Amniotic Membrane Transplantation With or Without Limbal Grafts in the Surgical Treatment of the Ocular Surface: Various Indications and Different Application Techniques

Nurettin Bayram[1,2], Ömür Uçakhan Gündüz[3]

ABSTRACT

Purpose: This study aimed to evaluate various indications and different application techniques of amniotic membrane transplantation (AMT) with or without limbal grafts in patients with ocular surface disorders.

Materials and Methods: Eighty-two eyes of 80 patients who underwent fresh AMT with various ocular surface disorders over 9 years were retrospectively analyzed.

Results: Of the 80 patients, 57 were male and 23 were female. The mean age and follow-up were 45.1±17.7 years and 14.3±11.1 months, respectively. The most common surgical indication was limbal stem cell deficiency (LSCD) (43 eyes, 52.4%). The most common cause of LSCD was chemical/thermal injuries (58.1%). AMT combined with allograft or autograft limbal transplantation was performed in 25 eyes with total LSCD, whereas AMT alone in 18 eyes with partial LSCD. The second most common surgical indication was corneal ulceration (21 eyes, 25.6%). The most common causes were herpetic, bacterial, and peripheral ulcerative keratitis (66.7%). Other surgical indications were recurrent pterygium (8 eyes, 9.8%), large conjunctival tumors (7 eyes, 8.5%), and cystic blebs (3 eyes, 3.7%). Dura mater graft was combined with AMT in the cystic bleb surgery. Chelation was applied to 3 eyes with band keratopathy, and symblepharon resection and fornix reconstruction were performed in 8 eyes. In total, a second time AMT was performed in 13 eyes (15.8%).

Conclusion: Fresh AM performed in a wide variety of indication groups was found to be safe and effective. Fresh AMT combined with limbal grafts may increase surgical success in patients with total LSCD.

Keywords: Amniotic membrane transplantation, Limbal graft, Ocular surface diseases, Surgical techniques.

INTRODUCTION

The amniotic membrane (AM) is the innermost layer of the placenta, consisting of three different layers: the epithelium, basement membrane, and stroma. The AM is a stem cell source and has excellent regenerative properties. It has been shown that AM contains essential growth factors and cytokines for tissue healing, provides a matrix for cellular proliferation and migration, reduces inflammation, improves pain management, and has anti-bacterial, anti-fibrotic, and anti-angiogenic properties.[1-3] Because of its transparent structure without blood vessels, a lack of immunogenicity, as well as the biological properties mentioned above, it is being performed increasingly for ocular surface reconstruction with a wide range of surgical applications, including corneal disorders with or without limbal stem cell deficiency (LSCD) and conjunctival reconstruction.[1-4] These biological properties support and facilitate wound healing, it also forms an ideal material for combination surgeries, including limbal autograft/allograft transplantation or scleral grafts.

Fresh AM can be used as an alternative to preserved AM for the reconstruction of the ocular surface in several diseases.

1- MD, University of Health Sciences, Department of Ophthalmology, Ankara Ulucanlar Eye Education and Research Hospital, Ankara

2- University of Health Sciences, Kayseri City Training and Research Hospital, Eye Clinic, Kayseri

3- Ankara University Faculty of Medicine, Department of Ophthalmology, Ankara

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Correspondence Address:
Nurettin Bayram
University of Health Sciences, Department of Ophthalmology, Ankara Ulucanlar Eye Education and Research Hospital, 06250, Altındağ, Ankara, Türkiye

Phone: +90 312 312 6261
E-mail: drnbayram@gmail.com

Besides, the fresh AM does not go through a process of preservation and freezing/unfreezing cycles which may be deleterious for the biochemical function and loss of some of its anti-inflammatory properties.[5]

Depending on the indication of amniotic membrane transplantation (AMT), there were reported three surgical techniques that can be used over the ocular surface: (1) inlay technique (graft, epithelial side up) allows migration of the surrounding epithelial cells on the AM; (2) overlay (patch, stromal side up) allows the host epithelium to grow below the AM, and a combination of the graft and patch, also called the sandwich technique.[1, 2, 4] Depending on the depth of the underlying corneal defect, the AM can be used as a monolayer or multilayer.[6]

Management of patients with LSCD varies depending on the severity of LSCD. AMT alone seems to have a limited long-term effect in patients with total LSCD. AMT combined with limbal allograft (Lal) or limbal autograft (Lau) is commonly performed based on the presumption that AM optimizes the ocular surface and provides biological and mechanical support and protection to the transplanted limbal tissues and stem cells.[1, 2, 4]

Because AMT is commonly used in challenging ocular surface conditions to maintain eye integrity and reduce ocular surface problems, it is essential to be reemphasized to better clarify the clinical scenarios for which it is required in the ophthalmic community. Hence, this study aims to evaluate the outcomes of a wide spectrum of indications of AMT with or without limbal grafts in ocular surface disorders.

MATERIALS AND METHODS

Study design and characteristics

Eighty-two eyes of 80 patients with various ocular surface disorders were retrospectively analyzed, and a detailed review of the medical records over 9 years was performed. All patients who underwent AMT alone or combined with limbal autograft or allograft transplantation were included in the study. The study protocol was approved by the Institutional Review Board (#2021/387) and was conducted on the ethical principles for medical research involving human subjects outlined in the 1964 Helsinki declaration and its later amendments. After explaining the potential risks and complications of the procedures, informed consent was obtained from all patients and limbal allograft donors before surgery.

All participants were assessed with a detailed ophthalmic examination, including lid examination, ocular motility evaluation, uncorrected visual acuity (UCVA) in LogMAR, intraocular pressure measurement, and slit-lamp and fundus examination. Ocular ultrasonography was performed in patients with opaque ocular media. The tear film break-up time test and the Schirmer tear test with anesthesia were used to evaluate tear function.

