Living-Related Conjunctival–Limbal Allograft for Chronic or Delayed-Onset Mustard Gas Keratopathy

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Purpose: To determine the long-term outcomes of living-related stem cell transplantation in patients with delayed or chronic mustard gas keratopathy (MGK).

Materials and methods: In this noncomparative interventional case series, 21 consecutive patients with advanced delayed or chronic MGK received living-related conjunctival–limbal allograft and were followed up for at least 1 year. All subjects received immunosuppression with systemic cyclosporine. Main outcome measures were reduction of subjective complaints, corneal epithelial healing, and regression of corneal neovascularization adjacent to the transplant area.

Results: Twenty-five eyes of 21 patients (all male), including 4 patients who received bilateral grafts, were operated. Mean age at the time of surgery was 35.8 ± 3.8 years, mean interval between mustard gas exposure and surgery was 12.2 ± 3.5 years, and mean follow-up was 37.2 ± 18.5 months. Average size of the donor lenticule was 71.16 ± 17.34 degrees. Simultaneous penetrating and lamellar keratoplasty were performed in 5 and 2 eyes, respectively. All patients consistently reported marked subjective improvement. Mean time for epithelial healing was 7.76 ± 3.2 days. Visual acuity was 1.35 ± 0.81 LogMAR before surgery, which improved to 0.59 ± 0.34 LogMAR 3 months after the procedure ($P < 0.001$). Mean visual acuity at final examination was 0.82 ± 0.49 LogMAR ($P = 0.001$). Acute stem cell rejection was observed in 10 (40%) eyes, which improved by increasing the dose of topical and systemic steroids. Chronic stem cell rejection was diagnosed in 8 (32%) eyes, which led to failure in 5 (20%) eyes.

Conclusions: Living-related conjunctival–limbal allograft is effective in stabilizing the ocular surface in patients with delayed or chronic MGK.

Key Words: mustard gas, conjunctival–limbal allograft, stem cell deficiency

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The corneal epithelium is constantly renewed every 4–6 days by continuous shedding of superficial cells. Preservation of corneal epithelial integrity is dependent on cells with self-renewal properties. Limbal stem cells are the source of regeneration for the corneal epithelium in normal and pathologic states.[1–4]

Limbal stem cell deficiency (LSCD), which can be caused by the destruction of limbal epithelial stem cells and/or by a dysfunctional limbal stroma (niche), manifests as conjunctivalization, vascularization, chronic ocular surface inflammation, and poor epithelial integrity.[1,3,5] A variety of disorders can lead to stem cell deficiency including chemical and thermal burns, Stevens–Johnson syndrome, ocular cicatricial pemphigoid, and multiple surgeries at the limbus.[1,3,5]

Mustard gas, which was extensively used by Iraqi forces against Iranian troops during the Iraq–Iran war (1980–1988), especially between 1984 and 1988, can cause severe stem cell deficiency.[6–8] This agent can progressively destroy limbal stem cells and induce severe keratopathy. Ocular manifestations are categorized into early and late, the latter being more destructive and severe in terms of visual morbidity.[6] Patients with chronic or delayed-onset mustard gas keratopathy (MGK) suffer from decreased vision, severe photophobia, foreign body sensation, and tearing. Distinctive features common to most cases include chronic blepharitis, meibomian gland dysfunction, dry eye, perilimbal conjunctival ischemia, stem cell deficiency, epithelial irregularity, recurrent or persistent epithelial defects, corneal neovascularization and thinning, stromal scarring, and secondary degenerative changes including lipoid and amyloid deposition.[6–10]

The purpose of this study was to evaluate the long-term outcomes of living-related conjunctival–limbal allograft (lr-CLAL) in patients with advanced MGK, which to the best of our knowledge is the first report of its kind.

MATERIALS AND METHODS

This noncomparative interventional case series includes consecutive patients with severe chronic or delayed-onset MGK who received lr-CLAL with at least 1 year of follow-up. Patients with chronic irritation; redness and tearing with persistent epithelial defects and severe corneal thinning; and unresponsive to conservative treatments, including punctal occlusion, blepharorrhaphy, tarsorrhaphy, artificial teardrops, and lubricants underwent lr-CLAL. The study was conducted at the Labbafinejad Medical Center, Shaheed Beheshti Medical University, from September 2000 to July 2007 and

was approved by the Institutional Review Board and Ethics Committee of the Ophthalmic Research Center.

