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Amniotic membrane transplantation: Indications and results

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PURPOSE. *To describe amniotic membrane transplantation indications and results at the authors' institution.*

METHODS. *In this study, chart review of 108 patients who underwent amniotic membrane transplantation between January 2002 and April 2006 was performed. The survival rate of corneal integrity was compared, using Kaplan-Meier survival analysis, as a measure of success rate.*

RESULTS. *The mean age of the patients was 55.2±20.1 (6–87 years, 75 female, 51 male). The patients underwent amniotic membrane transplantation for six different diagnoses: non-traumatic corneal perforation (32 eyes, Group 1), persistent epithelial defect (29 eyes, Group 2), aphakic/pseudophakic bullous keratopathy (18 eyes, Group 3), infectious ulcer resistant to treatment (14 eyes, Group 4), necrotizing keratitis secondary to endophthalmitis (10 eyes, Group 5), and caustic injury (5 eyes, Group 6). The mean survival of corneal integrity was similar in all groups (p=0.156).*

CONCLUSIONS. *Amniotic membrane transplantation is a successful adjunctive method in achieving corneal epithelization in the study indications. (Eur J Ophthalmol 2008; 18: 685-90)*

KEY WORDS. *Amniotic membrane transplantation, Corneal ulcer, Epithelization, Bullous keratopathy, Corneal perforation*

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INTRODUCTION

Amniotic membrane is the innermost layer of the fetal membrane. It consists of a thick basement membrane and an avascular stromal matrix (1). It is unique in its ability to promote epithelization using various mechanisms (2).

Despite the early first use of amniotic membrane transplantation (AMT) in ophthalmology (3), it has not been reported again until the early 1990s. The concept of AMT in ophthalmic surgery has been further developed and advanced by Tseng and colleagues, and its role has been re-established (2, 4). Today, AMT has been used and accepted for the treatment of various disorders, including primary and recurrent pterygium with or without symblepharon (5, 6), symptomatic postoperative bullous keratopathy (7), persistent epithelial defects (8), conjunctival surface reconstruction, and acute chemical and thermal burns (9, 10).

The objective of this study is to describe the clinical diagnoses and success rate in patients who underwent AMT to enhance epithelialization and sustain anterior chamber integrity at our institution.

METHODS

Chart review of all patients who underwent AMT between January 2002 and April 2006 was performed retrospectively. The study included 108 eyes from 108 patients who underwent AMT in order to provide epithelization and/or anterior chamber integrity. Patients

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who underwent AMT for primary and recurrent pterygium treatment and for conjunctival surface reconstruction were not included in this study.

The patients were divided into six groups according to the AMT indication. Group 1 included 32 eyes with nontraumatic corneal perforation secondary to noninfectious necrotizing keratitis (descemetocele and/or corneal melting), Group 2 included 29 eyes with persistent epithelial defect, Group 3 included 18 eyes with phakic/pseudophakic bullous keratopathy, Group 4 included 14 eyes with infectious ulcer resistant to medical treatment, Group 5 included 10 eyes with necrotizing keratitis secondary to endophthalmitis, and Group 6 included 5 eyes with caustic injury to the cornea (Tab. I).

Amniotic membrane was processed and prepared, as described by Lee et al (8).

In Groups 1, 2, and 3, the amniotic membrane was attached to peripheral cornea with the epithelial surface up, to secure the corneal surface and the amniotic membrane, thus it acted as a graft. In Groups 4, 5, and 6, the amniotic membrane was attached with the epithelial surface down, and acting as a patch to prevent the deleterious effect of tear inflammatory cells and proteins from the corneal stroma (2). The impending or recent perforation of the cornea was covered with at least two layers of amniotic membrane by using a continuous technique and 10-0 nylon suture. The preferred technique was the overlay technique, as described by Letko et al (11). After surgery, a bandage contact lens was applied to ensure safety of the graft and to facilitate corneal epithelial healing. In Groups 2 and 3, epithelial debridement was performed before the AMT. In Group 4, debris and necrotic tissue from the base of the ulcer was removed before AMT. In Group 6, where symblepharon is more likely, amniotic membrane was anchored by 10-0 nylon suture to the lid margin and to the fornix for a better coverage of the damaged area, starting from the upper lid margin to the lower lid margin. A conformer was also placed to prevent symblepharon.

After AMT, topical antibiotics (Ciloxan®, Alcon, USA), topical steroids (Predforte®, Allergan, USA) and intensive lubrication with artificial tear drops (Tears Naturale Free®, Alcon, USA) were administered. Patients were followed up daily after the AMT procedure for the first week, and thereafter weekly for the first 2 postoperative months. Surgical success was defined as the cessation of aqueous leak, formation of a deep anterior chamber, complete re-epithelialization of the cornea, and an increase in corneal stromal thickness at the operated site by the first month of follow-up. The need to perform a subsequent surgical procedure was considered a failure if the indication was tectonic support or sealing of a persistent leakage.

