The Results of Amniotic Membrane Transplantation for Symptomatic Bullous Keratopathy

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Objectives: *To evaluate the outcomes of amniotic membrane transplantation for symptomatic relief in patients with bullous keratopathy.*

Material and Method: *This retrospective study included 17 eyes (17 patients) with bullous keratopathy presenting with intractable pain or discomfort. The patients were enrolled from January 2000 to December 2004. Amniotic membrane transplantation was performed. Symptomatic relief, epithelial healing, and visual changes were analyzed.*

Results: *During the follow up period of 14.1 $\pm$ 11.9 months (range 1-36 months) after amniotic membrane transplantation, 14 eyes of 17 eyes (82.4%) with intolerable pain preoperatively had pain relief postoperatively. Corneal epithelial healing was complete in all except 2 eyes one of which had evisceration because of severe corneal ulcer, and the other underwent penetrating keratoplasty soon after amniotic membrane transplantation.*

Conclusion: *Amniotic membrane transplantation is a safe and effective treatment modality for pain relief associated with chronic bullous keratopathy. It can be an alternative to conjunctival flap, with better cosmetic appearance for the management of patients with bullous keratopathy.*

Keywords: *Bullous keratopathy, Amniotic membrane transplant*

J Med Assoc Thai 2005; 88 (Suppl 9): S57-62
Full text. e-Journal: *http://www.medassocthai.org/journal*

Bullous keratopathy is a disorder caused by corneal endothelial decompensation which is characterized by corneal stromal edema. If the bullae present clinically, the term "bullous keratopathy" is used.

Insults leading to corneal endothelial decompensation can come from various causes, such as intraocular surgery, nonsurgical trauma, uncontrolled glaucoma, and any diseases of endothelial dystrophy.

When the disease progresses until occurring of bullous keratopathy, patients may suffer from some symptoms. Especially, ruptured epithelial bullae can irritate corneal nerve terminals, producing pain and ocular surface discomfort[1]. Ocular pain is a characteristic complaint of most patients with advanced bullous keratopathy.

In histopathologic changes, there is increasing of stromal hydration, keratocyte loss[2], attenuated or ruptured Bowman layer and epithelial basement membrane, including decreasing of glycosaminoglycans in stroma[3,4]. All of these events lead to intraepithelial edema.

Prolonged epithelial edema results in poor epithelial adhesion and recurrent or persistent erosion which can become ulcerative keratitis. The ulcerative keratitis may be complicated by infectious organisms leading to ulcers. This is the most severe complication, which occurs in 4.7% of patients with bullous keratopathy[5]. Furthermore, prolonged corneal edema and ulceration cause deep and superficial vascular ingrowth

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and scarring which will complicate future surgical treatments[5].

The main purposes of treatment of bullous keratopathy are reducing pain and improved vision if possible. Currently, several choices of treatment are reported[6-12]. However, appropriation depends on severity of symptoms, cause of bullous keratopathy, and visual potential. The treatments include medical and surgical methods.

When there is good visual potential, penetrating keratoplasty is the only curative treatment. This treatment can alleviate pain and also restore vision. However, bullous keratopathy can still occur in transplanted cornea because of graft failure.

In eyes with poor visual potential and severe pain, corneal transplantation is no longer a feasible choice. Other therapies might avoid the unnecessary risks of open-eye surgery and the use of precious corneal tissue. Many options for this condition such as bandage contact lenses[6], anterior stromal puncture[7], annular keratotomy[8], epikeratophakia[9], excimer laser phototherapeutic keratectomy[10,11] or conjunctival flaps[12].

Many studies reported the usage of human amniotic membrane in the treatment of many ophthalmologic conditions. It has been shown to be effective in treating persistent corneal epithelial defects[13] and corneal[14,15] and conjunctival[16,17] surface reconstruction. Amniotic membrane facilitates epithelialization, reduces inflammation, vascularization and scarring.

From the advantage of amniotic membrane as mentioned above, it was chosen to be an alternative treatment in painful recurrent epithelial defects in bullous keratopathy, especially with poor visual potential patients. This choice of treatmant was effective in reducing pain for 88% (60% pain disappeared in the first postoperative day) and 67% free of symptoms at the final follow up of the study (mean time follow up 25.1 months)[18].

Moreover, this method is also safe. It does not induce limbal stem cell deficiency and provide better cosmetic result when compared with a conjunctival flap.

