

## Intermediate outcomes of therapeutic penetrating keratoplasty for severe microbial keratitis using glycerol-preserved donor corneas during the COVID-19 pandemic

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**Purpose:** To report the intermediate outcomes of therapeutic penetrating keratoplasty (TPK) performed for severe microbial keratitis using glycerol-preserved corneas during the Corona virus diseases of 2019 (COVID-19). **Methods:** Retrospective non-comparative case series from April to August 2020 in a network of tertiary eye care centers. Glycerol-preserved tissues were used for therapeutic keratoplasty (TPK). We reviewed the demographics, microbiology, surgical outcomes such as wound integrity, recurrence, graft melt, epithelialization, and complications. **Results:** A total of 49 eyes that underwent TPK with glycerol-preserved corneal tissues were analyzed. The primary indication was severe microbial keratitis in 47 eyes. The majority was a fungal infection in 33 eyes (67.3%). The mean age was  $53.8 \pm 12.2$  years, with male predominance (3:1). The corneas were stored for an average of  $85.5 \pm 53$  days prior to transplant. The median donor age was 65 years. The grafts were tectonically stable in 32/36 eyes (88.9%) at 1 month and 20/24 eyes (83.3%) at 3 months. The graft melt was noted in three eyes at 1 and 3 months. The recurrence of the infection was noted in four eyes and all were of fungal etiology. The graft epithelialization was delayed with a mean duration of  $48.9 \pm 25$  days after surgery. Post-TPK, raised intra-ocular pressure ( $>21$  mm Hg) was noted in 51.2% at 1 week, 17.4% at 1 month, and 11.8% at 3 months. **Conclusion:** Glycerol preservation is a reliable alternative with good therapeutic outcomes in the short and interim postoperative period. Delayed epithelialization and secondary glaucoma were the commonest postoperative complications.

**Key words:** Corneal transplant outcomes, glycerol-preserved corneas, severe microbial keratitis, therapeutic keratoplasty

Glycerol corneal preservation was first described in 1957 by King *et al.*<sup>[1]</sup> as a viable technique for preserving human cornea in an experimental model for an indefinite period. The corneal tissue had acceptable tensile strength and provided tectonicity in therapeutic implants.<sup>[2,3]</sup> However, these corneas are devoid of endothelial cells and are not viable for optical keratoplasty. Due to the highly successful cornea retrieval programs in the recent past, the eye banks did not have to look beyond corneas preserved in short and intermediate storage media like McCarey-Kaufman medium, Optisol, or Cornisol. However, a severe crisis due to the limited operational capacity of all eye banking activities during the COVID-19 pandemic and the subsequent lockdown culminated in an extreme shortage of donor tissues.<sup>[4]</sup> The preservation of corneas by glycerol was thus revived for long-term storage by eye banks

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for emergency use, especially when the donor corneal supply is erratic, e.g., the ongoing COVID-19 pandemic.<sup>[5]</sup> From April 2020 to August 2020, we utilized such corneas for emergency transplantations across our network of eye care services. In this report, we aim to evaluate the short-term outcomes and complications of using the glycerol-preserved corneal tissues in therapeutic keratoplasty during the COVID-19-related lockdown.

### Methods

The study was approved by the Institutional Ethics Committee (LEC/BHR-R-09-20-510) and adhered to the tenets of the Declaration of Helsinki.

### Glycerol preservation

The donor tissues were procured, processed, and stored as per regular practice.<sup>[6]</sup> The procedure for glycerol preservation has

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been reported earlier.<sup>[7]</sup> Briefly, the available corneal tissues which were stored in the McCarey-Kaufman (MK) medium (prepared at our eye bank) or Cornisol medium (AuroLab, Madurai, India) but not utilized yet at the time of the lockdown were transferred to the autoclaved vials containing glycerol (Arihant Traders, Hyderabad, India) under a sterile biological laminar flow hood and stored at  $-80^{\circ}\text{C}$ . Prior to transferring the donor corneas, a microbiological evaluation of a portion of the glycerol fluid was performed to ensure sterility. The glycerol-preserved corneas were then released for TPK by the eye bank. Post-lockdown, any retrieved donor tissue which was unlikely to be utilized within 15 days was shifted to glycerol-preservation mode.

