Human Amniotic Membrane Transplantation for Ocular Surface Reconstruction*

Oküler Yüzey Rekonstrüksiyonunda Amniyon Membranı Transplantasyonu

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Original Article *Klinik Çalışma*

ABSTRACT

Purpose: To investigate potential safety and efficacy of nonpreserved amniotic membrane transplantation (NP-AMT) in ocular surface reconstruction.

Materials and Methods: NP-AMT was performed on 18 eyes (17 patients) having persistent epithelial defect due to chemical injury, postinfectious keratitis, band keratopathy (BK), and topical anesthetic abuse. All patients underwent detailed ophthalmic examination including lid and ocular motility, extraocular examination, Schirmer tear test, visual acuity, tonometry, slit-lamp and fundus examination which were carried out before and after the treatment. Main outcome measures were complete surface epithelialization and epithelialization time.

Results: NP-AMT was considered successful in 16 cases (88.9%) with complete surface epithelialization. Two eyes (11.1%) had persistent corneal epithelial defect and stromal lysis despite NP-AMT and considered as surgical failure. Of these patients, one had nonherpetic postinfectious keratitis and one had topical anesthetic abuse. The overall visual improvement was detected in 11 (61.1%) of 18 eyes. The visual improvement was observed in 4 (50%) patients with postinfectious keratitis, 3 (60%) patients with chemical injury, and 3 (100%) patients with BK. Seven eyes (38.9%) maintained same visual acuity, and none of the eyes had a decrease in visual acuity. There were no infectious, inflammatory, immunologic, or toxic/allergic reactions related to NP-AMT.

Conclusion: NP-AMT is a useful treatment modality in patients with persistent epithelial defects due to infectious keratitis and chemical injury. Moreover, it is a safe and effective method to restore a stable corneal epithelium in eyes which underwent a primary surgical removal of BK.

Key Words: Nonpreserved amniotic membrane transplantation, Chemical injury, Postinfectious keratitis, Band keratopathy, Topical anesthetic abuse.

ÖZ

Amaç: Oküler yüzey rekonstrüksiyonunda taze amniyon membranı transplantasyonun (NP-AMT) güvenlik ve etkinliğini değerlendirmek.

Gereç ve Yöntem: Kimyasal yanık, postenfeksiyöz keratit, band keratopati (BK) ve topikal anestezik suiistimali sonucu oluşan kalıcı epitel defektine sahip 18 göze (17 hasta) NP-AMT uygulandı. Hastalara tedavi öncesi ve sonrasında kapak ve göz hareketliliği, göz dışı muayene, Schirmer gözyaşı testi, görme keskinliği, göz içi basıncı ölçümü, yarıklı lamba ve arka segment muayenesi dâhil tam bir göz muayenesi yapıldı. Ana parametreler tam yüzey epitelizasyonu ve epitelizasyon süresiydi.

Bulgular: NP-AMT'nin tam yüzey epitelizasyonunun oluştuğu 16 olguda (%88.9) başarılı, 2 olguda ise (%11.1) kalıcı korneal epitel defekti ve stromal lizis gelişmesi nedeniyle başarısız olduğu görüldü. Başarısız olunan olgulardan biri herpetik olmayan postenfeksiyöz keratit, diğeri ise topikal anestezik suiistimali idi. Görmede düzelme 18 gözün onbirinde (%61.1) [postenfeksiyöz keratitli hastaların dördü (%50), kimyasal yanıklı hastaların üçü (%60) ve BK'lı hastaların üçü (%100)] izlendi. Yedi gözde (%38.9) görme keskinliği değişmedi, hiçbir gözde görme keskinliği azalmadı. NP-AMT ile ilgili enfeksiyöz, inflamatuar, immünolojik veya toksik/allerjik reaksiyon yoktu.

Tartışma: NP-AMT enfeksiyöz keratite ve kimyasal yanığa bağlı kalıcı epitel defekti olan hastalarda yararlı bir tedavi yaklaşımıdır. Üstelik, BK'nın primer cerrahi olarak uzaklaştırıldığı gözlerde stabil kornea epitelini sağlamak için güvenli ve etkili bir yöntemdir.

