

## Glycerol-preserved corneal tissue in emergency corneal transplantation: An alternative for fresh corneal tissue in COVID-19 crisis

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**Purpose:** Due to the COVID-19 pandemic, most of the eye banks have limited/stopped corneal collection, as this is a highly contagious disease. This has led to shortage of donor corneas worldwide. Glycerol preservation of tissue remains a viable option in this scenario. The objective is to compare fresh corneal tissue (FCT) with glycerol-preserved cornea (GPC) in emergency corneal transplantation. **Methods:** This was a retrospective cohort study conducted in a tertiary care centre of Uttarakhand. Medical records of the patients who underwent therapeutic penetrating keratoplasty (TPK) were reviewed. FCT group included patients who underwent TPK with fresh corneal tissue and GPC group included patients who underwent TPK with glycerol preserved cornea. The indications and outcomes of TPK in the terms of therapeutic success were analysed and compared between both the groups. **Results:** A total of 94 eyes of 91 patients underwent TPK from October 2011 to August 2017. FCT group included 60 eyes of 57 patients and GPC group included 34 eyes of 34 patients. The primary indication of TPK was infectious keratitis in both the groups (FCT-81.6%; GPC - 91.2%) There was no significant difference in the therapeutic success in both the groups ( $P = 0.741$ , Odds ratio- 1.59 with 95% CI- 0.39-6.44). Complications included glaucoma (FCT-21.7%; GPC- 35.2%) graft infection (FCT- 18.33% GPC- 2.9%); graft rejection (FCT-11.66%, GPC- 0%); and graft failure (FCT-88.33%, GPC-100%). **Conclusion:** The GPC is comparable to FCTs in therapeutic transplant and can be a useful interim procedure in saving the eyes in cases of infective keratitis in the time of crisis.

**Key words:** Corneal transplant, fresh corneal tissue, glycerol-preserved cornea, keratitis, therapeutic keratoplasty

At a time of global crisis when the world is facing COVID-19, measures like social distancing and lock down are being practised universally to control spread. Most of the eye banks of the world have stopped or have limited donor corneal collection due to the risk of disease transmission, thereby leading to severe shortage of fresh corneal tissues. Due to the lock down, eye banks cannot share their tissues with the corneal transplant surgeons. All elective surgeries have stopped but patients with corneal infections and perforations continue to report to emergency.<sup>[1]</sup>

In centres with a low turnover of fresh donor corneal tissue (FCT), non-optical grade tissues are used with poor results or evisceration/enucleation remains the only choice. In an emergency corneal transplant setting, restoration of globe integrity and removal of infectious process is deemed as therapeutic success and is the primary goal of therapeutic keratoplasty (TPK); functional success or visual rehabilitation is a secondary consideration.<sup>[2]</sup>

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Using Glycerol-preserved donor cornea (GPC) in situations of emergency corneal transplant may circumvent the problem of corneal shortage, as an intermediary procedure to save the eye for a definitive optical transplant with fresh tissue at a later date. The preservation of cornea in glycerol is a simple technique of long-term storage of tissue and can be preserved for up to 5 years<sup>[3]</sup> and different techniques of preservation have been described.<sup>[4]</sup> Studies have shown good results in removal of infection and restoration of globe integrity with GPC in TPK.<sup>[5-8]</sup>

The purpose of this study was to compare therapeutic success, in terms of maintenance of anatomical integrity of the eyeball between GPC and FCT in non-healing corneal infections or perforations requiring an emergency corneal transplant. To the best of our knowledge, this is the only comparative study between fresh corneal tissue and glycerol preserved tissue in emergency corneal transplants.

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## Methods

This was a retrospective cohort study which included patients who underwent emergency TPK from October 2011 to August 2017. The study was approved by the Institutional ethics committee and is according to the tenets of the Declaration of Helsinki. The demographic details, indications of TPK, microbiological investigations, size of the donor cornea, recipient graft size, outcomes of surgery and complications were compiled from the hospital records. The patients were categorized as FCT group, who underwent TPK with Fresh corneal tissue preserved in McCarey-Kaufman (MK) medium and GPC group, who underwent TPK with Glycerol Preserved cornea. The GPC was used only in emergency situation when the eye bank did not have fresh donor corneal tissue for transplant.

