The Efficacy of Human Amniotic Membrane Transplantation in the Management of Different Ocular Surface Disorders

Dr Veena Pilli¹, Dr Deepika²

¹MBBS, MS, Visakhapatnam
²DO, DNB, MS, Visakhapatnam

¹Asst. Prof. of ophthalmology, GIMSR, Gitam University, Visakhapatnam, 2. Sankar foundation eye hospital, Visakhapatnam

Abstract: **Aim:** To study the efficacy of human amniotic membrane transplantation in the management of different ocular surface disorders. **Study centre:** It is a single centre study conducted at Sankar Foundation Eye Hospital, Tertiary eye care center, Visakhapatnam. **Type of study and design:** Prospective : Non-randomized, interventional study. **Study Population:** All the patients, who were diagnosed with various ocular surface disorders and not responding to maximum medical treatment, and needed ocular surface reconstruction/surgical treatment were selected for the present study by applying the spectrum of ocular inclusion and exclusion criteria. **Sample Size:** Sixty two patients with OSD of various etiologies, who underwent AMT, were included in this study which was done from May 2014 to June 2015. **Inclusion Criteria:** Primary progressive pterygium with 2 mm or more infiltration of cornea, recurrent pterygium, Chemical injury, Neurotrophic corneal ulcer, Systemic mucocutaneous disorders, Symptomatic bullous keratopathy, Persistent epithelial defect, Ocular neoplasia, Moorsen ulcer. **Exclusion Criteria:** Infective ulcers, Minimal defect due to injury or mucocutaneous lesions tending to heal with medical management. **Methodology:** A clearance from the Institutional Research Board of Sankar Foundation Eye Hospital and Institute of Ophthalmology was obtained prior to the commencement of the study. Participants were enrolled after prior informed consent. **Study Definitions:** All patients were examined on postoperative day 1, then at the end of the first week, second week, and monthly thereafter. The subjective symptoms of patients, such as pain, were recorded at every follow-up examination. Pain was evaluated using a 5-point scale from Lim-Bon-siong et al.5. A total of 62 patients with different ocular surface disorders(fig10) were operated with AMT during our study period. The outcome following AMT in different ocular surface disorders was studied in terms of relieving pain, irritation, and ocular discomfort, reducing inflammation of the ocular surface, in promoting epithelialization and preventing recurrence. In this study, AMT was performed most commonly for primary pterygium (n=32, 51.6%) followed by chemical injury. Ocular surface neoplasia and persistent epithelial defects not responding to conventional treatment underwent AMT. Bullous keratopathy and PBK were less commonly opted for AMT. **Results:** Majority of the patients (37.09%) in the preoperative period have a best corrected visual acuity of 6/36 to 6/18 whereas more than half of the patients (58.06%) have a best corrected visual acuity of 6/12 or better in the post operative period. Only one patient of chemical injury, in the preoperative period had a best corrected visual acuity of PL+ PR accurate which improved to 5/60 following AMT. 3 patients (4.83%) had a visual acuity of HM to CFCF preoperatively, 2 of them had chemical injury and one patient with psudohallic bullous keratopathy. More than half of the eyes (64.5%) had a BCVA of 6/36 or better preoperatively, which indicates that ocular surface disorders can affect vision to a lesser extent. Only 2 patients had BCVA of CFCF. following AMT in our study. 86% of the patients had no pain, postoperatively after one month. **Complications:** Significant reduction of symptoms like ocular pain, is observed in cases of PED, Mooren’s ulcer, chemical injury and PBK. There is moderate improvement in visual acuity following AMT for different indications. It is observed that the recurrence rate in primary pterygium is less than that in recurrent pterygium cases following AMT. In cases with chemical injury, AMT relieves pain, suppresses inflammation, promotes epithelialization, and prevents tissue necrosis with scarring sequelae and resulting vision loss. In cases with PED and Mooren’s ulcer not responding to medical treatment, it reduces pain and inflammation and it promotes epithelialization and healing. In cases with Ocular surface neoplasia undergoing excision biopsy, AMT covers the bare sclera and acts as a shield and prevents stromal melting. In PBK cases, it causes significant and rapid healing from pain. In severe cases of OCP, AMT combined with lamellar patch graft is more successful than AMT alone.

Keywords: AMG, Pterygium, Chemical injuries, Ocular surface diseases

1. Introduction

The management of patients with severe ocular surface disease has always been a challenge for ophthalmologists. Many times, these ocular surface disorders (OSD) are refractory to medical treatment and surgical interventions such as lamellar keratoplasty and therapeutic penetrating keratoplasty (TPK), to prevent corneal scarring, thinning, perforation or extension of infection and loss of vision. Although keratoplasty might eliminate residual pathology and preserve globe integrity, it depends on the availability of good donor cornea.

The rediscovery of the virtues of amniotic membrane has provided another surgical tool for restoring the damaged milieu in severely damaged ocular surface disorders. Amnion alone does not restore normal ocular surface epithelium. Residual islands of stem cells or transplanted limbal stem cells are more successful in covering the cornea surface in presence of amnion membrane.

2. Mechanism of Action of Amniotic Membrane

AM has several properties that renders it extremely useful in ocular surgery:

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Promotes Epithelialisation

- The presence of a normal substrate in the cornea is essential for normal proliferation and differentiation of epithelial cells.
- AM acts like a basement membrane and facilitates the migration of epithelial cells.
- It reinforces adhesion of basal epithelial cells, promote epithelial differentiation, prevents epithelial apoptosis, and improves corneal sensitivity and tear film stability, although the exact mechanism is not known.
- Amniotic membrane produces various growth factors such as basic fibroblast growth factor, hepatocyte growth factor, and transforming growth factor α, that can stimulate epithelialisation.

2) Inhibits Fibrosis:

Several factors are involved in the antifibrotic effect of the amniotic membrane.
- It has been shown that amniotic membrane induces a downregulation of transforming growth factor β1, responsible for fibroblastic activation in wound healing.
- The amniotic membrane may also function as an anatomical barrier, keeping the potentially adhesive surfaces apart.
- The stroma of the amniotic membrane is normally avascular and is believed to inhibit the incursion of new vessels.

3) Antiinflammatory and Antiangiogenic Factors

- There are several reports of reduction of inflammation with AM. The exact mechanism is not known.
- The AM probably acts as a barrier against tear film resulting in reduced amount of inflammatory mediators.
- Tissue inhibitors of metalloproteinase inhibitors (TIMPS); interleukin (IL)-10, IL-1 receptor antagonist (Anti-inflammatory factors) along with endostatin (inhibits endothelial cell proliferation, angiogenesis and tumour growth) are present in HAM.
- IL-α, IL-1β, highly potent proinflammatory cytokines are also suppressed by AM stromal matrix. In addition Presence of proteinase inhibitors may promote healing.

4) Antimicrobial and Antiviral Properties:

AM has anti microbial properties that decrease risk of postop infection. It also contains cystatin E, analogue of cysteine proteinase inhibitors, which has complimentary antiviral properties.

5) Non Immunogenic:

AM doesn’t express HLA-A,B/DR antigens and hence doesn’t undergo rejection.

