Use of glycerol-preserved corneas for corneal transplants

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Purpose: This study was carried out to see the results of glycerol-preserved cornea (GPC) in emergency situation when fresh corneal tissue was not available. The aim was to study the outcome of corneal transplantation using GPC. Methods: This was a retrospective study. The medical records of all the patients were reviewed, who underwent keratoplasty using “GPC” during the period from October 2011 to December 2015. The indication of keratoplasty, duration of preservation of the GPC, and its outcome were analyzed. Descriptive statistics were applied. Results: Out of the 222 penetrating keratoplasty (PKP) performed over the study period, the GPC was used in 34 patients (males = 31, 91.2%) aged 15–74 years. Therapeutic keratoplasty was performed in all cases in this cohort except one in which tectonic keratoplasty was done. The primary indication of PKP (91.2%) was infectious keratitis. Of these, 20 (64.5%) patients presented with perforated corneal ulcers. Post-PKP, ocular anatomy was preserved in 91.2%, and visual acuity of perforation of light positive and accurate projection of rays in all the quadrants was obtained in 76.5% cases. Complications included glaucoma (n = 12, 35.1%), phthisis bulbi (n = 2, 5.9%), and graft reinfection and endophthalmitis after PKP (n = 1, 2.9%). The secondary procedure post-GPC and PKP were trabeculectomy with mitomycin C (n = 7, 58.3%) in patients not controlled on topical antiglaucoma medication. Optical keratoplasty was performed in (n = 3) 8.8% patients and triple procedure in (n = 2) 5.8% patients with good visual acuity postprocedure. Conclusions: Acellular GPCs are useful in emergency keratoplasty to avoid loss of vision and can save the eye.

Key words: Corneal transplant, glycerol-preserved cornea, keratitis, keratoplasty

Globally, corneal diseases are the third leading cause of blindness after cataract and glaucoma. Approximately 7–9 million people become blind due to corneal diseases. Of this, 90% reside in developing countries. Despite huge burden, corneal blindness remains one of the treatable causes of blindness, if treated appropriately and on time. The causes of corneal blindness vary geographically, and social and economic factors play a significant role. Corneal scarring is the most common indications of corneal transplant in the developing world. In contrast, pseudophakic bullous keratopathy, keratoconus, and Fuchs endothelial dystrophy are the leading indications of corneal transplants in the developed countries.

Corneal transplant is the most successful organ transplant and is the definitive treatment of corneal diseases that may cause blindness. There is a severe shortage of donor eyes in India. The current cornea procurement rate according to eye bank association of India statistics in 2015–2016 was 52,758 corneas with a utilization rate of 43% (unpublished data). A significant number of donor cornea is unsuitable for corneal transplant. We would need approximately 277,000 donor eyes to perform 100,000 corneal transplant in a year in this country. This is approximately 20-fold from the donor eyes currently available in India. Limited tissue availability and deficiency of trained corneal surgeons are the factors that make eliminating cornea-related blindness challenging, especially in the developing countries. The problem is further compounded by poor compliance and follow-up of patients. The ever-increasing gap in the demand and supply of the fresh corneal tissue (FCT) may possibly be circumvented by the utilization of glycerol-preserved cornea (GPC) tissue. Glycerol preservation is a simple technique of long-term storage of acellular corneal tissue, and one of the major benefits of this method is the fact that corneal tissue can be safely preserved up to 5 years after harvesting. GPC has no viable endothelium and can be used for lamellar corneal transplant that does not require high-quality tissue. GPC can be used in emergencies like perforated corneal ulcer or globe rupture to maintain the globe integrity by therapeutic or tectonic keratoplasty.

The study was undertaken to analyze the indications and outcomes of cases of infectious keratitis managed using a GPC tissue when FCT was not available.

Methods

This was a retrospective study approved by the Institutional Ethics Committee according to the tenets of the Declaration of Helsinki. Hospital records of patients who underwent keratoplasty to avoid loss of vision and can save the eye.
Donor corneal preparation
Experts in the eye bank evaluated all donor corneas. Cornea, which was deemed unsuitable for optical penetrating keratoplasty (PKP), was preserved at 4°C in a sterile glass bottle having sterile glycerin solution. The duration of storage of GPC ranged from 1 to 3 months. At the time of surgery, the donor corneal button was soaked for 10 min in sterile basal salt solution (BSS) to wash away the residual glycerin and then trephined as per the requirement.