### Clinical data

We performed a retrospective chart review of all the cases of therapeutic penetrating keratoplasty (TPK) at our network of hospitals using glycerol-preserved corneal tissues from April 2020 to August 2020. The demographic details, history such as duration of symptoms, and ocular examination details such as size, location of infiltrate, presence of thinning, impending or actual perforation, presence of hypopyon, scleral, and intra-ocular extension if present, were noted. The results of microbiology and the details of medical therapy were noted. The indications for transplant, complications during surgery, and postoperative medical or surgical treatment, and post-transplant clinical outcomes of therapeutic keratoplasty were evaluated. Severe microbial keratitis was defined by our group as the presence of one or more of the following: infiltrate size  $>6$  mm, presence of stromal thinning of  $>50\%$ , presence of descemetocoele or perforation irrespective of the infiltrate size, presence of endothelial exudates, hypopyon more than half of the anterior chamber, intraocular involvement such as lens abscess, endophthalmitis, and scleral involvement.

### Microbiological data

The corneal scrapings were taken from the ulcers and subjected to microbiological examination as per the institutional protocol.<sup>[8]</sup> Smears were made on glass slides for direct microscopy (Gram's stain, potassium hydroxide 10% wet mount with 0.1% calcofluor white) and culture media were inoculated for bacteria, fungi, *Pythium* and *Acanthamoeba*. The culture plates were incubated for 7 days if the smears were negative or positive for bacteria and 14 days if the smears were positive for fungal filaments.

### Medical therapy

For cases where the initial microbiology report was either smear-negative or bacteria reported on smears, fortified vancomycin 5% with topical ciprofloxacin 0.3% every 1 hour was commenced. When the fungal filaments were identified on smears, 5% natamycin eye drops every 1 hour and when indicated oral tablet ketoconazole 200 mg (or itraconazole 100 mg) twice daily were started. For *Acanthamoeba*, 0.02% polyhexamethylene biguanide (PHMB) and 0.02% chlorhexidine were started every 1 hour.

### Therapeutic keratoplasty

Those cases that worsened on medical treatment (noted as an increase in the size of the infiltrate, thinning, perforation, scleral extension, and/or endophthalmitis) were advised TPK. The donor glycerol-preserved cornea was removed from the storage media, washed thoroughly with the ringer lactate solution, and allowed to rehydrate in a bowl filled with sterile

ringer lactate for 10 min. The glycerol-preserved corneas were observed to be thicker and of firm consistency as opposed to the MK- or Cornisol-preserved tissue. This increased the surgical time in view of the difficulties in trephination and suturing. Intraoperatively, the exudates over the lens and iris surface were gently removed and two surgical peripheral iridectomies were made. The donor tissue was oversized by 0.5–1 mm based on the recipient trephination ( $<10$  or  $>10$  mm) and secured with 16 or 24 interrupted sutures using either 10-0 nylon or a combination of 9-0 and 10-0 nylon. The postoperative regimen constituted of topical corticosteroids that was initiated immediately after TPK in bacterial keratitis and variably after *Acanthamoeba* and *Pythium* keratitis.

Postoperatively, the corticosteroids were initiated 2 weeks later even in fungal keratitis for the control of severe inflammation in these eyes.

The primary therapy was continued for 2 weeks in TPK after fungal and bacterial keratitis and up to 1-month post-*Acanthamoeba* and *Pythium* keratitis. The topical corticosteroids were initiated after 2 weeks in the fungal keratitis after an observation period of 2 weeks devoid of residual or recurrent infection. The patients were followed up on day 1, 1 week, 2 weeks, and then, monthly for the first 3 months. More frequent follow-ups were required in cases with complications.

### Postoperative follow-up

The postoperative data were reviewed at three time points: 1 week, 1 month, and 3 months. The visual acuity, graft epithelialization, graft apposition, intraocular pressure (IOP), and complications such as secondary glaucoma, cataract, poor epithelialization, graft melt, recurrence of primary infection, or endophthalmitis were reviewed. The raised IOP was defined as IOP  $>21$  mmHg, or elevated IOP on digital assessment, and these patients were treated with antiglaucoma medications (oral topical carbonic anhydrase inhibitors, topical  $\beta$ -blockers, and  $\alpha 2$  agonists).