The diagnosis of LSCD was confirmed by impression cytology. A complete ophthalmologic examination evaluation including visual acuity measurement, biomicroscopic examination, intraocular pressure measurement, and fundoscopy was performed. Integrity of the corneal epithelium was evaluated by fluorescein staining. Informed consent was obtained from all patients. All procedures were performed by 1 surgeon (M.-A.J.). Patients were visited on postoperative days 1, 3, 7, 14, 28, and then every 2 weeks until 3 months, monthly up to 1 year and every 3 months thereafter. Patients were requested to score their subjective complaints including tearing, photophobia, and foreign body sensation from 1^+ to 4^+ before surgery and 3, 6, and 12 months after surgery.

Schirmer test was not conducted before or after surgery because of a variety of reasons including previous ocular surgeries (punctal occlusion, blepharorrhaphy and/or tarsorrhaphy, amniotic membrane transplantation, and penetrating or lamellar corneal transplantation), simultaneous use of systemic antibiotics and broncholytics (secondary to associated advanced pulmonary problems), and tranquilizers (because of associated psychological problems such as post-traumatic stress disease), reflex tearing, and application of artificial tears by many of these patients.

Main outcome measures were subjective complaints, epithelial healing, and regression of corneal neovascularization adjacent to the transplant area. Healing of epithelial defects and its long-term integrity was evaluated by fluorescein staining at each visit. Changes in corneal neovascularization and thinning were evaluated by slit-lamp biomicroscopy.

Stem cell rejections were classified as acute and chronic. Acute rejection was diagnosed in eyes with limbal and perilimbal vascular engorgement and conjunctival chemosis in the transplant area and treated by increasing the dose and frequency of topical and systemic steroids. The dose was gradually tapered with elimination of vascular engorgement and local chemosis. Chronic rejection was diagnosed in cases with progressive corneal vascularization with or without epithelial disintegrity manifesting as recurrent epithelial erosions adjacent to the limbal lenticule. Decrease in subjective complaints, healing of corneal epithelial defects, and appearance of a smooth epithelial surface in the adjacent cornea together with regression of corneal neovascularization were considered as success. Failure was considered when persistent epithelial defects (more than 2 weeks) occurred adjacent to the graft with or without progressive corneal vascularization and thinning; this type of failure was assumed to be the consequence of chronic stem cell rejection.

Donor Selection

Potential donors (first-degree siblings) were screened for risk factors that might render them unsuitable for donation, including human immunodeficiency virus (HIV), hepatitis B, hepatitis C, human T-cell lymphotropic virus, and cytomegalovirus infections. Eyes with any ocular disease or chronic contact lens use were also excluded. A complete ocular examination was performed on all potential donors. Serologic screening tests for HIV-I, HIV-II, herpes simplex virus antigen, and hepatitis C virus antibody were performed. Because of the limitation of donors, ABO and HLA matching were not performed. The donor's consent for donation was obtained.

Surgical Technique

Recipient Eye

Surgery was performed under general anesthesia in 13 patients and under retrobulbar anesthesia in 8 patients. Limbal areas adjacent to the epithelial defects and thinnest peripheral corneal areas were selected as potential surgical sites. After local peritomy, shaving, and mild cauterization of the sclera, superficial keratectomy was performed. Using a #15 Bard-Parker blade, a half-thickness rectangular corneoscleral bed including 1 mm of cornea and 2 mm of the adjacent sclera was prepared. Then, a donor lenticule with nearly the same dimensions was transferred to the prepared bed and separately sutured to the corneal and scleral bed with 10-0 nylon sutures. Finally, donor and recipient conjunctivae were sutured. Simultaneous penetrating or lamellar keratoplasty was performed in a conventional manner when indicated. At the end of surgery, punctal occlusion and lateral blepharorrhaphy were done in most cases. All removed specimens were sent to the pathology laboratory.