Anahtar Kelimeler: Taze amniyon membranı transplantasyonu, kimyasal yanık, postenfeksiyöz keratit, band keratopati, topikal anestezik suiistimali.

Glo-Kat 2010;5:18-24

Geliş Tarihi : 23/02/2010
Kabul Tarihi : 22/03/2010

Received : February 23, 2010
Accepted : March 22, 2010

* This manuscript was presented as a poster in 9th Congress of the International Society of Dacriology and Dry Eye, Istanbul, Turkey (16-18 May 2008).

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INTRODUCTION

Ocular surface diseases resulting from physical and chemical injuries, infections or systemic disorders may be responsible for scarring of the conjunctiva, give rise to persistent ocular irritation and involve the cornea. If severe, ocular surface diseases may lead to significant visual impairment. Treatment of such a condition is challenging, often unsatisfactory and claims combining approach. The ideal treatment should restore the anatomic and physiologic structure of the ocular surface, with reconstruction of the corneal and conjunctival epithelium.

The amniotic membrane (AM) is a thin, semitransparent tissue from the inner layer of the placenta, consisting of a thick basement membrane composed of collagen type IV and laminin with a single layer of epithelium and an avascular stromal matrix.[1] Amniotic membranes have been previously used for conditions such as chronic ulcers of the leg, skin wounds and burns, and to prevent tissue adhesion in surgeries of the abdomen, head, or pelvis.[2] De Rotth first used a live fetal membrane including both amnion and chorion in 1940 as a graft for conjunctival surface reconstruction.[3] In 1995, Kim and Tseng[4] reintroduced amniotic membrane for ophthalmic uses. Since then, amniotic membrane transplantation (AMT) has been performed in many indications such as persistent epithelial defects,[5] neurotrophic ulcers,[6] conjunctival defects,[7] pterygium surgery,[8] trabeculectomy,[9] after photokeratectomy,[10] symblepharon,[11] bullous keratopathy,[12] limbal stem cell deficiency,[11,13] ocular cicatricial pemphigoid,[14] leaking filtering blebs,[15] Stevens-Johnson Syndrome,[14] and chemical or thermal injuries of the eye.[16]

All of these cited studies were performed by using preserved AM. To the best of our knowledge, few studies using nonpreserved AM for the restoration of ocular surface diseases have been reported so far.[17-19] Based on these clinical data, we aimed to investigate potential efficacy of NP-AMT in ocular surface reconstruction.

MATERIAL AND METHODS

This study was approved by the local ethics committee. All the procedures followed the principles of the Declaration of Helsinki. Eighteen eyes of 17 patients having persistent epithelial defect due to chemical injury, postinfectious keratitis, band keratopathy (BK), and topical anesthetic abuse who underwent NP-AMT from February 2005 to February 2007 were included in this study. Patients younger than 13 years old, patients with perforated corneal ulcers, acute infective keratitis, and patients unwilling to give a consent form were excluded from the study. The ocular surface destruction was unilateral in all but one patient and surgical procedures were performed by the same surgeon (A.A.).

All patients underwent detailed ophthalmic examination including lid and ocular motility, extraocular examination, visual acuity, slit-lamp and fundus examination which were carried out before and after the treatment. IOP was measured by using Tonopen XL (Medtronic, Solan) and was performed after topical instillation of 0.4% oxybuprocaine HCl. The Schirmer tear test with and without anesthesia was used to evaluate tear function.

Patients with herpes simplex keratitis received oral acyclovir 1000 mg/day pre and postoperatively. Patients with non-herpetic postinfectious keratitis received sufficient systemic and topical antibiotic to eradicate the causative organisms. None of these patients had clinical or microbiologic signs of acute corneal infection at the time of NP-AMT. All patients had persistent inflammation, epithelial breakdown with or without limbal ischemia at presentation.

The human placenta was harvested at the time of cesarean section after obtaining oral informed consent from donor who had negative serologic tests for hepatitis B and C, syphilis and human immunodeficiency virus (HIV) before surgery. All patients were also known to be serologically negative at the beginning of pregnancy. Under sterile conditions, a piece of membrane was separated from the placenta and placed in sterile saline solution. After the transportation to our department, the membrane was amply washed with saline solution and separated from chorion by blunt dissection. It was aseptically stored in a vial with saline solution. No antibiotics or other substances are used for its storage. It was refrigerated without freezing and used within 24 hours. The rest of AM was thrown away. The new AM was prepared for each patient who is fitted the indications for AMT. The rest of AM was not used repeatedly and was thrown away.