Appropriate medical therapies were given to all patients until surgery. In patients with dry eyes, preservative-free lubricant eye drops (Refresh single doses, polyvinyl alcohol 1.4% and povidone 0.6%, Allergan Pharmaceuticals Ltd.) 12 times a day and ointments (Lipotears 0.2% ophthalmic gel, carbomer 980, Liba Pharmaceuticals Ltd.) 4 times a day were given, and/or silicone punctal plugs were performed until surgery. Antiviral treatment (Virgan 0.15% ophthalmic gel, 1.5 mg ganciclovir, Thea Pharmaceuticals Ltd., and/or Asiviral tablets, Aciclovir 400 mg, Terra Pharmaceuticals Ltd.) 5 times a day was administered for herpes simplex keratitis, and topical fortified antibiotics (vancomycin 50 mg/ml and ceftazidime 50 mg/ml) every 1 hour were given for bacterial keratitis. The patients were divided into four groups based on the surgical indications: (1) total LSCD, (2) partial LSCD, (3) corneal ulceration or persistent epithelial defect, and (4) conjunctival reconstruction. The surgical approach was chosen based on the patient's diagnosis and findings, and a fresh AM obtained on the same day of surgery was applied to all patients. The fresh AMs were taken from hepatitis B-, hepatitis C-, syphilis- and HIV-seronegative women who underwent elective cesarean section, and their preparations were done under sterile conditions. The combination surgery, AMT with Lau or Lal transplantation, was performed in patients with LSCD. The diagnosis and stage of the severity of the LSCD were made based on the degree of corneal vascularization and opacity, conjunctival epithelial invasion, chronic ocular surface inflammation, the absence of palisades of Vogt, and the presence of recurrent/persistent epithelial defects. LSCD was accepted to be partial if there was any clock hour of the intact limbus or clear corneal phenotype and total if there were 360 degrees of corneal vascularization and opacity. Lal from living-related donors was performed in patients with total bilateral LSCD, and those with a healthy contralateral eye underwent Lau. HLA class I-matching (HLA-A, -B, and -C) was performed between donor-recipient before Lal surgeries using a standard serological method. Two or more HLA matches were accepted as an adequate degree of matching. AMT alone was performed in patients with partial LSCD.

Surgical technique

Surgery was performed under general anesthesia in pediatric patients. The rest of the patients underwent the operation

under topical and peribulbar anesthesia. In patients with total LSCD, a 360-degree conjunctival peritomy was performed, followed by a superficial keratectomy by a Beaver blade to remove fibrous pannus from the cornea (Figure 1, a and b). A piece of fresh AM graft was placed on the denuded ocular surface and secured with a suture, then Lau or Lal was performed in the same session. After the suturing of the limbal graft, a piece of AM patch was applied to cover the limbal grafts and the entire ocular surface. Interrupted 10-0 nylon was used for the corneal fixation, and interrupted 8-0 vicryl sutures were applied to the episclera. Limbal tissue was obtained either from the contralateral healthy eye or from a healthy-related donor's eye. Three clock hours of limbal tissue were obtained from the 6 and 12 o'clock positions of the limbus of the healthy cornea and sutured to the original limbal area of the damaged cornea. In the presence of symblepharon, it was resected, and the conjunctiva was retracted as much as possible, then the AM graft was performed on the bulbar episclera to recreate the eyelid fornix (Figure 1, c and d). A symblepharon ring was placed over these eyes for 2-6 weeks. In patients with band keratopathy, a superficial calcium deposit was removed with a superficial keratectomy and a 4% EDTA solution; then, the AM was performed (Figure 1, e and f).

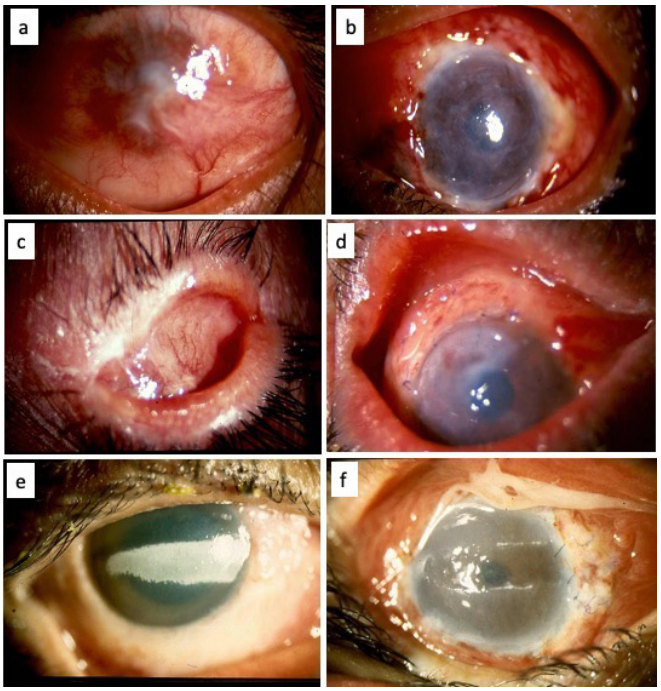

Figure 1: *A complete conjunctivalization of the entire corneal surface is seen in a patient with total limbal stem cell deficiency (LSCD) due to chemical injury in his left eye (a). Since the patient's other eye is also affected, keratectomy, limbal allograft, and amniotic membrane transplantation (AMT) with the sandwich technique were performed simultaneously in the same eye (b). A symblepharon formation is observed in a patient with partial LSCD due to ocular cicatricial pemphigoid in the right eye (c). After symblepharon resection and reform of the upper eyelid fornix, an amniotic membrane graft alone was performed for ocular surface reconstruction (d). Band keratopathy developed in one of our patients who had previously undergone AMT combined with a limbal autograft for total LSCD treatment due to a chemical injury in his right eye (e). A superficial keratectomy with a 4% EDTA solution was performed to remove the calcium deposits; then, the AMT was performed (f).*

In patients with partial LSCD, a keratectomy and peritomy were performed on the affected areas, then the AM graft was positioned to cover the damaged corneal surface, and sutured to the cornea using interrupted 10-0 nylon and to the episclera using interrupted 8-0 vicryl sutures. At the end of the surgery, a bandage contact lens was placed over the cornea and kept in place until corneal re-epithelialization.

