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A COMPARATAIVE STUDY OF CONJUNCTIVAL LIMBAL AUTOGRAFT AND AMNIOTIC MEMBRANE TRANSPLANTATION IN MANAGEMENT OF PTERYGIUM

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HOW TO CITE THIS ARTICLE:

Pramod Kumar Chhawania, Prashant Raj Pipariya. "A Comparataive Study of Conjunctival Limbal Autograft and Amniotic Membrane Transplantation in Management of Pterygium". Journal of Evolution of Medical and Dental Sciences 2014; Vol. 3, Issue 15, April 14; Page: 3908-3918, DOI: 10.14260/jemds/2014/2375

ABSTRACT: Pterygium is a common worldwide external eye disease especially affecting population in tropical and subtropical areas. It is a wing shaped fold of conjunctiva encroaching upon the cornea from either side within the interpalpebral fissure. The present study was conducted in the Department of Ophthalmology, G.R. Medical College and JAH Gwalior (M.P.) from January 2009 to June 2010. The study was conducted in 50 eyes of 50 patients of pterygium. They were randomly divided into two groups, Group A and Group B. In each Group 25 cases were included. Group A patients were surgically treated by Conjunctival limbal autograft and Group B patients by Amniotic membrane Graft. Maximum number of patients belongs to < 40 years of age (56.6%). Male were 34% and females were 16% in CLAG Group. In AMT Group 38% were male and 18% were female. Recurrences were 4(16%) cases in CLAG Group and 6(24%) cases in AMT Group. Study concluded AMT preserved the superotemporal conjunctiva and easy procedure, it has high recurrence rate. Conjunctival limbal autografts are technically demanding and time consuming, but they are more effective in preventing recurrences when compared with Amniotic membrane graft.

KEYWORDS: Conjunctiva, limbal, autograft, pterygium, transplantation.

INTRODUCTION: Pterygium is a common worldwide external eye disease especially affecting population in tropical and subtropical areas. It is a wing shaped fold of conjunctiva encroaching upon the cornea from either side within the interpalpebral fissure. Pterygium has a worldwide distribution with multiple predisposing factors such as ultraviolet radiation, tear film abnormalities, hot and dry climate and outdoor work. Recent studies suggest that it is also associated with stem cell deficiency. Stem cells are located in the limbus, the transition zone between cornea and conjunctiva. Limbus acts as a functional zone between cornea and conjunctiva. Defect in renewal and repair of ocular surface as a result of limbal stem cell deficiency are known to cause various ocular surface disorders in which pterygium is one of the cause. Localised corneal stem cell dysfunction as a loss of limbal barrier against conjunctival invasion has been proposed as a pathogenic factor in pterygium growth and recurrence. Most recently new etiologies have been suggested such as gene p53 mutation at the chromosome 17. Surgical removal is the treatment of choice. The indication for surgery includes cosmetic blemishes, chronic irritation, recurrent inflammation, redness and reduced vision due to irregular astigmatism. Today a variety of options are available for the management of pterygium including irradiation, CLAG, AMT and application of mytomyacin C. Amniotic membrane graft (AMG) and Conjunctival Limbal Autograft (CLAG) are safe and effective procedures in preventing recurrence rate.

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AIMS & OBJECTIVES:

1. To study the efficacy and safety of amniotic membrane graft and conjunctival limbal autograft as an adjunctive therapy after removal of pterygium.
2. To compare the surgical outcome between conjunctival limbal autograft and amniotic membrane graft.
3. To study and compare the recurrence rate between CLAG & AMT.
4. To study any major complications.

MATERIAL & METHODS: The present study was conducted in the Department of Ophthalmology, G.R. Medical College and JAH Gwalior (M.P.) from January 2009 to June 2010. The study was conducted in 50 eyes of 50 patients of pterygium. They were randomly divided into two groups, Group A and Group B. In each Group 25 cases were included. Group A patients were surgically treated by Conjunctival limbal autograft and Group B patients by Amniotic membrane Graft.

Patient's Selection: Patients were selected on the basis of symptomatology and clinical features such as redness, F.B. sensation, watering, irritation, cosmetic problem, decreased vision and discomfort. Affected eyes presenting with these symptoms and signs were included in the study whose condition did not improve despite medical treatment.

Inclusion Criteria:

- Nasal Pterygium.
- Unilateral.
- At Least 3mm growth over the cornea.
- Whose Condition did not improve despite topical medication.