Amniotic membrane transplantation was applied by using ''Overlay'' technique in all cases, as described previously (Figure 1).[20]

All surgeries were performed with peribulbar anaesthesia using 2% lidocaine and 0.5% bupivacaine. First, fibrovascular and inflammed tissue together with

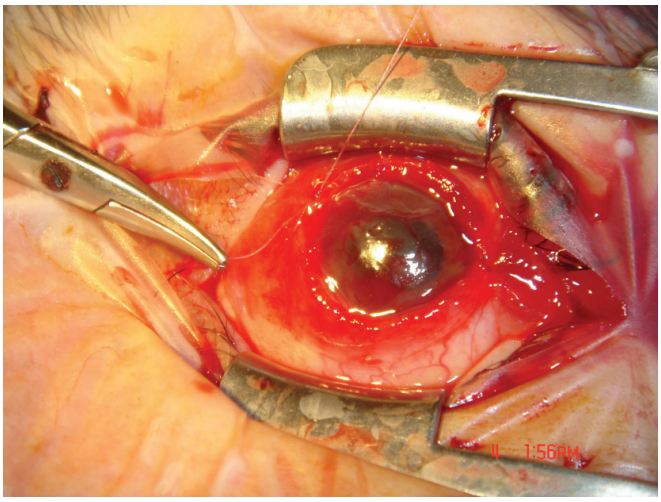

Figure 1: Intraoperative appearance of amniotic membrane transplantation (case 8).

Table 1: The patients' demographic data and clinical characteristics.

Patient no./ Case no.	Age (yrs)	Sex	Eye	Primary Dx	Associated Dx	Persistent Inflammation/ Epithelial Breakdown / Limbal Ischemia	Previous Surgeries	Stroma	Fluorescein	Time (days) b/w Injury and Surgery
1	39	M	OS	Postinfectious herpetic		+/+/-		Th/V	ED	Ø
2	74	M	OD	Postinfectious nonherpetic		+/+/-	Keratoplasty, pterygium surgery	Th/V	PK	Ø
10	77	F	OS	Postinfectious nonherpetic	Bullous keratopathy	+/+/-	Cataract surgery	V	ED	Ø
11	45	M	OD	Postinfectious herpetic		+/+/-		V	ED	Ø
13	81	F	OD	Postinfectious nonherpetic		+/+/-		Th	ED	Ø
15	69	M	OS	Postinfectious herpetic	Secondary glaucoma	+/+/-		V	ED	Ø
16	78	F	OS	Postinfectious nonherpetic	trachoma, distichiasis, entropion	+/+/-		Th/V	ED	Ø
5	41	M	OS	Postinfectious nonherpetic	Diabetes Mellitus	+/+/-	Pars plana vitrectomy	V	ED	Ø
12	65	M	OD	Topical anesthetic abuse	Chronic myeloid leukemia	+/+/-		Th/V	ED	Ø
12	65	M	OS	Topical anesthetic abuse	Chronic myeloid leukemia	+/+/-		Th/V	ED	Ø
6	25	M	OD	alkali burn		+/+/+		Th/V	ED	7
7	28	M	OD	alkali+ acid burn	Secondary glaucoma	+/+/-		Th/V	ED	7
8	19	F	OS	alkali burn		+/+/+		Th/V	PK	16
9	38	M	OS	alkali burn		+/+/+		Th/V	ED	5
3	25	M	OS	alkali+ acid burn		+/+/-		Th/V	ED	15
4	37	F	OD	band keratopathy	Neurotrophic keratitis, exotropia	+/+/-		V	ED	Ø
14	53	M	OD	band keratopathy		+/+/-		V	ED	Ø
17	65	F	OS	band keratopathy	Diabetes Mellitus	+/+/-		Th	PK	Ø