3. Action Mechanisms and Observed Effects of Amniotic Membrane Transplantation

3.1 Action Mechanisms

1) Prolong life span and maintain clonogenicity of epithelial progenitor cells
2) Promote non-goblet cell epithelial differentiation
3) Promote goblet cell differentiation when combined with conjunctival fibroblasts
4) Exclude inflammatory cells with anti-protease activities
5) Suppress TGFβ signaling system and myofibroblast differentiation of normal fibroblasts

3.2 Observed Clinical Effects

1) Facilitate epithelialization
2) Maintain normal epithelial phenotype
3) Reduce inflammation
4) Reduce vascularization
5) Reduce scarring

3.3 Surgical Technique

The main objectives of AMT are ocular surface reconstruction, promotion of epithelialization, providing symptomatic relief and reducing inflammation. Informed consent is obtained from each patient before surgery.

After general, peribulbar or topical anesthesia the diseased tissue is excised (depending on indications such as tumors, pterygium excision and symblepharon) or surface debrided (Persistant epithelial defects (PED), shield ulcer of vernal keratoconjunctivitis and bullous keratopathy).

Bleeders are cauterized with bipolar cautery and AM (Basement Membrane up) is sutured in place with 10-0 monofilament nylon, circumferential interrupted sutures on the corneal side.

Interrupted 8-0 polyglactin sutures anchor the AM to the conjunctival edge. The specific surgical technique is tailored depending on the clinical situation. In patients with pterygium, the head is lifted off the corneal surface by blunt dissection and trimmed from the rest at 3–5 mm from the limbus at its body. A thorough removal of subconjunctival fibrous tissue then is performed in an area much greater than the pterygium body itself. The epithelial defect (corneal as well as conjunctival) is measured. The defect is covered with amniotic membrane graft (AMG) For symblepharon the surgical goals include excision of all inflamed ocular surface epithelium and subconjunctival fibrous tissue, release of symblepharon, fornix formation, AMT and insertion of a symblepharon ring. There are three basic principles upon which the final technique is individualized.
1) **Inlay or Graft Technique**
When the AMG is tailored to the size of the defect and is meant to act as a scaffold for the epithelial cells\(^6\), which then merges with the host tissue, it is referred to as a graft. The AM is secured with its basement membrane or epithelial side up to allow migration of the surrounding epithelial cells on the membrane. Fig 1.

![Figure 1: Inlay or Graft Technique\(^7\)](image1.png)

**Figure 1**: Inlay or Graft Technique\(^7\)

Figure 1 Depicts amniotic membrane sutured to the cornea and covering a paracentral corneal epithelial defect.

2) **Overlay Technique**
The amniotic membrane is sutured to perilimbal episclera and to the edge of the conjunctiva (after peritomy) covering the whole corneal surface. Entire corneal surface including the limbus is covered with the amniotic membrane graft. Fig 2. Here the amniotic membrane functions primarily as a biological contact lens (BCL). The graft protects regenerating epithelium from the frictional forces of the eyelid and palpebral conjunctiva while at the same time appears to allow adequate oxygen permeability and moisture to the epithelium. Corneal transparency remains when the graft eventually detaches or dissolves. This method has been used successfully in cases of stem cell deficiency of various causes and in cases of persistent epithelial defect unresponsive to medical therapy and surgically induced epithelial defects. Gris, et al. propose that this method may be a safe alternative that some patients may prefer over tarsorrhaphy when medical treatment has failed.

![Figure 2: Overlay Technique\(^8\)](image2.png)

**Figure 2**: Overlay Technique\(^8\)

3) **Filling-In Or Layered Technique**
In this technique the entire depth of an ulcer crater is filled with small pieces of AM trimmed to the size of the defect. A larger graft is sutured to the edges of the defect in an inlay fashion and an additional patch may help in preserving the deeper layers for a longer duration. Its indicated in deep stromal ulcers.\(^11\) The entire depth of the crater is filled with small bits of AM\(^12\).

4) **Multilayered Graft**
Indicated in ulcers of cornea and sclera, it is similar to fill in technique, but here multiple layers of AM are used, one over the other.

4. **AMG Orientation**

The preferred surgical orientation of the AM on the ocular surface is with the epithelial side up. The stromal surface (fig; 3, 4) can be identified by the presence of vitreous-like strands that can be raised with a sponge. Intraoperative staining with lissamine green B may be a simple and effective way to assist surgeons in the proper handling of AM.

![Figure 3: Components and orientation of preserved human AM\(^9\)](image3.png)

**Figure 3**: Components and orientation of preserved human AM\(^9\)
5. Surgical Indications of Amniotic Membrane Transplantation

1) As A Graft for Conjunctival
   - Pterygium
   - Bulbar Conjunctival Reconstruction
   - Symblepharon Lysis
   - Conjunctivochalasis
   - Bleb Leakage or Revision
   - Scleral Melt
   - Lid Reconstruction
   - Orbit Reconstruction

2) As A Graft for Corneal Diseases
   Persistent Corneal Epithelial Defect with or without Ulceration
   - Partial Limbal Stem Cell Deficiency
   - Total Limbal Stem Cell Deficiency (with Limbal Transplantation)
   - For Chemical burns,
   - Stevens–Johnson Syndrome
   - Painful Bullous Keratopathy with Erosion
   - Band Keratopathy

3) As A Patch
   - Acute Stage of Chemical or Thermal burns, Stevens–Johnson Syndrome
   - Preventing Scar after PRK or PTK
   - Refractory or Recalcitrant Inflammatory or Ulcerative Keratitis: HSV, HZO, and Vernal keratoconjunctivitis.

4) As A Carrier for Expanding Epithelial Stem Cells Ex Vivo.

Corneal Surface Reconstruction
Non-absorbable sutures are used to anchor AMGs to the cornea. A single sheet of AM may be applied as an inlay graft or overlay patch and anchored to the superficial cornea with multiple interrupted 10-0 nylon monofilament sutures. A Weckcel sponge or blade is used to remove all cellular debris or exudates from the base of the defect in case of PEDs, shield ulcers and ulcerative keratitis. Loose epithelium surrounding an epithelial defect or over an area of bullous keratopathy is debrided using a fine forceps and a straight crescent blade. The size of the graft should be at least 1 mm larger than the defect. The sutures must be placed circumferentially or parallel to the cut edge of the graft in an interrupted or continuous manner. The suture knots must be cut short and knots buried in corneal tissue. If AM is used to fill in deep corneal ulcers, descemetocoeles or perforations, a multilayered approach is preferred. Small pieces of AM may be layered into the defect or a single sheet may be folded on itself twice (blanket fold). In either case a larger patch is anchored over the entire defect in an overlay fashion.

Conjunctival Surface Reconstruction
Vicryl sutures are used to anchor AM to the conjunctiva. Given the rapid healing ability of the conjunctiva, 8-0 or, 9-0 or 10-0 vicryl may be used for this purpose. The essence of the surgical technique in each of the indications is adequate dissection and removal of pathological subconjunctival tissue. In order to anchor a sheet of AM to the fornix two sets of double armed 4-0 chromic cat gut sutures on a cutting needle may be used. The needles are passed from the AM surface through the inferior fornix, via the full-thickness of the eyelid and exit through the eyelid skin. The two needles of each of the two sets of sutures are passed through two segments of an encircling band and then tied.

Ocular Surface Reconstruction
Extensive ocular surface damage seen in severe grades of chemical injury, Stevens Johnson syndrome (SJS) and ocular cicatricial pemphigoid warrants sequential surface reconstruction. It is important to ensure that all fibrotic tissue is meticulously dissected and removed from the corneal and conjunctival surfaces. The AMG must be a continuous sheet devoid of buttonholes. The lower lid is everted with a large chalazion clamp. A large sheet of AM is placed on the ocular surface and it is first anchored to the inner surface of the everted lower lid close to the lid margin using multiple interrupted 10-0 vicryl sutures. The anchorage to the inferior fornix is as described above.

