Limbal Stem Cell Transplantation for Gelatinous Drop-like Corneal Dystrophy

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Purpose: To report the outcomes of allograft limbal stem cell transplantation for recurrent gelatinous drop-like corneal dystrophy (GDLD).

Methods: In this non-comparative interventional case series, 4 eyes of 3 consecutive patients with recurrent GDLD underwent allograft limbal stem cell transplantation. Two eyes underwent concomitant penetrating keratoplasty while the other two underwent simultaneous superficial keratectomy. Main outcome measures were best spectacle corrected visual acuity, intraocular pressure and corneal clarity.

Results: Patient age ranged from 28 to 63 years. Mean follow-up after surgery was 23±10 (median, 22; range, 12-36) months. Mean visual acuity improved from 2.70±0.61 logMAR preoperatively to 1.05±0.06 logMAR at final postoperative visit (P=0.066). Intraocular pressure was normal in all eyes at baseline and remained within normal limits at all postoperative visits. All corneas remained smooth and clear during the follow-up period with no episode of rejection or recurrence. All patients maintained ambulatory vision until final follow-up.

Conclusion: The present study provides further evidence that limbal stem cell transplantation may be an effective therapeutic alternative in patients with GDLD.

Keywords: Corneal Dystrophy; Gelatinous Drop-Like Dystrophy; Limbal Stem Cell Transplantation

INTRODUCTION

Gelatinous drop-like corneal dystrophy (GDLD), also called subepithelial amyloidosis of the cornea, is an early-onset corneal stromal dystrophy with incomplete autosomal recessive pattern. The disease is rare and most reported cases in the literature are from Japan. A genetic abnormality in chromosome 1p and mutations in the M1S1 gene have been linked to the disorder. There is no associated systemic abnormality. Some studies have reported high epithelial permeability in corneas with GDLD which supports the epithelial and consequently limbal stem cell origin of this condition.

Symptoms may begin in the first or second decades of life with foreign body sensation, severe photophobia, lacrimation, and gradual visual loss. At this time, biomicroscopic examination of the cornea shows amyloid deposition as elevated mulberry-like (gelatinous) lesions in the superficial cornea. As a result, the corneal surface becomes irregular. With time, the cornea becomes vascularized and deep corneal involvement by the deposition develops which lead to profound visual loss. Although the mulberry appearance is typical for GDLD, a spectrum of clinical presentations
with four different variants has been introduced comprising of band keratopathy type, stromal opacity type, kumquat-like type, and typical mulberry type.

With severe corneal involvement, the conventional treatment is lamellar or penetrating keratoplasty (PK). However, almost all eyes are complicated by recurrence on the graft and repeat grafting does alleviate the problem. Therefore, it seems that a novel approach is necessary to address the high recurrence rate of graft failure in GDLD.

Limbal stem cell transplantation (LSCT) was first introduced by Kenyon and Tseng in 1989. Since then, encouraging results with LSCT using autografts or allografts have been reported for a variety of conditions including chemical and thermal burns, Stevens–Johnson syndrome, ocular cicatricial pemphigoid, aniridia, carcinoma in situ, contact lens–associated epitheliopathy, and chronic keratoconjunctivitis. In their case series, Shimazaki et al. reported successful outcomes with LSCT for treatment of GDLD. However, to popularize this approach in clinical practice, more reports are required from different centers/regions. In the present study, we report four consecutive eyes with recurrent GDLD receiving allograft LSCT.

METHODS

In this non-comparative interventional case series, 4 eyes of 3 consecutive patients with recurrent GDLD underwent allograft LSCT. Two eyes underwent concomitant PK and the other 2 underwent simultaneous superficial keratectomy (SK). Preoperatively, informed consent was obtained from all patients after a complete explanation of possible complications of the procedure and need for immunosuppressive therapy. This study adhered to the tenets of the Declaration of Helsinki. Table 1 presents demographic and baseline characteristics of the patients.

Surgical procedure

The surgical technique consisted of 360° limbal peritomy in the host eye and excision of perilimbal and limbal tissues providing a smooth bed for proper positioning of the donor stem cell crescent. For harvesting donor stem cells, the central donor cornea (obtained from a cadaver eye) was punched with a 7.5 mm trephine. The corneoscleral rim was then sectioned into equal halves to make surgery easier in patients with deep-set eyes. The anterior one-third of each hemisection was sharply dissected using a crescent blade. After PK (2 eyes) or SK (2 eyes), the crescents were positioned and secured at the superior and inferior limbus using 10-0 nylon sutures. We tried to approximate the ends of the crescents (at the 3 and 9 o’clock positions) as much as possible to avoid any gap between them.