M, Male; F, Female; Dx, Diagnosis, OD, right eye; OS, left eye; ED, Epithelial Defect; PK; Punctate Keratopathy, b/w; between, Th; stromal thinning, V; Neovascularization, PAAG; Primer Open Angle Glaucoma, DM; Diabetes Mellitus, KML; Chronic Myeloid Leukaemia.

the poorly adherent epithelium near to the lesion were removed. In eyes with BK, surgical removal of BK was achieved by superficial keratectomy with or without the use of 3% EDTA. Second, 360-degree conjunctival peritomy was done and NP-AMT was clipped and placed over the denuded surface epithelium side up and sutured to the episclera using interrupted 8-0 vicryl sutures. Thus the whole corneal surface was covered by NP-AMT. Subconjuctival gentamicin sulfate and dexamethasone sodium phosphate were administered at the end of the operation.

0.1% dexamethasone sodium phosphate and 0.3% ciprofloxacin eye drops were given every 6 hours and both tapered off in 1 month. Preservative-free artificial tears were given every 2 hours. Patients were followed-up on postoperative day 1, 7, and weekly for the first 2 months, and monthly after then.

Success was defined as healing of ulcer as evidenced by complete epithelialization of corneal surface, which was demonstrated by negative fluorescein staining on slit-lamp examination. Failure was defined as persistence of corneal ulcer, or incomplete epithelialization.

The statistical analysis was performed using SPSS (SPSS for Windows, version 11.5; SPSS, Chicago, IL, USA). Each categorical variable was demonstrated as frequency (%). Continuous variables were pointed out as mean±standard deviation.

RESULTS

Overall 18 eyes of 17 patients including herpetic (3 eyes, 16.7%) and nonherpetic (5 eyes, 27.8%) postinfectious keratitis, chemical injury (5 eyes, 27.8%), BK (3 eyes, 16.7%) and topical anesthetic abuse (2 eyes, 11.1%) were enrolled to the study. The patients' demographic data and clinical characteristics are demonstrated in Table 1. Of the patients, 6 were female (35.3%) and 11 were male (64.7%). The mean age at the time of surgery was 51.3±20.7 (range, 19-81) years. The mean follow up period was 12.3±7.9 (range, 5-24) months. The mean interval time between injury and surgery was 10.0±5.1 (range, 5-16) days in 5 eyes with chemical injury.

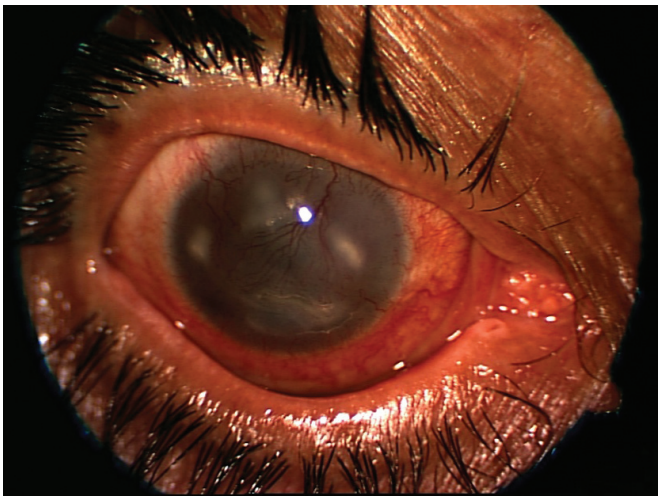

Figure 2: Preoperative appearance of persistent epithelial defect (case 12, OD).

Table 2: Surgical results following nonpreserved amniotic membrane transplantation.