Exclusion Criteria:

- Dry eyes.
- Temporal pterygium.
- Active infection.
- Collagen vascular disorders.
- Less than 3mm pterygium growth over the cornea.

Preoperative Evaluation: Patients data was collected which included age, sex, occupation, any previous ocular, medical and surgical history and duration of onset of the disorder was noted.

Local Examination of Eye: Examination of eye was commenced with recording of visual acuity in all cases.

Lid: Lid and surrounding areas were examined for evidence of rounding of eyelid margin, trichiasis resulting from fibrosis of lid tissue along with ectropion and entropion.

Conjunctiva: Particular attention was paid to evidence of congestion, xerosis, scarring and presence or absence of symblepharon.

Limbus: Limbus was especially examined for superficial and deep vascularization conjunctivalization and also for any ischemic areas following chemical injury.

Cornea: Corneal surface was meticulously examined for epithelial defects, conjunctivalization, keratinization and vascularization; density of opacity (nebular, macular or leucomatous) and corneal sensation were also assessed.

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Anterior chamber: Anterior chamber depth was assessed.

Iris: The examination of iris was done for evidence of synechiae, loss of iris pattern and presence of atrophic patches.

Pupil: Pupil was examined size, shape position and reaction.

Lens: Lens was examined to rule out subluxation, dislocation and any opacity.

Lacrimal system: Lacrimal drainage system was examined by syringing.

Intraocular tension: Assessed in all patients with the help of either schiotz's tonometer or clinically.

Special investigations: Following special investigation were also done to rule out any condition of dry eyes.

Schirmer's I and Basic secretion test: Schirmer's test-I is done to measure the tear production or tear flow:

- Whatman's filter paper strip No. 41, 35 mmx5mm is inserted under the lower lid at the junction of middle and lateral thirds.
- Patient is allowed to blink normally.
- The amount of wetting is measured after 5 minutes.

Tear Film Break up Time (TFBUT): It assess the ability of the precorneal tear film to maintain its integrity:

- No topical anaesthesia is used.
- Test is performed by instilling 2.5µl(1%) fluorescein solution or inserting commercially available fluorescein paper strips in the conjunctival sac.
- The patient is asked to blink three times and then look straight forward without blinking.
- The tear film is observed in red-free light through the slit lamp (Cobalt blue filter) and the time elapsed between the last blink and appearance of the first break (dark spot) in the tear film is recorded.

SURGICAL TECHNIQUE:

Excision of Pterygium: Excision of pterygium head is done bluntly from the underlying cornea by simply rotating and pulling the head using a multiple tooth forceps by reverse peeling technique. Resection of the pterygium along with involved conjunctiva and Tenon's capsule is done after freeing it from the sclera taking care not to damage the medial rectus muscle. The adjacent cornea and limbus are smoothened by scraping using a surgical knife. The length and width of excised area is measured using Castroviejo calipers. Adequate hemostasis is usually obtained with bipolar wet field cautery.

Preparation & Placement of Conjunctival Limbal Autograft (CLAG): Preparation of donor tissue consists of limbal conjunctival graft that is harvested from superior limbus. In our study graft was taken from the same eye. Appropriate measurement is done according to the size of recipient bed or a little larger using the Castroviejo calipers. After marking with cautery or ink, hydrodissection was done to separate underlying tenons from the conjunctiva. Conjunctival graft was dissected peripherally by simple corneoscleral scissors, 3-4mm from the limbus by thin dissection technique to avoid removal of excessive Tenon's and episcleral tissue. Graft was placed over the excised area and secured with 10-0 silk sutures.

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Placement of Amniotic Membrane Graft: Preserved amniotic membrane graft was dipped in sterile normal saline for 15 min at room temperature prior to the surgery.

- Amniotic membrane graft is placed over the surface with epithelial side up.
- The stromal side is identified by the presence of vitreous like strands.
- It is sutured with 10-0 silk suture.
- Amniotic membrane graft is trimmed to the size of the defect after placement of suture.
- Subconjunctival gentamicin and dexamethasone is injected.
- Pad and bandage applied.

Post-Operative Treatment:

- Post-operative treatment consists of antibiotic steroid eye drop six times a day which is gradually tapered over the next six weeks.
- Preservative free tear substitute q.i.d. daily for 1 month.
- Systemic antibiotics and analgesics were given b.d. for seven days.

