

## Determination of surgical outcomes with a novel formulation of intrastromal natamycin in recalcitrant fungal keratitis: A pilot study

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**Purpose:** To evaluate the outcomes of water-soluble intrastromal natamycin (IS-NTM) as an adjunct therapy for recalcitrant fungal keratitis. **Methods:** This was a prospective interventional pilot study in the setting of a tertiary eye-care center. Twenty eyes of 20 consecutive patients with microbiologically proven recalcitrant fungal keratitis (ulcer size >2 mm, depth >50%, and not responding to topical NTM for 2 weeks) were recruited. The selected patients were injected with a novel composition of IS-NTM (10 ug/0.1 mL, soluble natamycin) prepared aseptically in the ocular pharmacology department. All the patients continued using topical NTM suspension 5% 4-hourly until the ulcer healed. Repeat injections were undertaken after 72 h depending on the clinical response and all the patients were followed till 6 months. **Results:** The mean age of the patients was  $40.42 \pm 10.09$  years. The mean duration of the presentation was  $20.8 \pm 5.1$  days. The most commonly isolated organisms were *Aspergillus sp.* (12/20, 60%) and *Fusarium sp.* (8/20, 40%). No patient had iatrogenic perforation or precipitate formation after IS-NTM injection. The overall cure rate with IS-NTM was 95% (19/20 patients). The number of patients who healed with the 1<sup>st</sup>, 2<sup>nd</sup>, and 3<sup>rd</sup> injection was 13, 5, and 1, respectively. One (5%) had no response to treatment and was subjected to penetrating keratoplasty. The average time taken for the resolution of the epithelial defect, stromal infiltrates, and hypopyon was  $34 \pm 5.2$  days,  $35.3 \pm 6.4$  days, and  $15 \pm 2.5$  days. Healing with deep vascularization and cataract was noted in 6/19 eyes (31%) and 13/19 eyes (68.42%), respectively. **Conclusion:** Intrastromal injection of a novel formulation of NTM holds a promising role as adjunctive therapy to topical NTM in the management of recalcitrant filamentous fungal keratitis. The preliminary results are encouraging and further studies are required to validate the results.

**Key words:** Intrastromal injections, natamycin, recalcitrant fungal keratitis, voriconazole

Fungal keratitis is a visually devastating corneal condition that needs urgent and appropriate management to prevent consequent ocular morbidity.<sup>[1]</sup> Natamycin (NTM), a tetraene polyene amphoteric macrolide, is the only United States Food and Drug Administration (FDA)-approved topical antifungal agent. The drug has broad-spectrum antimycotic activity, is particularly active against *Fusarium* and *Aspergillus sp.* and is presently the most commonly preferred drug for the management of filamentous mycotic keratitis.<sup>[2,3]</sup> It has a dose-related fungicidal effect and blocks fungal growth by binding to ergosterol, an essential component in the fungal cell wall. The agent is commercially available for ophthalmic use as 5% (50 mg/mL) suspension with benzalkonium chloride (0.02%) added as a preservative. The safety and efficacy of topical NTM suspension for filamentous fungal keratitis are already well-established in the literature.<sup>[2,3]</sup> However, the topical application has to be repeated frequently for a prolonged time to ensure an adequate drug delivery to the site of infection. This

may result in irritation, congestion, epithelial toxicity, tearing, and hypersensitivity reactions. Besides these complications, poor corneal penetration and precipitate formation, attributed to the high molecular weight and suspension form of the presently available commercial ophthalmic preparations, respectively, might decrease the efficacy of NTM for deep mycotic infections and also prevent effective patient monitoring.

The targeted delivery of the antifungal agents at the ulcer site employing intrastromal injections remains an effective alternative method of dealing with deep and recalcitrant fungal infections. Multiple clinicians, including us, have reported satisfactory results with intrastromal voriconazole and amphotericin B injections for deeply infiltrating fungal abscesses.<sup>[3-5]</sup> However, the failure of the researchers to obtain much success with intrastromal NTM (IS-NTM) injections in animal studies due to its physicochemical limitations has ceased its further application in the human eyes.<sup>[6,7]</sup>

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Presently, we describe treatment outcomes with a novel formulation of NTM employed as an intrastromal injection for recalcitrant fungal keratitis. Sterile water-soluble natamycin complex (Natasol\*) was prepared at the Ocular Pharmacology and Pharmacy Division of our center.<sup>[8,9]</sup> The complex was further used to prepare the injection under strict aseptic conditions and supplied for clinical use in individually sealed ampules. To the best of our knowledge, this remains the first study describing the application and success of IS-NTM in recalcitrant fungal keratitis in human eyes and the first of its kind to employ a novel formulation of NTM (\*patent pending).