SPSS 11.5 (Chicago, IL, USA) program was used for the statistical analysis of the data. Continuous variables were expressed as mean ± standard deviation and categorical variables were expressed as percentages. The differences between the groups' means were compared using one-way analysis of variance. Chi-square test was used for comparison of age and gender distribution. The epithelization rates between the groups were compared using Kaplan-Meier survival analysis. For each group, survival rates at months 6, 12, 18, 24, and 36 were analyzed. p Values less than 0.05 were considered significant.

RESULTS

There were 32 patients (29%) in Group 1, 29 patients (27%) in Group 2, 18 patients (17%) in Group 3, 14 patients (14%) in Group 4, 10 patients (9%) in Group 5, and 5 patients (4%) in Group 6. The mean age of 108 subjects (57 female, 51 male) was 55.2±20.1 (6–87) years. The gender distribution was similar in all groups (p=0.849). Group 6 had the youngest mean age (25.2±10.4) (Tab. I). The mean follow-up period after surgery was 20.8±10.0 (6–45) months. There was no difference between the groups in terms of follow-up period (p=0.309) (Tab. I).

The mean survival time for epithelial integrity was 34.5±1.82 (31.0–38.0). The mean survival time for epithelial integrity was similar among all groups (p=0.156). Table I shows the mean survival time for each group. The survival time of epithelial integrity was similar in the 6th, 12th, 18th, and 24th months (Tab. II).

The surface integrity within the early postoperative period was successfully achieved in all eyes in Group 6. In 3 eyes (60%), however, the epithelial integrity was broken because of chronic limbal stromal inflammation and limbal stem cell deficiency, when the amniotic membrane disappeared.

In the patients in whom the AMT failed, secondary procedures were thus performed. While AMT was re-

peated in three patients three times and in six patients twice, penetrating keratoplasty (PKP) was performed in 11 patients. The other secondary procedures required were conjunctival patch in four patients, lateral tarsorrhaphy in three patients, limbal stem cell transplantation in two patients, and evisceration in two patients.

DISCUSSION

The first ophthalmic use of amnion was for replacement of lost conjunctival tissue (12) and as a biologic bandage in the treatment of caustic burn to the eye (13, 14). The presence of anti-angiogenic and anti-inflammatory factors in amniotic membrane helps decrease inflammation, neovascularization, and pain and improves its function as a biologic barrier (15).
Several laminin isoforms that are not characteristically present in corneal basement membrane are present in amniotic basement membrane (16, 17). These laminin isoforms encourage rapid adhesion and enhanced spreading of corneal epithelial cells (17) and are an important reason why the amniotic membrane is effective as a substrate transplant (2). The amniotic basement membrane usually survives the processing and storage procedures and affords a more suitable substratum for epithelial cell growth (2). This

TABLE I - DEMOGRAPHIC FEATURES AND MEAN FOLLOW-UP FOR EACH GROUP

	No. of subjects (percentage in the total group)	Mean ± SD age, yrs (range)	Female/male	Mean ± SD follow-up, mo (range)	Mean ± SD survival time, mo (range)
Group 1 (nontraumatic corneal perforation)	32 (29)	49.5±18.08 (22–80)	19/13	23.06±10.9 (7–40)	38.1±2.7 (32.9–43.5)
Group 2 (persistent epithelial erosion)	29 (27)	49.8±21.8 (6–78)	13/16	17.7±9.4 (7–38)	33.1±2.18 (28.8–37.4)
Group 3 (bullous keratopathy)	18 (17)	70.5±8.4 (53–79)	9/9	18.7±7.1 (9–33)	28.6±1.75 (24.2–32.0)
Group 4 (treatment resistant infectious ulcer)	14 (14)	57.9±16.20 (26–83)	8/6	22.5±10.03 (6–39)	29.7±3.0 (23.7–35.6)
Group 5 (endophthalmitis with corneal melting)	10 (9)	72.8±10.5 (65–87)	6/4	22.1±12.1 (6–45)	25.6±4.5 (16.7–34.4)
Group 6 (chemical burn)	5 (4)	25.2±10.4 (10–35)	2/3	24.0±11.64 (12–43)	26.4±5.3 (15.9–36.8)
Total	108 (100)	55.2±20.13 (6–87)	57/51	20.81±10.06 (6–45)	34.5±1.82 (31.0–38.0)

TABLE II - KAPLAN-MEIER SURVIVAL ANALYSIS OF EPITHELIAL INTEGRITY IN THE FOLLOW-UP PERIOD

Follow-up, mo	Group 1	Group 2	Group 3	Group 4	Group 5	Group 6	p
6	100	96.6	100	100	90	100	0.156
12	96.7	92.5	100	92.3	90	80	
18	96.7	86.4	100	83.9	50	80	
24	90.6	72.0	71.1	61.2	40	26.7	

Values are percentages

function is used typically for epithelial growth in the management of persistent epithelial defects following infection, chemical injuries in neurotropic corneas, and persistent epithelial defects associated with cicatricial conditions (8, 18-20).