The recurrent infection was defined as the reappearance or recurrence of the primary infection either in the graft or host or intraocularly or when there were persistent exudates in the anterior chamber, with or without associated intraocular spread such as endophthalmitis or lens abscess within 5 weeks of TPK. The delayed epithelialization of the graft was defined as an epithelial defect persisting beyond 2 weeks after post-keratoplasty. The graft melt was defined as the progressive thinning of the graft stroma with an overlying epithelial defect with or without concurrent graft infiltrate. The primary graft failure was defined as non-clearance of graft edema leading to the loss of graft clarity with poor visualization of the anterior chamber details.

All the grafts were expected to undergo primary failure and we have defined this for the sake of clarification but we have not considered it as a study outcome.

### Outcomes

For the purpose of the study, success was defined as *anatomic success* and implied *restoration* of globe integrity and successful removal of the primary infection. Failure was defined as a *therapeutic failure* if any one of the following was present: the presence of residual infection or recurrence of the primary infection or lack of globe integrity with dehiscence of the graft-host junction or graft melt and/or requirement of second surgical

intervention for maintaining the graft tectonicity such as wound revision (repeat suturing) or repeat transplant or evisceration for the end-stage disease and surgical interventions such as amniotic membrane transplant, tarsorrhaphy, or tissue adhesives for persistent epithelial defect (PED) which showed rapid worsening despite medical therapy or even repeat penetrating keratoplasty for the graft melt or perforation following progressive PED. The loss of graft clarity (primary or secondary graft failure) was not considered as the primary outcome measure.

### Statistical analysis

The statistical analysis was performed using software Origin v2019b (OriginLab Corporation, Northampton, MA, USA) and STATA v14.2 (StataCorp, College Station, TX, USA). The normality of the distribution of the continuous data was assessed by the Shapiro–Wilk’s test. Data with normal distribution were described in mean  $\pm$  standard deviation and data with non-normal distribution in the median and inter-quartile range (IQR). The categorical data were described in proportions. A mixed-effects model with maximum likelihood estimation was used in the analysis of IOP during the postoperative period and marginal linear predictions were compared among the visits. The Kaplan–Meier survival analysis was used in the estimation of therapeutic success probabilities. A *P* value of  $<0.05$  was considered statistically significant.

## Results

During the study period, a total of 49 eyes of 49 patients underwent TPK with glycerol-preserved corneas. The mean age was  $53.8 \pm 12.2$  years. There was a male predominance (3:1) with 37 males (75.5%) and 12 females (24.5%). The median duration of the symptoms was 20 days (IQR, 10–30 days). The geometric mean of the vertical and horizontal diameters of the corneal infiltrate was  $7.5 \pm 2.7$  mm. The details of the clinical presentation are described in Table 1. Figs. 1 and 2 depict the representative clinical and microbiology images from the series.

The features of severe microbial keratitis were noted in 47 eyes (95.9%) on presentation, and the two cases initially not falling under the severe category subsequently worsened, ultimately requiring TPK. The presenting visual acuity was worse than the accurate projection of rays (PR) in 30 eyes (61.2%) and the ability to see hand movements in the rest. The fungus was the predominant organism as noted in microbiology or histopathologic examination of the corneal buttons in 33 eyes (67.4%). The details are as follows: *Aspergillus flavus* 12, *Fusarium* spp. 9, *Fusarium solani* 3, *Aspergillus fumigatus* 1, *Candida guilliermondii* 1, *Curvularia* sp. 1 *Scedosporium apiospermum* 1, no growth 5.

The primary intervention was TPK in 30 eyes (61.2%). Table 1 mentions the details of all the interventions. The median waiting time for the patients after advising TPK was 1 day (IQR, 1–2 days) and the median time to surgery since the presentation was 6 days (IQR, 1–18 days).

The glycerol-preserved corneas were stored for a median duration of 82 days (IQR, 46–132 days).