Donor Eyes

Surgery on donor eyes was performed under retrobulbar anesthesia in all cases. A 60- to 120-degree arc of tissue was harvested from the superior limbus, starting with lamellar dissection in the cornea 1 mm anterior to the limbus, extending 2 mm posterior to the limbus, leaving behind tenon's capsule as much as possible. At the end, conjunctiva was dissected, advanced, and fixed to the limbal area with 10-0 nylon sutures. Only 1 limbal lenticule was removed from each eye of the donor. The maximum lenticule size in donor eyes was limited to 120 degrees.

Immunosuppression Regimen

Systemic cyclosporine A (Sandimmune; Novartis Pharma, Tokyo, Japan) 5 mg kg/d was started 1 week before surgery. The dose was reduced to 3 mg kg/d after 2–3 months and discontinued after 2–3 years according to the condition. Regular follow-up examinations were performed. No case of nephrotoxicity, hypertension, or neurotoxicity was detected. The dose of cyclosporine was adjusted in collaboration with a kidney transplant expert according to the clinical condition and serum cyclosporine level. Acceptable serum trough cyclosporine levels (2 hours after morning dose) were 200–300 ng/mL in the first 3 months and 100–200 ng/mL thereafter.

Postoperative Management

After surgery, oral prednisolone 1 mg kg^{-1} d^{-1} was started and tapered over 6–8 weeks. Topical antibiotic and steroid drops were started 4 times a day. The former was discontinued when corneal epithelialization was complete, whereas the latter was tapered off according to ocular surface inflammation 6–8 weeks after surgery. Most patients received topical 20% autologous serum drops every 2 hours. Serum drops were tapered over 1–2 months. Sometimes, instead of

serum drops, topical preservative-free artificial teardrops were administered.

FINDINGS

Overall, 25 eyes of 21 patients (all male) including 4 bilateral cases underwent lr-CLAL (Table 1). Mean age at the time of surgery was 35.8 ± 3.8 years (29–46 years). Mean duration from mustard gas exposure to surgery was 12.2 ± 3.5 years (8–16 years) and mean follow-up period was 37.2 ± 18.5 months (12–78 months). Previous procedures included punctal occlusion, blepharorrhaphy, tarsorrhaphy, penetrating or lamellar keratoplasty, superficial keratectomy, and amniotic membrane transplantation.

Eighteen eyes underwent only limbal stem cell transplantation. Simultaneous penetrating or lamellar keratoplasty was performed in 5 and 2 eyes, respectively. Penetrating and lamellar keratoplasty were performed 3–6 months after lr-CLAL in 3 and 5 eyes, respectively. Overall, 43 lenticules with mean size of 71.16 ± 17.34 degrees (60–120 degrees) were excised from 25 donors including 14 brothers and 11 sisters. Average size of limbal transplantation in each eye was 122.4 ± 25.5 degrees (75–200 degrees).

Persistent epithelial defects that were evident in all eyes before surgery healed in all cases over 2 weeks. Mean period of epithelial healing was 7.76 ± 3.2 days (3–14 days). Peripheral corneal neovascularization regressed at the transplant site in all eyes. No primary failure was observed. There was no case of persistent epithelial defect/recurrent epithelial erosion or recurrent corneal neovascularization in the first 6 months after surgery.

Three months after surgery in the 18 eyes without concomitant corneal transplant surgery, visual acuity improved in 15 eyes and remained unchanged in 3. At final examination, visual acuity improved in 12 eyes, was unchanged in 3 eyes, and decreased in 3 eyes. In this group, mean visual acuity before surgery was 1.19 ± 0.80 LogMAR, which improved to 0.63 ± 0.39 LogMAR 3 months thereafter ($P = 0.001$). Mean visual acuity at the last examination was 0.9 ± 0.51 LogMAR ($P = 0.049$). In 7 eyes with simultaneous corneal grafting, 3 months after surgery and at the last examination, visual acuity improved in 6 eyes and was unchanged in 1 eye. In total, visual acuity was 1.35 ± 0.81 LogMAR before surgery, which improved to 0.59 ± 0.34 LogMAR 3 months after the procedure ($P < 0.001$). Mean visual acuity at final examination was 0.82 ± 0.49 LogMAR ($P = 0.001$).