Amniotic basement membrane promotes epithelization by using the following mechanisms: it facilitates migration of epithelial cells (21, 22), reinforces adhesion of basal cells (22-24), promotes epithelial differentiation (26-28), and is important in preventing apoptosis (29, 30). It also prolongs the life span of corneal and conjunctival progenitor cells in vitro (22, 31) and maintains slow cycling limbal label retaining cells (21). The presence of mRNA for several growth factors and growth factor receptors has been demonstrated in preserved amniotic membrane by Koizumi et al (32). These may play important roles in ocular surface wound healing.

In this study, amniotic membrane successfully enabled epithelization in the majority of patients in Groups 1, 2, and 3. These three groups consisted of patients with relatively low levels of local inflammation. In a similar study, Azuara-Blanco et al found that AMT effectively promotes corneal healing in patients with persistent epithelial defect. However, it was pointed out that AMT fails to stabilize the cornea in patients with severe stromal thinning and impending perforation (18). AMT was performed as a single layer by Azuara-Blanco et al but was used as multiple layers in other studies (20, 33, 34).

AMT successfully heals epithelial defect and decreases ulcer size in infectious corneal ulcer, as shown in several studies (35-37). The inhibitory effect of amniotic membrane on proteinase activity and the barrier effect of amniotic membrane against the infiltration of polymorphonuclear leukocytes from the tear film are proposed mechanisms for this healing effect. The epithelization rates were lower, though not significantly, in Groups 4 and 5 of this study. Though antimicrobial properties of amnion and chorion have been shown against a variety of microorganisms (hemolytic streptococcus group A, *S aureus*, *E coli*, and *P aeruginosa*) (38, 39), the antimicrobial properties of amniotic membrane are less well known. Furthermore, Walsh et al (40) demonstrated that amniotic fluid is unable to inhibit the growth of five common bacteria isolated from the vagina. The antimicrobial property of amniotic membrane may reside solely in its ability to adhere intimately to the underlying substrate, as proposed by Talmi et al (41). In our study, the survival time for epithelial integrity was significantly lower for patients with endophthalmitis (Group 5) than patients with bullous keratopathy (Group 3) ($p=0.017$). In our opinion, the low success rate of epithelization in Group 5, compared with Group 3, is the result of the high bacterial load and the rich inflammatory medium present at the area of AMT. Endophthalmitis itself is a challenge to most ophthalmologists and may be very difficult to treat even in the absence of secondary necrotizing keratitis. Amniotic membrane may have a role in decreasing inflammation in these cases.

In cases with bullous keratopathy, we found that amniotic membrane transplantation is an effective treatment modality for re-epithelization in parallel with previous studies (42, 43). Additionally, according to a recent study with small series, performing amniotic membrane transplantation along with anterior stromal micropuncture for treatment of bullous keratopathy is more effective than performing amniotic membrane transplantation alone (44).

In the caustic injury, the leukocyte infiltration with persistent inflammation prevents epithelization and causes melting in the acute stage. The chemical damage also causes granuloma and scar formation in the chronic stage (30). Chronic inflammation in limbal stroma is presumably the major factor causing limbal stem cell deficiency (45). In acute chemical burns, AMT helps promote re-epithelization, reduces limbal-stromal inflammation, and restores conjunctival surface by limiting symblepharon formation (9, 10). In the early stage of chemical burns, matrix metalloproteinase (MMP-1 and MMP-2) induces apoptosis of inflammatory cells that contribute to destruction of corneal stromal collagen and corneal melting. The tissue inhibitors of matrix metalloproteinase have been demonstrated in amniotic membrane and this property may be helpful in the protection of corneal melting following acute chemical burns (46). However, the role of AMT in prevention of limbal stem cell deficiency in severe chemical burns seems limited and depends on the extent of limbal involvement (9). In our study, all of the subjects with caustic burn had at least 12 clock hours of involvement and survival rate of epithelization integrity at the end of the follow-up period was 26.7%. This ratio is lower when compared to Tsubota et al's series of 14 chemical and thermal injuries (with an epithelization ratio of 71% with a mean follow-up of 1163

days) (47). However, the limbal stem cell involvement is very extensive in our series and we believe this accounts for the relatively low rate of success in our patients. This result supports the general consensus that AMT alone is likely to succeed in partial limbal stem cell deficiency but limbal stem cell transplantation is required in patients with total stem cell deficiency (2). One weakness of our study is the low number of patients in Groups 5 and 6, which makes comparison difficult with other groups. We believe that a higher number of patients could make useful changes to the statistical study.

In conclusion, AMT is an effective tool to promote epithelization in persistent epithelial defect, nontraumatic corneal perforation, and bullous keratopathy. The effect of AMT is limited in the eyes with caustic injury and is dependent on the extent of limbal involvement. The success of AMT to promote epithelization with the presence of infectious ulcer resistant to treatment is lower than the above indications, though not significantly. Finally, the role of AMT to heal corneal epithelium in the presence of necrotizing keratitis secondary to endophthalmitis is limited and needs further evaluation in animal models. The major limitation of this study is its retrospective nature.

None of the authors has any proprietary interest.

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