### Donor corneal preparation

All donor corneas were evaluated by the cornea specialist in the eye bank. The corneas were graded as per NEI criteria.<sup>[9]</sup> All the non-optical grade tissues were first preserved in MK medium for 4 days at 4°C for therapeutic/tectonic transplants. If these tissues were not used for therapeutic use within 4 days, they were transferred to a sterile glass bottle having sterile glycerol solution and preserved at 4°C. The MK medium was evaluated microbiologically to exclude any infection after transferring the tissues to glycerol. The duration of storage of GPC ranged from 1-3 months. Prior to surgery, the donor corneal button was soaked for 10 minutes in sterile balanced salt solution (BSS) to wash away the residual glycerol.

### Surgical technique

The TPK was performed under general anaesthesia. Trephination of donor cornea was done with a disposable hand-held trephine as per the requirement. The recipient cornea was trephined gently to cover the edge of the infiltration completely. A 15° side port was used to enter the anterior chamber (AC) from the trephined area. The AC was formed using a viscoelastic device. Corneal scissors were used to cut the rest of the recipient cornea. The excised recipient cornea was

sent for microbiological and histo-pathological examination. AC was washed with sterile BSS Solution to remove the debris and infiltrates. Anterior and posterior synechiae were released with the viscoelastic device. The donor button was oversized by 0.5-1.0 mm. The graft was sutured by interrupted suturing using 10-0 nylon suture in all cases. At least 2 iridectomies were performed before suturing of the graft. AC was carefully washed with BSS and reformed with air. Crystalline lens was not removed in any of the cases, except one, where there was a corneal perforation with lens extrusion.

### Postoperative medication

Patients were prescribed topical and oral antibiotics, based on the clinical picture and microbiological reports. Topical steroids were started in only proven cases of bacterial keratitis where sensitivity was known after 48 hours of TPK. In cases of fungal infections, steroids were started after 2 weeks when recurrence of infection was clinically ruled out.

### Outcomes

*Therapeutic success* was defined as anatomical restoration and removal of infectious process while *Therapeutic failure* was considered if the integrity of eyeball could not be maintained (e.g. phthisis bulbi or evisceration/enucleation). *Functional success* was defined as a clear graft with best corrected visual acuity of more than 6/60 on Snellen visual acuity chart. *Graft failure* was defined as a graft that did not retain optical clarity or if there was high astigmatism that could not be optically corrected, resulting in recommendation of re-grafting. A minimal follow-up of 1 year was included to define therapeutic or functional success or failure.

### Statistical analysis

SPSS software version 22.0 (SPSS Inc, Chicago, IL) was used for statistical analysis. For description, quantitative variables have been expressed as mean $\pm$  SD and qualitative variables have been expressed as a percentage. Mann-Whitney U-test was applied for non-normally distributed continuous variables. Differences between FCT and GPC were analysed using unpaired t-test for continuous variables and Chi-square test for nominal variables. *P* value  $\leq 0.05$  was considered significant.