Follow Up: Follow up was done daily till epithelialization was complete, then weekly for 2 weeks, then fortnightly for 1 month, then monthly for 3 months and quarterly for 1 year.

Following Signs were Examined on First Post op day:

- Condition of the graft.
- Condition of the sutures.
- Reduction in inflammation.

On the 7th Post op Day:

- Corneal epithelial defect by fluorescein stain.
- Condition of the graft.
- Condition of the sutures.
- Reduction in inflammation.

On Subsequent Follow Up: In addition to the above findings patients were also looked for complications like haematoma, subconjunctival haemorrhage, graft rejection, graft oedema, graft retraction, granuloma and recurrences.

OBSERVATIONS:

AGE GROUP	CLAG		AMT	
	NO. OF CASES	%	NO. OF CASES	%
30-40	11	44	12	48
41-50	9	36	8	32
51-60	3	12	4	16
61-70	2	8	1	4
Total	25	100	25	100

Table 1: Age distribution of cases

Table no. 1 shows distribution of cases according to age of the patients. Total numbers of 50 cases were studied. The mean age of population was 47+26 years. However, In both group, maximum no. of cases were less than 40 years of age.

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Group	Male		Female		Total	
CLAG	17	68%	8	32%	25	100%
AMT	19	76%	6	24%	25	100%

Table 2: Sex distribution of cases

Table no. 2 shows sex distribution of cases in both groups. In present study 17(68%) cases were male and 8(32%) cases were female in CLAG Group and in AMT Group 19 (76%) cases were male and 6(24%) cases were female.

SL. NO	Symptoms	CLAG	AMT
1	F.B. Sensation	25 (100%)	25(100%)
2	Redness	24(96%)	18(72%)
3	Itching	14(56%)	18(72%)
4	Recurrent inflammation	12(48%)	18(72%)
5	Watering	6(24%)	4(16%)

Table 3: Pre-Operative ocular symptoms

The above table depicts pre-operative ocular symptoms. In the present study most common symptoms were F.B. sensation 100%, Redness 96%, Itching 56%, Recurrent inflammation 48%, Watering 28% in CLAG Group and F.B. sensation 100%, Redness 72%, Itching 72%, Recurrent inflammation 72%, Watering 16% in AMT group.

SL. No.	Symptoms	CLAG			AMT		
		Pre-Op	Post-Op Improvement	Post-Op Persistent (>6mnth)	Pre-Op	Post-Op Improvement	Post-op Persistent (>6 mnth)
1.	F.B. sensations	25(100%)	22(88%)	3(12%)	25(100%)	23(92%)	2(8%)
2.	Redness	24(96%)	22(88%)	2(8%)	18(72%)	16(64%)	2(8%)
3.	Itching	14(56%)	12(48%)	2(8%)	18(72%)	17(68%)	1(4%)
4.	Recurrent inflammation	12(48%)	10(40%)	2(8%)	18(72%)	17(68%)	1(4%)
5.	Watering	6(24%)	5(20%)	1(4%)	4(16%)	3(12%)	1(4%)

Table 4: Post-operative Improvement in Symptoms

In present study F.B. sensation decreased from 100% to 88%, Redness from 96% to 88%, Itching from 56% to 48%, Recurrent Inflammation from 48% to 40% and watering 24% to 20% in CLAG Group and FB sensation from 100% to 92%, Redness 72% to 66%, Recurrent inflammation 72% to 68%, Itching from 72% to 68% and Watering from 16% to 12% in AMT Group.

Post-operative complication	No of Patients (25)				
	Day 1	Day 7	Day 15	3 month	>6 month
1. F.B. sensation	25	10	5	3	3
2. Graft edema	20	10	-	-	-
3. S/C Haemorrhage	20	15	2	-	-
4. Granuloma	-	2	-	-	-

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5. Graft retraction	2	-	-	-	-
6. Graft rejection	2	2	2	2	2

Table 5: Post-Op complication in CLAG group

*Granuloma was excised on 15th post op day.

*Graft resuturing done on 2nd post op day.

Table no. 5 shows post-operative complications in CLAG Group. Early post op complications were FB sensation, graft oedema, S/C hemorrhage. Graft oedema and haemorrhage was subsides within one month but FB sensation was persistent after six month months.