The purpose of the present studies was to determine whether amniotic membrane transplantation (AMT) can be used to treat symptomatic bullous keratopathy.

Material and Method

This retrospective descriptive study was approved by the Ethics Committee, Faculty of Medicine, Prince of Songkla University.

The study included 17 eyes in 17 patients, presenting with symptomatic bullous keratopathy, complained of intractable pain, tearing and foreign body sensation. The patients were enrolled from January 2000 to December 2004. Every patient who underwent AMT was included.

Clinical data on age, sex, visual acuity changes, etiology, epithelialization, complication and symptoms at final follow-up were recorded. Their demographic data and clinical characteristics are summarized in Table 1.

Informed consent was obtained from each patient. AMT was performed by ophthalmologists. Amniotic membrane was prepared and preserved as standard process in Songklanagarind Hospital. The human placenta was obtained shortly after elective cesarean delivery when human immunodeficiency virus, hepatitis virus type B, hepatitis virus type C, and syphilis had been excluded by serologic tests. Under a lamellar-flow hood, the placenta was cleaned of blood clots with sterile Earle's balanced saline solution containing 50 μg/ml of penicillin, 50 μg/ml of streptomycin, 100 μg/ml of neomycin, and 2.5 μg/ml of amphotericin B. The amnion was separated from the rest of the chorion by blunt dissection through the potential spaces situated between these two tissues and flattened, with the epithelium/basement membrane surface up, onto nitrocellulose paper having a pore size of 0.45 μm. The paper, with the adherent amniotic membrane, was then cut into 2.0 x 2.0 cm disks and stored before transplantation at -80 °C in a sterile vial containing Dulbecco modified Eagle medium and glycerol at the ratio of 1:1 (v/v).

A retrobulbar block was administered. The loose epithelium was first removed with sponge, sparing 1.0 to 2.0 mm from limbus. The preserved amniotic membrane was removed from the storage medium. After thawing, the amniotic membrane was placed over the area of epithelial defect with the basement membrane side facing up. This fashioned membrane was then secured to the edge of the defect by 10-0 nylon sutures, which were interrupted. The membrane was flattened tightly on to the corneal surface and approximate to the epithelial edge. In some cases a bandage contact lens was applied.

Postoperatively, all patients were treated with topical steroid for 2-4 weeks, depending on the inflammatory response. Topical antibiotics was given for the first 2 weeks or until complete epithelialization.

Table 1. Demographic data and etiology

Patient no.	Age (yr)	Sex	Eye	Duration of BK (months)	Etiology
1.	81	F	R	6	Pseudophakia
2.	75	M	L	12	Pseudophakia
3.	80	F	R	24	Pseudophakia
4.	78	F	L	24	Pseudophakia
5.	74	M	L	24	Glaucoma
6.	75	M	R	24	Aphakia
7.	71	F	R	5	Pseudophakia
8.	81	M	L	24	Glaucoma
9.	74	F	L	15	Pseudophakia
10.	34	M	R	24	Glaucoma
11.	66	F	R	3	DesM split
12.	77	F	R	24	Glaucoma
13.	24	M	L	6	Glaucoma
14.	67	F	R	24	Pseudophakia
15.	66	M	R	24	Pseudophakia
16.	77	M	R	8	Glucoma
17.	73	F	L	6	ICE syndrome
median (range)	74 (24-61)			12 (3-24)	

Results

Seventeen patients (8 men and 9 women) with a mean age of 69 $\pm$ 15.9 years (range 24-81 years) were enrolled in the present study (Table 1). Before AMT, bullous keratopathy in these 17 patients had lasted for 16.3 $\pm$ 8.8 months(range 5-24 months). All patients suffered from intractable pain, foreign body sensation, and tearing. Etiologies of the bullous keratopathy were pseudophakia in 8 patients (47.0%), Descemet membrane split during cataract surgery in 1 patient (5.9%), glaucoma in 6 patients (35.3%), aphakia in 1 patient (5.9%), and ICE syndrome in 1 patient (5.9%).

Their preoperative visual acuities were all worse than or equal to 20/200 of ETDRS chart: 20/200 (1 eye), finger counting (6 eyes), hand movement (7 eyes), light projection (1 eyes), and no light percertion (2 eyes). All eyes had been treated with topical artificial tears, lubricants, or 5% saline solution.