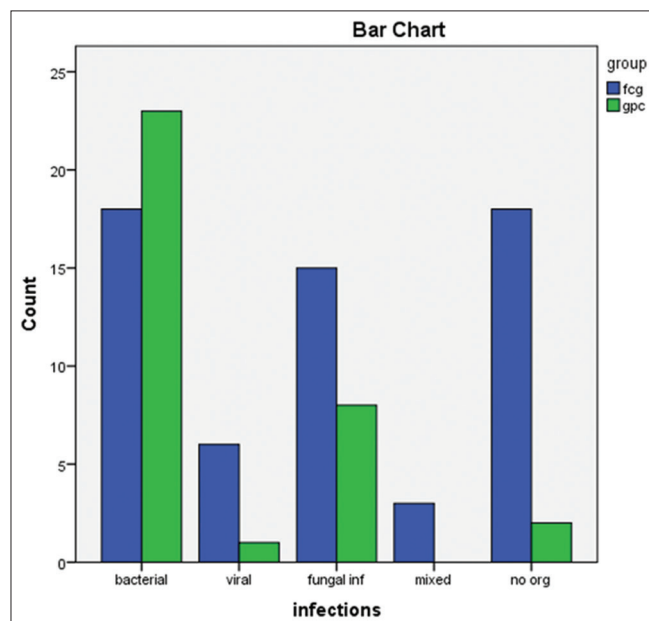


Figure 1: Cause of infectious keratitis in both the group

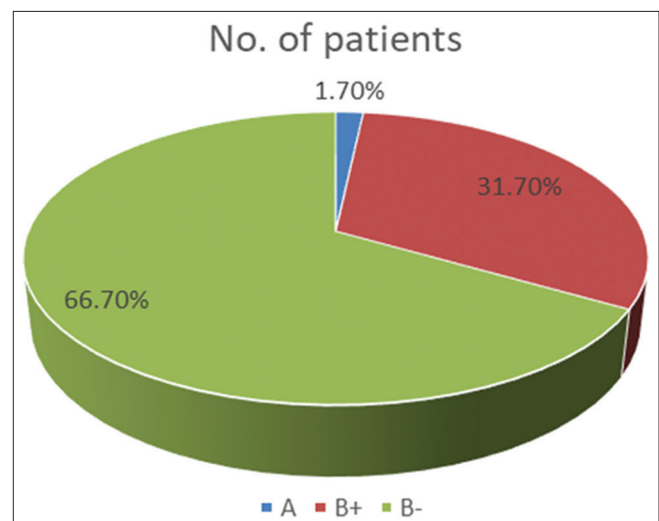


Figure 2: Grade of fresh corneal tissue used in FCT group

**Table 1: Baseline characteristics for both the study groups**

Characteristics	Fresh corneal tissue (FCT) group (n=60)	Glycerol preserved cornea (GPC) group (n=34)
Age (years)(mean±SD)	51.38±15.76	49.05±16.8
Sex (M:F)	2:1	16:1
Donor graft size (mm) (mean±SD)	9.57±0.91	9.52±1.02
Recipient graft size (mm) (mean±SD)	9.05±0.92	9.04±1.05
Time of presentation (days)	4-270	6-270

**Table 2: Indications of therapeutic keratoplasty in both the study groups**

Indication	FCT (n=60)	GPC (n=34)
Infectious keratitis	49 (81.66%)	31 (91.2%)
Non Healing corneal ulcer	25 (51%)	6 (19.3%)
Perforation	20 (40.8%)	20 (64.5%)
Desmatocele	4 (8.1%)	5 (16.1%)
Graft Infection	11 (18.3%)	-
Post-op Endophthalmitis with corneal ulcer	-	1 (2.9%)
Globe perforation with retained IOFB*	-	1 (2.9%)
Thermal burn with infectious keratitis	-	1 (2.9%)

\*IOFB – Intra ocular foreign body

**Table 3: Post TPK Complications in both the study groups**

	FCT (n=60)	GPC (n=34)
Graft Failure	53 (88.33%)	34 (100%)*
Graft rejection	7 (11.66%)	NIL
Graft Infection	11 (18.33%)	1 (2.9%)
Secondary Glaucoma	13 (21.7%)	12 (35.2%)

\*Viable endothelium is not present in GPC

**Table 4: Treatment outcome Post TPK in both the groups**

Outcome	FCT (n=60)	GPC (n=34)	P
Therapeutic success	52 (86.7%)	31 (91.2%)	P=0.741*
Therapeutic failure	8 (13.3%)	3 (8.8%)	
Visceration	3 (5.0%)	1 (2.9%)	
Phthisis bulbi	5 (8.3%)	2 (5.8%)	

\*Fisher exact test

## Results

TPK was performed in 94 eyes of 91 patients from October 2011 to August 2017. The minimum follow up period was 1 year in all the cases. All the patients hailed from the rural areas and time of presentation ranged from 4 to 270 days (median-30 days, IQR = 14.7-74.2 days).