Patient no./ Case no.	Follow-up (months)	UCVA Visual acuity (Snellen)		ED Healing (weeks)	Outcome Persistent Epithelial Defect/ Vascularization/ Inflammation)	Postop Schirmer's Test
		Preop	Postop			
1	24	CF 2 m	20/40	6	-/+ (1cl.hrs)/-	5 mm/ 5 min
2	24	LP	LP	4	-/+ (9 cl.hrs)/-, phthisis bulbi	5 mm/ 5 min
10	12	LP	CF 1 m	5	-/+ (1cl.hrs)/-	13 mm/ 5 min
11	5	CF 1 m	20/200	4	-/-/-	20 mm/ 3 min
13	5	HM	HM	4	-/+ (12 cl.hrs)/+	15 mm/ 3 min
15	7	HM	HM	2	-/-/-	30 mm/ 3 min
16	5	HM	HM	PED	+/+ (1cl.hrs)/+, impending perforation, keratoplasty.	15 mm/ 3 min
5	22	CF 2 m	20/200	8	-/-/-	15 mm/ 3 min
12	7	HM	CF 4 m	3	-/-/-	5 mm/ 5 min
12	5	CF 1 m	CF 1 m	PED	+/+ (4 cl.hrs /-, hypopyon, impending perforation, keratoplasty.	5 mm/ 5 min
6	18	CF 1 m	CF 1 m	4	-/-/+	15 mm/ 3 min
7	18	CF 1 m	CF 1 m	3	-/+ (3cl.hrs)/+	20 mm/ 3 min
8	6	20/200	20/50	5	-/-/+	30 mm/ 3 min
9	7	CF 1 m	20/20	4	-/-/-, nebula	25 mm/ 3 min
3	23	CF 4 m	0.6	4	-/-/-	21 mm/3 min
4	22	HM	CF 2 m	3	-/-/-, corneal edema, haze	35 mm/ 3 min
14	7	HM	CF 3 m	2	-/-/-, corneal edema, haze	20 mm/ 5 min
17	5	CF 2 m	20/200	2	-/-/-	20 mm/ 3 min

UCVA; Un Corrected Visual Acuity, CF; Counting Fingers, LP; Light Perception, HM; Hand Motions, m; meter, PED; Persistent Epithelial Defect, cl.hrs; clock hours.

Surgical results following NP-AMT are showed in Table 2. The amniotic membrane was completely dissolved within 2-4 weeks in all cases. Out of 18 eyes with corneal ulceration, NP-AMT was considered to be successful in 16 cases (88.9%) with complete surface epithelialization (Figure 2-4). The mean epithelialization time of these cases was 3.9±1.6 weeks (range, 2-8 weeks). In eyes with postinfectious keratitis, the success rate was 87.5% (7 of 8 cases) and the epithelialization was completed within 4.7±1.9 (range, 2-8) weeks. In eyes with chemical injury, the success rate was 100% (all of 5 cases) and the epithelialization was completed within 4.0±0.7 (range, 3-5) weeks. There was no difference in the results of patients with limbal ischemia operated early or late following the chemical burn. The etiologies for BK were systemic disease (diabetes mellitus) in one case, long-term use of lubricating drops and ointment in one case having neurotrophic keratitis, and idiopathic in another case. The epithelialization was achieved in all cases with BK no matter the cause and the mean epithelialization time was 2.3±0.6 (range, 2-3) weeks. On the other hand, two eyes (11.1%) had persistent corneal epithelial

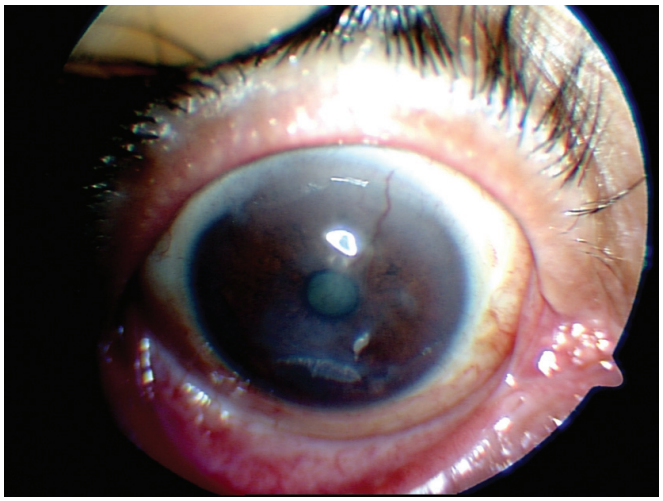

Figure 3: Postoperative appearance of the same patient 3 weeks after the amniotic membrane transplantation (case 12, OD).