A continuous encircling 10-0 nylon suture is used to anchor the membrane at the limbus or the peripheral 360° cornea. In addition, multiple interrupted vicryl sutures are placed to attach the membrane to the inner lid surface, beyond the inferior fornix and onto the bulbar conjunctiva.

Postoperative Care
A large hydrophilic bandage contact lens may be placed after surgery. Topical steroids and antibiotics are used until epithelialization is complete and inflammation subsides. The translucent membrane enables observation of the healing epithelial defect beneath it. In the presence of excessive inflammation, it disintegrates faster and may have to be repeated several times. Treating the AM with glutaraldehyde in vitro crosslinks the AM, increasing its stiffness and resistance to degradation.
6. Aim

To study the efficacy of human amniotic membrane transplantation in the management of different ocular surface disorders.

7. Objectives

To evaluate the efficacy of amniotic membrane-
- In reducing ocular symptoms
- In promoting epithelialization
- In minimizing inflammation of the ocular surface
- In preventing recurrence

8. Materials & Methods

**Study centre:** It is a single centre study conducted at Sankar Foundation Eye Hospital, Visakhapatnam

**Type of study:** Prospective study.

**Study design:** Non-randomized, interventional.

**Study Population:** All the patients, who were diagnosed with various ocular surface disorders and not responding to maximum medical treatment, and needed ocular surface reconstruction/surgical treatment were selected for the present study by applying the spectrum of ocular inclusion and exclusion criteria.

**Sample Size:** Sixty two patients with OSD of various etiologies, who underwent AMT, were included in this study which was done from May 2014 to June 2015

**Inclusion Criteria**

**Cases in which AMT Procedure done**

- Primary progressive pterygium with 2 mm or more infiltration of cornea

The pterygia were graded during the examination, according to the grading system used by Tan et al\(^{13}\)

Type 1-atrophic (type 1),

Type 2- minimal inflammation (type 2), and

Type 3- moderate/severe inflammation or showing active growth.

- Recurrent pterygium.
- Chemical injury
- Neurotrophic corneal ulcer,
- Systemic mucocutaneous disorders
- Symptomatic bulous keratopathy
- Persistant epithelial defect
- Ocular neoplasia
- Moorens ulcer

**Exclusion Criteria**

- Infective ulcers
- Minimal defect due to injury or mucocutaneous lesions tending to heal with medical management.

9. Methodology

A clearance from the Institutional Research Board of Sankar Foundation Eye Hospital and Institute of Ophthalmology was obtained prior to the commencement of the study. A predesigned pro forma was used for data recording of all the participants and follow up. Participants were enrolled after prior informed consent.

Preoperatively, detailed medical history was obtained including the presence of systemic diseases like diabetes mellitus or other collagen vascular diseases. Complete ophthalmic examination including visual acuity, intraocular pressure, slit lamp examination, dry eye assessment, adnexa evaluation and fundoscopy were performed.

The patient was prepared for surgery. A peribulbar anaesthesia was given. Amniotic membrane transplantation done in the selected patients using processed and preserved deepithalized amniotic membrane obtained from an eye bank which was stored in Dulbecco’s medium. The ocular surface prepared by peeling vascular membrane on corneal surface or excision biopsy in case of ocular surface neoplasia or releasing symblepharon or de epithelizing the cornea in moorens ulcer

The amniotic membrane was gently separated from the nitrouscelleulose paper with blunt tipped forceps. The membrane was then placed on the diseased part of ocular surface. The techniques appropriate to the particular case was performed, and the membrane was placed on the cornea to cover the defect and excess of AM was trimmed and was glued or sutured with interrupted 10-0 nylon on the cornea and 8-0 vicryl was used to suture onto sclera whenever required.

Postoperatively, antibiotic -steroid combination eye drops administered six times a day and lubricating eye drops eight to ten times a day along with supportive treatment of Vitamin C 2000mg per day orally Vit B12 1500ug/day. Patients were examined on first few consecutive days until epithelial healing. All patients were followed up on 1, 3, and 7 days and at 1, 3, and 6 months after the surgery. In each visit, visual acuity (VA) and tonometry with non contact tonometer were checked. Slit-lamp examination was performed and a digital anterior segment photograph was taken to monitor graft attachment, carefully looking for any evidence of graft retraction, graft dehiscence, recurrence, chemosis, or development of complications such as infection, graft loss, pyogenic granuloma, inclusion cysts, epithelial defects, dellen formation, scleral thinning and excessive photophobia.

10. Study Definitions

Assessment of surgical outcome was determined by patients’ symptoms (particularly pain, discomfort, and irritation), inflammation of the cornea and amniotic graft, healing time of the area covered by the membrane, and cosmetic appearance. Fluorescein staining was used to detect epithelial defects. All patients were examined on postoperative day 1, then at the end of the first week, second week, and monthly thereafter. The
subjective symptoms of patients, such as pain, were recorded at every follow-up examination. Pain was evaluated using a 5-point scale from Lim-Bon-siong et al. as follows:

0, was none, no pain;
1, very mild, presence of pain but easily tolerated;
2, mild, pain causing some discomfort;
3, moderate, pain that partially interferes with usual activity or sleep;
4, severe, pain that completely interferes with usual activity or sleep.

Delayed corneal epithelialisation is defined as a corneal epithelial defect that failed to heal within 7 days of surgery. Recurrence in case of Pterygium was defined as any fibrovascular growth of conjunctival tissue extending more than 1.5 mm across the limbus. Recurrence in other diseases is the occurrence of the primary lesion at the operated area. Surgical success was defined as complete epithelialisation of the ocular surface at the operated site as demonstrated by biomicroscopy and negative fluorescein staining and without any recurrence at the same area within 6 months of followup. Failure was defined as incomplete epithelialization, or recurrence of the pterygium or vascularisation of cornea.

**Data Collection techniques**

Primary data is collected from patients by direct questionnaire and data entered into the study proforma. Secondary data is collected from literature in the journals, articles from internet. Historical data and symptoms were recorded by verbal interviews. Visual acuity is recorded by Snellen's chart. Slit-lamp examination with fluorescein staining of cornea, drawing and recording of ulcer or lesion or affected area size, and followup photographs were the measures taken to observe changes like epithelial healing, decrease in density of stromal haze. Same measures helped to record evidence of complications like corneal vascularization, graft retraction, recurrence of primary disease and microbial infection. These observations helped to assess success of the procedure.

**11. Analysis**

**DATA RECORDING**

The results were recorded in tabulated form in Microsoft Excel 2013. Charts and graphs were prepared and analyzed by using IBM SPSS statistics version 20.0 for Microsoft and Microsoft Word 2013.