After treatment, contact lenses were applied for 12 patients (70.6%). Visual acuity improved in 3 patients, deteriorated in 2 patients, and did not change in 12 patients. The changing in visual acuity was not much in both groups. Pain relief was achieved in 14 patients (82.4%) with a mean follow-up of 14.1 $\pm$ 11.9 months (range 1-36 months). The defect covered by amniotic membrane healed rapidly with the mean time of 2.7 $\pm$ 1.0 weeks (range 1-4 weeks) of 15 patients. In 2 cases, the epithelium was not healed. In one patient, the symptoms were relieved after AMT but developed corneal ulcer 5 months later, and eventually underwent evisceration because of uncontrolled infection. The other (patient 11) received preserved cornea and underwent penetrating keratoplasty in 4 months after AMT that the epithelium still was not completely healed. She had a small persistent epithelial defect about 1.5 mm throughout 4 months follow-up. The other 3 patients, who developed recurrent bullous keratopathy with significant pain, underwent regraft. All of them were regrafted at 3 months, 7 months and 19 months. After the second time of AMT, the symptoms were also relieved.

The fading of amniotic membrane was also recorded. The membrane at last follow up remained intact in 4 eyes, became partially dissolved in 5 eyes, and completely dissolved in 8 eyes (Table 2, 3).

Discussion

In the present study, the authors found AMT to be an effective treatment modality for the relief of intractable pain and restoration of epithelial integrity associated with chronic bullous keratopathy. The symptoms were relief in 14 of 17 (82%) patients after AMT. Three eyes had recurrent pain due to recurrent bullous keratopathy and underwent regraft. After regraft, the pain was relieved in all of them. The results are consistent with a previous paper of Pires TFP & Tseng CGS[18],

Table 2. Clinical data

Patient at first (wks)	VA healing AMT	Epithelial after	VA use	CL dissolved	Membrane (mo)	Follow-up after AMT	Symptoms
1.	HM	-	Same	No	Partial	5	Same
2.	FC1	3	Same	Yes	Intact	29	Better
3.	HM	2	Same	Yes	Partial	24	Better
4.	FC1	2	Same	No	Complete	6	Better
5.	FC1	2	Worse	Yes	Complete	17	Better
6.	HM	4	Same	Yes	Intact	24	Better
7.	FC1	1	Worse	Yes	Complete	10	Same
8.	No PL	3	Same	Yes	Complete	36	Better
9.	HM	4	Same	Yes	Partial	7	Better
10.	FC1	2	Better	No	Partial	19	Better
11.	20/200	-	Same	No	Partial	4	Same
12.	HM	3	Same	Yes	Intact	1	Better
13.	PJ	2	Same	Yes	Complete	36	Better
14.	HM	2	Better	Yes	Intact	4	Better
15.	FC1	4	Better	Yes	Complete	2	Better
16.	No PL	4	Same	Yes	Complete	12	Better
17.	HM	2	Same	No	Complete	3	Better
Range		(1-4)				(1-36)	

Table 3. Clinical Data

Patient	Status of cornea at final	Further management
1.	Corneal ulcer	Evisceration
2.	No BK	Tear supplement
3.	Band K	Remove band K
4.	Mild BK	Tear supplement
5.	No BK	Tear supplement
6.	No BK	No medication
7.	No BK	PKP
8.	Conjunctivalization	Tear supplement
9.	Recurrent BK	Regraft
10.	Recurrent BK	Regraft
11.	Persistent epithelial defect	PKP
12.	Mild BK	Tear supplement
13.	Band K	Weak steroid
14.	No BK	Tear supplement
15.	Mild BK	Tear supplement
16.	PEE	Tear supplement
17.	Recurrent BK	Regraft

BK = Bullous keratopathy, Band K = Band keratopathy,
PEE = Punctate epithelial erosion, PKP = Penetrating keratoplasty procedure

who reported ocular pain relief in 90% of eyes. These results support that amniotic membrane transplantation can be considered an alternative surgical choice for treating this disorder. The technique is easier to perform with better cosmetic result compared with conjunctival flap[19].

Furthermore, amniotic membrane transplantation provides an additional advantage in that the resultant cornea does not induce limbal stem cell deficiency, which is created by a conjunctival flap. Such corneas are amendable for corneal transplantation if necessary.

