EXTENDED REPORT

Randomised controlled study of conjunctival autograft versus amniotic membrane graft in pterygium excision

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Aim: To determine whether amniotic membrane can be used as an alternative to conjunctival autograft after pterygium excision.
Methods: 287 eyes with either primary or recurrent pterygium were included in this study. All eyes were randomised to undergo conjunctival autograft or amniotic membrane transplantation after pterygium excision by a single surgeon. 106 eyes in primary pterygium and 14 eyes in the recurrent group were treated with conjunctival autograft, and 148 eyes in primary pterygium and 19 eyes in the recurrent group were treated with amniotic membrane transplantation. Patients were followed up at 6 weeks and 6 months after operation. The main outcome measurement was recurrence rate after surgery.
Results: In the conjunctival group, the recurrence rate was 12.3%, 21.4% and 13.1% for primary, recurrent and all pterygia, respectively. In the amniotic membrane group, the recurrence rate was 25.0%, 52.6% and 28.1% for primary, recurrent and all pterygia, respectively. The recurrence rate for all pterygia in the amniotic membrane group was significantly higher than that in the conjunctival group ($p = 0.003$).
Conclusions: Amniotic membrane graft had a higher recurrence rate than conjunctival autograft. However, it is an alternative choice, especially for advanced cases with bilateral heads or patients who might need glaucoma surgery later.

Pterygium, a wing-shaped encroachment of the cornea by the conjunctiva, which may be atrophic, stationary or progressive, is a common eye problem, especially in tropical areas such as Thailand. Numerous surgical approaches have been attempted.[1–5] All procedures can be classified according to the method of excision and the method of dealing with the defect created.

After excision, the resulting defect can be left exposed (bare sclera excision),[6 7] or covered by surrounding conjunctiva (primary closure)[4 6 8 9] or a pedicle flap,[6 10] or by transposition of the pterygium head.[11] The defect can also be covered by a conjunctival autograft without the limbus,[8 11–17] or with the limbus,[4 18–21] or using other tissue sources such as buccal mucous membrane grafts, lamellar keratoplasty,[22 23] penetrating keratoplasty[4] or sclerokeratoplasty.[24] The other techniques include yttrium–aluminium–garnet (YAG) laser treatment[25] and a polishing technique as advocated by Barraquer.[26]

Recently, Koranyi *et al*[27] published a cut-and-paste technique in primary pterygium with fibrin glue, which showed markedly less postoperative pain and shortened surgery time. The recurrence rate was only 5.3%.

Without covering the defect, adjunctive treatment such as β-radiation,[8 28 29] thiotepa,[4] mitomycin C,[15 30–35] 5-fluorouracil,[36] ciclosporin A[37] or daunorubicin[38] is used to reduce the recurrence rate. These adjunctive treatments are associated with complications, including poor epithelial healing,[32] superficial punctate keratitis,[33] late-onset scleral ulceration, microbial infection, glaucoma and endophthalmitis.[39 40] Owing to these potential complications, conjunctival autografting has been widely adopted in the management of pterygium. Although this method has reduced the recurrence rate,[12 16 41 42] questions have been raised whether conjunctival autografts should be reserved solely for recurrent pterygia because of the risk of compromising the outcome of glaucoma-filtering surgery if it should be required at some future date in the donor eye.[12] Moreover, for those with advanced pterygia with wide conjunctival involvement or multiple heads, conjunctival autografts might be limited by the lack of remaining healthy tissue in the same or fellow eye. For these reasons, an alternative tissue source has been sought. Many authors have reported that amniotic membrane grafts are a viable alternative to conjunctival autografts in reducing recurrences after pterygium excision.[43–49] The possible mechanisms of preventing pterygium recurrence include promotion of conjunctival epithelium, inhibition of inflammation by inhibiting chemokine expression by fibroblasts[50 51] and interleukin-1 expression by epithelial cells, and inhibition of neovascularisation by inhibiting vascular endothelial cell growth.[52] As there has been no randomised study comparing the recurrence rate between conjunctival autografts and amniotic membrane grafts in both primary and recurrent pterygium, especially in tropical areas, we conducted a randomised controlled study to examine this question.

MATERIALS AND METHODS

Patients

We carried out excision of pterygia at Srinagarind Hospital (Khon Kaen, Thailand) from 2000 to 2001 under protocol #I 44034 approved by the Medical Science Subcommittee for the protection of Human Subjects in Research of Faculty of Medicine, Khon Kaen University, Khon Kaen, Thailand. The inclusion and exclusion criteria were proposed as follows.

The inclusion criteria

1. Patients who were diagnosed with pterygium (both primary and recurrent pterygia) at Srinagarind Hospital and met the indication for surgical treatment.
2. Patients with pterygium who signed the informed consent to enrol into the study.