In patients with corneal ulceration, AM was performed either as a monolayer graft or a multilayer graft after de-epithelialization of a ring-shaped area around the cornea ulcer. In the deep corneal ulcers, the entire depth of the ulcer crater is filled with small pieces of AM graft, then a larger graft was positioned over the defect in an inlay fashion and sutured to the surrounding cornea with 10-0 nylon sutures. Finally, an even larger piece of AM patch was covered on the entire cornea in an overlay fashion and fixated to the conjunctiva with 8-0 vicryl sutures.

In patients with recurrent pterygium, the recurrent pterygium was removed with a surgical blade, and the residual tissue was scraped from the corneal surface with a crescent knife. AM graft was applied at the proper size to cover the bare area with the stroma facing down and was sutured to the conjunctiva and episclera using 8-0 vicryl sutures.

In patients with large conjunctival tumors, the tumors were totally removed with adjunctive cryotherapy to the surgical margins. Corneal epitheliectomy was performed with absolute alcohol in patients with corneal involvement, and lamellar sclerectomy in patients with episcleral involvement. AM graft was applied at the proper size to cover the bare area with the stroma facing down and was sutured to the conjunctiva and episclera using 8-0 vicryl sutures.

In patients with leaking cystic filtering blebs and ocular hypotony, leaking blebs were excised, and the sclera was reconstructed with an allogenic dura mater graft. AM graft was applied at the proper size to cover the bare area with

the stroma facing down and was sutured to the conjunctiva and episclera using 8-0 vicryl sutures.

Postoperative regimen

Postoperatively, all patients received topical 0.1% dexamethasone (Onadron eye drops, İ.E. Ulagay, Pharmaceuticals Ltd.) 5 times a day, 0.3% ciprofloxacin (Ciloxan ophthalmic solution, 3.5 mg/1mL, Alcon Pharmaceuticals Ltd.) 5 times a day, and preservative-free lubricating eye drops (Refresh single doses, polyvinyl alcohol 1.4% and povidone 0.6%, Allergan Pharmaceuticals Ltd.) 12 times a day. Drops were tapered and discontinued in 3 months or until healed. All allograft patients received systemic cyclosporine A (Sandimmun Neoral 50 mg Capsule, Novartis Pharmaceuticals Ltd.) 5 mg/kg/day in 2 divided doses for immunosuppression. It was started 3 days before the surgery and tapered and discontinued in 3 to 6 months according to the corneal surface stability.

Outcome measures

The primary outcome measures were to investigate the regression of corneal vascularization and opacity, resolution of the inflammation, restoring a smooth ocular surface, and rapid ocular surface healing with no recurrent erosions or persistent epithelial defects. The secondary outcome measure was an improvement of UCVA.

Statistical analysis

The study data were analyzed using the SPSS program for Mac OS version 26.0 (SPSS Inc, Chicago, IL, USA). Descriptive statistics were specified as means±standard deviations, frequency distributions, and percentages. The normal distribution of variables was examined by the Shapiro–Wilk test. The paired 2-tailed t-test was used to assess the significance of differences between baseline UCVAs and final UCVAs. Wilcoxon signed-rank test was performed in nonparametric data. In patients with penetrating keratoplasty, the final UCVA used for statistical analysis was obtained pre-penetrating keratoplasty. A p-value < 0.05 was accepted as a significance level.

RESULTS

Demographic Features

Of the included 80 patients, 57 (71.2%) were male and 23 (28.8%) were female, with a mean age of 45.1±17.7 years (range: 5–84 years). The AM was performed on the right eye in 40 (50.0%) patients, the left eye in 38 (47.5%) patients, and both eyes in 2 (2.5%) patients. The mean follow-up period was 14.3 ±11.1 months (range: 5-48 months). All patients' clinical characteristics and classifications based on the AMT indications are summarized in Table 1.

Patients with total LSCD who underwent AMT and Lau or Lal

In total, 25 eyes of 24 patients had total LSCD. All eyes developed total corneal opacity with vascularization and/or conjunctivalization, 3 eyes had band keratopathy, 1 eye presented with symblepharon, and 1 eye developed corneal stromal thinning. All patients underwent AMT and Lau or Lal. The surgery was performed on the right eye in

Table 1: *Clinical characteristics and classification of the patients based on the indications for amniotic membrane transplantation*

Indication of AMT	n of patients/n of eyes (%)	Mean age±SD (years)	Gender (male/female)	Eye laterality (right/left)	Surgery	Follow-up period (mean±SD)
Total LSCD	24 /25(30.5%)	43.4±16.8	5/19	11/14	Combined with Lau or Lal	21.2±12.0
Partial LSCD	17/18(21.9%)	41.2±17.3	2/14	8/9	AMT alone	14.9 ±11.5
Corneal ulcer or PED	21 /21(25.6%)	43.3±20.8	9/13	12/10	AMT alone mono-/multilayer	7.9±4.8
Conjunctival reconstruction						
- Recurrent pterygium	8 /8(9.8%)	45.7±7.4	4/4	5/3	AMT alone	7.9±4.6
- Large conjunctival tumor	7 /7(8.5%)	55.6±19.4	1/6	5/2	AMT alone	7.1±2.2
- Leaking cystic filtration bleb	3 /3(3.7%)	62.0±10.1	1/2	1/2	Combined with dura mater graft	23.3±9.1

Abbreviations: AMT: amniotic membrane transplantation, LSCD: limbal stem cell deficiency, Lal: Limbal allograft, Lau: limbal autograft, n: number, PED: persistent epithelial defect

10 (41.7%) patients, on the left eye in 13 (54.2%) patients, and on both eyes in 1 (4.1%) patient. Limbal tissue was obtained as Lau in 7 (28.0%) eyes and as Lal in 18 (72.0%) eyes. The mean follow-up was 21.2±12.0 months (6-48 months), and epithelialization was achieved in 21 (84.0%) eyes with a mean of 3.0±0.8 weeks (2-4 weeks).

AMT was performed for the second time in 4 (16.0%) eyes where corneal epithelialization could not be achieved, and in 1 eye with recurrent symblepharon. At the end of the follow-up, corneal vascularization and inflammation were decreased in all patients (Figure 2, a-c). The mean preoperative UCVA improved significantly compared to final measurements, from 2.4±0.68 to 1.9±0.72 logMAR (p=0.0003). No systemic side effects related to cyclosporine therapy were observed in any patient. Ten patients underwent penetrating keratoplasty during the follow-up period (Figure 2, c). Demographic and clinical characteristics of patients with total LSCD are summarized in Table 2.