## Methods

The study was approved by the Institutional Ethics Committee Board of our center, IECPG-106/30.12.2015, and was conducted adhering to the tenets of the Declaration of Helsinki. The research has also been registered under the Clinical Trials Registry, India (CTRI/2021/01/030360), National Institute of Medical Statistics, ICMR, New Delhi ([www.ctri.nic.in](http://www.ctri.nic.in)).

Twenty adult consecutive patients presenting with unilateral smear-positive or culture-proven recalcitrant fungal keratitis (ulcer size >2 mm, depth >50% stroma, and not responding to topical NTM suspension for 2 weeks) were selected for IS-NTM injection. All patients were offered all presently available treatment methods for fungal keratitis and written informed consent was obtained before each injection. The patients with poor compliance, follow-up, mixed infection on smear or culture analysis, impending perforation, stromal thinning with involvement of >50% depth, bilateral ulcers, and non-ambulatory visual acuity in the fellow eye, patients who were pregnant or breastfeeding, and had a history of known drug allergies, were excluded.

At the initial presentation, a thorough history regarding the onset, progression, and duration of corneal ulcer, predisposing factors such as trauma, steroid use, recent surgery, and diabetes was obtained and all the patients were subjected to the assessment of best-corrected visual acuity (BCVA) and detailed slit-lamp examination by an experienced corneal specialist (NS) to note the size of the epithelial defect, stromal infiltrate, and the hypopyon. The ulcer area was calculated from its maximum diameter and the dimension perpendicular to it. The posterior segment was evaluated either clinically or on B-scan ultrasonography to rule out coexisting endophthalmitis. The corneal infiltrates were scraped under topical anesthesia in a standardized manner and sent for microbiological evaluation including Gram staining, 10% potassium hydroxide wet-mount preparation, and cultures on blood agar, chocolate agar, and Sabouraud dextrose agar. All the patients received topical NTM suspension 5% 2 hourly, and homatropine 2% eye drops four times a day and assessed biweekly. The ulcer was defined as 'healing' if the area and size of the epithelial defect and the infiltrate were reduced by >20% from the last presentation. If no response was recorded in 2 weeks, then IS-NTM was added to the given regimen.

IS-NTM was prepared at the Ocular Pharmacology and Pharmacy Division at our center.<sup>[8,9]</sup> The lyophilized Natasol was reconstituted in the sterile zone, passed through 0.22 µm sterile filters, filled, and sealed in ampules for single use. Quality control parameters such as sterility, drug content, pH, and osmolality were analyzed at the quality control laboratory and the levels were found to be within the acceptable range.

The drug was injected by the treating clinician (NS) under local anesthesia. The reconstituted drug in its solution form was loaded in a 1 mL tuberculin syringe attached to a bent 30-gauge needle. Under full aseptic conditions, the needle, with its bevel up, was inserted obliquely from the relatively uninvolved site and progressed centripetally to reach the corneal infiltrate at the mid-stromal level. The amount of corneal hydration was used as a guide to assess the area covered with each injection. Once the desired amount of hydration was achieved, the plunger was withdrawn slightly to ensure discontinuation of the capillary column, thus, preventing back-leakage of the drug. The reconstituted formulation was injected circumferentially in five divided doses at the boundaries of the infiltrate to form a barrage of drug deposits around the lesion.<sup>[5]</sup>

All the patients were followed up on days 1, 3, 7, 14, 21, and monthly or according to the discretion of the treating clinician till 6 months. Repeat intrastromal injections were administered after 72 h if there were no reduction in the size of the epithelial defect and stromal infiltrates. At each follow-up, the size of the epithelial defect, infiltrate, and hypopyon, BCVA, and complications, if any were noted. All the patients were continued on topical NTM therapy till 2 weeks after the resolution of the ulcer. The ulcer was considered healed where a complete resolution of the epithelial defect, hypopyon, and stromal infiltrate was seen. At the final follow-up, the scar size was also calculated in the greatest dimension and along an axis perpendicular to it. The patient who developed a corneal perforation or did not show any signs of improvement or showed signs of worsening after three IS-NTM injections was considered a treatment failure and was started on an oral antifungal (tab voriconazole 200 mg twice a day). The patient was planned for therapeutic keratoplasty as early as possible based on the availability of donor tissue.