Surgical technique
Corneal trephination was done very gently as most of the recipient corneas were perforated at the time of surgery or had an impending perforation. A 15° side port was used to enter the anterior chamber (AC) from the trephined area. The AC was formed using viscoelastic (either from the limbal side port if the lesion was small or from the trephined area if the lesion involved the entire cornea). Corneal scissors were used to cut the rest of the recipient cornea. All the infiltrates and debris in AC were removed and washed with BSS, and anterior synechiae were released with viscoelastic. Posterior synechiae were also released, and the posterior chamber was washed thoroughly taking care not to injure the lens. The corneal button was trephined 0.5 mm oversize in lesions involving <1 mm of limbus and 1 mm oversize in lesions up to the limbus or involving sclera. The graft was sutured using 10-0 nylon suture. Interrupted suturing was done in all cases. At least two iridectomies were performed before the suturing of graft, due to the difficulty in visualizing the AC through the rehydrated donor cornea. AC was very carefully washed with 10-0 nylon suture. Interrupted suturing was done in all cases. At least two iridectomies were performed before the suturing of graft, due to the difficulty in visualizing the AC through the rehydrated donor cornea. AC was very carefully washed with BSS and reformed with air. We did not remove the crystalline lens in any of the cases, except one, where there was a corneal perforation with lens extrusion.

Postoperative medication
Based on the clinical picture and microbiological reports, all the patients received topical and oral antimicrobials.

Results
Out of the 255 keratoplasty performed over 4 years, 34 patients underwent PKP with a GPC. All patients hailed from rural areas, their age ranged from 15 to 75 years, and 91.2% (31) were males. Time to seek specialist care from the onset of symptoms ranged from 1 to 5 months. All the patients either received topical or systemic medications or both at the time of consultation. Few patients were continuing topical steroids, or they had instilled topical steroids in the past.

We did 33 (97.1%) therapeutic penetrating keratoplasty (TPK) and one (2.9%) tectonic PK using GPC. The length of follow-up post-PKP ranged from 12 days to 56 months (median = 195 days; interquartile range = 342.5 days). The primary indication (91.2%) for PKP was infectious keratitis and two-third of these patients presented with corneal perforation [Table 1]. Clinically, 12/31 (38.7%) patients had a bacterial infection; 9/31 (29%) had fungal infections, while 1 (3.3%) patient was HIV positive and had developed posterior zoster keratitis. Remaining (n = 12) had mixed infection.

The graft size ranged from 8.5 to 11 mm. All grafts showed delayed epithelialization. Reepithelialization was achieved in all the cases with a bandage contact lens and five patients underwent amniotic membrane transplant. All but one graft healed with vascularization [Fig. 1a and c]; the odd patient developed reinfection of the graft with endophthalmitis warranting enucleation.

After PKP, 12 (35.1%) patients developed glaucoma, out of which 7 underwent trabeculectomy with intraoperative application of mitomycin C 0.02% for 3 min. Remaining five patients maintained normal intraocular pressures on the topical antiglaucoma medications. Five patients out of 34 patients underwent optical keratoplasty (OPK) with FCT [Fig. 1b and d] 3–5 months after TPK following resolution of their ocular infection. The best-corrected visual acuity in glasses in these five patients ranged from 6/36 to 6/12 Snellen acuity [Table 2].

Ocular integrity was maintained in 31 (91.2%) patients and visual function was maintained with light perception and accurate projection of rays in all the quadrants in 30 (88.2%) patients [Fig. 1e and f] post-transplant with GPC. All 31 patients had a normal ocular ultrasound on follow-up. Five (14.7%) patients regained good visual acuity after OPK with FCT. As mentioned above, one patient had to undergo enucleation following the development of infection and endophthalmitis after corneal grafting. Phthisis bulbi developed in 2 (5.8%) patients (post pars plana vitrectomy with TPK for endophthalmitis and post pars plana vitrectomy with intraocular foreign body removal with tectonic keratoplasty) [Table 3].