Patients with Partial LSCD who underwent AMT alone

Eighteen eyes of 17 patients presented with partial LSCD. All patients developed partial corneal vascularization and/or conjunctivalization with opacity, 6 eyes had PED, and 7 eyes had symblepharon (Figure 2, d-f). All patients underwent AMT without Lau or Lal. Symblepharon formations were resected at the same session. The surgery was performed on the right eye in 8 (47.1%) patients, on the left eye in 8 patients, and on both eyes in 1 (5.8%) patient. The mean follow-up period was 14.9±11.5 months (5-37 months). Epithelialization was achieved in 13 (72.2%) eyes with an average of 3.4±1.5 weeks (2-5 weeks). AMT was performed for the second time in 5 (27.8%) eyes where corneal epithelialization could not be achieved, and in 1 eye with recurrent symblepharon.

At the end of the follow-up, corneal vascularization and inflammation were decreased in 16 eyes (88.9%) (Figure 2, d-f). In the remaining 2 eyes, a combination of AMT and limbal graft transplantation was planned. The mean preoperative UCVA improved significantly compared to final measurements, from 2.5±0.81 to 2.1±0.99 logMAR (p=0.007). Two patients underwent penetrating keratoplasty during the follow-up period. Demographic and clinical characteristics of patients with partial LSCD are shown in Table 3.

Corneal ulcer or PED

Of the 21 patients, 14 (66.7%) had stromal thinning, and 7 (33.3%) presented with PED. Monolayer AMT was applied in 19 eyes (Figure 3, a-c), and multilayer AMT was performed in 2 eyes with severe stromal thinning (Figure 3, d-f). The surgery was performed on the right eye in 11 (52.4%) patients and the left eye in 10 (47.6%).

The mean follow-up was 7.9±4.8 months (5-27 months). During the follow-up, corneal healing and epithelialization were achieved in 17 (80.9%) eyes with a mean of 3.4±0.9 weeks (2-5 weeks). However, the mean preoperative UCVA did not differ significantly compared to final measurements, from 1.92±0.97 to 1.76±0.96 logMAR (p=0.383). Two patients needed a secondary monolayer AMT, and another

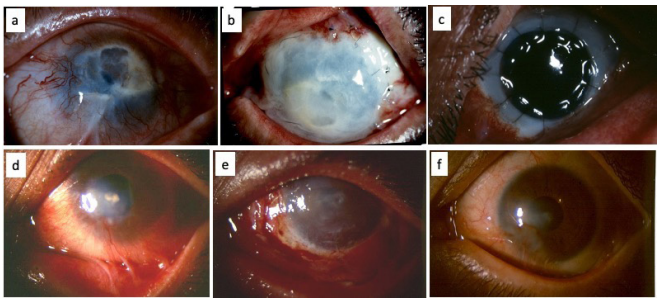

Figure 2: *Corneal opacity and diffuse conjunctivalization are observed in a patient with total limbal stem cell deficiency (LSCD) due to chemical injury in his left eye (a). Keratectomy, limbal autograft, and amniotic membrane transplantation (AMT) were performed simultaneously in the same eye (b). In the follow-up of the patient, although the cornea opacity remained, it was observed that the conjunctivalization and vascularization of the cornea completely regressed, and penetrating keratoplasty was performed (c). In a patient with partial LSCD due to chemical injury in the left eye, a reasonable result was obtained with only keratectomy and AMT (d-f).*

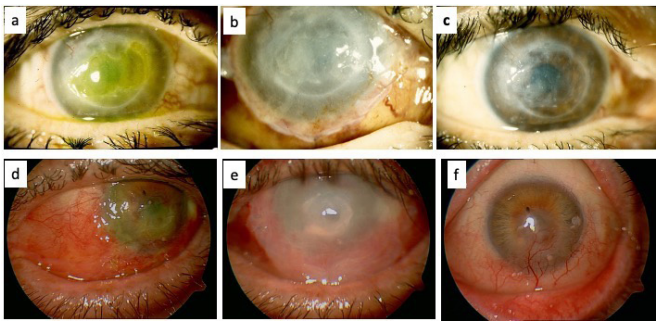

Figure 3: *In a patient with herpetic keratitis sequelae who underwent penetrating keratoplasty due to corneal leukoma in the right eye, the persistent epithelial defect was completely resolved with monolayer amniotic membrane transplantation (AMT) (a-c). In another patient with a deep central corneal ulcer in the left eye, the ulcer healed with multilayer AMT (d-f).*

Table 2: *Demographic and clinical characteristics of patients with total limbal stem cell deficiency*

Diagnosis	Number of eyes	Age (mean ±SD)	Gender (male/ female)	Eye (right/ left)	Clinical Findings	Primary procedure	Epithelization and healing time (mean ±SD)	Second Procedure	Follow-up period (mean ±SD)	Preop UCVA (LogMAR)	Final UCVA* (LogMAR)
Chemical eye injury	12 (48%) Alkali burn in 11 eyes Acid burn in 1 eye	46.5±9.4 years	10/2	6/6	C and/or CV with opacity in all eyes PED in 2 eyes Symblepharon in 1 eye Band keratopathy in 2 eyes	AMT combined with Lau or Lal Symblepharon resection	Epithelization in 11 eyes Healing time: 2.9±0.94 weeks	AMT in 1 eye Symblepharon resection with AMT in 1 eye PK in 6 eyes	22.4±13.2 months	2.36±0.72	1.94±0.69
Herpetic keratitis	6 (24%)	44.3±23.3 years	4/2	2/4	C and/or CV with opacity in all eyes PED in 3 eyes Band keratopathy in 1 eye	AMT combined with Lau or Lal	Epithelization in all eyes 2.8±0.75 weeks	PK in 2 eyes	16.5±8.2 months	2.30±0.79	1.65±0.85
Ocular cicatricial pemphigoid	3 (12%)	57.7±2.3 years	1/1	1/2	C and/or CV with opacity in all eyes PED in 2 eyes	AMT combined with Lal	Epithelization in 1 eye Healing time: 4 weeks	AMT in 2 eyes PK in 1 eye	32.7±17.9 months	2.67±0.58	2.03±0.95
Stevens-Johnson syndrome	2 (8%)	26.5±2.1 years	1/1	1/1	C and/or CV with opacity in both eyes PED in 1 eye	AMT combined with Lal	Epithelization in all eyes Healing time: 3.5 weeks	PK in 1 eye	12.5±3.5 months	2.50±0.71	1.90±1.41
Radiation keratopathy¥	2 (8%)	14.0±11.3 years	2/0	1/1	C and/or CV with opacity in both eyes Corneal stromal thinning and PED in 1 eye	AMT combined with Lal	Epithelization in 1 eye Healing time: 3 weeks	AMT in 1 eye	30.0±4.2 months	2.50±0.71	2.25±1.06