Table 1 Demographic data

	Conjunctival autograft			Amniotic membrane graft			
	Primary	Recurrent	Total	Primary	Recurrent	Total	p Value
No of eyes	106	14	120	148	19	167	0.881
OD	51	9	60	72	13	85	
OS	55	5	60	76	6	82	
No of patients							0.583
Male	106	14	120	148	19	167	
Female	40	4	44	52	4	56	
	66	10	76	96	15	111	
Age* (years)	44.75 (11.44) (19–72)	50.50 (10.73) (30–68)	45.42 (11.47) (19–72)	45.31 (12.84) (18–80)	55.63 (16.34) (19–76)	46.49 (13.63) (18–80)	0.178
Size* (mm)	3.50 (1.42) (1–9)	4.14 (0.95) (3–6)	3.58 (1.38) (1–9)	3.65 (1.49) (1–9)	3.79 (1.58) (1–6.5)	3.67 (1.49) (1–9)	0.837
Site							0.025
Nasal	103	14	117	133	19	152	
Temporal	3	0	3	15	0	15	

OD, right eye; OS, left eye.
Data are given as mean (SD) (range).

The exclusion criteria

1. Patients who had glaucoma in the study eye.
2. Patients who had an intraocular pressure >21 mm Hg in the study eye.
3. Patients who had a history of allergy to steroid eye drops.
4. Patients enrolled in another study, that might affect this study.
5. Patients who had not cooperated during pterygium excision surgery.

This study was designed as a single-blind randomised control trial. The patients were randomised into two groups by a simple randomisation technique. The sample size in this study was calculated based on the recurrence rate in the study of Prabhasawat *et al*.[43]

Sample size $= [(Z_\alpha+Z_\beta)^2 2P(1-P)]/D^2$

where $P = (P_1+P_2)/2$; $D = P_1-P_2$; Z_α = type-I error (5%); Z_β = type-II error (20%); P_1 = recurrence rate in the amniotic membrane group = 0.143 (14.3%); P_2 = Recurrence rate in the conjunctival autograft group = 0.049 (4.9%)

The results with respect to age, sex, size of pterygium and recurrence rates were compared between those of 120 patients (120 eyes) receiving excision of pterygia followed by conjunctival autograft and those of 167 patients (167 eyes) receiving excision of pterygia followed by amniotic membrane graft. These two procedures were carried out by the same surgeon to ensure that similar amounts of pterygial and surrounding fibrovascular tissue were removed. The results at 6 weeks and 6 months were examined by the same investigator in a blind assessment to grade the final appearance based on the criteria given by Prabhasawat *et al*.[43] Grade 1 indicates that the appearance of the operated site was not different from the normal appearance; grade 2 indicates that there were some fine episcleral vessels in the excised area extending up to, but not beyond, the limbus and without any fibrous tissue; grade 3 indicates that there were additional fibrous tissues in the excised area that did not invade the cornea; grade 4 represents a true recurrence, with fibrovascular tissue invading the cornea.

Preparation of preserved human amniotic membrane

Human amniotic membrane was prepared and preserved using the method of Kim and Tseng,[53] with minor modification. Human placenta was obtained shortly after elective caesarean section and exclusion of human immunodeficiency virus, hepatitis B and C viruses, and syphilis by serological testing. In a sterile operating room, the placenta was cleaned of blood clots with 0.9% normal saline and Earl's balanced salt solution (Gibthai, Bangkok, Thailand) containing 50 mg/ml penicillin, 50 mg/ml streptomycin, 100 mg/ml neomycin and 2.5 mg/ml amphotericin B. The amnion was separated from the rest of the chorion by blunt dissection to open the intervening potential space. The isolated amniotic membrane was then flattened on to a nitrocellulose paper with a pore size of 0.45 μm (Gibthai) with the epithelium or basement membrane surface facing up. The sheet, combined with the amniotic membrane and the filter, was then cut into 3×4-cm^2 disks, and stored at −80°C in a sterile vial containing Dulbecco modified Eagle's medium (Gibthai) and glycerol (Gibthai) at a ratio of 1:1 (vol/vol) before transplantation.