### Table 1: Indication of penetrating keratoplasty (n=34)

| Indication                                | n (%)                      |
|-------------------------------------------|----------------------------|
| Infectious keratitis                      | 31 (91.2)                  |
| Perforation                               | 20 (64.5)                  |
| Descemetocele                             | 5 (16.1)                   |
| Postoperative endophthalmitis with corneal ulcer | 1 (2.9)                   |
| Thermal burn with infectious keratitis    | 1 (2.9)                    |
| Globe perforation with retained intraocular foreign body | 1 (2.9)                   |

### Table 2: Secondary procedures done after therapeutic penetrating keratoplasty

| Secondary procedure                                      | n (%)   |
|----------------------------------------------------------|---------|
| Trab + MMC                                               | 7 (20.5) |
| OPK                                                      | 3 (8.8)  |
| Triple procedure (OPK + cataract extraction + PCIOL implantation) | 2 (5.8)  |
| AMT                                                      | 5 (14.7) |

Trab + MMC: Trabeculectomy with mitomycin C, OPK: Optical penetrating keratoplasty, PCIOL: Posterior chamber intraocular lens, AMT: Amniotic membrane transplantation
Discussion

Infectious keratitis is one of the important reasons for visual loss in developing countries. Therapeutic and tectonic keratoplasties constitute a significant number of corneal transplants in India[^1^-^4] and are effective in decreasing the infectious load, maintaining globe integrity, and preserving visual function.[^5^-^16] GPC can be used safely in infectious keratitis for preserving visual acuity and globe integrity with acceptable complication rate and reasonably good visual outcomes, especially when FCT is not available. In the present study, globe integrity was preserved in more than 90% patients with successful eradication of infection and restoration of visual function. Delayed diagnosis, inappropriate management, and the lack of modern medical facility in rural areas are some of the reasons that result in a delay in seeking specialist’s opinion and optimal treatment. In turn, it makes the corneal transplant inevitable. Moreover, access to sight-restoring keratoplasty is limited in some of the developing countries due to shortage of FCT, lack of developed eye bank network, and prohibitively high cost of imported tissue.[^17]

| Outcome | n (%) |
|---------|-------|
| Ocular integrity maintained | 31 (91.2) |
| Visual function preserved | 30 (88.2) |
| Good visual acuity after second corneal transplant with fresh corneal tissue | 5 (14.7) |
| Enucleation | 1 (2.9) |
| Phthisis bulbi | 2 (5.8) |

Table 3: Outcome of corneal transplant with glycerol-preserved cornea

Figure 1: Outcome of glycerol-preserved cornea (a) Case 1: Vascularized, opaque graft 3 months posttherapeutic penetrating keratoplasty with a glycerol-preserved cornea (b) Case 1: 2 years after reopical penetrating keratoplasty with fresh corneal tissue and cataract extraction with posterior chamber intraocular lens implantation with best-corrected visual acuity of 6/24. (c) Case 2: Failed opaque graft 4 months after therapeutic penetrating keratoplasty with glycerol-preserved cornea. (d) Case 2: 9 months after optical penetrating keratoplasty with fresh corneal tissue with trabeculectomy with best-corrected visual acuity of 6/12. (e) Case 3: Nonhealing fungal corneal ulcer. (f) Case 3: 1-day posttherapeutic penetrating keratoplasty with a glycerol-preserved cornea tissue
Utilizing GPCs may substantially alleviate the problem of availability, cost, storage, and transportation.\textsuperscript{37} Glycerol is a chemical compound commonly called glycerin. It is a colorless, odorless, and viscous liquid. As a dehydrating agent, it has antimicrobial and antiprotease properties, and it is known to maintain the corneal structure. These properties make it suitable for long-term storage of cornea and useful for those clinical indications where viable cell layer is not required.\textsuperscript{18,19} Although cornea preserved in glycerol does not have a viable endothelium. Still, it can be used for globe-salvaging surgeries as seen in the present study. Another benefit of glycerol-preserved tissue is readily available for emergency condition and its lower cost compared to the FCT. Furthermore, it can be preserved at room temperature and can be used for transplantation for 5 years.\textsuperscript{17} Thus, GPC is a suitable alternative in infective keratitis with impending perforation or in cases of patients presenting with perforations or globe rupture postinfections or trauma.