The median donor age was 65 years (IQR, 48–70 years). The geometric mean of the infiltrate, range 1.4–12 mm; donor size range, 8.5–15 mm, and recipient size range, 8–14 mm.

**Table 1: Demographics, clinical, and microbiological presentation of 49 patients included in the study**

Parameter	Value
Age (years)	53.75 $\pm$ 12.22
Gender (eyes)	
Female	12 (24.4%)
Male	37 (75.5%)
Laterality	
Right eye	22 (44.89%)
Left eye	27 (55.1%)
Clinical features at presentation	
Mean infiltrate vertical diameter	7.36 mm
Mean infiltrate horizontal diameter	7.63 mm
Impending perforation	3 (6.1%)
Corneal melt	13 (26.5%)
Perforated ulcer	21 (42.8%)
Scleral extension	5 (10%)
Presenting visual acuity (eyes)	
PL PR inaccurate	23 (46.9%)
PL PR accurate	7 (14.2%)
Hand movements	19 (38.77%)
Primary intervention (eyes)	
Tissue adhesive application	13 (26.5%)
TPK	6 (12.2%)
Intrastromal antifungal injection	2 (4.08%)
Intracameral antifungal injection	1 (2.04%)
Superficial keratectomy	1 (2.04%)
Tarsorrhaphy	1 (2.04%)
Vitreous biopsy	1 (2.04%)
Initial microscopy of corneal scraping (eyes)	
Fungal elements	33 (67.3%)
Gram positive cocci	3 (6.12%)
Gram negative bacilli	2 (4.08%)
Mixed fungal and bacterial	2 (4.08%)
<i>Acanthamoeba</i>	1 (2.04%)
No organisms	8 (16.32%)
Secondary intervention (eyes)	Number of eyes: 12
Amniotic membrane transplantation	5 (41.66%)
Cataract surgery	3 (25%)
Repeat therapeutic keratoplasty	2 (16.66%)
Evisceration	1 (8.33%)
Wound resuturing	1 (8.33%)