## Results

The mean age of the patients was  $40.42 \pm 10.9$  years (14 males, 6 females). The mean duration of the presentation was  $20.8 \pm 5.1$  days. The most common causative organisms were *Aspergillus sp.* (12/20, 60%) followed by *Fusarium sp.* (40%). All the organisms were found sensitive to NTM *in vitro*. None of the patients developed any iatrogenic perforation, precipitate formation, new foci or worsening of infection, endophthalmitis, allergic reactions, glaucoma, or systemic adverse effects after IS-NTM injections in our series. The overall success rate was 95% with 19/20 patients responding successfully to one or more than one IS-NTM injection. The number of patients who healed with the 1<sup>st</sup>, 2<sup>nd</sup>, and 3<sup>rd</sup> injections was 13 (65%), 5 (25%), and 1 (5%), respectively. Only one patient did not respond to the treatment and underwent penetrating keratoplasty with a clear graft till 6 months of follow-up. In the other 19 patients, the mean time taken for resolution of an epithelial defect, stromal infiltrates, and hypopyon was  $34 \pm 5.2$  days,  $35.3 \pm 6.4$  days, and  $15 \pm 2.5$  days, respectively. The mean final scar size was  $2.6 \pm 0.7$  mm [Figs. 1 and 2]. The visual acuity improved from  $2.23 \pm 0.35$  Logarithm of Minimum angle of resolution (LogMAR) to  $1.0 \pm 0.1$  LogMAR at a 6-month follow-up. Deep vascularization and cataract were noted in 6/19 eyes (31%) and 13/19 eyes (68.42%), respectively [Table 1].

**Table 1: Clinical features of the patients receiving IS-NTM, counting fingers close to face -1.9, Hand movements close to face-2.3, Light perception-2.7, and no light perception-3<sup>[10]</sup>**

Case	Age	Gender	Duration of symptoms (days)	Size of epithelial defect	Depth of ulcer	Visual acuity Log MAR	Organism isolated	Duration of Healing (days)	Outcome
1	32	M	20	3.5*3.5	>75%	1.9	<i>Fusarium</i>	35	Healed
2	55	F	14	2.75* 3	>75%	2.7	<i>Aspergillus</i>	30	Healed
3	39	M	28	4.25 *4.5	50%	2.3	<i>Aspergillus</i>	35	Healed
4	40	F	24	2.25 *3	>75%	2.7	<i>Fusarium</i>	28	Healed
5	44	M	30	4.5 *5	50%	1.9	<i>Fusarium</i>	35	Healed
6	42	M	26	2.25* 2	>75%	2.7	<i>Aspergillus</i>	49	Healed
7	36	M	24	4 *4	>75%	2.3	<i>Aspergillus</i>	35	Healed
8	43	F	20	2.5* 2	50%	1.9	<i>Fusarium</i>	35	Healed
9	58	F	28	2 *1.5	>75%	2.7	<i>Fusarium</i>	35	Healed
10	47	M	20	3 *2	50%	1.9	<i>Aspergillus</i>	28	Healed
11	24	M	14	1.5* 2	50%	1.9	<i>Fusarium</i>	35	Healed
12	60	M	24	3 *2.5	>75%	1.9	<i>Aspergillus</i>	35	Healed
13	39	M	24	4 *4.5	50%	1.9	<i>Fusarium</i>	42	Healed
14	32	M	14	2 *2	50%	2.3	<i>Aspergillus</i>	28	Healed
15	26	F	20	3 *2	50%	1.77	<i>Aspergillus</i>	35	Healed
16	36	M	14	5 *5	>75%	2.3	<i>Fusarium</i>	-	Perforation of ulcer on day 3
17	30	M	21	3 *2.5	50%	1.9	<i>Aspergillus</i>	28	Healed
18	25	F	18	4 *4.5	>75%	2.7	<i>Aspergillus</i>	35	Healed
19	58	F	14	4 *4	>75%	2.7	<i>Aspergillus</i>	35	Healed
20	38	M	20	3.25* 3	>75%	2.3	<i>Aspergillus</i>	28	Healed

