"TO STUDY THE EFFICACY OF AMNIOTIC MEMBRANE TRANSPLANTATION IN OCULAR SURFACE DISORDERS"

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The hardest arithmetic to master is that which enables us to count our blessings.
Eric Hoffer

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LIST OF ABBREVIATIONS

AM	–	Amniotic Membrane
AMT	–	Amniotic Membrane Transplantation
LSCD	–	Limbal Stem Cell Deficiency
TGF	–	Transforming Growth Factor
HAM	–	Human Amniotic Membrane
BM	–	Basement Membrane
HIV	–	Human Immunodeficiency Virus
PAM	–	Primary Acquired Melanosis
CIN	–	Conjunctival Intraepithelial Neoplesia
PED	–	Persistent Epithelial Defect
BCL	–	Bandage Contact Lens
FCS	–	Fetal Calf Serum
PBS	–	Phosphate Buffer Saline
OSSN	–	Ocular Surface Squamous Neoplasia
TEN	–	Toxic epidermal necrosis
SJS	–	Steven Johnson syndrome
OCP	–	Ocular cicatricial pemphigoid
PRK	–	Photo refractive keratectomy
PTK	–	Photo therapeutic keratectomy

ABSTRACT

INTRODUCTION

Preserved human amniotic membrane (AM) is currently being used for a wide surface disorders of the eye. The AM has a basement membrane, which promotes epithelial cell migration and adhesion. The presence of a unique avascular stromal matrix reduces inflammation, neovascularization and fibrosis. The basic tenets of amniotic membrane transplantation (AMT) are to promote re-epithelialisation, to reconstruct the ocular surface and to provide symptomatic relief from surface aberrations. AMT is a useful technique for reconstruction of surface defects resulting from removal of surface tumors and symblepharon. AMT has effectively restored a stable corneal epithelium in eyes with persistent epithelial defects(PED) and corneal ulcers. In the setting of acute ocular burns and SJS, AMT has satisfactorily reduced scarring and inflammation. AMT alone may be an effective alternative for partial Limbal stem cell defiency(LSCD). However, remarkable improvements in surface stability have resulted from concurrent use of AMT and limbal stem cell transplantation, wherein the limbal grafts are obtained from the normal fellow eye, living relative or cadaveric eye.

MATERIALS AND METHODS

All patients attending outpatient department of ophthalmology including referred cases from KLE's PKH & MRC. Subjects for present study were selected by applying the inclusion and exclusion criteria.

RESULTS:

Among 30 eyes of different ocular surface disorders selected in the study, AMT was successful in 86.6% cases of Primary progressive pterygium . Seventy five percent of the cases of Cicatrising conjunctivitis presenting in acute stages, showed good acceptance of the graft . However immunosuppressant's played a crucial role in maintaining ocular surface integrity. In only 33.3% cases of Chemical burns, the graft showed satisfactory results. In Symblepharon cases,the traumatic symblepharon showed success with no recurrence. In the 2 cases of PED, Persistant Epethilial Defect secondary to KCS, AMT was successful. Lastly a single case of OSSN ,showed no recurrence of the growth post AMT.

INTERPRETATION AND CONCLUSION:

In this study of AMT for various ocular surface disorders were observed that there was overall success rate of 66.66 % which is comparable to earlier studies by various authors with rapid healing and reduction of ocular surface inflammation following AMT which can be explained by various mechanisms, as a result AMT becomes an attractive alternative for country like our's where there is shortage of tissues. Hence AMT is used either as a substrate or patch graft, to replace ocular surface. Lastly efficacy of AMT is better in ocular surface disorders.

KEY WORDS: Amniotic membrane Transplantation; Explant Culture; Ex-vivoExpansion; Limbal Stem Cell Deficiency; Ocular Surface Reconstruction; Persistent Epithelial Defect; Preserved Human Amniotic Membrane; Pterygium

CONTENTS

SL NO.	PARTICULARS	PAGE NO.
01	INTRODUCTION	1
02	OBJECTIVES	23
03	REVIEW OF LITERATURE	24
04	METHODOLOGY	29
05	RESULTS	32
06	DISCUSSION	42
07	CONCLUSION	52
08	SUMMARY	55
09	BIBLIOGRAPHY	56
10	ANNEXURES	
	I: PROFORMA	62
	II: INFORMED CONSENT	69
	III: PHOTOGRAPHS	74
	IV: MASTER CHART	80

LIST OF TABLES

Sl. No.	Tables	Page No.
1	Frequency distribution – Sexwise	32
2	Frequency distribution – Based on diagnosis	33
3	Distribution of surgical outcome	34
4	Outcome vis a vis Diagnosis – Cross tabulation	35
5	Primary Progressive pterygium: Distribution and Outcome	36
6	Chemical injury	36
7	Outcome of chemical burns	37
8	Distribution and outcome of Cicatrising conjunctivitis	38
9	Outcome of cicatrizing conjunctivitis	39
10	Distribution and outcome of OSSN	40
11	PED(Persistent Epithelial Defect)	40
12	Symblepharon	41
13	Symblepharon Outcome	41

LIST OF PHOTOGRAPHS

Sl. No.	Photographs	Page No.
1	Amniotic membrane being peeled from nitrocellulose sheet	74
2	Amniotic membrane stored in Dulbecco's Modified Eagle's Medium (DMEM)	74
3	Amniotic Membrane	74
4	Primary Pterygium	75
5	Pterygium excision and AMT	75
6	Chemical burns – Pre-operative photograph	76
7	First day post AMT showing well placed graft	76
8	First1week post AMT showing early disintegration of graft in grade 3 burns	76
9	Cicatrising Conjunctivitis (Steven Johnson)	77
10	Post AMT	77
11 & 12	Cicatrising Conjunctivitis (OCP)	78
13	Fluorescent staining of the epithelial defect secondary to Kerato Conjuctivitis Sicca	79
14	Symblepharon	79

LIST OF GRAPHS

Sl. No.	Graphs	Page No.
1	Frequency distribution – Sexwise	32
2	Frequency distribution – Based on diagnosis	33
3	Distribution of surgical outcome	34
4	Outcome vis a vis Diagnosis – Cross tabulation	35
5	Outcome of chemical burns	37
6	Outcome of cicatrizing conjunctivitis	39

INTRODUCTION

The management of patients with severe ocular surface disease has always been a challenge for ophthalmologists. Ocular surface reconstruction techniques have advanced considerably during the last few years with the advent of amniotic membrane transplantation (AMT) and limbal stem cell transplantation.[1]

AMT is the latest approach to limbal stem cell transplantation for reconstruction of the ocular surface.[2] This new technology has great potential for a group of diseases otherwise considered to be incurable. Recently AMT has been used for a variety of ocular surface problems including persistent corneal epithelial defects, for pterygium surgery, conjunctival defects after removal of surface tumors, LSCD as in acute and chronic chemical injuries, Steven Johnson syndrome and shield ulcer of vernal keratoconjunctivitis.[1]De Roth (1940) was the first to describe live placental membranes to repair conjunctival defects.[3] Inclusion of the highly immunogenic chorion resulted in a low success rate. Sorsby et al. (1946) used chemically processed dry amniotic membrane (AM) termed amnioplastin as a temporary patch for the treatment of ocular burns. However, the need for repetitive applications made the technique rather unpopular until its revival in 1995, by Kim and Tseng. When appropriately processed and preserved, amniotic membrane can be used as a graft to replace the damaged ocular surface stromal matrix or as a patch to prevent unwanted inflammatory insults from gaining access to the damaged ocular surface.

One of the promising applications of the amniotic membrane is in the generation of a cultivated epithelium, using it as a substrate or a vehicle to culture the stem cells of limbal origin. The cultivated corneal epithelium thus generated on the denuded AM can be used for reconstructing the ocular surface in severe stem cell

deficiencies.[4] In this review we summarize the current applications of processed human amniotic membrane for ophthalmic indications.

AMT offers the advantage over conventional corneal transplantation – relative ease of surgery and avoidance of allograft rejection, has become attractive alternate for countries where there is shortage of corneal tissue. Even if corneal transplantation is required it can be performed after AMT where ocular surface is not inflamed.