All patients consistently reported marked subjective improvement. Three months after surgery, mean scores for tearing, photophobia, and foreign body sensation were 2.5^+, 2.4^+, and 2.0^+, respectively. Six and 12 months later, they were 2.5^+, 2.2^+, and 1.5^+ and 2.1^+, 1.8^+, and 2^+, respectively.

Acute stem cell rejection reaction occurred in 10 (40%) eyes. Mean time interval between surgery and stem cell rejection reaction was 1.5 ± 0.3 months (21–60 days). These patients complained of ocular pain and photophobia. On slit-lamp examination, local conjunctival hyperemia, edema, and vascular engorgement were evident (Fig. 1). These cases were treated by increasing the dose and frequency of topical or systemic steroids. Acute stem cell rejection reaction improved in all eyes. Chronic stem cell rejection reaction was diagnosed in 8 (32%) eyes and was treated by increasing the dose of systemic cyclosporine to 5–10 mg/kg but led to limbal failure in 5 (20%) eyes, which occurred 60 (case 3), 18 (case 4), 23 (case 5), 34 (case 9), and 20 (case 15) months after surgery. Tectonic corneal graft was performed in one of these eyes because of an infected persistent epithelial defect, which was unresponsive to medical therapy (case 4). Stem cell transplantation was repeated for all failed grafts during the follow-up period. At final follow-up (before repeating stem cell transplantation), the initial graft was successful in 20 (80%) eyes and failed in 5 (20%) eyes. A Kaplan–Meier graft survival diagram is depicted in Figure 2. All stem cell grafts were successful at 1 year; the cumulative probability for a successful graft was 77% and 51% at 3 and 5 years after stem cell transplantation, respectively. There were 7 episodes of endothelial rejection in 4 (16%) eyes with simultaneous corneal graft surgery, which led to corneal graft failure in 1 eye (case 10).

Histopathologic Findings

Histopathologic examination of the conjunctival–limbal specimens at the time of stem cells transplantation disclosed an epithelium of irregular thickness and including occasional goblet cells but no evidence of dysplasia. Intrastromal telangiectasia, vasculitis, and scar formation along with lymphocytic infiltration and dilation of lymphatic vessels were other abnormal findings. Examination of the peripheral corneal lenticules revealed focal presence of goblet cells in peripheral corneal epithelium, superficial stromal vascularization, spheroidal degeneration, lipid and band keratopathies, and scar formation.

CASE REPORT

Case 3

A 32-year-old man with a history of mustard gas exposure 15 years ago presented with severe photophobia, tearing, and foreign body sensation. His right eye had been eviscerated because of warfare injury. Best-corrected visual acuity in the left eye was 2/10. There were 2 areas of peripheral corneal thinning in the superior nasal and inferior temporal quadrants with lipoid deposition and neovascularization (Fig. 3). Lr-CLAL was performed from both eyes of his sister. One month after surgery, the eye was quiet without photophobia and tearing (Fig. 4). Three months later, visual acuity was 5/10, and 3 years later, it was 4/10 (Fig. 5).

DISCUSSION

Our study shows that living-related limbal stem cell transplantation is an effective procedure for stabilizing an inflamed ocular surface in patients with advanced MGK. This procedure can markedly decrease subjective complaints, heal persistent corneal epithelial defects, and lead to regression of peripheral corneal vascularization in the affected segments. Despite these facts, other reconstructive ocular surface procedures such as penetrating or lamellar keratoplasty may be needed.[6,7]

The late manifestations of MGK seem to be the results of 2 mechanisms. (1) Chronic and progressive perilimbal conjunctival ischemia, mainly in the palpebral fissure leading