Surgical technique for pterygium excision

All patients received topical anaesthesia of 0.4% oxybuprocaine hydrochloride (Novesin; Novartis, Thailand). The eye undergoing surgery was prepared and draped in the usual sterile fashion. After insertion of a lid speculum, the pterygium tissue was measured from the limbus to the head of the pterygium. The pterygium was then injected with 0.5 ml of 2% xylocaine with epinephrine 1:80 000. The pterygium was cut near the limbus using Wescott's scissors. The head of the pterygium was removed from the surface of the cornea. Subconjunctival fibrous tissue was then

Table 2 Recurrence rate at 6 weeks

	Recurrence rate, n (%)		
	Conjunctival autografts	Amniotic membrane grafts	p Value
Primary	7/106 (6.6)	8/148 (5.4)	0.690
Recurrent	1/14 (7.1)	2/19 (10.5)	0.738
Total	8/120 (6.7)	10/167 (6.0)	0.815

Table 3 Recurrence rate at 6 months

	Recurrence rate, n (%)		
	Conjunctival autografts	Amniotic membrane grafts	p Value
Primary	13/106 (12.3)	37/148 (25.0)	0.012
Recurrent	3/14 (21.4)	10/19 (52.6)	0.070
Total	16/120 (13.3)	47/167 (28.1)	0.003

Table 4 Grading at 6 weeks

	Grading, n (%)				
	1	2	3	4	p Value
Primary					
CA	67/106 (63.2)	25/106 (23.6)	7/106 (6.6)	7/106 (6.6)	<0.001
AM	56/148 (37.8)	40/148 (27.0)	44/148 (29.7)	8/148 (5.4)	
Recurrent					
CA	7/14 (50.0)	4/14 (28.6)	2/14 (14.3)	1/14 (7.1)	0.180
AM	6/19 (31.6)	2/19 (10.5)	9/19 (47.4)	2/19 (10.5)	
Total					
CA	74/120 (61.7)	29/120 (24.2)	9/120 (7.5)	8/120 (6.7)	<0.001
AM	62/167 (37.1)	42/167 (25.1)	53/167 (31.7)	10/167 (6.0)	

AM, amniotic membrane; CA, conjunctival autografts.

completely removed in an area much greater than the pterygium body itself. The completeness of episcleral tissue removal was judged by exposing all the tortuous episcleral blood vessels extending from the nasal rectus muscle insertion for nasal pterygium. Any abnormal scars on the cornea surface were removed with a no 15 blade.

For conjunctival autografts, a free graft of size similar to the defect area was obtained from the superotemporal bulbar conjunctiva.[54 55] For amniotic membrane grafts, the preserved amniotic membrane was removed from the storage medium after thawing and cut into the same size as the defect. Both types of graft were secured, flattened and approximated to the recipient episcleral tissue edge by 10 interrupted 10-0 nylon sutures.

After surgery, all patients received dexamethasone phosphate 0.1% (Sang Thai Medical, Bangkok, Thailand) eye drops four times per day, and tapered off within 1 month. Sutures were removed 2 weeks after surgery.

Statistical analysis

All demographic data including age, sex, occupation and diagnosis were compared between conjunctival autografts and amniotic membrane grafts using the χ^2 test. The recurrence rates between the two groups at 6 weeks and 6 months were also analysed using the χ^2 test.

RESULTS

A total of 346 eyes of 346 patients were included in the study. Although some of the patients had bilateral pterygium in our series, patients allowed us to carry out surgery only monocularly. All patients were Asian (Thai). In total, 59 patients were lost to follow-up (one patient died from a car accident and the remainder did not show up in the clinic owing to unknown reasons). Of 59 patients lost to follow-up, there were 45 patients (76.27%) in the primary pterygium group and 15 patients (23.73%) in the recurrent group. The total number of eyes in the conjunctival group was 120, including 106 eyes in the primary group and 14 eyes in the recurrent group. The total number of eyes in the amniotic membrane group was 167, including 148 eyes in the primary group and 19 eyes in the recurrent group. The number of recurrences was one time in both conjunctival autograft and amniotic membrane groups before enrolment into this study. The ratio of the right and left eyes, male and female, and the age range and size of pterygia were similar in both groups (table 1).

Complications included increase in the intraocular pressure in 14 patients, of which 13 patients returned to normal level (eight patients from the conjunctival autograft group and five patients from the amniotic membrane group) and 1 patient (from the conjunctival autograft group) received trabeculectomy. Pyogenic granuloma occurred in seven patients (four patients from the conjunctival autograft group and three patients from amniotic membrane group). No symblepharon was found in any of the patients.

The recurrence rate in the conjunctival group at 6 weeks was 6.6% for primary pterygia and 7.1% for recurrent pterygia, and the corresponding rates for the amniotic membrane group were 5.4% and 10.5%. We found no significant differences in the recurrence rate between the two groups ($p = 0.815$; table 2).

The total recurrence rate in the conjunctival group at 6 months was 13.3% (primary pterygia 12.3%, recurrent pterygia 21.4%), and that in the amniotic membrane group was 28.1% (primary pterygia 25.0%, recurrent pterygia 52.6%). Thus, at 6 months, amniotic membrane graft had significantly higher recurrence rate than conjunctival autograft ($p = 0.003$; table 3).