ANATOMY OF OCULAR SURFACE

OCULAR SURFACE

Functional unit comprised of

1. Tear film

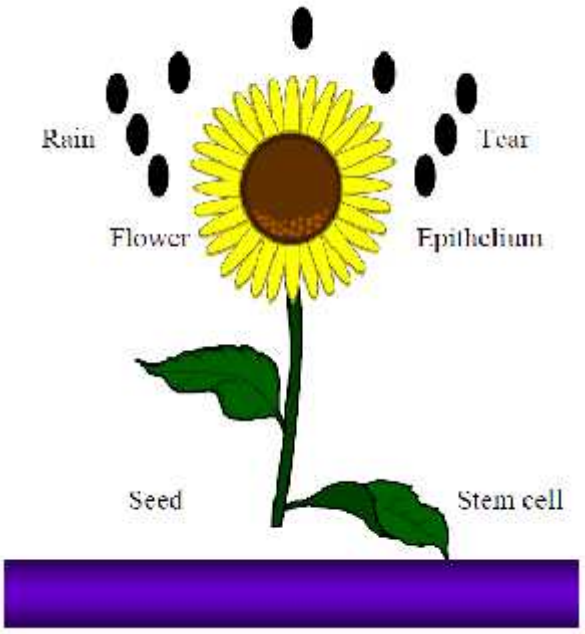
2. Corneal epithelium

3. Limbal epithelium

4. Conjuctival epithelium

5. Meibomian glands

6. Lacrimal glands Seed Stem cell



Nelson (1980) Described ocular surface as mucosal lining between upper and lower lids

* Functions of ocular surface are

Ocular protection

Providing smooth, clear surface for optical purpose

ELEMENTS OF OCULAR SURFACE DEFENSE

Stable preocular tear film consists of

Lipid layer secreted by Meibomian glands

Aqueous layer secreted by Lacrimal glands and accessory lacrimal glands

Mucins layer secreted by Ocular surface epithelia and Goblet cells

Lid blinking helps in Tear spread

Tear clearance

Lid closure helps in preventing evaporation of tears

LIPID LAYER

• Secreted by meibomian glands

• Is the outer most layer of tear film

• Prevents evaporation and stabilizes tear film

• Provides smooth optical surface

AQUEOUS LAYER

• Secreted by main and accessory lacrimal glands

• Helps in clearing debris, toxins and foreign bodies

• It is a source of growth factors EGF, TGF-α, HGF and immune and inflammatory mediators

MUCIN LAYER

• Secreted by conjunctival goblet cells: MUC5AC (gel forming) and ocular surface epithelium, i.e. MUC1 & MUC4

• Ocular surface is rendered hydrophilic

• Contributes to tear viscosity

• Inter blink protection decreases microbial adhesion

CORNEAL EPITHELIUM

- It is multilayered (5-6 layers) with basal cells, wing cells, superficial nonstratified squamous epithelium
- Microvillae are present superficially, with cytokeratin K_3 - K_{12} are present

CONJUNCTIVAL EPITHELIUM

- It is present from mucocutaneous junction to conjuctival sac then bulbar conjuctival where the epithelium changes from non-keratinized stratified squamous epithelium to columnar and cuboidal epithelium.
- Goblet cells are present maximally in the inferonasal aspect.
- Has micrtovilli and cytokeratin K_{19}

LIMBAL EPITHELIUM

- It is multilayered (8 to 10 layers) has Palissade of Vogt, Cytokeratin P_{53} with Melanocyte in the basal layer.

AMNIOTIC MEMBRANE

(a) ANATOMY

The human amniotic membrane (HAM) is the inner most layer of the placenta, composed of the outer chorion of maternal origin and the inner amnion of fetal origin The HAM is devoid of any vasculature and is 0.02 to 0.5 mm in thickness.

Histologically, the amniotic membrane is composed of the following layers.

(i) Epithelial layer

(ii) Thick basement membrane

(iii) Avascular, hypo cellular stromal matrix

a) Compact layer

b) Fibroblast layer

c) Spongy layer

(i) Epithelium

The amniotic membrane epithelium consists of a single layer of cuboidal cells with a single nucleus and a number of cytoplasmic vacuoles uniformly arranged on the basement membrane. They are differentiated from the ocular surface epithelium by the presence of greater number of microvilli on the apical surface. Current cryopreservation techniques of AM storage tend to devitalize these cells by cell membrane disruption, leaving an intact basement membrane and stromal matrix. It has been established that LSCs expanded on intact AM do not form adhesion complexes as strong as those expanded on epithelially denuded AM. Presence of amniotic epithelium may hinder the uniform expansion of explant cultures on the membrane and delay formation of strong hemidesmosomal attachments.

(ii) Basement membrane (BM)

Thick layer composed of network of reticular fibers. These are interdigitations of short, blunt processes from the basal region of epithelial cells with similar basement membrane processes. This is a tough layer and resistant to current cryopreservation techniques for AM storage. It is one of the thickest membranes found in human tissue and the support provided to the fetus during gestation stands testimony to the structural integrity of this remarkable tissue. This structural integrity, transparency and elasticity of the AM make it currently the most widely accepted tissue replacement for ocular surface reconstruction. Both collagen IV and VII, components of the corneal epithelial BM, are present in the basement membrane of the AM.[5] In addition, collagens I, II, III and V are also present in the AM. Basement membrane is known to promote epithelial cell migration, adhesion and differentiation. It also suppresses epithelial cell apoptosis.[6,7,8] Histochemically, the basement membrane more closely resembles that of the conjunctiva.[5] It is an ideal substrate for supporting the growth of the epithelial progenitor cells by prolonging their life span and maintaining their clonigenicity.[6] This action explains why AMT facilitates epithelialization for persistent epithelial defects with stromal ulceration.[9] In tissue cultures, AM supports epithelial cells grown from explant cultures and maintains their normal morphology and differentiation.[10] The resultant cultured epithelium can be transplanted with the AM to reconstruct damaged corneas.[9] The amniotic membrane can also be used to promote non-goblet cell differentiation of the conjunctival epithelium. These data support why goblet cell density is promoted following AMT in vivo. Amniotic epithelium produces basic fibroblast growth factor, hepatocyte growth factor and transforming growth factor β. These growth factors may modulate proliferation and differentiation of stromal fibroblasts.

(iii) Stromal Matrix

Compact layer -> (5-20 μm) thought to be strongest layer comprised of a complex network of reticular fibers. This layer is thought to contribute to the tensile strength of AM. Its acellular nature points to possible epithelial origin.

Fibroblast layer -> thickest layer of AM and is made of a loose fibroblast network embedded in a mass of reticulum.

Spongy layer -> outermost layer of the amnion, has wavy bundles of reticulin, made up of branching fibers with triangular shaped nodes at the junction. Scattered fibroblasts are present in this layer.

(b) CHARACTERISTICS

Certain characteristics of AM make it ideally suitable for its application in ocular surface reconstruction.

1) Easily obtainable.

2) Nearly unlimited availability.

3) AM does not express HLA-A, B or DR Ag and hence immunological rejection after its transplantation does not occur.

4) Believed to have antibacterial properties, reducing the risk of post-op infection.

5) Can be preserved at -80°C for several months, allowing sufficient time to plan surgery or consider a trial of other options.

6) Favours epithelial cell migration/growth promoting activity.

7) Reinforces adhesion of basal epithelial cells, diminishes their apoptosis and promotes their differentiation.

8) Has anti-adhesive properties.

9) Has anti-inflammatory effect not linked to its function as a basement membrane.

10) It is avascular and angiogenic (?).

(c) MECHANISM OF ACTION

Question arises whether corneal epithelium grows over or under the AMT. Both these possibilities exist.

When corneal epithelium grows beneath the HAM, the regenerating epithelium will cause the HAM to detach progressively from the ocular surface as healing continues; on the other hand, when regenerating epithelium grows over the HAM, this causes the HAM to be incorporated into host corneal stroma. In this instance HAM remnants have been detected as late as 13 months after transplantation.

The therapeutic effect of AM basically involves three key actions that work synergistically, namely promoting epithelialization, reducing inflammation and suppressing fibrosis. The AM stromal matrix, rich in fetal hyaluronic acid suppresses transforming growth factor β signaling, proliferation and myofibroblastic differentiation of normal corneal and limbal fibroblasts as well as normal conjunctival and pterygium fibroblasts.[10] This action explains why AMT helps reduce scars during conjunctival surface reconstruction, prevents recurrent scarring after pterygium removal and reduces corneal haze following photorefractive keratotomy. The AM stromal matrix also suppresses the expression of certain inflammatory cytokines that originate from the ocular surface epithelia, including interleukin 1α (IL - α), IL - 1β\ IL - 8, interferon ∞, tumor necrosis factor - α, β-fibroblast growth factor and platelet derived growth factor. The suppression of inflammation is the key element in prevention of conjunctival scarring, neovascularization and fibrosis. The AM attracts

and sequesters inflammatory cells infiltrating the ocular surface and contains various forms of protease inhibitors. This may explain some of the anti-inflammatory properties of the fetal tissue and how neovascularization is mitigated, actions important for preparing the stromal microenvironment to support subsequent limbal stem cell transplants.

(d) PROCUREMENT

1. Commercial source. Cryopreservation of Human Amniotic Membrane

The human amniotic membrane is prepared using the standard protocol proposed by Kim[11] after an informed consent was taken. The placenta obtained shortly after an elective Caesarian delivery was the preferred source of amniotic membrane. Placentas from vaginal deliveries or subsequent to premature rupture of membranes are known to be contaminated and unsuitable for transplantation.

The maternal donor is screened serologically for HIV, Hepatitis B and C, and syphilis. Under a laminar air hood, this placenta is cleared of blood clots with Earle's balanced salt solution containing 50 u/ml streptomycin, 100 ug/ml neomycin, and 2.5 ug/ml amphotericin B.[12] The amniotic membrane is separated from the chorion by blunt dissection through the potential spaces between the two tissues. The amnion with epithelial/basement membrane side up is flattened and spread on to a nitrocellulose paper. Then the nitrocellulose paper along with the amniotic membrane is cut in to required dimensions and stored in vials containing a 1:1 combination of Dulbecco's Modified Eagle's Medium (DMEM) and glycerol at - 80°C, can be stored up to 2 years.[13] Just before use, the amniotic membrane is thawed at 37°C for half an our and placed on to the cut glass slide. This thawing devitalizes all cells in amniotic membrane making it nonviable but biologically active.