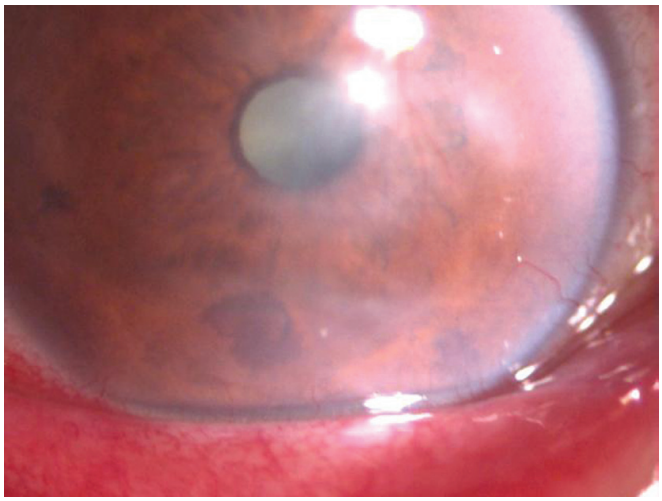

Figure 4: Postoperative appearance of amniotic membrane transplantation after 7 months (case 12, OD). Note progressive reduction of neovascularization, complete corneal epithelialization, increase of corneal transparency.

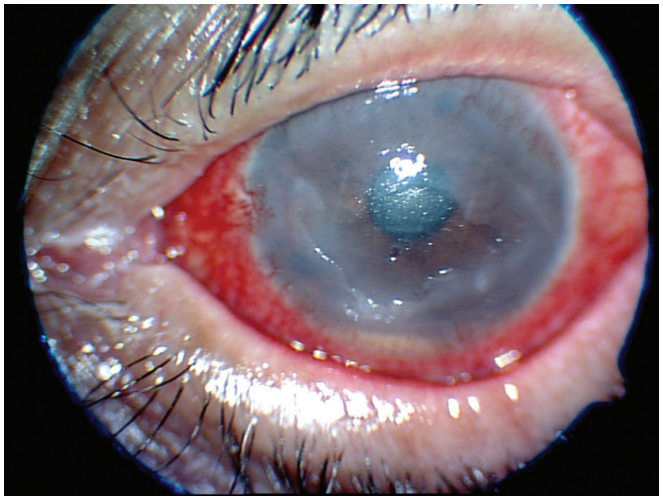

Figure 5: Preoperative appearance of persistent epithelial defect (case 12, OS). Note intense conjunctival neovascularization.

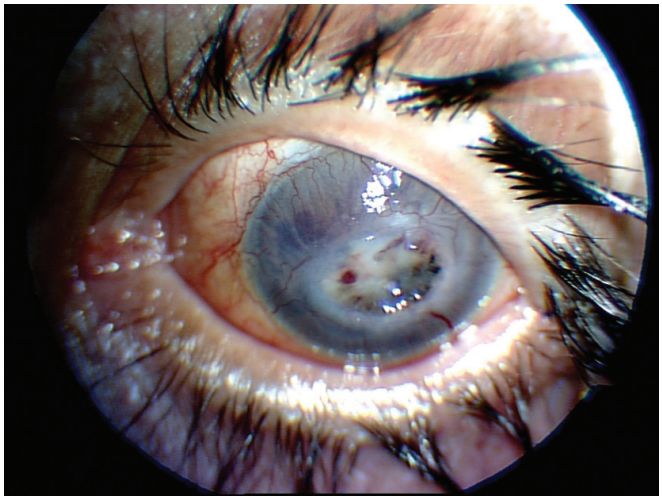

Figure 6: Corneal perforation necessitating keratoplasty, 6 months after the amniotic membrane transplantation (case 12, OS).

defect and stromal lysis despite NP-AMT and considered as surgical failure. Of these patients one had nonherpetic postinfectious keratitis and one had topical anesthetic abuse. Afterwards in these two patients keratoplasty was uneventfully necessary (Figure 5-6).

Pre- and postoperative uncorrected visual acuities (UCVA) of the eyes are summarized in Table 2. The overall visual improvement was detected in 11 (61.1%) of 18 eyes. The visual improvement was observed in 4 (50%) patients with postinfectious keratitis, 3 (60%) patients with chemical injury, and 3 (100%) patients with BK. Seven eyes (38.9%) maintained the same visual acuity, and none of the eyes had a decrease in visual acuity.