Preoperatively, donor corneas were ordered for all patients. Initially, SK was performed in each patient; if deep corneal vascularization was found intraoperatively, we proceeded with PK. For PK, the cornea was trephined using a Hessburg-Barron suction trephine and a 0.25 mm oversized donor button was sutured to the recipient cornea using 16 interrupted 10-0 nylon sutures. For SK, blunt dissection with a dry cellulose sponge was performed initially. Often, additional semi-sharp dissection with a rounded steel blade or sharp Westcott scissors

Table 1. Demographics, baseline characteristics, and surgical outcomes

| Case | Age (yrs) | Sex | Eye | Previous surgery (N) | Surgical method | Preop VA | Postop VA | Rejection | Recurrence | Clarity | F/U* (mo) |
|------|-----------|-----|-----|----------------------|----------------|----------|----------|-----------|------------|---------|----------|
| 1    | 53        | F   | L   | PK (2)               | LSCT+PK        | HM       | 20/250   | No        | No         | Clear   | 36       |
| 2    | 63        | M   | L   | PK (1)               | LSCT+PK        | 20/1200  | 20/200   | No        | No         | Clear   | 24       |
| 3    | 28        | F   | R   | DLK (1)              | LSCT+SK        | HM       | 20/200   | No        | No         | Clear   | 12       |
| 4    | 28        | F   | L   | PK (1) SK (1)        | LSCT+SK        | HM       | 20/250   | No        | No         | Clear   | 20       |

*Last follow-up.
Yrs, years; F, female; M, male; L, left; R, right; N, number; PK, penetrating keratoplasty; DLK, deep lamellar keratoplasty; SK, superficial keratectomy; LSCT, limbal stem cell transplant; preop, preoperative; VA, visual acuity; postop, postoperative; HM, hand motions; F/U, follow-up; mo, month
was required to create a smooth surface. Care was taken to ensure that the dissection remained in the anterior corneal stroma and that the deep layers were not disturbed. The purpose of this dissection was to remove the abnormal fibrovascular conjunctivalized surface that had replaced the normal corneal epithelium. The last step of surgery was placement of an amniotic membrane (stromal side down) over the entire cornea which was secured with 10-0 nylon sutures (Fig. 1). All eyes received a 360° allograft except case 2 who received a 120° superior limbal allograft due to milder involvement. We assumed that a segmental graft may be sufficient in this particular case with less corneal involvement and would decrease adverse events.

After surgery, all patients received topical antibiotics, steroids and preservative free artificial tears. Immunosuppressive therapy comprised of oral cyclosporine A, mycophenolate mofetil, and prednisolone. Oral prednisolone (1 mg/kg/day) was started on the day of surgery, gradually tapered after 1 week, and discontinued about 6 months after surgery. Cyclosporine A (200-300 mg/day) and mycophenolate mofetil (2 g/day) were started 5 days prior to surgery and continued up to 12-18 months postoperatively with monitoring of blood cell counts.

**Outcome measures**

Main outcome measures were recurrence of amyloid material, corneal vascularization and best spectacle corrected visual acuity (BSCVA). Intraocular pressure (IOP) was also measured before surgery and monitored at all postoperative visits.

**Statistical analysis**

Statistical analysis was performed using the SPSS for Windows 17.0 software (SPSS Inc., Chicago, IL, USA). Nonparametric Wilcoxon signed-rank test was used to compare pre- and postoperative BSCVA in logMAR notations. Counting fingers vision was converted to Snellen equivalents using the method described by Holladay. Snellen equivalent of 20/20000 (logMAR=3) was used for VA of hand motions at 2 feet. The differences were considered as statistically significant when P values were less than 0.05.

**RESULTS**

Patient age ranged from 28 to 63 years. All patients in this study had recurrent GDLD after previous penetrating or lamellar keratoplasty/keratectomy. Based on history, recurrences typically commenced about 1 year after each operation. All eyes had notable diffuse amyloid deposition and corneal vascularization (Fig. 2). The main complaint was profound decrease in vision, foreign body sensation, photophobia and lacrimation. Mean follow-up duration after surgery was 23±10 (median, 22; range, 12 to 36) months.