2. Non-commercial source

Preserved AM is not available in all developing countries because of expense incurred for a -80°C freezer, the media and the nitrocellulose paper. Hence fresh non preserved AM without a carrier sheet is used following elective CS. Washing of placenta free of blood clots under sterile conditions, section of membrane is obtained and amnion separated from chorion, AM is profusely irrigated and stored aseptically in a vial with saline solution. The AM is refrigerated and used within 24 hrs.

This donor AM can also be stored temporarily in a regular freezer (-20°C) upto 4 weeks (bio-tissue, Amniograft information summary). In contrast, freeze-dried AM, does not require deep freezing and can be stored at room temp and rehydrated prior to use in a operating room.

Differences between Preserved and unpreserved AM

	Preserved	**Unpreserved**
1.	AM epithelial cells are nonviable (The viability of amniotic epithelial cells has been associated with low-grade inflammatory response)	AM epithelial cells are viable
2.	Serological tests on donor are done AM at the time of procurement and six is months interval which adds the safety and effectively eliminates the increasing risk of disease transmission.	By contrast with fresh non-preserved the time procurement to transplantation short and prevents repeat of disease transmission test of donor thus the risk.
3.	Scheduled surgery	Short notice for AMT
4.	No wastage so grafts can be prepared.	Wastage of unused AM when prepared fresh.
5.	One has to have a deep freezer in institution were surgery is to be performed.	Freezer not required

(E) SURGICAL TECHNIQUE

Ocular surface reconstruction, facilitation of epithelialization, limbal stem cell replenishment and symptomatic relief are the basic objectives of amniotic membrane surgery. There are four basic principles upon which the final technique is individualized:

1. Inlay or Intrastromal graft technique – wherein the AM graft is tailored to fill in or plug when used on the cornea. Intrastromal graft combined with surface graft to cover entire surface of corneal defect is meant to act as a scaffold for the epithelial cells.[8] The AM is secured with its basement membrane side up (the less sticky side). The stromal surface is identified by the presence of vitreous like strands that can be raised by a sponge.

2. Overlay or patch technique – here the AM functions akin to a biological contact lens, as it protects the healing surface defect beneath.[14,15] The graft also reduces inflammation by its barrier effect against the chemical mediators from the tear film.

3. Filling-in or Layered technique – is indicated in deep stromal ulcers.[16] The entire depth of crater is filled with small bits of AM.[17] A larger graft is secured to the edges of the ulcer in an inlay fashion. An additional patch may help in preserving the deeper layers for a longer duration.

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.

Technique of Amniotic Membrane Transplantation

Informed consent was obtained from each patient before surgery. Under general, peribulbar or topical anesthesia, the diseased tissue was excised (depending on indications such as pterygium excision, and symblepharon) or surface debrided (persistent epithelial defect, shield ulcer of Vernal Keratoconjunctivitis and bullous keratopathy). Bleeders were cauterized with cautery and amniotic membrane with its stromal side down placed on the cornea (Identification of AM, which side up was done using a fine forceps, forceps was applied to the membrane and gently lifted, a fine strand of "vitreous like" layer was seen from stromal side and not from epithelial side. The orientation of AM was not important when doing fill-in technique) and sutured in place with 10-0 monofilament nylon, while 8-0 polyglactin was used to suture on to conjunctival edge. Subconjuctival gentamycin with dexomethasone was given and lids were closed.

Surgical Indications of Human Amniotic Membrane Transplantation[18]

1) SURFACE GRAFT

A) Corneal indications

- PED without ulceration
- Acute stage of chemical or thermal burn
- TEN , SJS
- Preventing scar after PRK or PTK
- Painful bullous keratopathy with erosion
- Band keratopathy after surgical removal of calcific deposits
- Partial limbal stem cell deficiency
- Total limbal stem cell deficiency (with limbal transplantation)

B) Conjunctival indications

- Symblepharon
- Conjunctivochalasis
- Bulbar conjunctival reconstruction following excision of large lesions or scars
- Bleb leak or revision

- Fat adherence syndrome after Retinal Surgery

C) Combined corneal and conjunctival indications

- Pterygium

- Limbal tumor involving cornea and conjunctiva

D) On eyelid

- Acute stage of chemical or thermal burn
- Acute TEN, SJS
- Entropion surgery

2) INTR-STROMAL GRAFT

A) Corneal indication

- Corneal ulceration
- Descemetocele
- Corneal perforation

B) Scleral indication

- Scleral melt

- Scleral perforation (small) without prolapse of intraocular contents

3) COMBINED SURFACE AND INTRASTROMAL GRAFT

- Corneal ulceration
- Descemetocele
- Corneal perforation

Pterygium

Pterygium surgery, even today remains one of the most controversial and challenging subject. Clinically, pterygium is characterized by a fleshy triangular portion of bulbar conjunctiva encroaching on to cornea. Also by causing limbal stem cell deficiency and fibrovascular proliferation of subconjunctival tissue. The goals of surgery are to remove the pterygium completely without much tissue loss or scarring and prevent recurrences. Prevention of recurrences has always been the end point of treatment success of any modality. Recurrence rate ranges from 5-90% in various studies. Recurrent pterygium is characterized by hyper-proliferation of subconjunctival fibrosis with more accelerated growth rate than primary pterygium. The resultant fibrosis can cause restriction of ocular movements and symblepharon formation. Treatment of recurrent pterygium requires both suppression of fibrosis and reconstruction of limbal barrier. Tseng et al. demonstrated that transferring growth factor beta signaling pathway in fibroblast was suppressed when AM was used with stromal side up. So anti-inflammatory effect of AMT contributes to post-operative healing leaving a quiet eye. Fibrotic tissue can be extensively excised as there is no limitation in terms of graft size and large defect is rapidly epithelialised. AMT in combination with conjunctival or limbal autograft appears to be a promising surgical

treatment for ocular surface reconstruction in patients with recurrent pterygium, by placing sutures on the conjunctival flap that adheres both AM and sclera.

AM functions as a mechanical barrier against fibrous tissue invasion.[19] Sangwan et al. (2003) reported that a combined surgical procedure of pterygium excision with simultaneous amniotic membrane transplant, conjunctival limbal autograft, and mitomycin C application may be beneficial in the management of chronically recurring pterygium in young patients.[20] Ma et al. (2000) showed that amniotic membrane graft is as effective as conjunctival autograft and mitomycin C in preventing pterygium recurrence, and can be considered as a preferred grafting procedure for primary pterygium.[21] The efficacy of AMT is comparable to the established method of conjunctival autograft transplantation (CG) and it is especially indicated when there exists a very large conjunctival defect to cover as in primary double-headed pterygium, or in the context of preserving superior bulbar conjunctiva for future glaucoma surgeries.

Ocular Surface Burns

Ocular burns are one of the blinding ocular emergencies, where the basic pathology revolves around the sequelae of chemical burns which include limbal stem cell deficiency, gross surface destruction, anterior segment ischemia and lid deformities. The outcome depends on the severity of ocular damage and warrants a planned multi-step approach. The limbal stem cell deficiency determines the extent of conjunctivalization, neovascularisation and persistent epithelial defects on the cornea. The other aspects of surface reconstruction that need attention are conjunctival cicatrisation and symblepharon formation. The results of AMT for ocular surface reconstruction have been favourable, according to most reports.[12,22,23] Amniotic membrane plays an important role in facilitating epithelial wound healing. AM differs

from other mucous membrane grafts in that it allows the normal conjunctival cells to grow uniformly, thereby being cosmetically more acceptable. Since the avascular matrix reduces the inflammatory, cicatricial and angiogenic processes, it is believed that the AM restores a non-inflamed perilimbal stromal microenvironment to support the transplanted limbal stem cells.

A combination of AMT with limbal transplantation offers improved results in cases with total limbal stem cell deficiency.[22,24] The limbal graft may be an autograft from the contralateral eye in unilateral cases and an allograft from living related donors or cadaver eyes for bilateral cases. The limbal tissue acts as an effective barrier against conjunctivalization of the cornea. It provides an active source of stem cells capable of differentiation into phenotypically distinct corneal and conjunctival epithelium. AMT alone seems to suffice for ocular surface reconstruction in chemical burns with Partial LSCD. Meller et al. (2000) have concluded that AMT is effective in promoting re-epithelialisation and reducing inflammation in the acute stages, thus preventing the chronic scarring sequelae.[25]

However randomized controlled trials are required to establish the exact role and specific benefits of AMT in acute ocular surface burns of varying degrees of severity.