The acellular glycerol-preserved tissue lacks antigen-presenting cells and therefore cannot directly sensitize the recipient T cells, making rejection a nonissue, which is associated usually with fresh cornea.\textsuperscript{22,23} The absence of graft rejection in the present study supports this theory. Li \textit{et al.}\textsuperscript{22} found significantly less ($P = 0.006$) rejection rate in their study in those receiving a glycerol-cryo-preserved corneal transplant as compared to corneal transplant done with a FCT.\textsuperscript{24} Furthermore, the use of GPC precludes the postoperative use of corticosteroids or immunosuppressive agents to prevent or suppress graft rejection and thus prevents reinfection and reduces the cost of treatment. This is of immense importance in a developing country like India where follow-up and compliance with long-term immunosuppression therapy is poor.

In our study, only one patient developed reinfection of graft and endothphalmatitis after TPK. This patient presented with globe perforation with lens extrusion at the time of presentation. We assume that in this case, the infection might have transmitted from anterior to posterior segment presumably due to the absence of barrier effect of the lens.

The incidence of post-PK glaucoma is around 10%–53%,\textsuperscript{25,22} being higher in cases of therapeutic keratoplasty due to larger graft size and inflammation. We observed that high intraocular pressures prevailed in a third of our cases, 41.6% of whom were successfully managed conservatively [Table 2].

Lin \textit{et al.}\textsuperscript{11} demonstrated good cosmetic results utilizing GPC for tectonic keratoplasty with anterior vitrectomy with/without conjunctival flaps as an adequate substitute for evisceration in patients with perforated keratitis. All the patients in their study had little or no visual potential. In our study, the ocular integrity with visual potential was maintained in 91.2% patients, and visual acuity of 6/36–6/12 in 14.7% patient was maintained after second corneal transplant with a FCT.

Anatomical integrity was maintained in 86.7% patients without recurrence of infection and complications using cryopreserved cornea (at $-20^\circ$C) in BSS with antibacterials and antifungals for therapeutic PKP in perforated fungal corneal ulcers in a study by Yao \textit{et al.}\textsuperscript{28} Comparable results were achieved using GPC that can be stored at room temperature or 4°C in the regular refrigerators in eye banks without any antibiotics given the antibacterial properties of glycerol.\textsuperscript{17}

There is a paradigm shift in the corneal transplant in recent years, from full-thickness corneal transplant to removing only the diseased layer of the cornea improving the graft survival rate and reduced the chances of rejection. Deep anterior lamellar keratoplasty (DALK) does not need donor endothelium and requires less rigid criteria for donor corneal tissue quality.\textsuperscript{25} Furthermore, the advantage of DALK over PKP is the elimination of endothelial rejection.\textsuperscript{22,24} However, there is a risk of epithelial and stromal rejection as epithelial cell, keratocytes, and bone marrow-derived cell are the antigens that might be responsible for rejection in DALK\textsuperscript{29,31} Glycerol-preserved tissue can be an effective method sans complications.\textsuperscript{32}

A study by Li \textit{et al.} compared DALK in the high-risk cornea by glycerol cryopreserved corneal tissue (GCCT) in one group with FCT in the other group. They found postoperative BCVA of 20/40 or better at the last follow-up in 57.6% in GCCT group and 54.8% in the FCT group. They found no episode of graft rejection in GCCT group versus ten episodes of stromal rejection in FCT group. They concluded that the overall rejection-free graft survival rate of 2 years was significantly higher ($P = 0.006$) in the GCCT group (100%) than FCT group (78%).\textsuperscript{22}

The limitation of this study is that this is a retrospective study with a small sample size. Furthermore, the second optical transplant could be performed in very few patients. Nevertheless, the paucity of literature from South Asia in general and particularly in India, on the subject given its potential to reduce the cost and morbidity, lends credence to our observations. Prospective randomized control trials can be done in future comparing other methods of long-term corneal preservation with glycerol preservation.

**Conclusion**

In our opinion, it is prudent to undertake keratoplasty with GPC (in the case of nonavailability of FCT) in emergency, instead of subjecting the patient to evisceration or enucleation given its cosmetic and psychological implications. The use of glycerol-preserved tissue for lamellar transplant can increase the success rate in high-risk transplant and can increase the corneal pool in developing countries with a paucity of FCT.

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**Conflicts of interest**

There are no conflicts of interest.

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