**Descriptive Analysis**

Descriptive statistics (mean, standard deviation, and range) were calculated for all parameters. Data presentation tools bars, pie charts and cross tabulations. Statistical techniques – hypothesis are formulated and tested for significance using z test (1 sample/2 sample test), Chi-square test A total of 62 patients with different ocular surface disorders (Table 1) were operated with AMT during our study period. The outcome following AMT in different ocular surface disorders was studied in terms of relieving pain, irritation, and ocular discomfort, reducing inflammation of the ocular surface, in promoting epithelialization and preventing recurrence. A total of 62 patients with different ocular surface disorders (Fig 10) were operated with AMT during our study period. The outcome following AMT in different ocular surface disorders was studied in terms of relieving pain, irritation, and ocular discomfort, reducing inflammation of the ocular surface, in promoting epithelialization and preventing recurrence. In this study, AMT was performed most commonly for primary pterygium (n=32, 51.6%) followed by chemical injury. Ocular surface neoplasia and persistent epithelial defects not responding to conventional treatment underwent AMT. Bullous keratopathy and PBK were less commonly opted for AMT. Majority of the patients come fall in to 40-60 years age group in our study. More than half of the patients (66.6%) belong to this group. Four patients are below 20 years age group those were among the operated. Around 20% of patients are between 21 – 40 years. Around 1/4th of the patients are above 60 years in our study who underwent AMT. Most of the patients are in the economically productive age group of 40-60 years followed by 20-40 years (Table 2). These age groups more prone for industrial accidents like chemical injuries and also increased risk of pterygium because of increased outdoor activity.

**Table 1: Different ocular surface disorders**

<table>
<thead>
<tr>
<th>Indications for AMG</th>
<th>Number of Eyes</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Primary Pterygium</td>
<td>32</td>
<td>51.61%</td>
</tr>
<tr>
<td>Recurrent Pterygium</td>
<td>3</td>
<td>4.8%</td>
</tr>
<tr>
<td>Chemical Injuries</td>
<td>11</td>
<td>17.4%</td>
</tr>
<tr>
<td>OSSN</td>
<td>9</td>
<td>14.51%</td>
</tr>
<tr>
<td>PED</td>
<td>5</td>
<td>8.06%</td>
</tr>
<tr>
<td>OCP</td>
<td>1</td>
<td>1.6%</td>
</tr>
<tr>
<td>PBK</td>
<td>1</td>
<td>1.6%</td>
</tr>
<tr>
<td><strong>TOTAL</strong></td>
<td><strong>62 EYES</strong></td>
<td><strong>100%</strong></td>
</tr>
</tbody>
</table>

The mean age of patients in the study population is 49 with a standard deviation of 14. Among the 62 patients operated, 32 patients were males and 30 patients were females. Ocular surface disorders can affect both males and females equally without any gender preference. While chemical injuries are

**Table 2: Age distribution**

<table>
<thead>
<tr>
<th>Age</th>
<th>Number</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt; 20 YEARS</td>
<td>4</td>
<td>6.45%</td>
</tr>
<tr>
<td>20-40 YEARS</td>
<td>10</td>
<td>16.12%</td>
</tr>
<tr>
<td>40-60 YEARS</td>
<td>40</td>
<td>64.51%</td>
</tr>
<tr>
<td>&gt;60 YEARS</td>
<td>8</td>
<td>1%</td>
</tr>
<tr>
<td><strong>TOTAL</strong></td>
<td>62</td>
<td><strong>100%</strong></td>
</tr>
</tbody>
</table>

The mean age of patients in the study population is 49 with a standard deviation of 14. Among the 62 patients operated, 32 patients were males and 30 patients were females. Ocular surface disorders can affect both males and females equally without any gender preference. While chemical injuries are
more common in males, disorders like pterygium and PED’s can affect both sexes. Among the patients operated for pterygium, majority are females because of increased farm work and outdoor activity.

All the patients who underwent AMT for chemical injuries are male, as they are mostly due to industrial accidents. Right eye was operated in 28 patients (45.16%) and remaining 34 (54.84%) patients were operated on left eye. There is no statistical significance between the right eye and left eye that was operated.

**Pre-Operative BCVA:** Majority of the patients (37.09%) in the preoperative period have a best corrected visual acuity of 6/36 to 6/18 whereas more than half of the patients (58.06%) have a best corrected visual acuity of 6/12 or better in the post operative period. Only one patient of chemical injury, in the preoperative period had a best corrected corrected visual acuity of PL+ PR accurate which improved to 5/60 following AMT. 3 patients (4.83%) had a visual acuity of HM to CF CF preoperatively, 2 of them had chemical injury and one patient with pseudophakic bullous keratopathy. More than half of the eyes (64.5%) had a BCVA of 6/36 or better preoperatively, which indicates that ocular surface disorders can affect vision to a lesser extent.

**Best Corrected Visual Acuity**

| Table 3, Fig8 |
Post-operative BCVA-(fig 9) Majority of patients (n=35, 58.06%) had a BCVA of 6/12 or better postoperatively. 85.2% of patients had a BCVA of 6/36 or better. Only 2 patients had BCVA of CFCF. One patient among the two has PBK for which the main purpose of performing AMT was pain relief and another patient had severe chemical injury in which AMT was not successful. Among the 15 eyes which showed no improvement in visual acuity, 5 eyes had a BCVA of 6/6 preoperatively. Four of them with OSSN and one eye with pterygium. Majority of the eyes had a 2 or 3 line improvement of BCVA. 13 eyes each showed an 2 or 3 line improvement.

Outcome of AMT in Pterygium Surgery
Thirty two eyes (86.7%) had primary and 3 recurrent pterygia (13.3%). The sex and age distribution of the patients that had pterygium surgeries are presented in Table 4

<table>
<thead>
<tr>
<th>Table 4: Sex and age distribution in pterygium group</th>
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<tbody>
<tr>
<td>Age Group</td>
</tr>
<tr>
<td>21-40 years</td>
</tr>
<tr>
<td>41-60 years</td>
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<tr>
<td>&gt;60 years</td>
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</table>

In this study, the mean age of the patients was 53.74, SD±12.01. This is similar to those of other studies. This may be a reflection of the active years when most people are involved in outdoor activities that exposes them to actinic degenerative changes on the conjunctiva. Table5, Grade 3 pterygium accounted for most of the cases (54.3%) followed by Grade 2 (37.14%) and Grade 1 (8.6%).

<table>
<thead>
<tr>
<th>Table 5: Grading of Pterygium</th>
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<tr>
<td>GRADE of Pterygium</td>
</tr>
<tr>
<td>GRADE 1</td>
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<tr>
<td>GRADE 2</td>
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<tr>
<td>GRADE 3</td>
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</table>

Preoperatively, best-corrected visual acuity for most patients (19) was between 6/12 to 6/36 Two or more lines of visual improvement occurred in 19 patients, visual improvement was less than 2 lines in 8 patients. No significant change in BCVA was seen in the remaining cases.

Following AMT, the improvement in visual acuity observed is shown in Table 6

<table>
<thead>
<tr>
<th>Table 6: Visual Improvement Following AMT in Pterygium Patients</th>
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<tbody>
<tr>
<td>No. of lines of improvement</td>
</tr>
<tr>
<td>0</td>
</tr>
<tr>
<td>1</td>
</tr>
<tr>
<td>2</td>
</tr>
<tr>
<td>3</td>
</tr>
<tr>
<td>4</td>
</tr>
<tr>
<td>5</td>
</tr>
<tr>
<td>6</td>
</tr>
<tr>
<td>TOTAL</td>
</tr>
</tbody>
</table>

- Two or more lines of visual improvement occurred in 19 patients (54.98%)
- Visual improvement is less than 2 lines in 8 patients (22.85%).
- No significant change in BCVA was seen in the remaining 8 (22.85%).

From this study, it can be observed the AMT following excision of the pterygium, improves visual acuity by at least two lines in at least 50% of patients with progressive pterygium. (Z test of Proportions 1 – sample test, α = 0.05).

Outcome following pterygium excision and AMT in terms of symptomatic improvement, epithelialization and recurrence is shown in Table 7.