OCULAR SURFACE NEOPLASIA

AMT is a useful technique for the reconstruction of both small and large surface defects that result from the excision of conjunctival malignant melanoma, primary acquired melanosis (PAM)[26] and other ocular surface squamous neoplasia (OSSN). The surgical management of surface tumours constitutes a clinical challenge especially when the tumour is large or arises multifocally. In these conditions a wide

conjunctivectomy may be indicated. Non-surgical methods to treat these lesions include cryotherapy, radiotherapy and topical chemotherapy. Exenteration is a more radical modality, which is now reserved for locally advanced malignancy. Paridaens et al. (2001) have demonstrated the beneficial effects of AMT for ocular surface reconstruction following surgical excision of conjunctival malignant melanoma and PAM.[26] The advantages AM include (1) the cosmetic appearance following surgery, (2) the absence of donor site morbidity complicating the harvest of mucosal autografts and (3) the ability to clinically monitor local recurrence of tumour beneath the transparent AMT. Tseng et al. (1998) suggest that the best results occur in limbal and epibulbar melanomas. In 16 eyes where excision of large OSN including conjunctival intraepithelial neoplasia (CIN), primary acquired melanosis, and malignant melanoma was followed by adjunctive cryotherapy and suturing of a single layer of amniotic membrane (AM) with the basement membrane side facing up to the healthy bordering tissue. Recurrence was noted in one case and pyogenic granuloma occurred in one.[27] Combined therapeutic approaches, consisting of extensive tumor removal, cryotherapy, amniotic membrane allograft, and topical mitomyin C, can be effective in the management of diffuse conjunctival and corneal melanoma arising from primary acquired melanosis.

Persistent corneal epithelial defects

Persistent epithelial defects (PEDs) signify varying degrees of limbal stem cell deficiency and are a common feature of chemical injury, SJS, OCP, neurotrophic keratitis and keratoconjunctivitis sicca. The common factor in all these conditions is prolonged inflammation of the ocular surface, damaging the stem cells and basement membrane. In addition the matrix metalloproteinases produced by keratocytes, epithelial cells and neutrophils can cause progressive stromal ulceration with risk of

corneal perforation. The use of AMT is usually reserved for cases in which removal of epitheliotoxic medications, aggressive lubrication and surface protection (BCL and tarsorrhaphy) and control of inflammation have failed to promote closure of the defect. Owing to the properties of the AM, which facilitate epithelialization, various studies have proved the efficacy of AMT in healing PEDs. Kim et al. (1995) and Shimura et al. (2001) have reported the benefits of using amniotic membrane as a temporary patch to cover the surface during wound healing and protect the defect from inflammatory cells in the tear film. Lee et al. (1997) have used the AM as a substrate substitute for the basement membrane of the cornea.[9] The success rates however seem to vary in different reports. Tseng et al. reported a healing rate of 90% after the first AMT as compared to 70% by Letko et al. (2001).[15] In the latter group there was also a higher rate of recurrence (29%) because PED was due to immunologic diseases.

CICATRISING KERATOCONJUNCTIVITIS

The most severe manifestations of ocular surface disease are encountered in Stevens Johnson syndrome, ocular cicatricial pemphigoid and toxic epidermal necrosis etc., characterized by chronic conjunctival inflammation that causes progressive conjunctival scarring, goblet cell depletion, conjunctival shrinkage, forniceal shortening, symblepharon formation and severe dry eye. This hostile corneal environment further leads to corneal scarring, thinning, ulceration and finally perforation over a period of time. Associated lid deformities from entropion and trichiasis continuously traumatize the surface epithelia. The spectrum of clinical manifestations in SJS and OCP are difficult to treat and warrant a multiple approach. The ideal sequence of ocular surface reconstruction and visual rehabilitation include 1) Management of meibomian gland dysfunction and dry eye, 2) ocular surface

reconstruction with release of symblepharon, 3) correction of lid deformities, 4) limbal stem cell transplantation, 5) penetrating keratoplasty for visual rehabilitation and 6) all of the above would work only if the ongoing immune-mediated inflammation is well controlled.

Honavar et al. (2000) have evaluated the role of AMT as a preliminary step in the sequential management of SJS.[12] Symblepharon release and surface reconstruction had secondary benefits in the form of reversal of cicatricial entropion, increased ocular motility and eyelid stability. Therefore AMT is an effective and essential step in restoring a stable and non-inflammed ocular surface to support the transplanted limbal and corneal tissue in the subsequent stages. Tsubota et al. (1996) and Tseng et al. (1998) have elucidated similar results, however in a smaller series. The response to AMT shown by patients with OCP is rather unpredictable owing to the chronicity of the immune mediated inflammation and the frequent recurrences. Barabino et al. (2003) noted a slight deterioration in the clinical effects with time.[24] Subsequent surgeries might be attempted, provided the ocular surface is stable and non-inflamed for at least four weeks.

BULLOUS KERATOPATHY

The corneal endothelium consists of a single layer of uniformly arranged hexagonal cells. The most important function of the corneal endothelium is to regulate the water content of the stroma and maintain corneal transparency. Since corneal endothelial cells do not replicate, a critical loss of cells may lead to compromised corneal clarity. Corneal endothelial decompensation is characterized by stromal edema with or without epithelial bullae. Endothelial insult may result from surgical trauma as in aphakic and pseudophakic bullous keratopathy or silicone oil keratopathy. Other causes are uncontrolled glaucoma, Fuch's endothelial dystrophy

and pseudo exfoliation syndrome. Regardless of the underlying etiology, ocular pain or discomfort is a characteristic complaint of most of these patients, along with reduced vision. Increased stromal and epithelial hydration leads to poor cell adhesion and recurrent erosions, which further increases the risk of infectious keratitis (4.7%).

In eyes with good visual potential, penetrating keratoplasty is the treatment of choice. However, in eyes with poor visual potential and intractable pain, other modalities are considered such as bandage contact lenses, anterior stromal puncture, conjunctival flaps, Bowman's membrane cauterization or phototherapeutic keratectomy.

Amniotic membrane transplantation can be considered as an alternative to these modalities in alleviating pain (in 88% on first post-operative day, 67% symptom free at the end of follow-up) promoting epithelial healing, and preserving cosmetic appearance in patients with symptomatic bullous keratopathy and poor visual potential.[28] It could also be performed for symptomatic relief in patients waiting for keratoplasty and are intolerant to bandage contact lenses. Pires et al. (1999) concluded that amniotic membrane transplantation is a viable alternative to the Gundrson's conjunctival flap, as it is technically easier, avoids complications of ptosis, and provides a better cosmesis and above all does not induce limbal stem cell deficiency.

DEEP CORNEAL ULCERS

Corneal epithelial are associated with defective wound healing due to tear film abnormality, lid abnormality, inflammation and infections which can result in persistent epithelial defects and stromal thinning. A multi step approach should be done to control inflammation and also to protect the surface. In such cases restoring

surface integrity gains priority over visual rehabilitation by keratoplasty with guarded visual prognosis.

Deep corneal and scleral ulcers have been successfully treated by multilayered AMT.[16,17] Since a monolayer disappears within a few weeks and a deep stromal defect is not filled by de-novo synthesis during this period, the multilayered technique offers a better option for resurfacing. There is a significant reduction in the inflammation, as the AM forms an effective barrier against the tear film. This seals the volume of inflammatory mediators that could invade the corneal stroma. It has also been proposed that grafted AM also has an effect on the stromal keratocytes i.e. modifies the proliferative and migratory behavior of stromal keratocytes and might down regulate the synthesis of chemokines.

Multilayered AMT allows rapid epithelial healing and long-term stability of the corneal surface, owing to its basement membrane properties. Supported by the maintenance of the stromal thickness even after dissolution of the AM with time. However AMT has little effect in influencing the corneal innervation, vascularisation or deep scarring.

SHIELD ULCERS OF VERNAL KERATOCONJUNCTIVITIS

Vernal keratoconjunctivitis is a common allergic ocular condition characterized by seasonal itching, photophobia and a ropy mucoid discharge. Corneal complications vary from micro erosions to larger epithelial defects coated with mucous plaques, generally located superiorly. Shield ulcers occur frequently in the severe forms of vernal catarrh, especially the palpebral form. The mechanical hypothesis proposes that the giant papillae on the upper tarsal conjunctiva are responsible for mechanically abrading the cornea. According to the toxin hypothesis, the inflammatory mediators derived from the eosinophills are thought to damage the

corneal epithelium and inhibit wound healing. Because of the delayed healing, shield ulcers are at increased risk of corneal scarring, vascularisation, and infectious keratitis. Generally the first line of treatment is frequent instillation of topical steroids and mast cell inhibitors along with surgical debridement of the mucous plaque to facilitate epithelial healing. In severe shield ulcers superficial keratectomy and excimer PTK with BCL have been tried. Amniotic membrane transplantation combined with surgical debridement is an effective alternative modality in the management of these ulcers.[29] The renewed basement membrane enhances epithelialisation, reinforces cellular adhesion and prevents epithelial apoptosis.

EXCIMER LASER SURGERY

Photorefractive keratectomy performed for high refractive errors may be associated with loss of corneal transparency (haze). The application of amniotic membrane after PRK reduces keratocyte proliferation and corneal haze during corneal wound healing, possibly by reducing infiltration of inflammatory cells and loss of keratocytes in the ablation area during the early postoperative period. To date there is no large human study to substantiate this.

AMNIOTIC MEMBRANE FOR EXPANSION OF LIMBAL STEM CELLS

Two specialized phenotypically different epithelial cells, called the central corneal epithelium and the peripheral conjunctival cells, separated by limbus, cover the human ocular surface. The limbus is lined by several layers of cells with rete ridges and contains the Langerhans cells as well as melanocytes, but is devoid of goblet cells. Stem cells are present in the peripheral cornea and limbus, which are harvested and grown on amniotic membrane either as autograft or allograft. It is transplanted wherever there is limbal stem cell deficiency.