FCT group comprised of 60 eyes of 57 patients with mean age of 51.3 ± 15.7 (14-76 years) and male to female ratio of 2:1 [Table 1]. The commonest indication for transplant was infectious keratitis [n = 49, 81.6%; Table 2]; bacterial keratitis was the most common infection [n = 18, 30%; Fig. 1]. Most of the fresh corneas used in this group were non-optical grade [66.7%; Fig. 2]. Graft failure was seen in 88.33% (n = 53) of patients and only 11.66% (n = 7) grafts survived which were

optical grade tissues with a best corrected visual acuity (BCVA) of 6/60 or better on Snellen acuity chart after the therapeutic transplant [Table 3]. All non-optical grafts failed after transplant within one year. Complications in this group included graft infection (18.33%, n = 11), graft rejection (11.66%, n = 7) and secondary glaucoma (21.7%, n = 13). At the final outcome, therapeutic success was achieved in 86.7% (n = 52) of eyes [Table 4].

GPC group comprised 34 eyes of 34 patients with a mean age of 49.05 ± 16.8 years (Age 74 years) with a male preponderance of 94.1% [Table 1]. The duration of preservation of donor GPC ranged from 1-3 months. The commonest indication of transplant in this group was also microbial keratitis seen in 91.2% (n = 31) of cases [Table 2]. The most common infection in this group was bacterial infection [n = 23, 67.6%; Fig. 1]. Graft failure was seen in all cases (100%). Complications include graft infection (2.9%, n = 1), secondary glaucoma (35.2%, n = 12) and delayed epithelialization in all cases. Amniotic membrane transplant was done in five cases for non-healing epithelial defect. There was no episode of graft rejection in this group. At the final outcome, therapeutic success was seen in 91.17% (n = 31) of eyes [Table 4].

The age in both the groups were comparable (P = 0.5) however, there was male preponderance in GPC group (94.1% vs. 66.7%). There was no significant difference in the size of the trephines used in both the groups [donor P = 0.82, recipient P = 0.9; Table 1]. Incidence of glaucoma post-surgery was clinically higher in GPC group but was statistically not significant (P = 0.34). Therapeutic success was better in GPC group as compared with that in FCT group; however, the difference was not statistically significant (P = 0.741, Odds ratio- 1.59 with 95% CI- 0.39-6.44). Rates of reinfection and rejection was more in FCT group and only one case of reinfection, and no case of rejection was noticed in GPC group [Table 3].

Re-transplant with optical grade tissue was done in 11 eyes in FCT group and 5 eyes in GPC group. Six out of these 11 grafts in FCT group had graft failure at the end of one year, whereas all the grafts survived for more than one year in GPC group.

## Discussion

Glycerol preserved corneal tissue is comparable to fresh corneal tissue for maintaining the ocular integrity of the eye in emergency corneal transplant as seen in our study. GPC can be used safely in infectious keratitis for preserving visual acuity and globe integrity, with an acceptable complication rate, when FCT is not available.

Therapeutic success seen in our study was 86.66% and 91.17% in FCT and GPC respectively (P = 0.513). Literature

**Table 5: Comparison of Glycerol Vs. Fresh corneal tissue for therapeutic transplant**

Glycerol preserved tissue	Fresh corneal tissue
Very Cheap (50 Rupees)	Cost of preservative media (400-1200 Rupees)
No risk of transmission of infection	Risk of transmission of infections esp. viral and fungal organisms
Failure of all the grafts	Graft survival is poor in TPK
No risk of rejection	Very high risk of rejection
Can be stored at room temperature	Strict temperature control required during storage and transportation
Corneal tissue can be preserved for years	Corneal Tissue can preserve for days only

search did not show any studies comparing the therapeutic success of FCT with GPC, however, non-comparative studies are available. Two studies from north and west India<sup>[10,11]</sup> had therapeutic success of 89.7% and 85%, respectively, when using FCT in infectious keratitis which is comparable to FCT group in our study. A study by Lin *et al.*<sup>[7]</sup> showed therapeutic success of 92.8% ( $n = 13/14$  eyes) with GPC in infectious keratitis which is again comparable with GPC group in our study (91.17%). However, another study by Thanathanee *et al.*<sup>[6]</sup> showed a therapeutic success of 59.1% using GPC. A lower anatomical success may be due to the fact that they had more recalcitrant cases in their study.