<table>
<thead>
<tr>
<th>Table 7: DURN * RESULT * DIAGNOSIS CROSSTABULATION</th>
</tr>
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<tbody>
<tr>
<td>Diagnosis</td>
</tr>
<tr>
<td>PPP Day 1</td>
</tr>
<tr>
<td>Week 1</td>
</tr>
<tr>
<td>Month 1</td>
</tr>
<tr>
<td>Month 3</td>
</tr>
<tr>
<td>Month 6</td>
</tr>
<tr>
<td>RP Day 1</td>
</tr>
<tr>
<td>Week 1</td>
</tr>
<tr>
<td>Month 1</td>
</tr>
<tr>
<td>Month 3</td>
</tr>
<tr>
<td>Month 6</td>
</tr>
</tbody>
</table>

On the postoperative day, 65.62% patients had ocular pain of grade 1 or less. 7 patients had pain and irritation of grade 2 and 4 patients of grade 3. Postoperatively, all transplanted Amniotic membranes maintained their graft integrity and were
well retained. In all eyes, complete conjunctival epithelialization over the transplanted AM was achieved within 1–2 weeks. The surrounding conjunctival inflammation began to gradually subside and slit-lamp examination confirmed that conjunctival fibrosis was successfully suppressed in all patients. On the first postoperative day, 8 patients had corneal epithelial defects. By one week, all epithelial defects healed completely and there was no conjunctival staining with fluorescein. One patients had PED and scleral melt which required repeat AMG at 2 weeks and healed well on follow up. The recurrence rate in primary pterygium was 9.37% (3 of 32 cases) over a minimum follow-up period of 6 months. No other adverse effects or complications occur throughout the study period. One patient (3.12%) developed amniotic membrane retraction and pigment epithelial defect and complained of progressive photophobia, pain and foreign body sensation which did not respond to medical therapy and finally required a second amniotic membrane transplantation leading to complete resolution. One patient (3.12%) developed corneal dellen with mild thinning which responded to medical therapy and resolved completely. In 3 cases of recurrent pterygium, who underwent AMT, one case (33.3%) developed recurrence at 3 months. None of the patients developed corneal ulcer, scleral melting, conjunctivitis, dellen, hypersensitivity to fibrinadhesives, symblepharon formation. we encountered no significant intra- or postoperative complications at the transplantation site and none of the 36 patients suffered loss of visual acuity.

### Outcome of AMT in Chemical Burns

Eleven patients with limbal stem cell deficiency caused by ocular chemical injury were included in the study. They were divided into 2 groups according to the severity of limbal deficiency: **Group 1** (partial limbal deficiency [PLD]), composed of eight eyes, which received AMT alone, **Group 2** (total limbal deficiency [TLD]), composed of three eyes, which received AMT and conjunctival and limbal stem cell transplantation. They were divided into above two groups based on clinical examination of persistent epithelial defects or completely keratinized epithelium accompanied by an absence of palisades of Vogt. The patients’ profiles are summarized in Tables 8 and 9.

### Table 8: Preoperative Characteristics of Patients with a Chemical Burn and Partial Limbal Deficiency

<table>
<thead>
<tr>
<th>S.No</th>
<th>MR No</th>
<th>AGE/SEX</th>
<th>Duration</th>
<th>Type of injury</th>
<th>Stroma</th>
<th>Schirmer 1</th>
<th>Fluorescein</th>
<th>Pre-op BCVA</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>41904</td>
<td>48/M</td>
<td>1 week</td>
<td>Acid</td>
<td>Corneal epithelial defect with stomal thinning</td>
<td>8</td>
<td>+</td>
<td>6/18</td>
</tr>
<tr>
<td>2</td>
<td>47685</td>
<td>44/M</td>
<td>5 days</td>
<td>Acid</td>
<td>Epithelial detachment</td>
<td>6</td>
<td>+</td>
<td>6/18</td>
</tr>
<tr>
<td>3</td>
<td>41434</td>
<td>21/M</td>
<td>1 week</td>
<td>alkali</td>
<td>Limbal ischemia&gt;270, necrose corneal epithelium</td>
<td>5</td>
<td>+</td>
<td>HM+</td>
</tr>
<tr>
<td>4</td>
<td>32456</td>
<td>34/M</td>
<td>1 year</td>
<td>alkali</td>
<td>Symblepharon of the lower lid</td>
<td>12</td>
<td>=</td>
<td>6/24</td>
</tr>
<tr>
<td>5</td>
<td>38919</td>
<td>30/M</td>
<td>3 weeks</td>
<td>alkali</td>
<td>Inferior corneal opacity with ectasia with symblepharon</td>
<td>8</td>
<td>-</td>
<td>CFCF</td>
</tr>
<tr>
<td>6</td>
<td>44698</td>
<td>19/M</td>
<td>4 days</td>
<td>Acid</td>
<td>Corneal epithelial defect</td>
<td>10</td>
<td>+</td>
<td>6/12</td>
</tr>
<tr>
<td>7</td>
<td>39476</td>
<td>52/M</td>
<td>1 week</td>
<td>Acid</td>
<td>Epithelial detachment</td>
<td>8</td>
<td>+</td>
<td>3/60</td>
</tr>
<tr>
<td>8</td>
<td>42341</td>
<td>35/M</td>
<td>2 weeks</td>
<td>Acid</td>
<td>Congestion, corneal edema</td>
<td>12</td>
<td>+</td>
<td>6/36</td>
</tr>
</tbody>
</table>

### Table 9: Preoperative Characteristics of Patients with Chemical Burn and Total Limbal Deficiency

<table>
<thead>
<tr>
<th>S.No</th>
<th>MR No</th>
<th>AGE/SEX</th>
<th>Duration</th>
<th>Type</th>
<th>Stroma</th>
<th>Schirmer 1</th>
<th>Fluorescein</th>
<th>Pre-op BCVA</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>29898</td>
<td>19/M</td>
<td>1 year</td>
<td>Alkali</td>
<td>LSCD with conjunctivalisation of cornea</td>
<td>7</td>
<td>-</td>
<td>PL+</td>
</tr>
<tr>
<td>2</td>
<td>16154</td>
<td>24/M</td>
<td>2 years</td>
<td>Acid</td>
<td>Corneal opacity with vascularisation with pseudo pterygium</td>
<td>10</td>
<td>-</td>
<td>6/18</td>
</tr>
<tr>
<td>3</td>
<td>32567</td>
<td>31/M</td>
<td>1 year</td>
<td>Alkali</td>
<td>LSCD, corneal neovascularisation and opacity</td>
<td>10</td>
<td>-</td>
<td>6/60</td>
</tr>
</tbody>
</table>

### Table 10: Surgical Results of Ocular Surface Reconstruction with Amniotic Membrane Transplantation in Chemical Burn with Partial Limbal Deficiency