AIMS AND OBJECTIVES

To assess the efficacy of Amniotic membrane graft, either as a "Substrate" to replace the damaged ocular surface or as a "Patch [biological dressing]" or a combination of both

REVIEW OF LITERATURE

(a) HISTORICAL PERSPECTIVE

Various Donor Materials used in Ocular Surface[18]

Skin—1893

Oral mucous membrane-1912

Vaginal mucous membrane-1922

Tarsal conjunctiva-1938

Fetal membrane, Amnion and Chorion-1940

Rabbit Peritoneum-1941

Amnion only-1946

Amnion with cultured autologus limbal epithelial cells-2000

Amnion with cultured allogenic and autologous epithelial cells-2000

Fetal membranes (amnion, or chorion or both) were used for transplantation purpose.

In 1900-fetal membranes were used for skin transplantation to treat burnt and ulcerated skin surface.

1912* Mucous membrane from mouth was used as a donor material to fill the defect created by excision of necrotic conjunctiva in acute phase, following severe conjunctival burn

1920* Mucous membrane from lip, following chemical injury by chlorinated lime.
1930 -> Fetal membrane were used to create artificial vagina.

1938- Von Blaskaics used tarsal conjunctiva strip of tarsus from one eye to the other to build the inner layer along with vaginal mucous membrane.

In 1940- DeRoth was of opinion that the ideal donor material should be thin, smooth, and transparent like conjunctiva. He felt fetal membranes had these qualities and used them in six patients of symblepharon caused by chemical injury, pemphigus, 2 cases to enlarge the socket where in he placed chorion surface onto ocular surface and amnion formed a free surface.

Alternatively Brown used rabbit peritoneum in human alkali bur, wherein he used double armed silk suture to anchor graft through fornix to full thickness lid to emerge at skin and was found to be irritant.

Sorsby and Symons first used AM possibly without chorion to treat 30 human eyes by alkali burns and reported few complications. 1983- Holtz used Thiersch skin graft.

1993- Bathe and Perdomo introduced human amniotic membrane preserved in 95% ethyl alcohol as a substitute for conjunctival membrane to treat recurrent pterygia, dermoids, Gunderson procedure, fornix reconstruction and alkali burns. 1995^ Tseng and Kim-AMT in rabbit.

Recently John used HAMT in acute toxic epidermal necrolysis.

Further it has been used as carrier tissue to transfer cultivated corneal epithelial stem cells to treat limbal stem cell deficiency.

Pires, Renato T.F. et al.[30] (1999) Amniotic membrane transplantation was performed at 5 centers on consecutive eyes with symptomatic bullous keratopathy and poor visual potential. Epithelial defects in 45 of 50 eyes created and covered by

amniotic membrane healed rapidly within 3 weeks. Only four showed recurrent surface breakdown and one eye developed pseudopterygium. Hence amniotic membrane can be considered as an alternative to conjunctival flap in alleviating pain, promoting epithelial healing, and preserving cosmetic appearance in patients with symptomatic bullous keratopathy and poor visual potential.

Friedrich E. Kruse et. al.[16] (1999) performed a prospective, non-comparative, interventional case series in eleven consecutive patients with deep corneal ulcers refractory to conventional treatment; six patients had herpetic keratitis and five had other forms of neurotrophic keratitis. Epithelium healed above all corneal ulcers within 4 weeks and remained stable in 9 of 11 patients for 1 year.

Ma Hui-Kang David, et al.[21] (2000) performed amniotic membrane graft for primary pterygia in 80 eyes of 71 patients. This was compared with 56 eyes of 50 patients receiving conjunctival autografts and 54 eyes of 46 patients receiving topical mitomycin C, where they showed amniotic membrane graft as an effective and preferred grafting procedure for primary pterygium.

Chen Hong-Jeng, et al.[31] (2000) amniotic membrane graft was performed in 16 eyes of 15 patients with neurotrophic corneal ulcers and vision equal to or worse than 20/200. During follow-up period of 18.8 months, 1 to 3 layers of amniotic membrane with or without additional membrane as a patch were used for 17 procedures in 16 eyes for persistent neurotrophic corneal ulcers. All but four instances of amniotic membrane transplantation achieved rapid epithelialisation. Hence amniotic membrane is considered an effective alternative for treating severe neurotrophic corneal ulcers.

Letko Erik et al.[15] (2001) In this study 30 patients underwent amniotic membrane transplantation for primary epithelial defects. The use of amniotic membrane transplantation was restricted to patients in whom all previous measures including bandage contact lens and tarsorrhaphy, had failed. Primary epithelial defects healed after first amniotic membrane transplantation in 21 eyes within an average of 25.5 days after surgery and recurred in 6 eyes. So it is used as treatment in which all other conventional management has failed.

Sridhar M.S. et al.[32] (2001) Amniotic membrane transplantation was performed in 4 patients (7 eyes) with grade 2 (ulcer with opaque base) and grade 3 (plaque like lesion) shield ulcers not responding to steroid therapy with or without surgical debridement. The ulcers healed with disintegration or retraction of the membrane in all patients within 2 weeks. Hence they concluded that amniotic membrane transplantation in combination with debridement is an effective surgical modality in the management of severe shield ulcer.

Handa K.A. et al.[17] (2001) All AMT performed in 11 patients: 4 patients (4 eyes) corneal perforation, 5 patients (5 eyes) with deep corneal ulcers and descemetocele and 2 patients (2 eyes) with a scleral ulcer. 8 eyes healed with epithelialisation with 5 and 3 eyes showing corneal and conjunctival epithelialisation. Persistent epithelial defect noted in one eye with corneal ulcer after limbal allograft transplantation for chemical burn and 2 eyes with corneal ulcer as a complication of rheumatoid arthritis. Hence they concluded that multilayered AMT may be effective for treatment of deep ulceration of cornea and sclera while in some eyes with total corneal limbal dysfunction or autoimmune disorders AMT alone is not effective.

Jose Alvaro Perira Gomes et al.[33] (2003) AMT performed for 20 consecutive patients with limbal stem cell deficiency secondary to ocular chemical injury, after 19 months ocular surface reconstruction was obtained in 15 eyes with reduced inflammation and vascularisation and mean epithelialisation time of 3.3 weeks. Success was observed in all four cases of partial LSD and 11 eyes of total LSD. Surgical failure was observed in five severe cases. Significant on improvement was observed in all cases after surgery except for 2 eyes that maintained pre-operative visual acuity and was concluded that AMT seems to be an effective adjuvant for ocular surface reconstruction in chemical burns with PLD. When performed in conjunction with LSCD, it is also effective in most cases of TLD.

Virendra S Sangwan et. al.[34] (2004) examined medical records of four patients with partial LSCD who underwent pannus resection and AMT for ocular surface stability and improvement in visual acuity. All the eyes exhibited stable corneal epithelial surface by an average of 7 weeks post-operatively with improvement in subjective symptoms. Best corrected visual acuity from pre-operative (range: 6/9p-6/20) to post-operative (range: 6/6p-6/15) by an average of 4.5 lines on Snellen visual acuity charts.

Ma Hui-Kang David et al.[22] (2005) performed AM graft alone in 48 patients and AM graft with Mitomycin (0.025%) for 3 minutes for recurrent pterygia, where six conjuctival and six corneal recurrences developed in AMT group four conjuctival and six corneal recurrences seen in AMT with mitomycin C group. No significant difference was found in conjuctival and corneal recurrences between two groups.

METHODOLOGY

1) Source of data

A. Outpatients attending the Dept. of Ophthalmology, KLE's Dr PK Hospital and MRC.

B. Inpatients attending the Dept. of Ophthalmology KLE's Dr PK Hospital and MRC.

C. Out-patients and in-patients of other departments from same hospital and Civil Hospital, Belgaum referred to Department of Ophthalmology.

2) Methods of collection of data

The subjects for the present study were selected from the above source using the following inclusion and exclusion criteria.

Inclusion Criteria

a) Primary progressive pterygium

b) Persistent epithelial defects

c)Cicatrising conjunctivitis (Steven johnson, Toxic epidermonecrolysis, etc)

d) Chemical injury (alkali and acid burns)

e) Symblepharon due to various cause

Exclusion Criteria

a) Infective ulcers

b) Recurrent pterygium

c) HIV and HBsAg +ve Patients

Study design: Descriptive type of study

Sample Size: 30

Study period:

Patients satisfying the above criteria were included in the study, which was done from Jan 2009 to December 2009. The number of patients were 25 and number of eyes were 30.

Analysis plan

It's a one shot case study. The intervention is done and the outcome is evaluated

Procedure

An informed consent was taken. Preoperatively, detailed medical history was obtained including the presence or otherwise of Diabetis Mellitus or collagen vascular diseases. Complete ophthalmic examination including visual acuity, intraocular pressure, slit lamp examination and fundoscopy were performed.

The processed and preserved amniotic membrane was obtained from an international eye bank which was stored in Dulbecco's medium.