Abbreviations: AMT: amniotic membrane transplantation, Lal: Limbal allograft, Lau: limbal autograft, PED: persistent epithelial defect, C: conjunctivalization, CV: Corneal vascularization, PK: Penetrating keratoplasty

*Please note that the patients' final UCVAs were obtained before keratoplasty and significantly increased when compared to preoperative UCVAs (paired sample t-test, p=0.0003).

¥Both patients with radiation keratopathy were previously diagnosed with bilateral retinoblastoma. One eye of each patient had previously been enucleated, and external radiotherapy was applied to the other eyes.

2 patients underwent penetrating keratoplasty during the follow-up period. Demographic and clinical characteristics of patients with a corneal ulcer or persistent epithelial defect are presented in Table 4.

Conjunctival reconstruction

Of the 8 patients with recurrent pterygium, 4 (50%) were female and 4 (50%) were male with a mean age of 45.7±7.4 years (34-55 years). The mean follow-up was 7.9±4.6 months (5-19 months). During the follow-up period, pterygium recurred in one patient. The mean preoperative UCVA improved from 0.15±0.19 to 0.05±0.05 LogMAR at the end of the follow-up (Figure 4, a-c).

Of the 7 patients (1 female, 6 male) with large conjunctival tumors, 4 (57.1%) patients had squamous cell carcinomas, 1 (14.3%) lymphoid hyperplasia, 1 (14.3%) malignant melanoma, and 1 (14.3%) granulocytic sarcoma (Figure 4, d). There was right eye involvement in 5 (71.4%) patients and left eye involvement in 2 (28.6%) patients. The mean age was 55.6±19.4 years (27-84 years). The mean postoperative follow-up time was 7.1±2.2 months (5-10 months). A rapid epithelialization was observed on the ocular surface postoperatively (Figure 4, d-f). No complications related to the AMT were observed. During the follow-up period, recurrence was observed in the patient with granulocytic sarcoma (14.3%) in the 2nd month postoperatively, and radiotherapy was applied.

Of the 3 patients (1 female, 2 male) with leaking cystic filtering blebs and ocular hypotony, all of them had previously undergone mitomycin-C trabeculectomy with the diagnosis of primary open-angle glaucoma. The mean age was 62±10.1 years (51-71 years). The mean preoperative intraocular pressure and cup/disc ratio were 6.7 mmHg (range: 5-8 mmHg) and 0.6 (range: 0.5-0.7), respectively. The mean postoperative follow-up time was 23.3±9.1 months (15-33 months). The mean postoperative

Table 3: *Demographic and clinical characteristics of patients with partial limbal stem cell deficiency*

Diagnosis	Number of eyes	Age (mean ±SD, years)	Gender (male/ female)	Eye (right/ left)	Clinical Findings	Primary procedure	Epithelization and healing time (mean ±SD)	Second Procedure	Follow-up period (mean, ±SD)	Preop UCVA (LogMAR)	Final UCVA* (LogMAR)
Chemical eye injury	8 (44.4%) Alkali burn in 5 eyes Acid burn in 3 eyes	33.4±12.2	6/1	4/4	CV and opacity in all eyes PED in 2 eyes Symblepharon in 4 eyes	AMT without Lau or Lal Symblepharon resection	Epithelization in 5 eyes Healing time: 3.0±0.7 weeks	AMT in 3 eyes Symblepharon resection with AMT in 1 eye Penetrating keratoplasty in 2 eyes	15.2±9.9 months	2.62±0.74	2.05±0.99
Thermal eye injury	5 (27.8%)	44.2 ±15.0	4/1	4/1	CV and opacity in 4 eyes PED in 2 eyes Symblepharon in 1 eye	AMT without Lau or Lal Symblepharon resection	Epithelization in 4 eyes 3.5±1.3 weeks	AMT in 1 eye	12.8±13.7 months	2.18±1.14	1.54±1.03
Ocular cicatricial pemphigoid	2 (11.1%)	46.5±9.2	2/0	0/2	CV and opacity in both eyes Symblepharon in both eyes	AMT without Lau or Lal Symblepharon resection	Epithelization in 1 eye Healing time: 3 weeks	AMT in 1 eye	24.5±13.4 months	3.0±0.0	3.0±0.0
Stevens-Johnson syndrome	2 (11.1%)	40.5±43.1	2/0	1/1	CV and opacity in both eyes PED in 1 eye	AMT without Lau or Lal	Epithelization in both eyes Healing time: 4.0±0.0 weeks	–	6.0±1.4 months	3.0±0.0	3.0±0.0
Herpetic keratitis	1 (5.6%)	39	1/0	0/1	CV and opacity PED	AMT without Lau or Lal	Epithelization achieved Healing time: 4 weeks	–	6.0 months	1.8	1.3

Abbreviations: AMT: amniotic membrane transplantation, Lal: Limbal allograft, Lau: limbal autograft, PED: persistent epithelial defect, CV: Corneal vascularization

*Please note that the patients' final UCVAs were obtained before keratoplasty and significantly increased when compared to preoperative UCVAs (Wilcoxon signed-rank test, p=0.007).