TABLE 1. Data of the Patients

No.	Age (yrs)	Eye	Donor	Concomitant Surgery	Epithelial Healing (d)	Visual Acuity		
						Before	After	Last Examination
1	29	OS	Sister		5	CF at 0.5 m	2/10	20/160
2	34	OD	Brother		5	9/10	9/10	20/15
3	32	OS	Sister		4	2/10	5/10	4/10
4	35	OD	Sister		8	CF at 1 m	3/10	CF at 2 m
5	34	OS	Brother	PKP	7	CF at 1 m	3/10	20/80
6	37	OD	Brother		6	CF at 3 m	2/10	CF at 4 m
		OS	Brother		7	20/120	4/10	2/10
7	34	OD	Brother	PKP	5	CF at 0.5 m	5/10	3/10
8	34	OS	Sister		14	1/10	3/10	CF at 2 m
9	39	OD	Brother	LK	3	HM	20/60	20/120
10	39	OD	Sister	PKP	8	HM	2/10	CF at 4 m
11	33	OD	Brother	LK	8	HM	2/10	CF at 1 m
12	37	OD	Brother		6	3/10	20/50	CF at 4 m
13	31	OD	Sister		13	2/10	2/10	2/10
14	33	OD	Sister		11	CF at 5 m	3/10	1/10
15	38	OD	Sister	PKP	11	HM	CF at 2 m	CF at 2 m
16	35	OD	Brother		7	20/200	20/120	CF at 2 m
		OS	Brother		9	CF at 1 m	CF at 1 m	CF at 1 m
17	46	OD	Sister		14	5/10	6/10	20/40
		OS	Brother		12	3/10	5/10	20/40
18	40	OD	Brother	PKP	7	4/10	4/10	4/10
		OS	Sister	PKP	7	HM	2/10	4/10
19	38	OD	Brother		5	CF at 2 m	2/10	2/10
20	40	OD	Sister		3	1/10	3/10	4/10
21	35	OS	Brother		9	CF at 4 m	3/10	5/10

No.	Follow-Up (mo)	Rejections		Complications	Previous Surgery	Size of Graft
		Cornea	Limbus			
1	46				Punctal occlusion	2 × 90°
2	43		1		AMT	2 × 75°
3	72			Limbal failure		2 × 90°
4	60			Limbal failure, corneal ulcer, lens opacity, ICA, ↑IOP		1 × 90°
5	38	2	1	Limbal failure		1 × 120°
6	78		1			1 × 120°
	78		1			1 × 120°
7	25	2		Lens opacity, ICA		2 × 60°
8	44		1		Punctal occlusion	2 × 60°
9	42			Limbal failure, lens opacity	AMT	2 × 60°
10	36	2	2	Corneal graft failure, lens opacity, ICA	LK, PKP	2 × 60°
11	36				LK	1 × 75°
12	28					2 × 75°
13	20		1		Punctal occlusion	2 × 75°
14	33					2 × 60°
15	30	1		Limbal failure	PKP, conjunctival flap, ECCE + PCIOL	2 × 60°
16	25		1			2 × 60°
	25		1			2 × 60°
17	22		1			2 × 60°
	20		1			2 × 60°
18	12			ICA, ↑IOP	Punctal occlusion/OU	2 × 60°
	12			ICA, ↑IOP	Punctal occlusion/OU	2 × 60°
19	34				AMT	2 × 60°
20	30					1 × 75°
21	29					1 × 90°

AMT, amniotic membrane transplantation; CF, counting fingers; ECCE, extracapsular cataract extraction; HM, hand motion; ICA, iridocorneal adhesion; IOP, intraocular pressure; LK, lamellar keratoplasty; OD, right eye; OS, left eye; PCIOL, Posterior chamber intraocular lens.

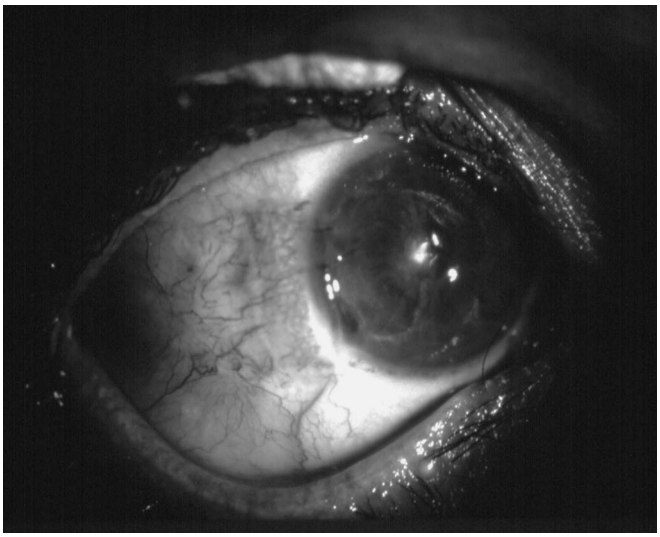

FIGURE 1. Acute graft rejection. Note the hyperemic chemosis and vascular engorgement in the transplanted area.