The patient was prepared for surgery. A peribulbar anaesthesia was given. The amniotic membrane was gently separated from the nitrocellulose paper with blunt forceps. The membrane was then placed on the cornea. The techniques appropriate to the particular case were performed, and the membrane was placed on the cornea to cover the defect . Excess of AM was trimmed and was sutured with interrupted 10-0 nylon on the cornea and 8-0 vicryl onto the sclera whenever required. Patients were prescribed steroid antibiotic drops and tear substitutes. In few cases of cicatrizing

conjunctivitis ,confirmer or symblepharon ring was placed and removed after 3 to 4 weeks of surgery. Patients were examined on postoperative day 1, and on 1stweek, 2ndweek and monthly thereafter for six months. The patients at follow-up were examined for evidence of vascularisation or recurrence of the primary condition, etc. The success or otherwise of the procedure was assessed.

RESULTS

Table 1: Frequency distribution – Sexwise

	No Of Patients	Percentage
Female	14	47
Male	16	53
Total	30	100

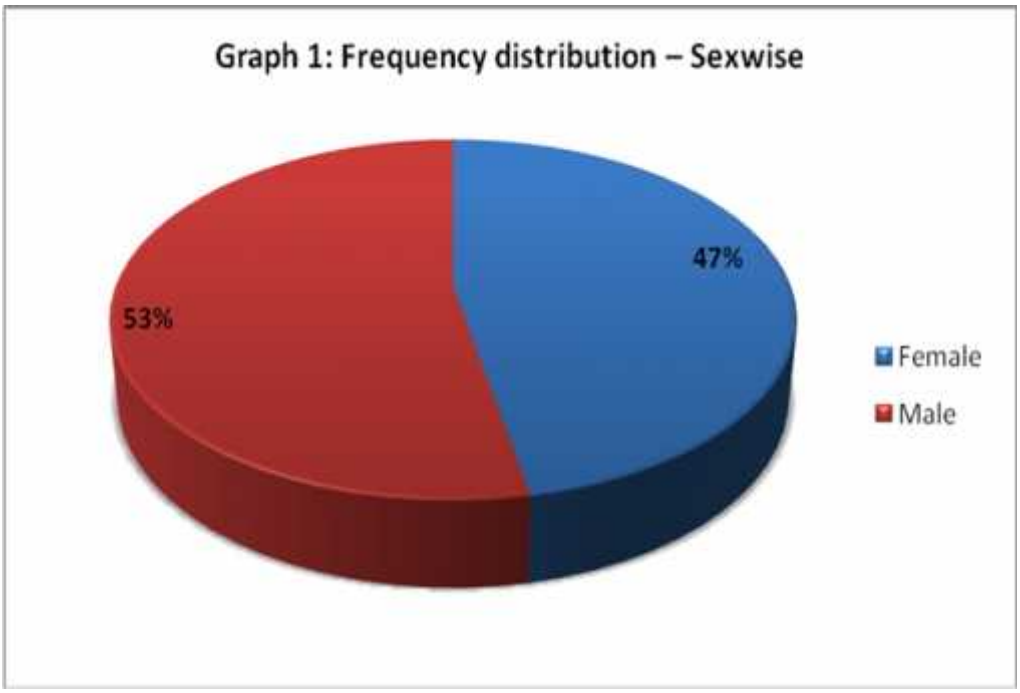


Table 2: Frequency distribution – Based on diagnosis

Diagnosis	Frequency	Percentage(%)
Primary progressive pterygium	15	50.
Chemical burns	6	20
Cicatrising conjunctivitis	4	13.3
Symblepharon	2	6.6
PED	2	6.6
OSSN	1	3.3
Total	30	100

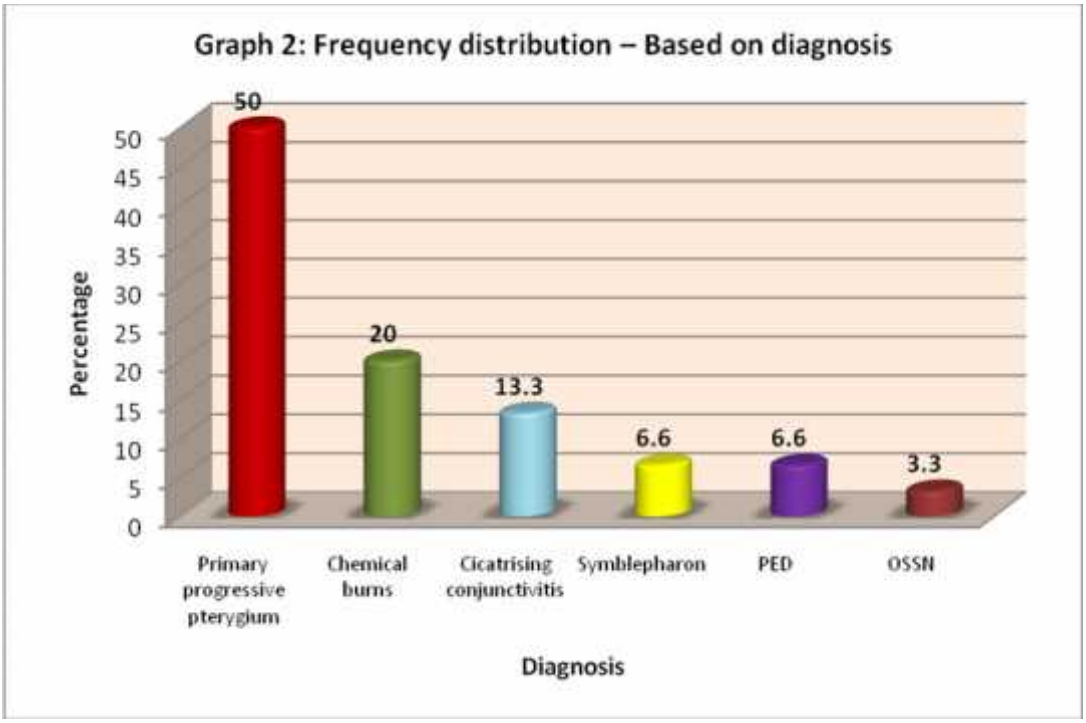


Table 3: Distribution of surgical outcome

	Frequency	Percentage
Failure	10	33.3
Success	20	66.66
Total	30	100

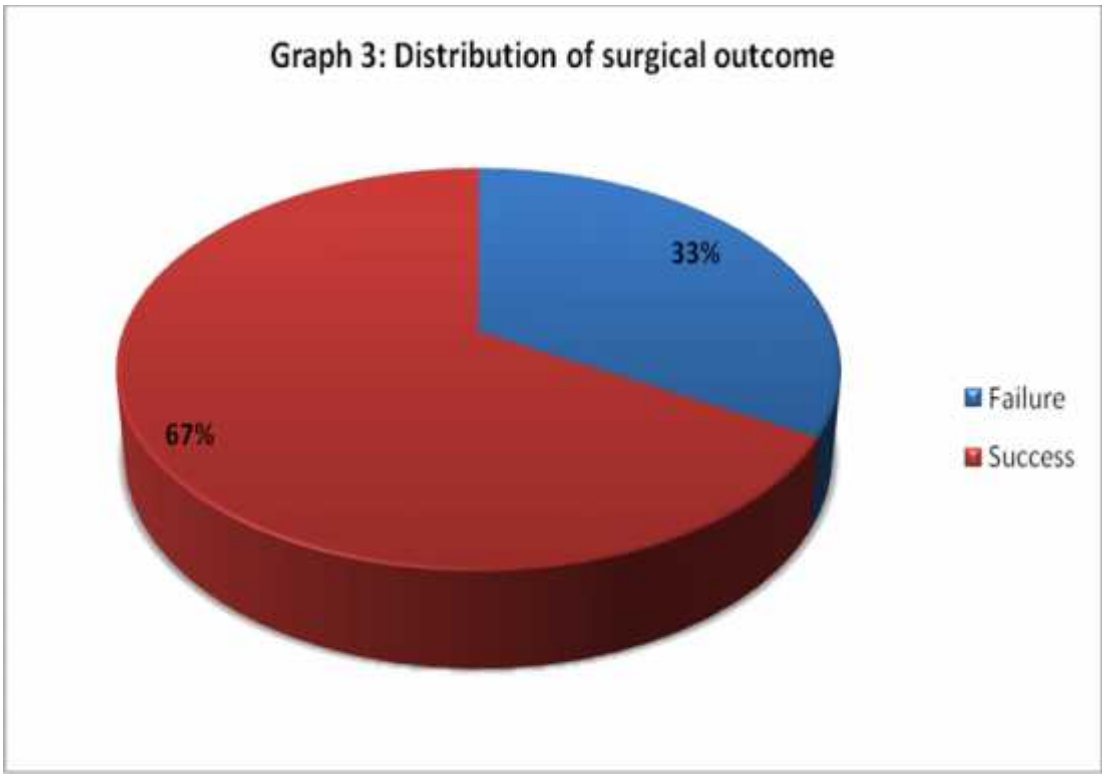


Table 4: Outcome vis a vis Diagnosis – Cross tabulation

Diagnosis	OUTCOME		
	No of Pts	Failure	Success
Primary progressive pterygium	15	2	13
Chemical burns	6	4	2
Cicatrising conjunctivitis	4	2	2
Symblepharon	2	1	1
PED	2	1	1
OSSN	1	0	1
Total	30	10	20