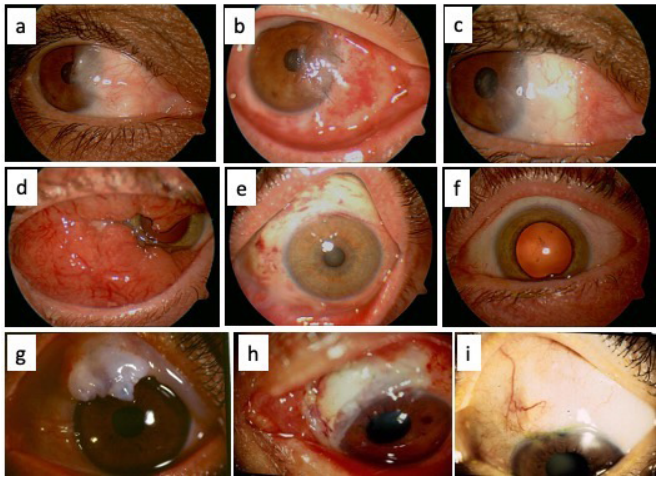

Figure 4: *Preoperative and post-operative early/late anterior segment photographs are observed in three patients with a recurrent pterygium (a-c), a large conjunctival tumor (d-f), and a cystic bleb with hypotonia (g-i). In these three cases, conjunctival defects were repaired with amniotic membrane transplantation (AMT). Please note that AMT was combined with a dura mater graft (h) in the surgery of the patient with cystic bleb.*

intraocular pressure was 14.3 mmHg (range: 12-17 mmHg). No complications were observed in any patient during the follow-up period (Figure 4, g-i).

DISCUSSION

The indications of AMT in ocular surface disorders are expanded with each passing year. This study presents a summary of current indications for AMT in ocular surface disorders. Several acute or chronic insults (chemical burns, ocular cicatrizing disorders, etc.) can destroy the limbal epithelial stem cells leading to a partial or total LSCD. Severe LSCD impairs corneal epithelium turnover, re-epithelialization fails and conjunctivalization occurs, leading to vascularization, persistent epithelial defects, and chronic inflammation. In these eyes, optimization of the ocular surface and restoration of the stem cell population are critical in final visual acuity and successful long-term outcome of the lamellar or penetrating keratoplasty. AMT can facilitate in vivo expansion of the remaining limbal stem cells[2, 4] and promote a higher success rate of the Lau or Lal transplantation.[7-9] A literature review showed that Lau transplantation (>120°) improved vision in 90% of patients with unilateral total LSCD and the ocular surface was restored in 94% of them.[10] Meallet et al.[11] performed

Table 4: *Demographic and clinical characteristics of patients with a corneal ulcer or persistent epithelial defect*

Etiology	Number of eyes	Age (mean±SD, years)	Gender (male/ female)	Eye (right/ left)	Corneal stromal involvement	Primary procedure	Epithelization and healing time (mean±SD, weeks)	Second Procedure	Follow-up period (mean±SD)	Preop UCVA (LogMAR)	Final UCVA* (LogMAR)
Herpetic keratitis	7 (33.3%)	48.1±13.0	4/3	5/2	Mild in 3 patients	Monolayer AMT	Epithelization in all eyes Healing time: 3.6±0.7	–	6.7±1.7 months	1.70±083	1.57±0.83
Bacterial keratitis	4 (19.1%)	51.7 ±22.7	2/2	1/3	Mild in 1 patient Severe in 2 patients	Monolayer AMT in 2 patients Multilayer AMT in 2 patients	Epithelization in all eyes Healing time: 3.0±0.8	–	6.7±1.5 months	2.20±0.97	2.07±1.11
Peripheral ulcerative keratitis	3 (14.3%)	34.3±12.0	1/2	2/1	Mild in 1 patient Moderate in 1 patient	Monolayer AMT	Epithelization in all eyes Healing time: 3.3±0.6	–	6.3±0.6 months	1.33±0.72	0.90±0.96
Neurotrophic keratitis- failed PK	2 (9.5%)	32.5±16.3	1/1	1/1	Mild in 1 patient	Monolayer AMT	Epithelization in 1 eye Healing time: 4	AMT in 1 eye	16.5±14.8 months	1.25±0.77	2.50±0.71
Keratoconjunctivitis sicca	2 (9.5%)	75.5±4.9	2/0	0/2	Mild in both patients	Monolayer AMT	Epithelization in 1 eye Healing time: 5	PK in 1 eye	6.5±0.7 months	2.00±0.00	1.70±0.42
Shield ulcer	2 (9.5%)	10.5±7.8	2/0	2/0	Mild in both patients	Monolayer AMT	Epithelization in 1 eye Healing time: 3	AMT in 1 eye	11.5±2.1 months	1.75±0.35	1.9±0.14
Stevens-Johnson syndrome	1 (4.8%)	25	0/1	0/1	Moderate	Monolayer AMT	Epithelization was not achieved	PK	5 months	3.00	2

Abbreviations: AMT: amniotic membrane transplantation, PK: penetrating keratoplasty
* Please note that the patients' final UCVAs were obtained before keratoplasty and did not differ significantly compared to preoperative UCVAs (paired sample t-test, p=0.383).

Lau with AMT in 5 eyes of five patients with total LSCD. During the mean follow-up of 22 months, all eyes experienced symptomatic relief and had an improvement in visual acuity. Uçakhan et al.[8] performed Lau with AMT in 3 eyes of three patients with total LSCD due to chemical eye injuries. The Authors reported 16 days of mean re-epithelialization time and visual acuity improvement in all eyes. Ivekovic et al.[12] assessed re-epithelialization time and visual acuity in patients with ocular burns after Lau transplantation alone, and a combination of AMT and Lau transplantation. The authors reported that there was no difference in the re-epithelialization time between Lau transplantation alone and Lau + AMT (14 and 15.3 days, respectively), and visual acuity improved in all patients. Cankaya[13] reported the promising outcomes of the combination of conjunctival limbal autograft and AMT in a patient with LSCD due to a chemical eye injury. In the present study, Lau or Lal with AMT was performed in 25 eyes of 24 patients with total LSCD. This study showed re-epithelialization in 84% of eyes after the first surgery with a mean of 3.0±0.8 weeks (2-4 weeks). At the end of the follow-up, corneal vascularization and inflammation were decreased in all patients, and UCVA improved significantly. The absence of Lal rejection in this study could be associated with an HLA class I tissue matching and systemic cyclosporine treatment.