## Discussion

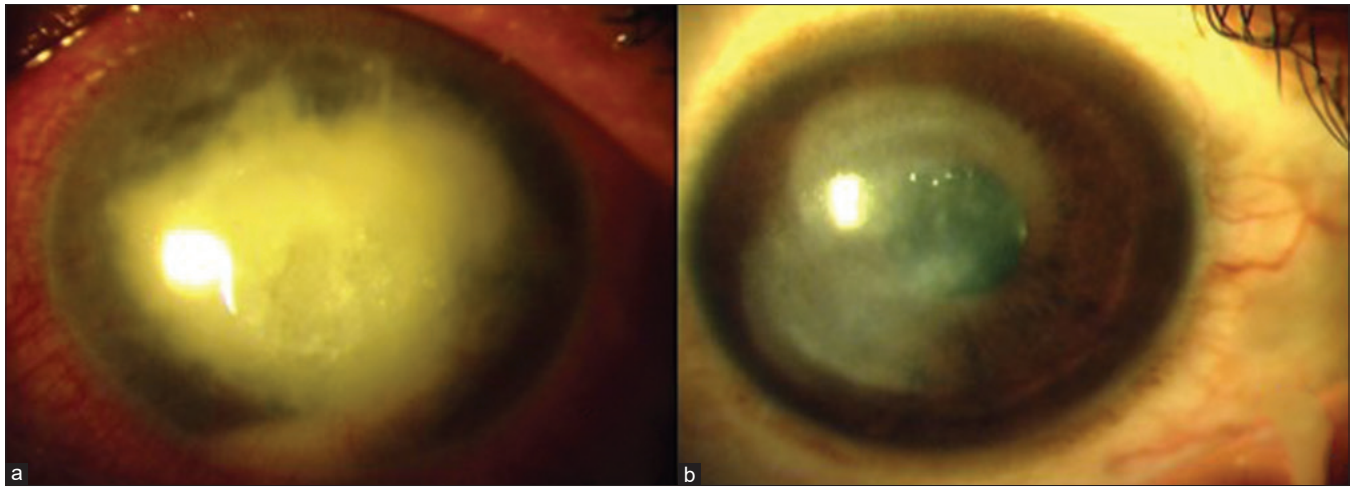
Natamycin is the most commonly advocated topical antifungal agent for the management of superficial fungal infections. However, the results are not very convincing with deep fungal infections due to their suboptimal stromal penetration, therefore, most of the clinicians add voriconazole to the treatment regime despite the organisms possessing *in vitro* sensitivity to NTM.<sup>[11]</sup> Various alternative routes to improve deeper penetration of NTM include epithelial debridement and encapsulation of NTM with lecithin/chitosan mucoadhesive nanoparticles.<sup>[12]</sup> While iatrogenically compromising epithelial integrity adds up to the epitheliotoxic effect of benzalkonium chloride preservative used in antifungal medications, the widespread utility of the latter is restricted by the cost, availability, strict and cumbersome preparation criteria, and difficult physicochemical characterization of the nanoparticles. The intrastromal injection, a feasible option of enhancing targeted drug delivery, has been only occasionally tried with NTM in the peer-reviewed literature mainly due to its dissatisfactory pharmacokinetic profile. Mimouni *et al.*<sup>[6]</sup> in a recent experimental animal study, failed to demonstrate a beneficial additive role of IS-NTM (5% suspension form) to topical 5% NTM therapy. Similarly, O'Day *et al.*<sup>[7]</sup> suggested poor intracorneal and aqueous levels of NTM (5% microfine suspension) in rabbit eyes after its intrastromal injection. Poor surgical results despite the high activity of NTM against *Fusarium*, as noted in the previous studies, could be attributed to its poor bioavailability and to the variability in *in vivo* and *in vitro* drug susceptibilities.<sup>[13]</sup> However, we experienced an overall cure rate of 95% with three injections and a 65% cure rate with the first IS-NTM injection along with an improved visual acuity in all the patients. Enhanced success rates in our series

could be attributed to our novel water-soluble NTM (Natasol) injection.