<table>
<thead>
<tr>
<th>S No</th>
<th>MR No</th>
<th>Pre op V/A</th>
<th>Post op V/A</th>
<th>Epithelialisation</th>
<th>Fluorescein</th>
<th>Result</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>41904</td>
<td>6/18</td>
<td>6/6</td>
<td>+</td>
<td>-</td>
<td>Success</td>
</tr>
<tr>
<td>2</td>
<td>47685</td>
<td>6/18</td>
<td>6/6</td>
<td>+</td>
<td>-</td>
<td>Success</td>
</tr>
<tr>
<td>3</td>
<td>41434</td>
<td>HM+</td>
<td>1/60</td>
<td>Stromal opacity with scleral necrosis</td>
<td>-</td>
<td>Failure</td>
</tr>
<tr>
<td>4</td>
<td>32456</td>
<td>6/24</td>
<td>6/6</td>
<td>+</td>
<td>-</td>
<td>Success</td>
</tr>
<tr>
<td>5</td>
<td>38919</td>
<td>CFCF</td>
<td>CFCF</td>
<td>Epithelial defect</td>
<td>+</td>
<td>Failure</td>
</tr>
<tr>
<td>6</td>
<td>44698</td>
<td>6/12</td>
<td>6/6</td>
<td>+</td>
<td>-</td>
<td>Success</td>
</tr>
<tr>
<td>7</td>
<td>39476</td>
<td>6/12</td>
<td>6/12</td>
<td>+</td>
<td>-</td>
<td>Success</td>
</tr>
<tr>
<td>8</td>
<td>42341</td>
<td>6/36</td>
<td>6/12</td>
<td>+</td>
<td>-</td>
<td>Success</td>
</tr>
</tbody>
</table>
Group 2: This group consisted of 3 patients with TLD. All subjects received AMT and conjunctival limbal autograft from the contralateral eye at the same time. Successful ocular surface reconstruction was observed in all eyes, with a mean epithelialization time of 3.4 weeks and a progressive decrease of the perilimbal inflammation and corneal vascularization. A significant visual improvement was observed in all the cases Table 11.

**Group 2: TLD - The results of this group are summarized in Table 11**

<table>
<thead>
<tr>
<th>S no</th>
<th>MR No</th>
<th>Simultaneous procedure</th>
<th>Preop V/A</th>
<th>Post op V/A</th>
<th>Epithelialisation</th>
<th>fluorocein</th>
<th>Result</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>29898</td>
<td>CLAG</td>
<td>PL+</td>
<td>5/60</td>
<td>+</td>
<td>-</td>
<td>Success</td>
</tr>
<tr>
<td>2</td>
<td>16154</td>
<td>CLAG</td>
<td>6/18</td>
<td>6/12</td>
<td>+</td>
<td>-</td>
<td>Success</td>
</tr>
<tr>
<td>3</td>
<td>32567</td>
<td>CLAG</td>
<td>6/60</td>
<td>6/24</td>
<td>+</td>
<td>-</td>
<td>Success</td>
</tr>
</tbody>
</table>

**Outcome of AMT in Ocular Surface Neoplasia**

Clinical findings and outcome of nine patients with conjunctival neoplasia following wide excision and amniotic membrane transplantation are summarized in the table-5. During the follow up, complete conjunctival epithelial healing assessed by fluorescein staining and corneal smoothness was achieved in all cases. No persistent defect, ulcer, or signs of partial or total limbal deficiency were noted. The healed corneal surface did not have blood vessels, nor did it show any late staining with fluorescein Table 12.

**Table 12: Clinical findings and outcome of Ocular surface neoplasia**

<table>
<thead>
<tr>
<th>S.no</th>
<th>MR No</th>
<th>Precursor lesion</th>
<th>location</th>
<th>Preop BCVA</th>
<th>Postop BCVA</th>
<th>epithelialisation</th>
<th>recurrence</th>
<th>Result</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>43245</td>
<td>Nevus</td>
<td>epibulbar</td>
<td>6/6</td>
<td>6/6</td>
<td>+</td>
<td>-</td>
<td>Success</td>
</tr>
<tr>
<td>2</td>
<td>32029</td>
<td>Nevus</td>
<td>epibulbar</td>
<td>6/6</td>
<td>6/6</td>
<td>+</td>
<td>-</td>
<td>Success</td>
</tr>
<tr>
<td>3</td>
<td>34891</td>
<td>OSSN</td>
<td>limbal</td>
<td>6/6</td>
<td>6/6</td>
<td>+</td>
<td>-</td>
<td>Success</td>
</tr>
<tr>
<td>4</td>
<td>52579</td>
<td>OSSN</td>
<td>epibulbar</td>
<td>1/60</td>
<td>2/60</td>
<td>+</td>
<td>-</td>
<td>Success</td>
</tr>
<tr>
<td>5</td>
<td>56389</td>
<td>OSSN</td>
<td>limbal</td>
<td>6/6</td>
<td>6/6</td>
<td>+</td>
<td>-</td>
<td>Success</td>
</tr>
<tr>
<td>6</td>
<td>53916</td>
<td>CIN</td>
<td>limbal</td>
<td>3/60</td>
<td>6/18</td>
<td>+</td>
<td>-</td>
<td>Success</td>
</tr>
<tr>
<td>7</td>
<td>41543</td>
<td>CIN</td>
<td>epibulbar</td>
<td>6/12</td>
<td>6/6</td>
<td>+</td>
<td>-</td>
<td>Success</td>
</tr>
<tr>
<td>8</td>
<td>41099</td>
<td>OSSN</td>
<td>epibulbar</td>
<td>6/12</td>
<td>6/12</td>
<td>+</td>
<td>-</td>
<td>Success</td>
</tr>
<tr>
<td>9</td>
<td>51121</td>
<td>OSSN</td>
<td>Limbal</td>
<td>6/60</td>
<td>6/24</td>
<td>+</td>
<td>-</td>
<td>Success</td>
</tr>
</tbody>
</table>

Visual improvement was evident in four patients (44.4%) who had corneal involvement before the surgery. All specimens were sent to the pathology department for histological evaluation. None of these cases was invasive to the surrounding structures. In our study we had 9 cases of ocular surface neoplasia which underwent tumour excision and the conjunctival defect was covered with AMG. Graft was taken up well with no complications or recurrence in 6 months follow up.

**Outcome of AMG in Persistent Epithelial defect (PED):** 5 patients with PED underwent AMT in this study. Criteria for recruitment were persistent corneal epithelial defect that failed to respond to conventional treatment and PED with desemetocoele or stromal thinning Table 13.

**Table 13: Outcome of AMG in Persistent Epithelial defect (PED)**

<table>
<thead>
<tr>
<th>S. No</th>
<th>Age/ Sex</th>
<th>Diagnosis</th>
<th>Asssociated disease</th>
<th>ED size (mm)</th>
<th>Preop BCVA</th>
<th>Surgery done</th>
<th>Epithelialization (weeks)</th>
<th>Postop BCVA</th>
<th>Result</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>55/M</td>
<td>Neurotrophic ulcer</td>
<td>Graft failure,post PKP</td>
<td>3</td>
<td>2/60</td>
<td>AM</td>
<td>3</td>
<td>6/60</td>
<td>S</td>
</tr>
<tr>
<td>2</td>
<td>70/F</td>
<td>Exposure keratitis</td>
<td>Lid deformity after tumour removal</td>
<td>4</td>
<td>2/60</td>
<td>AM+ Tarsoraphy</td>
<td>2</td>
<td>6/60</td>
<td>S</td>
</tr>
<tr>
<td>3</td>
<td>68/M</td>
<td>Moorens ulcer</td>
<td>-</td>
<td>10</td>
<td>1/60</td>
<td>AM 2layers</td>
<td>1</td>
<td>4/60</td>
<td>S</td>
</tr>
<tr>
<td>4</td>
<td>64/M</td>
<td>Moorens ulcer</td>
<td>-</td>
<td>5</td>
<td>6/60</td>
<td>AM 2 layers</td>
<td>1</td>
<td>6/24</td>
<td>S</td>
</tr>
<tr>
<td>5</td>
<td>45/F</td>
<td>Neurotrophic ulcer</td>
<td>Post trauma</td>
<td>4</td>
<td>6/60</td>
<td>AM</td>
<td>2</td>
<td>6/24</td>
<td>S</td>
</tr>
</tbody>
</table>

Out of the five patients,
- Two patients underwent AMG for Neurotrophic ulcer,
- Two patients for Moorens ulcer and
- One patient for exposure keratopathy.