Post-operative complications	No. of Patients (25)				
	Day 1	Day7	Day 15	3 month	>6 month
1. F.B. sensation	25	8	2	2	2
2. Graft edema	10	5	-		
3. S/C Haemorrhage	15	8		-	-
4. Granuloma	-	-	-	-	-
5. Graft retraction	1	1	1	1	1
6. Graft rejection	3	3	3	3	3

Table 6: Post-Op complication in AMT group

Table no. 6 shows post-operative complications in AMT Group. The most common post-operative complications were F.B. sensation, graft oedema and S/C haemorrhage.

NO. OF PATIENTS	PROCEDURE	RECURRENCE
25	CLAG	4 (16%)
25	AMT	6(24%)

Table 7: Recurrence rate

Table no. 7 shows post-operative recurrence rate in both CLAG and AMT group. In present study, recurrence of pterygium occurs in 4(16%) cases of CLAG and 6(24%) cases in AMT.

DISCUSSION: This study has been carried out on 50 eyes of 50 patients of pterygium, who presented themselves in the Department of Ophthalmology, G.R. Medical College and J.A. Group of Hospital, Gwalior (M.P.). 25 cases of pterygium were treated with CLAG and 25 cases with Amniotic Membrane Transplantation.

Preserved Amniotic membrane graft-Freeze, dried and irradiated measuring 5x3 sq. cm. from TATA Memorial Hospital tissue bank, Mumbai were used.

- 1. Age and sex incidence (Table 1&2):** The present study comprised of 50 patients of which 17(34%) were males and 8(16%) females cases in CLAG group and 19(38%) cases were males and 8(18%) were females in AMT group. The age distribution of patients varied between 30 years and 70 years, with a mean age of 47+26 years.

Similar observations were made by Gabric N. et al (1999) and D.H. Ma et al (2000) in their studies (mean age ranged from 42.7 + 14.5 years and 49+14.7 years respectively).

These findings were also consistent with studies conducted by E.M. Espana et al (2002) and Gris O et al (2003) they found that most of the patients of pterygium in the mean age group 63+ 13.3 years and 61+13.1 years.

- 2. PRE-OPERATIVE SYMPTOMS (TABLE 3):** The table 3 shows pre-operative ocular symptoms in the 50 cases. In the study most common symptoms were F.B. sensation 25(100%), Redness 24(96%), Itching 14(56%) Recurrent inflammation 12(48%) and Watering 6(24%) in CLAG group and F.B. sensation 25(100%), Redness 18(72%), Itching 18(72%) Recurrent inflammation 18(72%) and Watering 4 (16%) in AMT group.

These finding were in concordance with studies conducted by Lee SH and Tseng (1997) who found that the patients with Pterygium had features F. B. sensation, conjunctival epithelial defect and symptoms of chronic ocular irritation and photophobia.

These finding were consistent with CT Pillai and Harminder S dua (1999) who observed that all patients with Pterygium had FB sensation, red and inflamed eyes, non-healing epithelial defect, conjunctivization, Itching and watering.

- 3. IMPROVEMENT IN SYMPTOMS (Table 4):** Following Amniotic Membrane Transplantation (AMT) and conjunctival limbal autografting reduction in the severity of symptoms were noted. Post – operative improvement of symptoms in CLAG group were F.B. sensation reduced from 25(100%) to 22(88%), Redness from 24(96%) to 22(88%), Itching from 14(56%) to 12(48%), Recurrent inflammation from 12(48%) to 10(40%), Watering from 6(24%) to 5(20%). Post op improvement of symptoms in AMT group were F.B. sensation reduced from 25(100%) to 23(92%), Redness 18(72%) to 16(64%), Recurrent Inflammation 18(72%) to 17(68%), Watering 4(16%) to 3(12%).

Al Fayed et al (2000) noted that conjunctival limbal autograft showed improvement in redness, inflammation, irritation and improvement in vision.

D. H. Ma et al (2000) noted that within the follow up period of 12 month all the eyes showed redness, FB sensation, photophobia watering were reduced.

- 4. INTRA –OPERATIVE COMPLICATIONS:** Following CLAG and AMT, no major complications were noted intra-Operatively except button holding in 1 Case of CLAG which was repaired with 2(10-0) silk suture, while placing the graft on to the recipient bed.