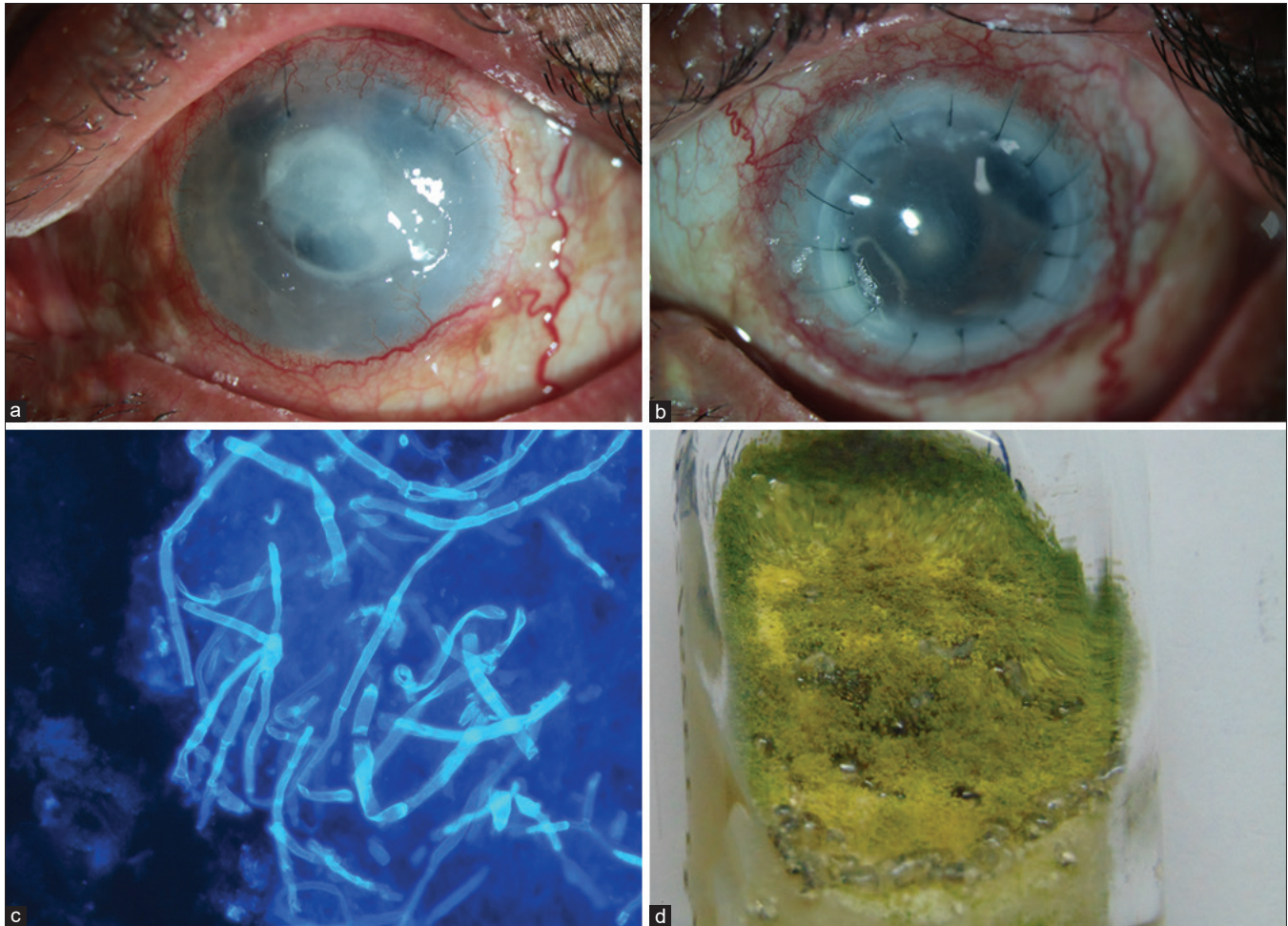
The median recipient and donor trephination sizes were 10 mm (IQR, 9–11 mm) and 11 mm (IQR, 10–12 mm). Intraoperative complications such as lens expulsion along with vitreous loss and Descemet’s membrane detachment were noted in four (8.2%) and three (6.1%) eyes respectively. Tarsorrhaphy was concomitantly performed in 20 eyes (40.8%). Twelve eyes (24.5%) underwent a second surgical procedure within the 3 months follow-up period which included amniotic membrane transplant in five eyes, cataract extraction in three, repeat TPK in two, evisceration in one, and wound resuturing in one. The associated endophthalmitis was noted preoperatively in seven eyes (14.3%). Two patients received

an intraocular antibiotic injection (in one case it was combined with pars plana vitrectomy and in the second case it was combined with cataract extraction).

Postoperatively, all 49 patients were available for the 1-week follow-up, whereas 36 (73.5%) were seen at 1 month and 24/49 (49%) were available for the 3-month follow-up. The loss-to-follow-up was noted in 13 patients (26.5%) at 1 month and 25 patients (51%) at 3 months of presentation. The graft epithelialization was complete in 2/49 (4.1%) eyes

at 1 week, 14/36 (38.9%) eyes at 1 month, and 10/24 (41.7%) at 3 months. In all, the graft epithelialization was complete in 21 eyes (42.9%) and the mean duration to re-epithelialization was  $48.9 \pm 25$  days (range, 8–97 days).

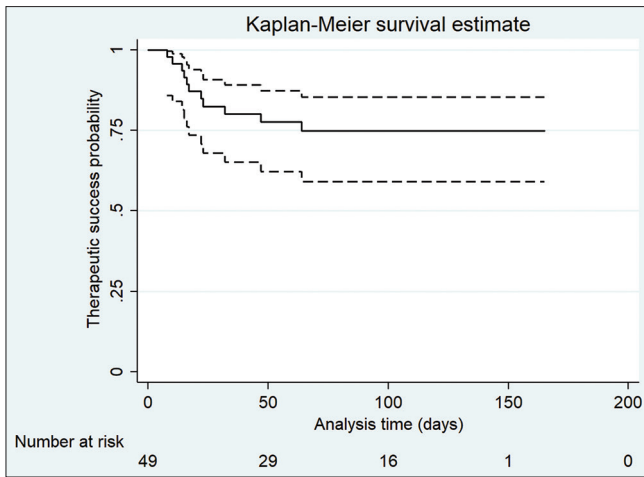
Recurrence of primary etiology was noted in six eyes (12.2%). All were of fungal etiology. One eye underwent repeat penetrating keratoplasty (PK), one eye was eviscerated, no intervention was performed in two eyes, and two patients were lost to follow-up.