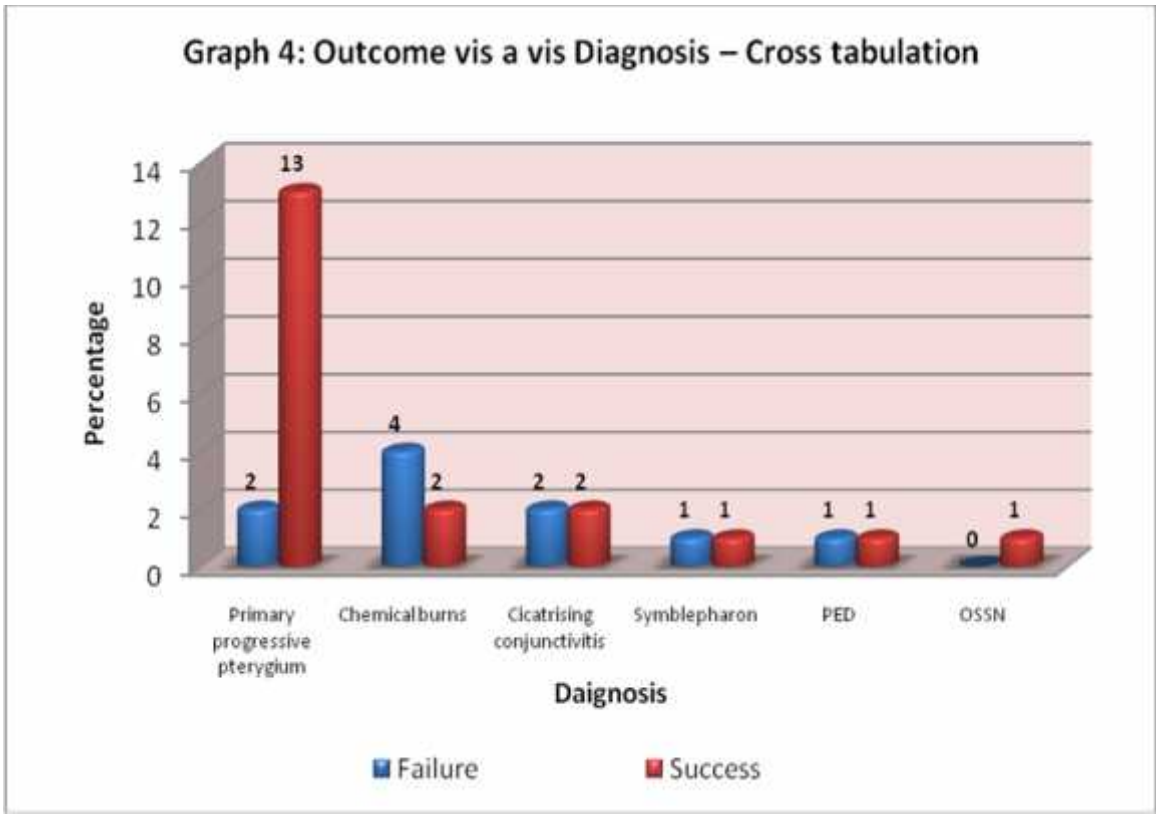


TABLE 5:- Primary Progressive pterygium: Distribution and Outcome

No of cases	Mean Corneal extension	Treatement -Excision and AMT	Mean healing time	Recurrence	Mean interval	Success (%)	Failure (%)
15	3.2mm	15	22 days	2	16 wks	13 (86.6%)	2 (13.4 %)

Fifteen cases of primary progressive pterygium with mean corneal extension of 3.2mm were treated with excision and AMT. The mean healing period was around 22 days, however there were recurrences noted in 2 cases with total success in 13 cases (86.6%)

Table 6: Chemical injury

Sl. No.	Eye affected	Hugh's classification (Grading)	Results	Resurgery
1	RE	IV	Unsuccessful	Done--- Failed
2	LE	III	UnSuccessful	
3	RE	III	Unsuccessful	
4	LE	III	Unsuccessful	
5	RE	II	Successful	
6	LE	II	Successful	

Table 7:- Outcome of chemical burns.

No of cases	Grading	Treatement	Success	Failure
4	III	AMT	0	4(66.6%)
2	II	AMT	2(33.3%)	0

Among 6 eyes treated, 4 eyes belonged to Grade III Hugh's classification and 2 eyes belonged to grade II . Graft was taken up by two eyes (33.3%) successfully with re-epithelialisation and no symblepharon , but the other 4 eyes showed failure, including resurgery done to one eye of grade IV. So there was a net failure of 4 eyes with grade 3 amounting to 66.6% failure

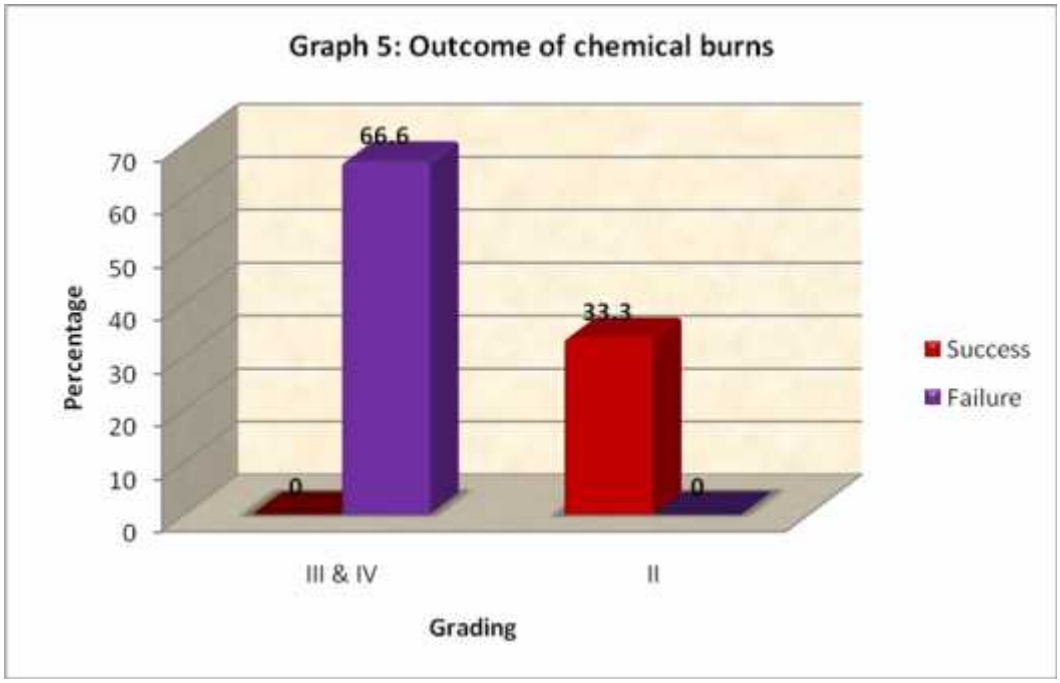


Table 8: Distribution and outcome of Cicatrising conjunctivitis

Sl. No.	Symblepharon	Etiology	Ocular motility	Surgery	Observations	Results
1.	Partial	Steven johnson	Restricted	Release and AMT	Ocular motility was restored fully and No recurrence of symblepharon,with healing of epithelial defect	Success
2	Partial	Steven johnson	Restricted	Release and AMT	Ocular motility restored with no recurrence of symblepharon & healing of epithelial defect	Success
3	Total	OCP	Fully Restricted	Epithelium debided,Forniceal reconstruction and AMT	LSCD, Symblepharon fully recurred with total conjunctivalisation of cornea	Failure
4	Total	TEN	Fully restricted	Release,conjunctiva over cornea debrided and AMT	Failed, Resurgery done, no recurrence of symblepharon, with healing of epithelial defect with partial clarity of cornea	Success

Table 9:- Outcome of cicatrizing conjunctivitis

No. of Cases	Treatment Done	Resurgery done	Outcome	
			Success (%)	**Failure (%)**
4	Release and AMT	1	03**(75.0)**	1**(25.0)**

Two cases of acute Steven Johnson syndrome and one case of acute TEN were treated with AMT which was successful in terms of re-epithelialisation, decreased inflammation and decreased vascularity. Healing in acute TEN case was obtained after repeat of surgery. However one case of OCP presented in late stages treated with AMT, showed failure.

Thus outcome of this study shows 3 success and 1 failure in cicatrising conjunctivitis group.