Srinivas et al (1998) also reported this complication in their cases which was repaired by 1(10-0) silk suture

POST-OPERATIVE COMPLICATIONS (Table 5&6):

- **Graft Edema:** Graft edema occurred in all cases in the early post-operative period due to handling of graft. However, this resolved within 15-30 days, following instillation of topical steroids and lubricants.

Our results were consistent with the studies conducted by Murat Gular et al (1994) who reported similar results; in their study graft oedema had been caused by allowing the graft to dry, by handling with forceps and by bending.

Our results were also similar with the results of Mutlu et al (1997), Wong et al (2000) and Al Fayed et al (2000).

- **SUBCONJUNCTIVAL HAEMORRAGE:** Another post-operative complication occurred in our series was S/C haemorrhage which due to inadvertent trauma to conjunctival vessels and inadequate hemostasis.

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Our finding corresponding with the study conducted by Murat Gular et al (1994) who reported haematoma in 1 case which was considered to due to inadequate hemostasis. It was drained through a one millimeter incision made on graft on first post-operative day.

Al Fayez et al (2002) in their studies showed S/C haemorrhage in 2(5.5 %)

Mutlu et al (1997) in their studies also reported 2(4.8%) cases of subconjunctival haemorrhage.

- **TENON'S GRANULOMA:** It was seen in 2(8 %) cases of CLAG in our study. It might be due to inadvertent removal of tenon's tissue along with graft and suturing of graft only to adjacent conjunctiva not with episclera. Granuloma was excised on 15th post-operative day.

This observation also reported by Murat Gular et al (1994) who found 5(16.1%) cases that 3 of these occurred at the graft donor site and 2 of them at recipient site. The granuloma occurring at recipient site, was due to suturing of graft only to adjacent conjunctiva and at donor site due to injury to Tenon's capsule during blunt dissection. No granuloma appeared in cases where graft was secured to both adjacent conjunctiva and episclera. These granuloma were excised on 15th post-operative day. To avoid this, hydrodissection of graft was accomplished by 0.9% NaCl with 26G needle, which was routinely done in our study during surgical procedures.

Mutlu et al (1997) in their studies reported this complication in 5(20%) cases.

- **GRAFT REJECTION:** In our series of patients, graft rejection was seen in 2(8%) cases of CLAG and 2(8%) cases of AMT which was comparable with Murat Gular et al (1994) who found this in 2(6.4%) of their cases, In one of their case the graft appeared pale and avascular probably because of the epithelial side of the graft might have been sutured to the sclera and in the other case it was considered that the graft had been sutured too tightly.

Tsuboto et al (1999) reported 13 cases (46%) of rejection.

In our study, it might be due to poor quality of graft or faulty surgical technique of transplantation of graft or improper removal of pterygium tissues.

- **Graft Retraction:** Graft retraction (probably due to small sized graft) were seen in 2 cases (8%) in CLAG and 1(4%) case of AMT (due to drying of the graft and cut through of sutures)in present study.

Similar observations were made by Srinivas et al (1998) who reported 2 cases (3.5 %) of this complication which was due to cut through of the sutured with retraction of conjunctiva at graft host junction.

- **RATE OF RECCURENCE:** After a mean follow up period of a 6+3 months, recurrence of pterygium was noted in 4 cases (16%) of CLAG and 4 cases in AMT 6(24 %) after 6 months in our series. The recurrence was 3mm inside the cornea and patient was symptomatic and further treatment was required. The recurrence might be due to the patient younger age with primary or faulty surgical technique which includes improper removal of pterygium tissue, excessive removal of tenons tissue along with graft, wrong method of placement of graft in CLAG group. In AMT Group it might be due to loosening of the sutures and removal of the graft or rapid shed off of the graft or less.

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Our results were consistent with the other studies done by Kenyon & Tseng et al (1989) who reported (5.3%) recurrence, Murat Gular et al (1994) 13% recurrence at 3-18 months, Shimazaki et al (1996) found (7.43%) recurrence at 16 weeks.

Mutlu et al (1997) reported a recurrence of (14.6%) at 16+-1.9 months, the reason behind this was possibly reflects surgical techniques and expertise and patients demographics.

Sriniwas et al (1998) observed (3.8%) at 3.5- 9.2 months and the recurrence were across the graft, asymptomatic and were detected on routine follow-up, they concluded that both patients were 40 years or younger in age and had primary pterygium.

