients, no history of trauma in 4 patients, insect trauma in 2 patients, industrial injury in 2 patients and 1 each with stone, sand and buffalo tail. In each of the thirty patients smears were positive for fungus and on
cultures Fusarium species was identified in 15 patients, Aspergillus species in 12 patients and Candida species in 3 patients.

As poor response was seen following two weeks of therapy with topical eye drops of 5% natamycin and tablet itraconazole 100mg bd, voriconazole was injected intrastromally around the infected area and 5% natamycin eye drops and tablet itraconazole 100mg bd were continued till the healing of the ulcer.

In each of the thirty patients the procedure was performed successfully and no intraoperative or postoperative complications were observed, perforation of the ulcer was noticed in two patients in the second week of follow up and ulcer was progressive in third week of follow up in one diabetic patient who underwent TKP, one patient did not come for follow up and the infection was resolved completely in the remaining twenty six patients after voriconazole injection. The mean healing duration was five weeks ± one week.

The improvement of BCVA in ten patients from PL+, PR+ to CF-1mt group to 6/60 to 6/24 group another ten patients from same group to CF-2mts to CF-4mts and one patient to 6/18 to 6/9 group. This shows great significance of intrastromal voriconazole injection. In some patients there was a minimal improvement in BCVA attributable to involvement of the abscesses and the resultant scar in the central cornea.

DISCUSSION: Corneal infections involving deeper parts of the stroma are not amenable to topical antimicrobial therapy. This is particularly true for mycotic keratitis because none of the present-day antifungal agents can optimally penetrates the deeper layers of cornea. To overcome these problems, modalities of targeted drug delivery are being evaluated. Similar attempts of site-directed drug deposit have been made in posterior segment pathologies in the form of intra-vitreal injections and posterior Sub-tenon injections of drug.[6]

In our series, none of thirty cases had responded to topical antifungal therapy and therefore we decided to proceed with intrastromal drug delivery. Intrastromal injections of amphotericin B have been used previously to treat recalcitrant mycotic keratitis.[7] We used voriconazole because previous experiences with it in ocular infections, using both topical and systemic routes, have been promising.[8,9,10,11] Furthermore, voriconazole has optimal activity against fungi that are resistant to amphotericin B and itraconazole and has a good safety profile.[12,13,14] The intrastromal injections of voriconazole helped in early and complete resolution of the ulcer with no adverse effects in 26 out of 30 cases of our study.

Our series of 30 cases provides some indication of a possible therapeutic role of intrastromal antimicrobial drug delivery by intrastromal injection in the management of recalcitrant fungal keratitis. We believe that if combined with topical therapy, a judicious use of intrastromal administration of antifungal drugs may be of immense benefit in such cases.

Gaurav Prakash et.al have done similar study wherein they have injected intrastromal voriconazole in three patients and in all those patients the infection was controlled.[15] Our study confirms the success rate of the study done by them.

Another study by Vandana Jain et.al.[16] one case of post-operative recalcitrant fungal tunnel infection treated with intrastromal injection of voriconazole, the infection was controlled.
Advantages of Intrastromal Voriconazole Injection:
I. High percentage of success in healing the recalcitrant mycotic fungal keratitis.
II. Because the drug is given as depot, the patient compliance in usage of other topical drugs is decreased in duration and dosage.

Limitations of Intrastromal Voriconazole:
I. The only reason why the voriconazole is not used as first line of the drug in fungal keratitis is the huge cost of the drug (rupees 2400 per 200mg vial).
II. There is a risk of corneal perforation while injecting the drug.

CONCLUSION: Targeted delivery of voriconazole by intrastromal injection may be a safe and effective way to treat cases of deep-seated recalcitrant fungal keratitis responding poorly to conventional treatment modalities.

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