to stromal thinning and epithelial disintegrity in the adjacent cornea. These areas of conjunctival ischemia are often surrounded by leaking telangiectatic vessels, which lead to lipoid deposition in the adjacent cornea. (2) Progressive LSCD, which is partial and asymmetric at onset but finally leads to total stem cell deficiency. This deficiency may be because of the direct and progressive effect of mustard gas or secondary to chronic limbal ischemia.

Corneal manifestations in MGK are not identical to the total LSCD seen in severe chemical burns. The cornea is not totally vascularized, and in contrast, localized corneal thinning with amyloid and lipoid depositions are prominent. In some areas, leaking telangiectatic vessels invade the peripheral cornea. Corneal involvement is often asymmetric, and

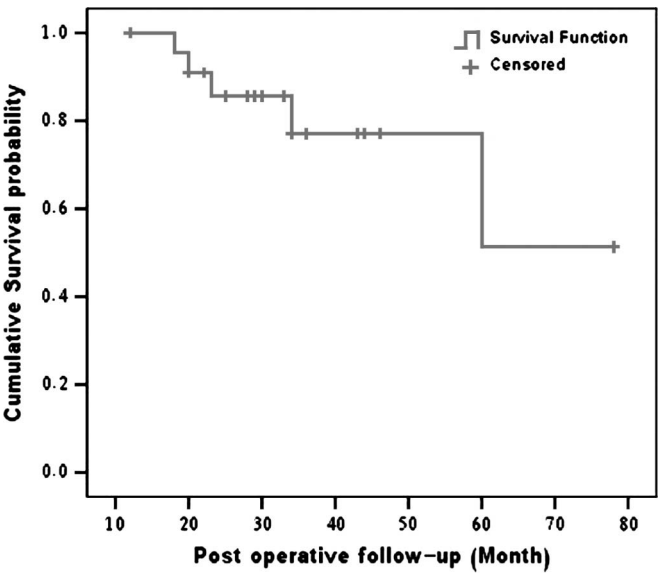

FIGURE 2. Kaplan–Meier diagram demonstrating cumulative graft survival probability.

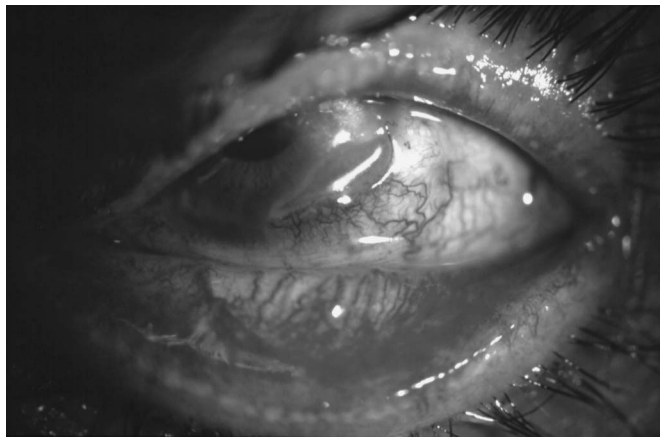

FIGURE 3. Peripheral corneal thinning (melting) in the inferior temporal quadrant.

different sectors may not be involved to the same extent. Most often, the interpalpebral (exposure) area is more severely damaged.[6,7] For these reasons, when stem cell deficiency is not severe and only mild perilimbal conjunctival ischemia with corneal thinning and amyloid /lipoid deposition is present, penetrating or lamellar keratoplasty may suffice.[6,7]

In the event of bilateral and total LSCD, transplantation of allogeneic limbal stem cells by keratolimbal allograft (KLAL) from a cadaver or by conjunctival–limbal allograft (CLAL) from a living-related donor seems to be indicated.[11,12] The main objective of KLAL and CLAL was to continue the supply of new corneal epithelium for a prolonged, if not indefinite, period.[11,12] In eyes with superficial corneal vascularization and pannus, a single procedure of KLAL or CLAL is frequently enough to achieve this goal. However, if there is concomitant deep corneal stromal scarring, penetrating or lamellar keratoplasty is needed to restore vision.[11,13,14]