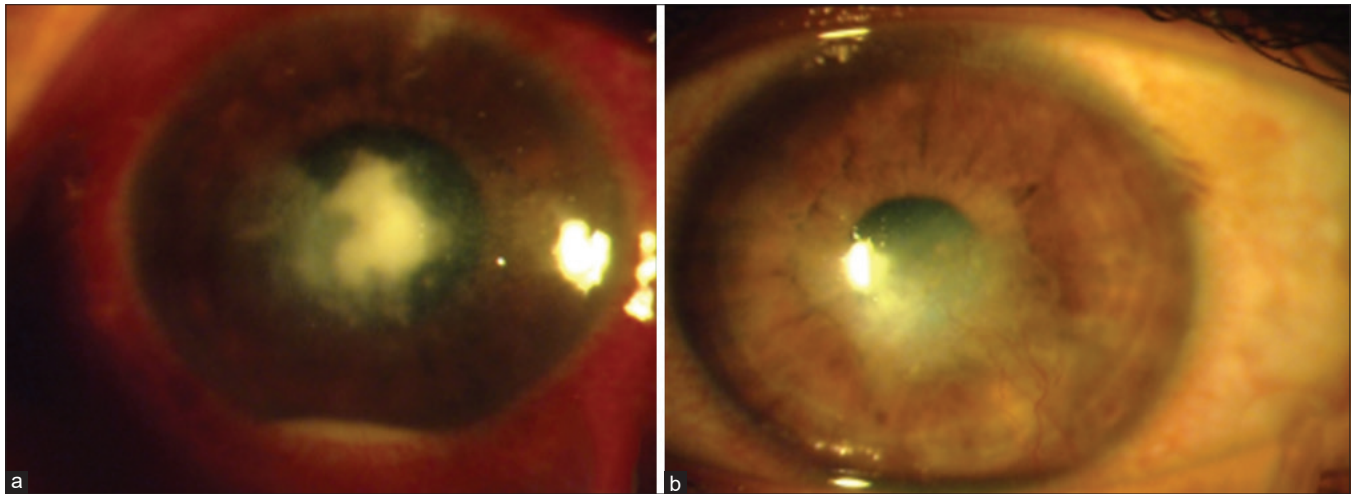
As NTM is insoluble in an aqueous solution, it is always used as a suspension for topical fungal keratitis. Making it soluble by using the inclusion complexation technique enabled it into a soluble form suitable for intrastromal injection. This process probably augmented the delivery of NTM to the site of infection for a prolonged period that ultimately resulted in an enhanced microbiological cure. Moreover, the optically clear and preservative-free intrastromal formulation further facilitated the healing of the epithelial defect and better patient monitoring. All these factors improved patient compliance by shortening the total duration and frequency of topical NTM. These encouraging preliminary results suggest that the novel NTM formulation could become the antifungal agent of choice for intrastromal injections besides presently being the drug of choice for topical administration.

Ocular pharmacokinetics and ocular safety studies of sterile soluble natamycin formulation (Natasol\*) have already been documented.<sup>[8,9]</sup> The pharmaceutical excipients used in the soluble natamycin injection have already been recognized as GRAS (Generally Recognized as Safe) and inactive by FDA for human use.<sup>[11,12]</sup> The only complications noted with this newer formulation were postoperative cataract formation and deep vascularization. At present, it is difficult to comment on any direct correlation between the drug formulation and these complications as they can be individually associated with intense inflammation due to recalcitrant keratitis and repeated intrastromal injections. The limitations of our current study include small sample size, short-term follow-up, absence of a control group, and lack of standard and clearly defined dose,





**Figure 1:** (a) Clinical photograph of recalcitrant fungal keratitis at day 0, case 18; (b) Clinical photograph of the same patient after healing.



**Figure 2:** (a) Clinical photograph at day 0 of case 8. (b) Clinical photograph of the same case after healing.

and clinical pharmacokinetics of IS-NTM. The minimum inhibitory concentration (MIC) clinical breakpoints for filamentous fungi against natamycin have not been established by the Clinical and Laboratory Standards Institute (CLSI), hence, without a defined guideline for classifying organisms as susceptible, intermediate, or resistant, it is currently challenging to assess the association between antifungal susceptibility and clinical outcomes in fungal keratitis. Also, *in vitro* and *in vivo* susceptibility of the drug varies. There is no denial of the fact that further larger long-term prospective comparative trials are required to validate our results. However, considering the paucity of the newly-added antifungal agents, encouraging preliminary results with a novel composition of an already existing drug may open up a new arena for the management of complex and recalcitrant fungal keratitis cases. With these encouraging results, we are currently optimizing the dose and dosage forms for making them widely available for clinical use.

## Conclusion

To conclude, novice NTM inclusion complex-enabled formulation holds a promising role as a useful adjunct to standard therapy in the management of recalcitrant filamentous

fungal keratitis. Regional differences in microbiological profiles should be considered before prescribing them. Also, larger, long-term randomized comparative trials are required to validate the current results.

## Declaration of patient consent

The authors certify that they have obtained all appropriate patient consent forms. In the form the patient(s) has/have given his/her/their consent for his/her/their images and other clinical information to be reported in the journal. The patients understand that their names and initials will not be published and due efforts will be made to conceal their identity, but anonymity cannot be guaranteed.

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Nil.

## Conflicts of interest

There are no conflicts of interest.

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