S.K.Rao et al (1999) reported 3.8% recurrence of pterygium.

Wong et al (2000) noted 5 (18.2%) recurrence in CLAG group of patients which could be because of the greater technical difficulty in harvesting a graft from the limbus. This was across the graft, non-progressive and did not require further surgery.

All recurrence ranged from 2-15% which is comparable with our results.

SUMMARY AND CONCLUSIONS: The present work "A Comparative Study of Conjunctival Limbal Autograft and Amniotic Membrane Transplantation in Management of Pterygium" has been carried on 50 eyes 50 patients of Pterygium. They were randomly divided into two groups, Group A- 25 Cases were treated by CLAG and Group B- 25 cases were treated by AMT, who presented, themselves in the Department of Ophthalmology, G.R. Medical College and JA Group of Hospital Gwalior (M.P.)

All patients were precisely observed and studied according to age, sex address, complaints with duration and detail history of pterygium. This comprehensive clinical examination as per proforma was done and necessary investigations were also performed. All surgical procedure was performed under local anesthesia. All the patients were followed up for the period of 6 months to 9 months. Various intra and post-operative complications of the eye on which surgery was performed were noted.

Following conclusions were drawn after this study:

- Maximum number of patients belongs to < 40 years of age (56.6%).
- Male were 34% and females were 16% in CLAG Group.
- In AMT Group 38% were male and 18% were female.
 - In CLAG Group –
 - FB sensation decreased from 25(100)% to 22(88%),
 - Redness from 24(96%) to 22(88%)
 - Itching from 14(56%) to 12(48%)
 - Recurrent inflammation from 12(48%) to 10(40%)
 - Watering from 6(24%) to 5(20%).
 - In AMT Group-
 - FB sensation decreased from 25(100%) to 23(92%),
 - Redness from 18(72%) to 16(64%),
 - Itching from 18(72%) to 17(68%),
 - Recurrent inflammation from 18(72%) to 17(68%) and
 - Watering from 4(16%) to 3(12%).
- Post-operative complications in CLAG Group.
 - FB sensation 100% on 1st day and 40% on 7th day,

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- Graft oedema 80% on 1 post-operative day which remains 40% in 7 post-operative days
- S/C hemorrhage 80% on 1 day and 60% in 7 post-operative day. Graft oedema and S/C haemorrhage subsides within one month of operation.
- Granuloma was seen on 7th post operation day.
- Graft retraction was seen in 2 cases and rejection in 2 cases.
- Post-operative complications in AMT Group
 - FB sensation 100% on 1st day and 32% on 7th day,
 - Graft oedema 40% on 1st day and 20% on 7th day,
 - S/C haemorrhage 60% on 1st day and 32% on 7th day which were subsides within one month of operation.
 - Graft retraction was seen in 2 cases and
 - Graft rejection in 3 cases.
- Recurrences were 4(16%) cases in CLAG Group and 6(24%) cases in AMT Group.

Loss of limbal barrier causes pterygium growth and recurrence. To treat this disorder, reconstruction of limbal barrier are important. Surgical removal of the pterygium and conjunctival autograft with limbal stem cells transplantation was done in present study. Conjunctival limbal autograft was appeared to be effective in preventing recurrence of pterygium.

In present study we used preserved Amniotic membrane as a graft to cover the conjunctival wound it provides a substrate for the migration of conjunctival epithelial cells. The advantages of AMT include superior postoperative cosmeses and absence of donor site morbidity.

The AM, being continuously moistened by tears, provides adequate hydration to the regenerating epithelium and protects it from the abrasive effect of an abnormal palpebral conjunctiva.

AMT shows promise in all pterygium cases for the restoration of the ocular surface and reduction of inflammation. Repeatability and freedom from intraocular intervention makes it an attractive surgical option. Amniotic Membrane Transplantation (AMT) using preserved Amniotic Membrane Graft is an effective and safe procedure for pterygium surgery.

In present study, it is concluded, although AMT preserved the superotemporal conjunctiva and easy procedure, it has high recurrence rate. Conjunctival limbal autografts are technically demanding and time consuming, but they are more effective in preventing recurrences when compared with Amniotic membrane graft.

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Date of Submission: 17/01/2014.
Date of Peer Review: 18/01/2014.
Date of Acceptance: 15/03/2014.
Date of Publishing: 08/04/2014.