Graft rejection of 11.66% was seen in FCT group at the end of one year. Incidence of graft rejection was 5.92% in a large series in TPK with FCT from New Delhi.<sup>[10]</sup> Previous studies report a rejection of 15% in 5 years and 50% in 2 years.<sup>[12,13]</sup> As is well known, graft rejections are more in TPK as a large corneal button is used that is closer to the limbus. Also there is more inflammation in therapeutic grafts which can trigger the rejection reaction.

No case of graft rejection in GPC group was seen in this study, although the graft size was comparable in both the groups [donor  $P = 0.82$ , recipient  $P = 0.9$ ; Table 1]. This can be explained by the fact that the acellular GPC lacks antigen-presenting cells and therefore cannot directly sensitize the recipient T cells, making rejection a non-issue.<sup>[14,15]</sup> No episode of rejection was seen by Lin *et al.*<sup>[7]</sup> and Yang *et al.*<sup>[16]</sup> in their studies when using GPC.

Graft reinfection in FCT (11 patients) groups was not related to the primary infection and occurred after the primary infection healed but within one year of transplant. The graft reinfection in GPC (1 patient) was recurrence of primary infection as the patient had endophthalmitis with corneal ulcer. Graft re infection seen in FCT group was 18.33% and was higher than the GPC group 2.9%. Topical steroids were stopped in GPC group once the inflammation subsided but was continued in the FCT group to prevent graft rejection. This would have possibly contributed for presence of more Reinfection in FCT group. Another possible reason could be larger number of primary viral infections in FCT group than in GPC group [Fig. 1]. In the study from New Delhi<sup>[10]</sup> reinfection of graft, where fresh cornea was used was 12.6% and 20% in another study from West Bengal.<sup>[11]</sup> Reinfection was not seen in the study by Lin *et al.*<sup>[7]</sup> where GPC was used. However, a higher incidence of reinfection (59.1%) was seen by Thanathanee *et al.*<sup>[6]</sup> in GPC for TPK. However, this study had large numbers of highly virulent organisms (Acanthamoeba and Pythium) and fungal keratitis which usually have poor prognosis.

The incidence of glaucoma reported in literature after penetrating keratoplasty has ranged from 9% to 35%.<sup>[17-19]</sup> The incidence of glaucoma in current study was 35.2% in GPC group and 26.3% in FCT group and this difference was not statistically significant ( $P = 0.34$ ) [Table 2].

In this study, functional success in FCT group was 11.66%. The functional success seen in a study by Sharma *et al.*<sup>[10]</sup> was 14.82% with fresh corneal tissue in TPK, which is comparable to our study. We cannot compare the functional success of FCT with GPC as there is no viable endothelium in GPC and post transplant corneal tissue becomes edematous with decreased graft clarity.<sup>[20]</sup>

Constraints of availability, cost, storage, and transportation of fresh corneal tissues may be alleviated by glycerol-preserved corneas [Table 5]. Glycerol has microbial growth inhibiting and anti-protease properties and as a dehydrating agent it is known to maintain the corneal structure. It is a simple, effective technique facilitating long-term storage of acellular corneal tissue for up to 5 years.<sup>[3]</sup> Due to the acellularity and low antigenicity of GPC, there appears to be minimal risk of rejection, thereby avoiding use of corticosteroids in post-transplant period. This further decreases the risk of re-infection and thus decreases the cost of treatment.

The limitations of the current study were that the sample size is small. Also there is the possibility of selection bias and incomplete data from the retrospective study.

In the current COVID-19 pandemic, most of the eye banks have limited/stopped corneal collection due to highly contagious nature of the disease, leading to shortage of donor corneas worldwide. Glycerol preservation of tissue remains a viable option in this scenario. This has recently been highlighted in the advisory issued by Eye bank association of India, where they have recommended transfer of corneal tissues from intermediate preservation media to glycerol on the last day of preservation for future use for tectonic purposes till eye banks restore their collection.<sup>[21]</sup>

## Conclusion

The authors recommend GPC as a good and an inexpensive alternative to a FCT for emergency corneal transplant in resource-limited settings or crisis situation as evident from our study. It can be effectively used for saving the eyes when fresh corneal tissues are not available and gives a good anatomical outcome instead of subjecting the patient to evisceration/enucleation.

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### Conflicts of interest

There are no conflicts of interest.

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