Two patients received pre- and postoperative topical β-blockers because of glaucoma. Symblepharon did not develop in any of the patients. The pain, foreign body sensation and photophobia were relieved immediately after NP-AMT. All eyes had stable tear films (Schirmer's test ≥5 mm) at the final examination. There were no infectious, inflammatory, immunologic, or toxic/allergic reactions for the vial amniotic cells when use for transplantation.

DISCUSSION

Persistent corneal epithelial defects may progress to persistent sterile corneal ulcer and, infrequently to perforation.[2] Common factors leading to the breakdown of the epithelial surface include infections, xerosis, trauma and chemical injuries. In cases intractable to medical treatment various approaches can be considered including AMT.[2]

Amniotic membrane is avascular and has unique properties including antiscarring and antiadhesive effects, induction of epithelial migration, reduction of inflammatory, cicatricial and angiogenic reactions, bacteriostatic properties, wound protection, pain reduction, and epithelialization.[17] It does not express histocompatibility antigens and wets ulcerated area to reduce surface exposure.[21] AM is a substrate upon which cells can migrate and regenerate, forming new and healthy tissue. It reinforces adhesion of basal epithelial cells, reduces their apoptosis and promotes their differentiation.[17] Furthermore, AM serves as a physical barrier and protects the corneal stroma from damaging effects of inflammatory cells and proteins in the tear film.[20] Recently, an experimental study on herpes simplex virus 1-related stromal keratitis showed that in corneas treated with AMT, levels of the proinflammatory cytokines had decreased comparing to untreated eyes or eyes treated with tarsorrhaphy.[20]

There are many preparation techniques of AM in the literature.[20] Amniotic membrane is generally cryopreserved in 50% glycerol at 80°C. It is unknown whether the AM epithelial and stromal fibroblasts, and associated growth factors, survive after cryopreservation. Kruse et al. suggest that AM cells are not viable after cryopreservation, as assessed by vital stain and inability to grow in cell culture.[22] Mejia et al.[17] suggested that the AM might even lose some of its anti-inflammatory properties during the preservation process and proposed the NP-AMT as a safe and valid technique, more advantageous than P-AMT. Furthermore, -80° C freezers, the media, and the nitrocellulose paper which are necessary to prepare preserved AM are expensive. Preparation of nonpreserved AM does not need a process of preservation and freezing/unfreezing. Therefore the cost and availability of NP-AMT are attractive. Literature review showed that studies using NP-AM for ocular surface reconstruction are few.[17-19]

Nonpreserved amniotic membrane is initially thicker than preserved AM. Therefore the manipulation and the orientation of the membrane are easier during the NP-AMT. However, their appearance is identical in the first postoperative week.[17] As the membrane has antibacterial properties, the risk of infection is very low when using NP-AM, in order to work in aseptic conditions.[23] Evidence suggests that AM obtained from vaginal delivery is more likely to be contaminated with bacteria species.[24]

Therefore, placenta obtained from a caesarean section was preferred in this study. As all pregnant women are routinely tested at the beginning of pregnancy, we think that testing donors one week before the elective caesarean section for HIV, hepatitis, and syphilis is safe enough to almost eliminate the risk of infection by these agents; however, the possibility that the donor might get infected during the last week, and the possibility of transmitting an unknown pathogen cannot be rule out. We did not observe postoperative infection in any of our cases. In addition, there have been few NP-AMT to date and no disease transmission has been reported. However, Mejia[17] stated, it is compulsory for surgeon to inform the patient about the risk of viral transmission associated with the use of tissue, organ, and blood and obtain informed consent to use them.

In previous studies, the percentage of eyes epithelialized with different techniques of AMT ranges from 30% to 91%.[20] In this study this ratio was found to be 88.9%. Furthermore, Tseng et al. reported that the use of AMT was beneficial to restore ocular surface in patients with partial limbal stem cell deficiency (PLSCD), while the total limbal stem cell deficiency (TLSCD) requires both the limbal transplantation and AMT.[13]

Amniotic membrane transplantation has been reported to be successful in treating persistent corneal ulcers due to herpetic infections.[25] According to Kim et al.[25], AMT acts as a viable method of treatment to promote healing and prevent progressive melting of refractory corneal ulcers induced by infectious keratitis. In our study, the epithelialization rate in eyes with postinfectious keratitis was 87.5% (7 of 8 cases) and there were no recurrences of microbial infection during the follow-up period.