Patients with Neurotrophic ulcer underwent single layer AMT and patients with Moorens ulcer underwent multi layered AMT technique. In Exposure keratopathy patient, tarsoraphy was performed along with AMT. All the cases showed complete epithelial healing within 3 weeks. Regarding the techniques performed, epithelial defects of the patients who underwent multilayer AMT healed significantly faster than those who underwent a single layer method. Visual improvement of three to four lines was observed in all the five cases.

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There were no complications or recurrence of the epithelial defect in the operated site during six months of follow up and it was observed that the graft was taken up well with epithelisation and had 100% success.

**Outcome of AMT in PBK**

<table>
<thead>
<tr>
<th>S.No</th>
<th>Age/Sex</th>
<th>Symptoms</th>
<th>Signs</th>
<th>Preop BCVA</th>
<th>Postop BCVA</th>
<th>Symptomatic relief</th>
<th>Epithelialization</th>
<th>Recurrence</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>66/F</td>
<td>Pain, photophobia, watering</td>
<td>Corneal edema + bullae</td>
<td>CFCF</td>
<td>CFCF</td>
<td>+</td>
<td>nil</td>
<td>3 weeks</td>
</tr>
</tbody>
</table>

Although there was no improvement in vision, it can be concluded that AMT can be performed for symptomatic relief in patients with PBK waiting for PBK.

**Outcome of AMT in OCP**

One case of OCP with symblepharon is studied. Table 15

<table>
<thead>
<tr>
<th>S.No</th>
<th>Age/Sex</th>
<th>Symptoms</th>
<th>Signs</th>
<th>Preop BCVA</th>
<th>Postop BCVA</th>
<th>Epithelialization</th>
<th>Recurrence</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>40/F</td>
<td>Pain, irritation, watering</td>
<td>Corneal epithelial defect with symblepharon lower lid</td>
<td>6/24</td>
<td>6/9</td>
<td>3 weeks</td>
<td>nil</td>
</tr>
</tbody>
</table>

Following AMT, epithelial defect healed with complete epithelialization within 3 weeks. There is improvement in visual acuity by 3 lines during the follow up for 6 months and there was no recurrence of symblepharon.

**12. Discussion**

The past two decades have witnessed the revival of amniotic membrane transplantation in ophthalmology. It has gained importance because of its ability to reduce scarring and inflammation, enhance wound healing and epithelialization and due to its anti-microbial properties. This study comprises various etiological groups with indication of AMT. Results are observed in the form of success rate of AMT. Majority of the patients in our study who underwent AMT are in the pterygium (51.6%) and chemical injury (17.4%) groups. Most of the patients belonged to the age group of 40-60 years. Mean age of the patients is 53.74±6.95 years. The sample profile is similar to a study done in Oman by Shreya Thatta (2011)\(^\text{[19]}\). In their study, majority of the patients are in the age group of 40-60 years and pterygium is the common indication for AMT. There is remarkable improvement in ocular symptoms of pain and photophobia following AMT in our study. 86% of the patients had no pain, postoperatively after one month. This increased the comfort level and improved the quality of life. This result is comparable to other studies where they reported a 90% reduction in ocular pain following AMT\(^\text{[16]}\). There was moderate improvement of visual acuity following AMT in our study. 50% of the patients showed 2 or more lines of improvement in Snellen’s visual acuity. 58.06% of the patients had a best corrected visual acuity of 6/12 or more. This is in contrast to the study conducted by Muhammad Salman Hamza et al\(^\text{[21]}\). In their study, 13.3% had a best corrected visual acuity of 6/12 while 67% of patients had a best corrected visual acuity of 6/60 or less. This can be attributed to the difference in the sample profile selected between the two studies.

**Comparison of present study and earlier studies for pterygium**- Majority of the patients with pterygium are in the age group of 40-60 years in our study. According to a study conducted by Alemwork Meseret et al\(^\text{[18]}\) prevalence of pterygium was more in middle and old age group. It typically develops in patients who have been living in hot climates and may represent a response to chronic dryness and exposure to ultraviolet light, dust and hot winds. Duke Elder\(^\text{[22]}\) observed that pterygium is more likely to occur in outdoor workers, and hence it is more common in men than in women. In the study conducted by Rao et al\(^\text{[23]}\) also prevalence is more in males. Our study had more number of females who underwent AMT for pterygium compared to males. This can be due to low socioeconomic status and increased agricultural work and outdoor activity for the females. In the present study, it is observed the AMT following excision of the pterygium, improves visual acuity by at least two lines in at least 50% of patients with progressive pterygium. In this study, in primary pterygium cases, 3 cases out of 32 (9.37%) had recurrence; but in recurrent pterygium, out of 3 cases one had recurrence (33.3%). They are started on Cyclosporine 0.05% eye drop four times a day to suppress inflammation. The recurrence rate in our study is comparable to the study conducted by Prabhasawat et al\(^\text{[24]}\). Ma et al\(^\text{[25]}\) have found that single-layered amniotic membrane was able to reduce the recurrence to 12.5%, which is comparable with our study.

**Comparison of present study and earlier studies for Chemical injury**- In this study we had 11 patients with chemical injuries for which AMT is performed. Out of 11 patients, we had 8 cases of chemical burns which presented with partial limbal stem cell deficiency. These patients showed remarkable symptomatic relief and rapid epithelialization. The
epithelialization is complete in 7 patients by 7–10 days. There is reduction in inflammation and cicatricial sequelae postoperatively. It was considered as an alternative primary procedure to limbal transplantation in these cases. Significant success rate was observed in all cases after surgery except for 2 eyes that maintained pre-operative visual acuity. One patient developed corneal stromal necrosis with scleral melt at 3 weeks which is treated with corneoscleral patch graft and repeat AMG leading to healing and scar formation. One patient who underwent AMT after 8 months of chemical injury, developed fibroblastic response two months after AMT is done. Cyclosporine eye drops 0.05% four times a day is started leading to suppression of the fibrous activity. Although limited in number, it is observed that patients with chemical injury who underwent AMT immediately within first two weeks of injury had a better success compared to those for whom AMT is done later. Although there is initial success, there is relapse of the fibroblastic response in patients for whom AMT is performed after months of injury. These patients are started on cyclosporine eye drops 0.05% to suppress inflammation. In our study, 3 patients with total limbal deficiency underwent LSCT along with AMG. Successful ocular surface reconstruction was observed in all eyes, with a mean epithelialization time of 2–3 weeks and a progressive decrease of the perilimbal inflammation and corneal vascularization is observed. There is no recurrence of corneal vascularization or cicatricial activity. Ricardo J R et al. 26 studied ten eyes which had chemical ocular burn. The epithelial defect was healed at an average of 27.8 ± 4.7 days (ranging from 20 and 35 days). They reported the efficacy of AMT in promoting epithelialization, reducing inflammation and its consequences in severe acute cases of chemical burns and stevens-johnson syndrome. Prabhawat P et al (84) reported the promotion of rapid epithelial healing and reduced complications by AMT in grade 2–4 acute chemical burns. Shimazaki et al. 25 obtained 100% surgical success in 6 cases of ocular burn using AMT associated with CLAU (four eyes) and keratolimbal allograft obtained from donor eyes (KLAL, two eyes). Although limited by the number of subjects, the results of the present study are similar to previous studies and showed that AMT was efficient for ocular surface reconstruction in cases of PLD secondary to chemical burn. It was also effective in cases of TLD, in association with LSCT. Hence it can be observed that AMT can be used to promote epithelialization and suppress the inflammation and that by preparing the eye for future penetrating keratoplasty with less chances of corneal graft failure due to reduced inflammation.