DISCUSSION

A pterygium is characterised by excessive fibrovascular proliferation on the exposed ocular surface, and is thought to be caused by increased ultraviolet light exposure from climatic factors and aggravated by microtrauma and chronic inflammation from environmental factors.[2–4 56 57] Despite the multifactorial pathogenesis, surgery is the mainstay of

Table 5 Results of grading at 6 months

	Grading, n (%)				
	1	2	3	4	p Value
Primary					
CA	37/106(34.9)	31/106(29.2)	25/106(23.6)	13/106(12.3)	0.010
AM	33/148(22.3)	32/148(21.6)	46/148(31.1)	37/148(25.0)	
Recurrent					
CA	4/14 (28.6)	4/14 (28.6)	3/14 (21.4)	3/14 (21.4)	0.255
AM	2/19 (10.5)	3/19 (15.8)	4/19 (21.1)	10/19 (52.6)	
Total					
CA	41/120 (34.2)	35/120 (29.2)	28/120 (23.3)	16/120 (13.3)	0.002
AM	35/167 (21.0)	35/167 (21.0)	50/167 (29.9)	47/167 (28.1)	

AM, amniotic membrane graft; CA, conjunctival autografts.

treatment. The primary concern in pterygium surgery is recurrence defined by regrowth of the fibrovascular tissue across the limbus and on to the cornea.[58]

The reported recurrence rates vary greatly not only among different surgical procedures but also between different groups carrying out the same procedure. To eliminate such variability, the same technique was carried out by the same surgeon throughout the study. With this variable controlled, and similar demographic data (table 1) and sufficient follow-up, we can compare the role of conjunctival autografts and amniotic membrane grafts in covering the pterygium excision defects to reduce the recurrence rate. Our result, which is similar to the result of the study by Prabhasawat *et al*,[43] confirms that conjunctival autografts achieve the best result, with a recurrence rate of 13.3% at 6 months in a total of 120 eyes, which consisted of 106 primary and 14 recurrent pterygia ($p = 0.003$; table 3). The recurrence rates in our study were higher in both groups than those previously reported,[43] possibly due to the race of our patients, amount of subconjunctival tissue removal, type of suture used and drug given after surgery. The recurrence rates in our study were similar to those found in a previous study in Thailand,[58] 4.76% and 40.9% in conjunctival autograft and amniotic membrane graft groups, respectively.

The recurrence rate in the amniotic membrane graft group was 28.1% at 6 months in a total of 167 eyes, which consisted of 148 primary and 19 recurrent pterygia. At 6 weeks, when we excluded patients with recurrent pterygia, who might have received different surgery or treatments before the study, and only looked at those with primary pterygia, the recurrence rate with conjunctival autografts was 6.6%, which was slightly more than the 5.4% with amniotic membrane grafts ($p = 0.690$). With the longer follow-up, the recurrence rate of amniotic membrane grafts at 6 months was 25.0%, which was significantly more than the 12.3% of the conjunctival grafts ($p = 0.012$).

The two procedures produce differences in final appearance not only with respect to the rate of frank recurrence (defined as grade 4 in this study) but also in the percentage of grade 1 (ie, normal appearance). As listed in tables 4 and 5, the percentage of grade 1 was higher in conjunctival autografts than in amniotic membrane grafts. Even when we examined those patients with final grades of 2 and 3 (ie, intermediate between normal appearance and frank recurrence), a similar trend emerged. The percentage of grades 2 and 3 for conjunctival autografts was less than that for amniotic membrane grafts. This result suggests that covering the defect area with normal conjunctival tissue also has a higher likelihood of promoting the restoration of a normal appearance.

The amniotic membrane is known to contain a thick basement membrane and a vascular streamed matrix,[50 60] and we theorise that these features are crucial to the observed success. The basement membrane facilitates migration of epithelial cells, reinforces adhesion of basal epithelial cells,[61] reinforces adhesion of basal epithelial cells,[64 65] promotes epithelial differentiation[66–69] and prevents epithelial apoptosis.[68 69] Collectively, these actions explain why the amniotic membrane permits rapid epithelialisation.

Although this study shows that amniotic membrane grafts are less proficient than conjunctival autografts in reducing recurrences after pterygium excision, it indicates that this technique could be considered as an alternative in the surgical management of pterygia, especially when the bare sclera technique alone has an unacceptably high recurrence,[70] and complications related to mitomicin-C as an adjunctive treatment are a concern.[71] Conjunctival autograft should be considered as the first choice for pterygium excision even if there is a recurrence. The amniotic membrane graft can also be considered to be the first choice for those with advanced and diffuse conjunctival involvement (bi-head) or those who might like to preserve the donor bulbar conjunctiva for a prospective glaucoma-filtering procedure. With these limitations, careful use of adjunctive treatment such as mitomycin C may be useful and needs further study.

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