**Figure 1:** Representative clinical pictures of a patient of fungal keratitis at presentation (a and b) edematous graft 11 days post-TPK, (c) the smear showed fungal filaments in 10% potassium hydroxide + Calcofluor white wet mount X40 magnification, Greenish-yellow velvety fungal colony on potato dextrose agar (d).



**Figure 2:** Representative clinical picture of a patient of Acanthamoeba keratitis at presentation (a) and 20 days postoperatively (b). The smear showed Acanthamoeba cyst in 10% potassium hydroxide + Calcofluor white wet mount X40 magnification (c).



**Figure 3:** Kaplan–Meir survival estimate of therapeutic success in 49 patients

**Table 2: Postoperative-intraocular pressure (at each visit)**

Postoperative visit	Intraocular pressure (IOP) (mm Hg), mean±standard error	Raised IOP (IOP >21 mmHg or digitally high IOP)*
Week 1	26.7±2.4	21/41 (51.2%)
Month 1	13.4±3.2	4/23 (17.4%)
Month 3	13.7±4.3	2/17 (11.8%)
<i>P</i>		
Week 1 vs. Month 1	0.001	0.003
Week 1 vs. Month 3	0.009	0.002
Month 1 vs. Month 3	0.96	0.69

\*Mean IOP measurements were taken from all the patients where recordings were available

The graft melt was noted in one eye (2%) at 1-week follow-up. The eye was managed medically, and the patient was lost to follow-up subsequently. At 1 month, three eyes (8.3%) had graft melt. They were managed with tarsorrhaphy in one and amniotic membrane with tarsorrhaphy in two eyes. At 3 months, three eyes (12.5%) had graft melt, managed with tarsorrhaphy in two and medical management in one eye. In total, seven eyes (14.2%) had graft melt during the 1-week, 1- or 3-months visit.

The raised intraocular pressure (>21 mmHg or digitally assessed) was managed with topical and oral antiglaucoma medications. Table 2 shows the IOP summary during the postoperative period among the patients whose IOP recordings were available.

Therapeutic failure was noted in 11 eyes (22.4%) (recurrence 6, scleral involvement 2, graft melt 1, graft ectasia 1, PED 1). The anatomical success was noted in 32/36 eyes (88.9%) at 1 month, with four eyes showing therapeutic failure due to graft melt in three and wound leak in one eye. At 3 months of follow-up, 20/24 eyes (83.3%) achieved anatomic success and three had a therapeutic failure, which included graft melt in two and ectasia in one. The anatomic success was achieved in 38 eyes (77.5%) during the follow-up. Table 3 shows the Kaplan–Meier survival estimates of therapeutic success [Fig. 3].

**Table 3: Survival estimates of graft success (legend too sketchy-can be elaborated for clarity)**

Days	Number at Risk	Success (%)	95% Confidence Interval
7	48	100%	-
15	44	91.5% ± 4.1%	78.9-96.7%
30	36	82.5% ± 5.6%	68.0-90.8%
60	30	77.6% ± 6.3%	62.3-87.3%
90	21	75.0% ± 6.6%	59.2-85.3%

The dotted lines represent the 95% confidence intervals of the Kaplan–Meier estimates [Fig. 3].

The visual acuity at the last follow-up ranged from the perception of the light present, inaccurate PR to finger counting at 1 m. Two patients underwent subsequent optical keratoplasty with fresh donor corneas and were stable postoperatively.