Comparison of present study and earlier studies for Ocular Surface Neoplasia- In our study we had a 9 cases of ocular surface neoplasia who underwent tumour excision with amniotic membrane transplantation. Graft is taken up well with complete epithelialization of the surgical site within an average time of 1 week. No complications or recurrence was observed after 6 months of follow up. Pardaeas et al 15 reported successful reconstruction of conjunctival surface in 3/4 eyes (75%) following the removal of malignant melanoma and primary acquired melanosis with atypia with amniotic membrane transplantation. Chen et al19 referred amniotic membrane transplantation as a very effective method to repair wound after conjunctival tumour removal in 26 patients (26 eyes) including 9 eyes with malignant tumours (conjunctival melanoma, corneal and conjunctival squamous cell carcinoma and conjunctival lymphoma), 17 eyes with benign tumours (conjunctival papilloma, conjunctival nevus, etc.). Dalla et al27 reported successful reconstruction of conjunctival surface in 4/4 patients with diffuse conjunctival melanoma after a minimum follow-up of 48 months. The success result of our study is comparable to other studies. In all the 9 cases the defect covered by amniotic membrane healed rapidly, and the resultant surface is less inflamed with minimal scarring. There was no recurrence of the primary lesion during the follow-up for 6 months.

Comparison of present study and earlier studies for Persistent Epithelial Defect- Three patients with persistent epithelial defects not responding to conventional treatment due to neurotrophic ulcer and exposure keratitis underwent AMT in our study. Following AMT the epithelial defects healed well and there is dramatic improvement of pain within 48 hours of undergoing AMT. No recurrence is seen during follow-up of 6 months. Grzetic-Lenac et al. 28 described 21 patients with corneal ulcer (n = 18) or non-healing epithelial defect (n = 3) unresponsive to conventional treatment. All patients are treated by AMT. H Chen et al. 29 studied the efficacy of amniotic membrane transplantation as an alternative treatment for neurotrophic corneal ulcers. They observed that all but four (76.4%) cases of amniotic membrane transplantation achieved rapid epithelialisation in 16.6 (9.0) days. Our study is comparable to the previous studies with success in all the 3 cases (100%) following AMT.

Comparison of present study and earlier studies for Moore’s Ulcer- Two patients with PUK due to Moore’s ulcer underwent AMT in our study. Both the cases improved symptomatically and amniotic membrane promoted epithelialization of the peripheral ulcer. There is rapid pain relief and healing of ulcer. Ngan ND, Chau HT 30 studied the outcome of surgery using amniotic membrane transplantation for Moore’s ulcer. Schallenberg M et al. 31 observed that Amniotic membrane transplantation is not able to cure severe forms of Moore’s ulcer. The results of our study although limited in number supports the role of AMT in management of Moore’s ulcer. Cases with corneal pathology in this study showed improvement in healing process and vision, reduction in inflammation and stromal haze, and good restoration of corneal surface. This healing process prevented further complications and thus delayed therapeutic keratoplasty.

Comparison of present study and earlier studies for Bullous Keratopathy- One case of PBK underwent AMT in our study. There is significant reduction of pain and photophobia following AMT. The result is similar to a study conducted by Pires RT et al 34. It is observed that epithelial defect healed rapidly and patient with intolerable pain preoperatively became pain free postoperatively.

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Comparison of present study and earlier studies for OCP-
In the present study we had one case of symblepharon due to ocular cicatricial pemphigoid (OCP). The patient underwent AMT two times with no improvement. There is corneal stromal melting >3mm with imminent perforation. Finally, lamellar patch graft with repeat AMG is done. Patient is started on oral steroids in consultation with a dermatologist. On follow up, corneal stromal melting started healing with resulting scar formation. Visual improvement is moderate. Our observation is similar to that seen in other studies.Solomon et al 32 described a 71% success rate in fornical reconstruction in a variety of conditions. Barbabino et al 33 suggested that AM may be used for ocular surface reconstruction in OCP. However, they observed that the effectiveness deteriorated over time, with 44% recurrence after 28 weeks.

13. Complications in our Study
One patient (1.5%) with Primary pterygium developed amniotic membrane retraction and complained of progressive pain and foreign body sensation. The patient did not respond to medical therapy and finally required a repeat amniotic membrane transplantation leading to complete resolution. One patient (1.5%) developed corneal dellen at second week with mild thinning which responded to medical therapy with lubricating eye drops and resolved completely. As commercially available preserved AM used, postoperative microbial infection was not seen in our study. It is observed in other series that postoperative microbial infection is very low with cryopreserved AM (1.6%), while with fresh AM it is as high as 8%. In this study, out of the sixty five patients with different ocular surface disorders for whom AMT is performed, majority of the cases (55 out of 62, 88.70%) showed success. Three cases with primary pterygium and one case with recurrent pterygium had recurrence. One case with chemical injury developed corneal stromal melt with scleral necrosis which required corneoscleral patch graft for scleral melt. Another case developed fibroblastic response and is started on Cyclosporine 0.05% eye drops. One case with OCP required lamellar patch graft along with AMT.

14. Limitations of our Study
The following limitations are present in our study-
1) Small sample size. There was no control group.
2) The sample selected is not random. All the cases of ocular surface disorder satisfying the inclusion criteria are included.
3) Single centre study.
4) Relatively less follow-up time, so long term results are not studied.
5) Sample has more number of pterygium cases compared to other cases of ocular surface diseases. So not truly representative of all the ocular surface disorders.

15. Conclusions
Our study is a prospective, single centre, non-randomized descriptive study. It included 62 eyes of 62 patients who underwent Amniotic Membrane Transplantation for different Ocular surface disorders.

Following AMT, the outcome of surgery is studied in terms of symptomatic relief, improvement of visual acuity, promotion of epithelialization and prevention of recurrence.

The main aim of using AMT is different in different ocular surface disorders. AMT is found to be effective in relieving pain in Chemical burn and Bullous keratopathy, Neurotrophic corneal ulcers and Mooren’s ulcer. It achieves healing and prevents complications like scleral/corneal melt in various corneal ulcers, after excision of ocular surface neoplasia, and pterygium. Visual improvement is moderate.

The following conclusion are drawn from the present study:
- Ocular surface disorders most commonly affect the economically productive age group of 40-60 years.
- Significant reduction of symptoms like ocular pain, is observed in cases of PED, Mooren’s ulcer, chemical injury and PBK.
- There is moderate improvement in visual acuity following AMT for different indications.
- It is observed that the recurrence rate in primary pterygium is less than that in recurrent pterygium cases following AMT.
- In cases with chemical injury, AMT relieves pain, suppresses inflammation, promotes epithelialization, and prevents tissue necrosis with scarring sequelae and resulting vision loss.
- In cases with PED and Mooren’s ulcer not responding to medical treatment, it promotes epithelialization and healing.
- In cases with Ocular surface neoplasia undergoing excision biopsy, AMT promotes healing of the defect and prevents stromal melting.
- In PBK cases, it causes significant and rapid healing of pain.
- In severe cases of OCP, AMT combined with lamellar patch graft is more successful than AMT alone.

References

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