In patients with advanced bilateral MGK, with varying degrees of corneal involvement, 360-degree coverage of the limbal area is not necessary. Due to accessibility of first-degree siblings, fresher donor tissue with closer genetic composition

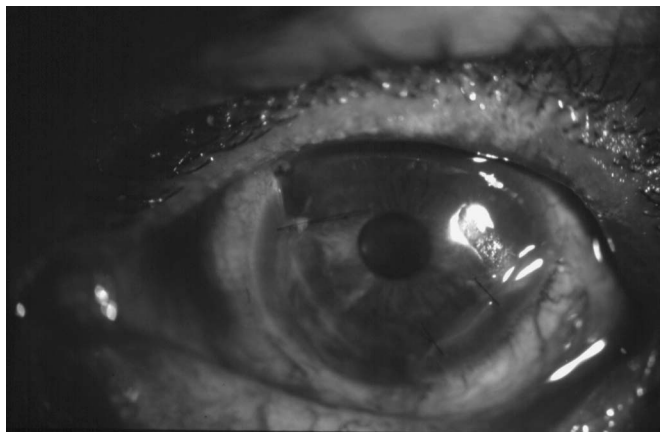

FIGURE 4. One month after CLAL in the inferior nasal and inferior temporal quadrants. Quiet appearance of the eye.

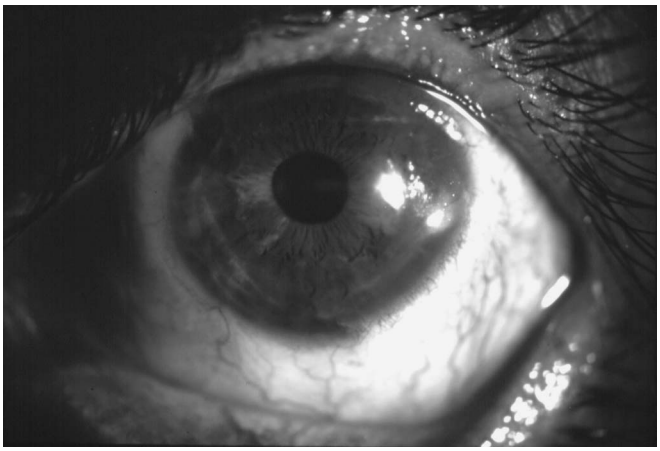

FIGURE 5. Appearance of the eye 3 years later without invading vessels in transplanted quadrants.

and more stem cells and theoretically lower chance for rejection and less need for intense immunosuppression,[12,15] living-related CLAL seems to be a good alternative in these patients.

Daya and Ilari[16] reported the results of best HLA-matched lr-CLAL in 10 eyes of 8 patients with stem cell deficiency. Mean follow-up period was 26 months. Eight grafts survived with restoration of corneal epithelium and reduction of vascularization. Visual acuity improved in 7 eyes and corneal opacification was reduced in 4 eyes. Graft rejection was seen in 2 cases, which was successfully treated with medical therapy. Samson et al[17] performed CLAL on 11 eyes of 9 patients with different ocular surface inflammatory disorders. Re-epithelialization of the corneal surface in the immediate postoperative period occurred in 10 eyes (91%) within an average period of 10 days. Long-term restoration of the corneal surface was achieved in 6 (55%) eyes and visual acuity improved in 6 (55%) eyes. Santos et al[18] reported the results of conjunctival–limbal grafts on 33 eyes of 31 patients with total LSCD. Ten (30%) eyes underwent CLAL and 23 (70%) underwent CLAL from living HLA-matched donors. Graft survival was seen in 13 (40%) eyes at 1 year and in 11 (33.3%) eyes at 2 years, with a cumulative survival of 33% after a mean follow-up of 33 months.