### Discussion

In the last few years with the success of hospital cornea retrieval programs, fresh donor cornea availability had improved at least in the larger eye banks in India. During the COVID-19 pandemic, many eye banks and institutions had to revive glycerol preservation. The ease of procuring glycerol, and the relatively inexpensive means of storage, make glycerol-preserved tissues a viable alternative for emergency use in developing countries where corneal tissue is in short supply.<sup>[8]</sup> Glycerol renders all vital cells of the cornea non-viable, therefore, there have been no reports of rejection following its use.<sup>[9,10]</sup> The authors have reported its use with varying results. Gupta *et al.*<sup>[11]</sup> compared the outcomes of TPK using fresh versus glycerol-preserved tissues and found no significant difference in the therapeutic success. Other studies report the anatomic integrity to range from 57.1 to 92.8%.<sup>[9,10,12,13]</sup> We noted similar results in our study, wherein the anatomic success was achieved in 38 eyes (77.5%).

Lin *et al.*<sup>[9]</sup> and Gupta *et al.*<sup>[13]</sup> reported nearly equal bacterial and fungal keratitis in their study populations, whereas we had a predominance of fungal infection (67%). Gupta *et al.*<sup>[13]</sup> reported an anatomic success of 91.2% as opposed to the present study where we had graft melt in six cases and recurrence was noted in four eyes. The higher incidence of recurrence can be attributed to the severity of the infection at the time of transplantation and the higher incidence of graft melt is due to severe postoperative inflammation and/or poor epithelial healing of the glycerol-preserved tissue, which has poor reparative properties.

Thanathanee *et al.*<sup>[12]</sup> reported fungus as the predominant etiologic agent in 72.7% (16/22) in their series of glycerol-preserved tissues. Evisceration was needed in 9 eyes and 13 could be saved. They report the lowest therapeutic success of 59.1% (13/22) among all the published reports on the outcomes of glycerol-preserved donor corneas. They also reported a high rate of recurrence (15/22 eyes) and wound leak (7/22). The authors strongly recommend against the use of glycerol-preserved corneas in severe keratitis. In contrast, we noted a relatively lower rate of recurrence in 4/49 (8.1%) eyes of which, in three eyes, it occurred within 1 week postoperatively (residual infection). We also had associated endophthalmitis in 6/49 (12.2%) eyes.

Glaucoma was reported in TPK with glycerol-preserved tissues in the range of 21.4–50%.<sup>[9,10,12,13]</sup> We noted a 42.8% raised IOP in the first week postoperatively and 12.5% at 3 months. None of these patients required surgery for secondary glaucoma.

Gupta *et al.*<sup>[13]</sup> reported delayed epithelialization in all the cases in their study. In a separate study, Yang *et al.*<sup>[10]</sup> reported a mean time to re-epithelialization of 10.2 days. Similar to the reported studies, in our series, we noted delayed epithelialization (median 46 days) with a gradual increase in graft epithelialization over the three follow-up periods (from 4% at 1 week to 38% at 1 month and 41% at 3 months). Even at 3 months, a significant number continued to show a lack of complete epithelialization. This delayed epithelial healing is one of the chief limitations of glycerol-preserved corneal tissues. The glycerol-preservation process renders all the cells to be non-viable and is a potential reason for poor postoperative recovery.<sup>[14]</sup> Our results have shown that the time to re-epithelialization is prolonged, up to 3 months in some of the cases. Therefore, a low threshold for early intervention with tarsorrhaphy or amniotic membrane is recommended.

Our surgical experience with the glycerol-preserved tissue has led us to propose some guidelines for using these corneas. (1) Glycerol storage causes the cornea to stiffen and thicken and this corneal tissue is no longer pliable and has handling properties similar to sclera. It is also recommended to thoroughly wash off the glycerol medium which is viscous and adheres to the tissue surface. (2) Placing the tissue in a bowl of balanced salt solution for 20 min prior to trephination allows the tissue to thaw and rehydrate while making it pliable for trephination and suturing. (3) Trephination is challenging and a sharp trephine is necessary to avoid incomplete trephination. (4) The tissue is clear for the initial part of the surgery, but within a few minutes, it becomes opaque and limits the view of the anterior chamber. Therefore, any procedures with the graft in place, such as forming the chamber or intracameral injections, should be done with caution to avoid Descemet's detachment. (5) Using 9-0 nylon for the initial 4–8 sutures followed by 10-0 nylon for suturing the thickened cornea can be considered. Only interrupted sutures are recommended. (6) Performing a paramedian tarsorrhaphy at the end of the case is recommended in anticipation of surface-related complications. (7) Overall, the surgical time is prolonged, and we recommend general anesthesia wherever possible for these cases.