Less satisfactory results can be seen when AMT alone is used to treat patients with severe LSCD.[12] However, based on a large number of studies,[1-4, 7-9, 14] AMT promotes the expansion of residual limbal epithelial stem cells and helps the ocular surface restoration in eyes with partial LSCD. AMT, without Lau or Lal, success is associated with the severity of the LSCD. In partial LSCD, mechanical debridement of the corneal conjunctivalization can be enough to restore a stable ocular surface. Removal of the conjunctival epithelium can be repaired with AMT, which may help the restoration of the microenvironment of the limbal stem cells and allow for faster healing of the ocular surface.[2, 4, 7-9, 14] Inconsistent with the literature, this study revealed that corneal epithelialization was achieved in

72.2% of eyes with an average of 3.4±1.5 weeks in eyes with partial LSCD who underwent AMT alone. Corneal vascularization and inflammation were decreased in 16 of the patients (88.9%), and UCVA improved significantly.

PEDs may occur due to a variety of mechanisms, including neurotrophic keratopathy following Herpetic keratitis, after penetrating keratoplasty, chronic inflammation, or mechanical factors. PEDs can progress to stromal collagenolysis, ulceration, perforation, or scarring when they are unresponsive to conventional therapy including medical therapy, bandage contact lenses, or tarsorrhaphy. Corneal ulcers are usually caused by keratitis, including infectious keratitis, traumatic keratitis, and keratitis related to autoimmune and systemic diseases. AMT can be incorporated into the treatment of nonhealing PEDs and corneal ulcers by any of the methods including monolayered inlay, multilayered inlay, and sandwich techniques depending on the ulceration depth. Asyali Altinok et al.[15] performed AMT in 17 patients with PED due to different etiological factors with an 88.9% successful rate for a stable corneal epithelium. AMT can be applied in the primary treatment of corneal ulcers, including deep ulcers with a high risk of perforation, to protect the integrity of the eye or to gain time until a suitable donor cornea is found for therapeutic-tectonic keratoplasty.[16] The results of a recent meta-analysis including 17 studies and 390 eyes of 385 patients who underwent AMT revealed that the pooled corneal epithelium healing rate was 97% and the pooled vision improvement rate was 53% in eyes with corneal ulceration.[17] The authors reported the highest corneal epithelium healing rate with the sandwich technique, whereas the lowest was with multilayered AMT graft. Considering the improvement in visual acuity, the highest improvement was observed with the monolayered AMT graft, whereas the lowest one was with the multilayered AMT graft. In our study, monolayer AMT was applied in 19 eyes, and multilayer AMT was performed in 2 eyes. Corneal healing and epithelialization were achieved in 19 (90.5%) eyes with a mean of 3.4±0.9 weeks, but the mean preoperative UCVA did not differ significantly compared to final measurements. The lack of significant improvement in visual acuity in our study may be due to etiological factors and ocular clinical feature differences between studies. Our study showed that AMT was great for corneal epithelialization in PEDs and corneal ulcers. However, when the AM is absorbed, it is replaced by a fibrotic stroma. Therefore, corneal transparency can be reduced compared with the normal cornea,[18] which might be why the efficacy of AMT for vision improvement in corneal ulceration is not so obvious.

Several techniques have been reported in pterygium surgery, including repeat conjunctival autograft, conjunctival-limbal autografting, AMT, and adjunctive use of subconjunctival 5-fluorouracil, mitomycin C, or bevacizumab application.[19] Solomon et al.[20] performed AMT in pterygium surgery and reported the true recurrence rate was 3.0% and 9.5% for primary and recurrent pterygium, respectively. A recent study compared conjunctival autograft, primary closure, and AMT in primary pterygium surgery. The authors reported 6.4%, 14.4%, and 14.7% recurrence rates for conjunctival autograft, primary closure, and AMT, respectively. The study revealed that conjunctival autograft was associated with a significantly lower recurrence rate.[21] Our study showed 12.5% recurrence rates for AMT in the recurrent pterygium. Although conjunctival autografts have shown successful results, they may be insufficient to cover large defects created in large recurrent pterygium. Also, it has given rise to concerns for those who may require future glaucoma filtering surgeries. Therefore, AMT may be a suitable treatment option in these scenarios.

AMT can be applied for the reconstruction of the conjunctival surface as a substitute for conjunctival grafts in any situation where the availability of autologous conjunctival tissue is limited, such as after removing of large conjunctival tumors and leaking filtering bleb excision.

Available literature[22-24] and results of this study showed that AMT is an effective method of reconstruction following a conjunctival and limbal tumor excision and ocular surface healing can be achieved without any clinically significant complications. Several patching techniques have been described for bleb reconstruction in the literature, including the donor scleral patch graft,[25] partial-thickness sclera,[26] donor cornea,[27] dura mater patch graft,[28] and pericardium.[29] After bleb repair, conjunctival advancement flaps can be used for conjunctival reconstruction.[28] Our study showed that AMT offers a good alternative in unsuitable eyes for conjunctival advancement flaps.

This study has some limitations because of the retrospective nature of the analysis and the lack of a control group. Another limitation is that the diagnosis of LSCD was made based on medical history and clinical features. Standardized quantitative methods for the staging of LSCD such as in vivo confocal microscopy, anterior segment optical coherence tomography, and impression cytology could not be applied.

In conclusion, we have demonstrated the wide-ranging applications of AMT in ocular surface disorders. AMT offers a good option in some challenging situations of the

ocular surface in the hands of ophthalmic surgeons. Fresh AMT combined with limbal grafts may increase surgical success in patients with total LSCD. Optimal outcomes can be achieved with the correct timing, appropriate indication, and transplantation technique, as well as patient compliance, medical therapy, and proper management of postoperative complications.

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Informed consent: Informed consent was obtained from all individual participants included in the study.

Ethical Approval: All procedures were performed following the ethical standards of the Local Ethics Committee, and the 1964 Helsinki declaration and its later amendments. The study protocol was approved by the Institutional Review Board (#2021/387).