There are a few reports on the results of KLAL in patients with total LSCD.[13,15,19,20] Mean follow-up period ranged from 12 to 39 months. Mean visual acuity improved in a significant group of cases after the surgery (53.6%–100%). Epithelial defect healed in 51%–100% of cases. But the report of Shimmura et al[21] with a mean follow-up of 18 months on 8 cases was disappointing. The difference between preoperative and postoperative visual acuities was not statistically significant. Corneal stromal vascularization was seen in all cases. Seven of 8 cases had persistent epithelial defects, which led to corneal infection in 4 eyes.

There is debate regarding the long-term survival of donor stem cells. In an experimental study, it was shown that there was no evidence of limbal stem cells on the recipient ocular surface 3–5 years after surgery. Despite this fact, there might be considerable clinical improvement and there was no correlation between objective signs and patients' symptoms.[22] Djalilian et al,[23] using polymerase chain reaction and immunohistochemistry, determined the long-term fate of donor epithelial cells in 3 cases after limbal allograft transplantation. Nonrecipient cells were consistently detected in all 3 cases up to 3.5 years after clinically successful limbal allograft transplantation. In our series, all cases experienced subjective improvement up to 1 year after surgery. We did not perform impression cytology after surgery; therefore, we could not objectively evaluate the survival of limbal stem cells and corneal conjunctivalization. Instead, we relied on clinical and biomicroscopic findings to judge our surgical success.

Patients with MGK do not have eyelid structural deformities. Lid-globe apposition is normal, and there is no exposure in these cases. Frequency of blinking seems to be within normal limits, and they actually suffer from reactive blepharospasm in severe cases. We did not observe even a single case of corneal keratinization in these patients. For these reasons, all failures were considered to be the consequence of chronic immune rejection, which might be because of insufficient immunosuppression. We observed 5 limbal failures, and there was no history of acute stem cell rejection in 4 of them.

All acute rejections occurred up to 60 days after surgery. Although episodes of acute stem cell rejection were relatively common in our patients (11 times in 10 eyes), none of them led to graft failure in the acute phase. Whether there is any correlation between acute stem cell rejection episode and chronic limbal failure remains an unresolved issue. Definite conclusions in this regard need larger numbers of patients with longer follow-up, definite classification of rejections, better understanding of the pathophysiology and mechanism of stem cell rejection, and more objective evidence for detecting rejections.

We performed 3 penetrating keratoplasties and 5 lamellar keratoplasties for rehabilitation of vision during the follow-up period. Also, we performed 5 and 2 concomitant penetrating and lamellar keratoplasties, respectively. We observed rejections in 4 concomitant penetrating keratoplasties. In the study of Solomon et al,[13] KLAL performed alone resulted in higher survival of ambulatory vision (visual acuity $\geq$20/200) at 2 years (86.1% $\pm$ 9.1%) compared with KLAL with penetrating keratoplasty (PKP) (46.9% $\pm$ 10.6%). They concluded that performing PKP simultaneously with KLAL may be associated with a less favorable outcome. Shimazaki et al[24] evaluated the immunologic rejection of the central graft after KLAL transplantation combined with PKP. They found that approximately one third of eyes had endothelial rejection in the central graft after simultaneous KLAL and PKP. Abnormalities suggestive of rejection in the limbal grafts were seldom observed in these eyes, suggesting that the immunologic response was different in central and limbal grafts. Whether concomitant PKP and stem cell transplantation is an independent risk factor for increasing the chance of graft rejection remains an unresolved issue. Whether stem cell rejection predisposes to corneal graft rejection or corneal graft rejection predisposes to simultaneous or old stem cell transplantation rejection are questions that future advances in the field of stem cell transplantation will hopefully answer.

There are some advantages in this study. First, all cases were affected by advanced MGK; the patients in other studies were nonhomogeneous with different types and severities of ocular surface diseases. Second, all donors were first-degree siblings with fresh materials. Third, the surgical technique and the immunosuppression protocol were the same in all. In other studies, different immunosuppressive regimens have been used and were changed during the follow-up period.

CONCLUSIONS

Lr-CLAL surgery is an effective procedure for stabilizing the ocular surface in patients with advanced chronic or delayed-onset MGK. Additional reconstructive procedures such as penetrating or lamellar keratoplasty might be needed to improve vision.

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