The current study has some limitations due to the inclusion of the lost to follow-up cases, with only 50% of the patients following up at 3 months. The relatively high number of loss to follow-up was due to the difficulty to travel with the ongoing COVID-19 pandemic. We did not perform a comparison with the outcomes of the TPK using fresh corneal tissues. The data on how many patients subsequently underwent visual restoration procedures and the consequent outcome are not included.

## Conclusion

Based on the results of our study, TPK using glycerol-preserved corneal tissue is a viable alternative for the eradication of infection and providing adequate tectonic support in severe microbial keratitis during times of severe donor tissue non-availability. This is of relevance in view of the resurfacing

of the pandemic as the second and subsequent waves strike across globally.

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## Conflicts of interest

There are no conflicts of interest.

## References

- King JH. Keratoplasty; experimental studies with corneas preserved by dehydration. *Am J Ophthalmol* 1957;43:353–80.
- Chen W, Lin Y, Zhang X, Wang L, Liu M, Liu J, *et al.* Comparison of fresh corneal tissue versus glycerol-preserved corneal tissue in deep anterior lamellar keratoplasty. *Invest Ophthalmol Vis Sci* 2010;51:775–81.
- Tripathi H, Mehdi MU, Gupta D, Sen S, Kashyap S, Nag TC, *et al.* Long-term preservation of donor corneas in glycerol for keratoplasty: Exploring new protocols. *Br J Ophthalmol* 2016;100:284–90.
- Busin M, Yu AC, Ponzin D. Coping with COVID-19: An Italian Perspective on corneal surgery and eye banking in the time of a pandemic and beyond. *Ophthalmology* 2020;127:e68–9. doi: 10.1016/j.ophtha.2020.04.031.
- Chaurasia S, Das S, Roy A. A review of long-term corneal preservation techniques: Relevance and renewed interests in the COVID-19 era. *Indian J Ophthalmol* 2020;68:1357–63.
- Chaurasia S, Mohamed A, Garg P, Balasubramanian D, Rao GN. Thirty years of eye bank experience at a single centre in India. *Int Ophthalmol* 2020;40:81–8.
- Roy A, Das S, Chaurasia S, Fernandes M, Murthy S. Corneal transplantation and eye banking practices during COVID-19-related lockdown period in India from a network of tertiary eye care centers. *Indian J Ophthalmol* 2020;68:2368–71.
- Gopinathan U, Sharma S, Garg P, Rao GN. Review of epidemiological features, microbiological diagnosis and treatment outcome of microbial keratitis: Experience of over a decade. *Indian J Ophthalmol* 2009;57:273–9.
- Lin H-C, Ong SJ, Chao A-N. Eye preservation tectonic graft using glycerol-preserved donor cornea. *Eye (Lond)* 2012;26:1446–50.
- Yang J-W, Lin H-C, Hsiao C-H, Chen PYF. TPK in severe infective keratitis using glycerol-preserved donor corneas. *Cornea* 2012;31:1103–6.
- Gupta N, Dhasmana R, Maitreya A, Badahur H. Glycerol-preserved corneal tissue in emergency corneal transplantation: An alternative for fresh corneal tissue in COVID-19 crisis. *Indian J Ophthalmol* 2020;68:1412–6.
- Thanathane O, Sripawadkul W, Anutarapongpan O, Luanratanakorn P, Suwan-Apichon O. Outcome of therapeutic penetrating keratoplasty using glycerol-preserved donor corneas in infectious keratitis. *Cornea* 2016;35:1175–8.
- Gupta N, Upadhyay P. Use of glycerol-preserved corneas for corneal transplants. *Indian J Ophthalmol* 2017;65:569–73.
- Li J, Shi S, Zhang X, Ni S, Wang Y, Curcio CA, *et al.* Comparison of different methods of glycerol preservation for deep anterior lamellar keratoplasty eligible corneas. *Invest Ophthalmol Vis Sci* 2012;53:5675–85.