Mean logMAR BSCVA improved from 2.70±0.61 preoperatively to 1.05±0.06 at last postoperative visit (P=0.066, Table 1). All patients maintained ambulatory vision (defined as being able to see objects and move around a room without stumbling or bumping into obstacles) until final follow-up. Intraocular pressure was normal in all eyes at baseline and remained within normal limits during all follow-up visits. All corneas remained smooth and clear during the follow-up period with no episode of rejection or recurrence. Complaints...
of photophobia and lacrimation decreased after surgery in all subjects.

**DISCUSSION**

In this study, LSCT combined with keratoplasty/keratectomy was effective in maintaining a clear and smooth cornea, and maintaining ambulatory vision in GDLD up to 2 years after the operation. No patient developed any significant complication such as rejection, recurrence, or glaucoma. No significant complication occurred due to immunosuppressive therapy.

There are a few reports on LSCT in GDLD. Shimazaki et al.\(^{11}\) reported 9 eyes with primary (n=4) or recurrent (n=5) GDLD undergoing LSCT combined with keratectomy/keratoplasty. With a mean follow-up of 4 years, 8 eyes (88.9%) were free of recurrence and glaucoma was detected in five of nine eyes (55.6%). Omoto et al.\(^{13}\) reported two cases of GDLD undergoing LSCT combined with deep anterior lamellar keratoplasty without recurrence up to 1.5-2 years postoperatively. Overall, LSCT showed reasonable long-term success for GDLD.

None of our patients developed glaucoma, likely due to the small number of cases. However, glaucoma is a known complication of LSCT,\(^ {14}\) and has been observed in a significant number of patients in the study by Shimazaki et al.\(^ {11}\)

Early diagnosis of this complication and prompt management is critical to preserve ambulatory vision provided by LSCT.

The pathogenesis of GDLD is not clear. Some investigators have suggested that abnormalities of the M1S1 gene may affect epithelial cell junctions, resulting in increased epithelial permeability in affected corneas. Accordingly, it has been shown that the corneal epithelium in GDLD has considerably increased permeability for fluorescein and horseradish peroxidase.\(^ {15,16}\)

In addition, the apical side of the corneal epithelium in GDLD demonstrates loosened cell junctions and an increased number of scarred cells as compared to normal corneas.\(^ {15}\) Overall, these findings suggest that the epithelial barrier function of the cornea may be compromised in GDLD.\(^ {17}\)

As a result, tear components and serum factors may penetrate the subepithelial tissues causing subepithelial amyloid deposition.\(^ {6,15,18}\)

This hypothesis is supported by the presence of lactoferrin, a product of lacrimal glands, in the amyloid deposits in GDLD.\(^ {19}\) After PK for GDLD, early signs of recurrence develop approximately 8 months postoperatively which roughly corresponds to the replacement of donor corneal epithelium by recipient epithelium.\(^ {7}\)

Figure 2. Preoperative slit lamp photograph of case number 2 shows diffuse amyloid deposition and corneal vascularization
GDLD, intensive postoperative management is a key for long-term success.\textsuperscript{11}

Since the limbal area contains numerous Langerhans cells,\textsuperscript{21} immunologic reactions are likely to be more frequent in LSCT than LK or PK alone.\textsuperscript{11} Previous studies showed that endothelial rejection in the central graft develops in 36\% to 46\% of eyes undergoing combined surgery.\textsuperscript{14,22} Similarly, Shimazaki et al\textsuperscript{11} reported immunologic rejection in the central graft in three out of nine eyes who underwent LSCT for GDLD despite receiving oral cyclosporine A. In the present study we found no rejection in the 2 cases that underwent simultaneous PK.Nevertheless, aggressive immunosuppressive therapy is mandatory for maintaining long-term visual improvement.

Previous studies showed that despite achieving acceptable long-term ambulatory vision after LSCT for a variety of indications, a progressive decline in vision and graft survival occurs with time.\textsuperscript{23} Additionally, concomitant PK may be associated with less favorable outcomes.\textsuperscript{23} However, LSCT can be repeated and long-term survival of the second LSCT has been shown to be better than the first one.\textsuperscript{23} Therefore, repeat LSCT may be considered in failed cases.

In conclusion, the present study provides further evidence that LSCT may be an effective alternative for treating patients with GDLD. This study has certain limitations; the number of cases was substantially small because of rarity of the condition in our region. In addition, this study and all previous reports have been non-comparative. Finally, follow-up period in our study was relatively short in some patients (particularly patient 3) to detect recurrences. Future long-term randomized controlled trials comparing LSCT plus keratoplasty to keratoplasty alone would provide more robust evidence for clinical practice.

Conflicts of Interest

None.

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