In acute chemical injury, leukocyte infiltration and persistent inflammation prevent epithelialization, contribute to stromal melting, and cause granuloma and scarring in chronic stage.[16,26] Conversely, despite the presence of wide ocular surface defects in the acute stage, some viable conjuctival and corneal stem cells believed to remain at the basal level.[16] Therefore, when used in the early stage of chemical burn, AMT is thought to promote healing of the ocular surface by preventing leukocyte infiltration, decreasing the duration and severity of inflammation, and protecting the epithelial progenitor cells. Eyes with TLSCD in which limbal transplantation is required have a higher risk of failure because of inflammation.[27] In such cases, AMT reduces the inflammation and prepares the eye for limbal transplantation.[20] In our study AMT was considered to be successful in all eyes (100%) with chemical injury. In these cases, NP-AMT was performed within two weeks after the injury. Results of this study demonstrated that AMT used in the early stage, reduces limbal and stromal inflammation, reconstructs the conjuctival surface, and prevents symblepharon formation in eyes with chemical injury.

The treatment of BK aims to remove the calcified plaque deposition and reconstructing the smooth corneal surface. To restore the corneal surface, NP-AM consisting of a thick basement membrane and avascular stromal matrix was used in this study. This matrix can supress the proliferation and myofibroblast differentiation of normal human corneal and limbal fibroblasts[28] and contains protease inhibitors,[29] which may inhibit corneal neovascularization.[30] Anderson et al.[31] and Kwon et al.[32] successfully used AM graft for the treatment of BK after removing the calcium deposits. They showed that the mean time for epithelialization was 15.2 and 9-10 days respectively with no pain and recurrence during the follow up period. Similarly, we observed that mean epithelialization time was 2.3 weeks and neither recurrence nor pain was observed during the follow up period. Despite different underlying causes, complete epithelialization occurred in all of our patients with BK.

Rosenwasser et al.[33] reported that topical anesthetic abuse was a serious disorder which includes persistent epithelial defects, corneal stromal ring infiltrates, anterior segment inflammation, disproportionate pain, visual loss, and a history of psychoactive substance abuse with poor prognosis. Previous studies have shown that all topical anesthetics reduce epithelial healing in animal model.[34,35] Similarly, our patient had all of these characteristics. Management of this disorder depends on the discontinuation of the anesthetic agent which is very difficult for the patients because of the psychoactive substance abuse. Since compliance to conservative approaches was poor, we performed NP-AMT in this patient. Despite NP-AMT, the left eye underwent penetrating keratoplasty and the right eye healed with residual corneal scarring. The poor visual outcome in our case confirms the results of previous reports.[33]Improvement of visual acuity in eyes with chemical injury and corneal ulcers which underwent AMT varies from one study to another. Visual acuity improved in 66.7% and 90% of eyes with chemical injury in Arora et al.[19] and Gomes et al.[37] studies respectively. Kim et al.[25] showed that visual acuity increased in 16 of 21 eyes (76.2%) with infectious corneal ulcers. According to our results, AMT improved the visual acuity in 11 cases (61.1%), owing to both corneal surface reconstruction and improvement of corneal transparency. The visual improvement was observed in 4 (50%) patients with postinfectious keratitis, 3 (60%) patients with chemical injury, and 3 (100%) patients with BK in our series.

The first drawback of this study is that there was no control group using P-AM to compare with the patients using the NP-AM. The other drawback of the present study is having a small study group.

In conclusion, our study confirmed that NP-AMT is a useful treatment modality in patients with persistent epithelial defects due to infectious keratitis and chemical injury. Moreover it is a safe and effective method to restore a stable corneal epithelium in eyes which underwent a

primary surgical removal of BK. Further controlled and randomized studies which investigate the changes and the differences in the levels of trophic factors between nonpreserved and preserved AM, compare their clinical outcomes, and evaluate safety and efficacy of NP-AM to treat ocular surface diseases are needed.

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