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REFERENCES

1. Malhotra C, Jain AK. Human amniotic membrane transplantation: Different modalities of its use in ophthalmology. World J Transplant. 2014;4:111-121.
2. Dua HS, Gomes JA, King AJ, et al. The amniotic membrane in ophthalmology. Surv Ophthalmol. 2004;49:51-77.
3. Tamhane A, Vajpayee RB, Biswas NR, et al. Evaluation of amniotic membrane transplantation as an adjunct to medical therapy as compared with medical therapy alone in acute ocular burns. Ophthalmology. 2005;112:1963-9.
4. Walkden A. Amniotic Membrane Transplantation in Ophthalmology: An Updated Perspective. Clin Ophthalmol. 2020;14:2057-72.
5. Mejía LF, Acosta C, Santamaría JP. Use of nonpreserved human amniotic membrane for the reconstruction of the ocular surface. Cornea. 2000;19:288-91.
6. Brücher VC, Eter N, Uhlig CE. Results of Resorbable and Running Sutured Amniotic Multilayers in Sterile Deep Corneal Ulcers and Perforations. Cornea. 2020;39:952-6.
7. Cauchi PA, Ang GS, Azuara-Blanco A, et al. A systematic literature review of surgical interventions for limbal stem cell deficiency in humans. Am J Ophthalmol. 2008;146:251-9.
8. Uçakhan OO, Köklü G, Firat E. Nonpreserved human amniotic membrane transplantation in acute and chronic chemical eye injuries. Cornea. 2002;21:169-72.
9. Santos MS, Gomes JA, Hofling-Lima AL, et al. Survival analysis of conjunctival limbal grafts and amniotic membrane transplantation in eyes with total limbal stem cell deficiency. Am J Ophthalmol. 2005;140:223-30.
10. Liang L, Sheha H, Li J, et al. Limbal stem cell transplantation: new progresses and challenges. Eye (Lond). 2009;23:1946-53.
11. Meallet MA, Espana EM, Grueterich M, et al. Amniotic membrane transplantation with conjunctival limbal autograft for total limbal stem cell deficiency. Ophthalmology. 2003;110:1585-92.
12. Ivekovic R, Tedeschi-Reiner E, Novak-Laus K, et al. Limbal graft and/or amniotic membrane transplantation in the treatment of ocular burns. Ophthalmologica. 2005;219:297-302.
13. Cankaya C. Conjunctival Limbal Autograft Combined with Amniotic Membrane Transplantation to Treat a Moderate Chemical Eye Injury. Beyoglu Eye J. 2019;4:46-50.
14. Matsumura T, Yamaguchi T, Higa K, et al. Long-Term Outcome After Superficial Keratectomy of the Abnormal Epithelium for Partial Limbal Stem Cell Deficiency. Am J Ophthalmol. 2021;231:134-43.
15. Ayse AA, Balikoglu Yilmaz M, Ozdal P, et al. Human amniotic membrane transplantation for ocular surface reconstruction. Journal of Glaucoma and Cataract. 2010;5:18-24.
16. Cevher S, Kocluk Y, Alyamac Sukgen E. Therapeutic and Tectonic Rekeratoplasty in a Case with Late Graft Abscess Resistant to Medical Treatment and Amniotic Membrane Transplantation. Journal of Glaucoma and Cataract. 2016;11:129-32.
17. Liu J, Li L, Li X. Effectiveness of Cryopreserved Amniotic Membrane Transplantation in Corneal Ulceration: A Meta-Analysis. Cornea. 2019;38:454-62.
18. Gris O, Wolley-Dod C, Güell JL, et al. Histologic findings after amniotic membrane graft in the human cornea Ophthalmology. 2002;109:508-12.
19. Hacıoğlu D, Erdöl H. Developments and current approaches in the treatment of pterygium. Int Ophthalmol. 2017;37:1073-81.
20. Solomon A, Pires RT, Tseng SC. Amniotic membrane transplantation after extensive removal of primary and recurrent pterygia. Ophthalmology. 2001;108:449-60.
21. Röck T, Bramkamp M, Bartz-Schmidt KU, et al. A Retrospective Study to Compare the Recurrence Rate After Treatment of Pterygium by Conjunctival Autograft, Primary Closure, and Amniotic Membrane Transplantation. Med Sci Monit. 2019;25:7976-81.
22. Palamar M, Kaya E, Egrilmez S, et al. Amniotic membrane transplantation in surgical management of ocular surface squamous neoplasias: long-term results. Eye (Lond). 2014;28:1131-5.
23. Asoklis RS, Damijonaityte A, Butkiene L, et al. Ocular surface reconstruction using amniotic membrane following excision of conjunctival and limbal tumors. Eur J Ophthalmol. 2011;21:552-8.
24. Goktas SE, Katircioglu Y, Celik T, et al. Surgical amniotic membrane transplantation after conjunctival and limbal tumor excision. Arq Bras Oftalmol. 2017;80:242-6.
25. O'Rourke M, Moran S, Collins N, et al. Bleb reconstruction using donor scleral patch graft for late bleb leak and hypotony. Eur J Ophthalmol. 2021;31:1039-46.

26. Clune MJ, Shin DH, Olivier MM, et al. Partial-thickness scleral-patch graft in revision of trabeculectomy. Am J Ophthalmol. 1993;115:818-20.

27. Bochmann F, Kaufmann C, Kipfer A, et al. Corneal patch graft for the repair of late-onset hypotony or filtering bleb leak after trabeculectomy: a new surgical technique. J Glaucoma. 2014;23:e76-80.

28. Al-Shahwan S, Al-Torbak AA, Al-Jadaan I, et al. Long-term follow up of surgical repair of late bleb leaks after glaucoma filtering surgery. J Glaucoma. 2006;15:432-6.

29. Niegowski LJ, Bravetti GE, Gillmann K, et al. Pericardium Patch Graft (Tutoplast) for Bleb Repair and Bleb Remodelling After Nonpenetrating Filtering Surgery: 6-Month Outcomes. J Glaucoma. 2020;29:347-50.