Many structural and molecular mechanisms may explain the action of amniotic membrane in improving ocular surface symptoms in patients with bullous keratopathy. It has been recognized that the basement membrane facilitated migration of epithelial cells, reinforced adhesion of basal epithelial cells, and promote epithelial differentiation[20,21]. Recently, the basement membrane has also been found to be important in preventing epithelial apoptosis[22], prolonging the life span of corneal and conjunctival proginator cells[23]. This action may explain why AMT can be used to facilitate epithelialization. AMT provides a new and non antigenic human basement membrane[24] for renewed expansion of epithelial cells. The new basement membrane restores a healthy environment to promote cell migration, differentiation, and most important, cell adhesion.

Ljubimov et al[1] described altered immunostaining for key matrix proteins such as fibronection, laminin, tenascin, and type IV collagen. In bullous keratopathy, in which the basement membrane is attenuated in quality, cell adhesion impairment may be reflected by continuous epithelial ulceration, persistent epithelial defect, and epithelial bullae formation. Normal cultured corneal epithelial cells adhere to different matrix proteins using integrins $\alpha 2\beta 1$, $\alpha 3\beta 1$, $\alpha 6\beta 1$[19]. It has been postulated that integrins that function as adhesion molecules of corneal epithelial cell to the matrix proteins are shattered in bullous kera-topathy[17].

Moreover, amniotic membrane posses anti-inflammatory effects. The membrane as a patch facilitates apoptosis of infiltrating PMNs and lymphocytes[25]. Its stroma contains growth factors, these may play a role in the process of accelerating corneal epithelial healing.

However, The present study has many limits. The study was a retrospective descriptive study, had a small number of cases and evaluated the symptoms subjectively. A larger group of cases and randomized controlled trial may be required.

In conclusion, amniotic membrane transplantation seems to have the advantage of relief in symptoms in bullous keratopathy. It may be considered as an alternative treatment for this condition. In countries in which there is a shortage of corneal tissue, such as Thailand, amniotic membrane transplantation may serve as a temporary alternative to corneal transplantation in bullous keratopathy with good visual potential for patients who are on a long waiting list.

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ผลการรักษาอาการที่เกิดจากภาวะกระจกตาบวมด้วยการปลูกถ่ายเยื่อหุ้มรก

อรทัย ชาญสันติ, อรสา ห่อรัตนาเรือง

วัตถุประสงค์: *เพื่อรายงานผลการรักษาอาการที่เกิดจากภาวะกระจกตาบวมด้วยการปลูกถ่ายเยื่อหุ้มรกของผู้ป่วยที่คลินิกตาในโรงพยาบาลสงขลานครินทร์*

วัสดุและวิธีการ: *เป็นการวิจัยเชิงพรรณนา รวบรวมผู้ป่วยที่มีอาการปวดจากภาวะกระจกตาบวมตั้งแต่เดือนมกราคม พ.ศ. 2543 ถึง ธันวาคม พ.ศ. 2547 โดยผู้ป่วยทั้งหมด 17 คน (17 ตา) ได้รับการผ่าตัดด้วยการปลูกถ่ายเยื่อหุ้มรก รายงานผลการรักษาด้วยการติดตามอาการหลังการผ่าตัด ระยะเวลาการหายของแผลที่ผิวชั้นนอกของกระจกตา และการเปลี่ยนแปลงการมองเห็นหลังผ่าตัด*

ผลการศึกษา: *อาการปวดจากภาวะกระจกตาบวมลดลงหลังการผ่าตัดปลูกถ่ายเยื่อหุ้มรกใน 14 ตาจากทั้งหมด 17 ตา คิดเป็นร้อยละ 82.4 ระยะเวลาในการติดตามการรักษาหลังการผ่าตัดเฉลี่ย 14.1 $\pm$ 11.9 เดือน (ตั้งแต่ 1-36 เดือน) เกือบทั้งหมดของตาที่ศึกษามีการหายของแผลที่ผิวชั้นนอกของกระจกตา ยกเว้น 2 ตาที่ไม่มีการหายของแผล โดย 1 ตาเกิดภาวะติดเชื้อรุนแรงที่กระจกตาและได้รับการผ่าตัดเอาลูกตาออก อีก 1 ตาได้รับการปลูกถ่ายกระจกตา ซึ่งแผลที่ผิวชั้นนอกของกระจกตายังไม่หายสนิท*

สรุป: *การปลูกถ่ายเยื่อหุ้มรกเป็นการผ่าตัดที่ปลอดภัยและได้ผลดีในการลดอาการปวดจากภาวะกระจกตาบวม*