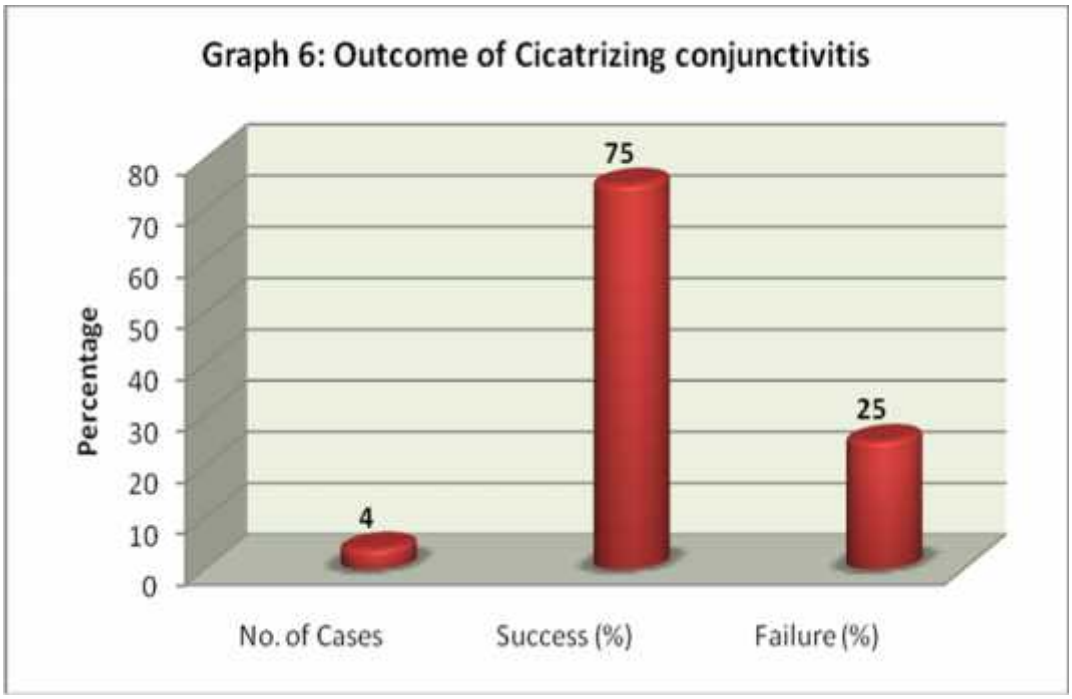


Table 10: Distribution and outcome of OSSN

Sl.No.	Affected Eye	Surgery Done	Follow Up
1.	LE	AMT	No recurrence

In a case of ocular surface squamous neoplasia of LE, Excision of the mass lesion with AMT was done, after a follow up 24 weeks, no recurrence was observed. Since a single case of OSSN was included in this study it cannot be statistically compared and no recurrence of the lesion was observed in subsequent follow ups for 24 weeks

Table 11: PED(Persistent Epithelial Defect)

Sl. No.	Schirmers	Surgery Done	Observation	Result
1.	10	AMT	No Recurrence of epithelial defects	Success
2	08	AMT	Recurrence of epithelial defects	Failure

Two cases of persistent epithelial defect was treated with amniotic membrane and a followed up for 24 weeks along with lubrication therapy. No recurrence of the defect was observed in the first case, however second case showed superficial vascularization with recurrence of epithelial defects amounting to failure of the graft.

Table 12: Symblepharon

Sl. No.	Etiology	Symblepharon	Affected eye	Ocular motility	Surgery	Results
1.	Traumatic	Partial	RE	Restricted	Release and AMT	Ocular motility was restored fully and No recurrence of symblepharon
2	Chemical	Partial	LE	Restricted	Release and AMT	Recurred

Table 13: Symblepharon Outcome

No. of Cases	Treatment Done	Outcome	
		Success	Failure
1 traumatic 1 chemical	Release and AMT	01	01

In One case having traumatic symblepharon, release of symblepharon and AMT was done which showed complete acceptance of graft with no recurrence observed upto 24 weeks . In another case of symblepharon due to chemical injury etiology, release of symblepharon and AMT was done, however in subsequent follow ups there was recurrence of symblepharon amounting to failure

DISCUSSION

Management of patients with severe ocular surface disease has always been a problem for ophthalmologists. Ocular surface reconstruction techniques have advanced considerably during the last years, moving away from bare sclera techniques, through free conjunctival autograft , oral and nasal mucosal grafts, and the more potent and physiological weapon- the limbal autograft first proposed by Dr. Jose I Barraquer. However there are cases that cannot be solved with the mentioned techniques and their prognosis is dismal. It is in these complicated cases where the Amniotic Membrane Transplantation has proven to be helpful.

The ophthalmologic literature describes a multitude of surgical procedures for conjunctival and cornea reconstruction, most of which have either never gained widespread use or have been abandoned. One of the procedures is the use of AMT, described by de Roth in 1940 and subsequently by Sorsby and Symons in 1946 for conjunctival reconstruction for symblepharon or chemical burns of conjunctiva. Limited by the state of microsurgery at that time, as well as by preparative procedures that deprived the amniotic membrane of its biological properties, the technique never gained widespread attention. Recently, Kim and Tseng have put AMT in a totally different perspective. Lee and Tseng were the first to propose the use of AM for the treatment of epithelial defects with corneal ulcers, subsequently; AMT was successfully applied to human patients with pterygium, conjunctival reconstruction, and ocular surface reconstruction following chemical, thermal burns and cicatricial eye diseases.

Human placental amnion is composed of single epithelial cell layer, a basement membrane (BM) and an avascular stroma. Both collagen IV and VII, components of corneal epithelial basement membrane, are present in the BM of amniotic membrane. In addition, collagen I, III and V are also present in amnion. Amniotic epithelium produces basic fibroblast growth factor, hepatocyte growth factor, and transforming growth factor β. Amnion prevents inflammatory cell infiltration and reduces apoptosis in keratocytes after transplantation onto the corneal surface. All of these properties explain its usefulness in reconstruction of the ocular surface, without ever being vascularised and conjunctivalised but by serving as a scaffold for host epithelium. Because of its property of not expressing human leucocyte antigens, it is well tolerated and does not cause any rejection reaction in the host. Amniotic membrane is also found to have anti-inflammatory and anti-scarring effect.

With the objective of determining the efficacy of AMT in ocular surface disorders, the above study was conducted and the following outcomes do support the use of AM as the graft material.

COMPARATIVE STUDIES

Table 14: Comparison between present study and earlier study for primary progressive pterygium

Present study		**PinnitaPrashasawat**
No. of Cases	15	46
Success	13 (86.6%)	41 (89.1%)
Failure	2 (13.4 %)	5 (10.9%)

There is a very good correlation in the success rate of present study (86.6%) and the study of Pinnita et al. (89.1%), thus demonstrating the efficacy of AMT in primary progressive pterygium.

Out of 15 cases, 13cases did not show any recurrences until 24 weeks of follow up, however 2 cases showed superficial vascularisation and re-growth upto 2mm crossing the limbal margin.

Conjuctival autograft including the limbus may yield a better result by acting as a barrier against fibrovascular invasion of cornea and supplying stem cells to the corneal epithelium. Although conjunctival autograph can achieve a lower recurrence rate,there are some limitations in advanced and recurrent pterygia,scarred conjunctiva and glaucoma patients who may need future filtering procedures. In such cases amniotic membrane has an advantage and is simpler to perform.

Amniotic membrane transplantation gives better results than the bare sclera technique and has lesser complications as compared to the use of Mitomycin-C

According to Ma Hui-Kang David, et al.[21] (2000) performed amniotic membrane graft for primary pterygia in 80 eyes of 71 patients. This was compared

with 56 eyes of 50 patients receiving conjunctivalautografts and 54 eyes of 46 patients receiving topical mitomycin C, where they showed amniotic membrane graft as an effective and preferred grafting procedure for primary pterygium.

According to PinnitaPrabhasawat et al(39). (1997) group A with AMT included 46 eyes with primary pterygia and 8 eyes with recurrent pterygia (the recurrence rate was 10.9%, 37.5% and 14.8% for primary, recurrent, group B with primary closure had 20 eyes with primary pterygia (recurrence was significantly lower than 45%) and group C with conjuctivalautograft consisted of 78 eyes with primary and 44 eyes with recurrent pterygia (the recurrence rate was 2.6%, 9.1% and 4.9%).

It was thus concluded that amniotic membrane transplantation after pterygium excision is a safe and effective procedure with no significant postoperative complication.

Table 15: Comparison between present study and earlier study for Chemical Burns

Present study		**Jose Alvaro, et. al. (2003)**	**Annie Joseph (2001)**
No. of Eyes	06	20	4 (100%) 3 (chemical) and 1 (thermal)
Success	02 (33 %)	15 (75%)	0
Failure	04 (66.6%)	05 (25%)	4 (100%)

Among 6 eyes treated, 4 eyes belonged to Grade III Hugh's classification and 2 eyes belonged to grade II , Graft was taken up by two eyes (33.3%) successfully with re-epithelialisation and no symblepharon. In 4 cases who presented in acute phase, amniotic membrane was transplanted. In the first week of follow up there was

early degradation of membrane along with extensive corneal neovascularisation and subsequently symblepharon was observed in all 4 cases in 3 rd week of follow up for which surgery was undertaken, which again showed no success.

These 4 eyes exhibited an intense and prolonged post operative inflammatory response. The failure in these eyes is mainly due to deficient limbal stem cells of > 1/3-1/2 of limbus may thus also be related to the elaboration of collagenases as a result of inflammation that can cause early degradation of the Amniotic membrane before the epithelialisation is complete.

In this study, success was obtained only in grade II category and in grade III and IV AMT alone showed no success and further management with Limbal stem cell transplantation is needed.

Lastly there was net failure of 4 eyes in this study accounting to 66.6%.

According to Jose Alvaro Perira Gomes et al.[40] (2003) AMT performed for 20 consecutive patients with limbal stem cell deficiency secondary to ocular chemical injury, after 19 months ocular surface reconstruction was obtained in 15 eyes with reduced inflammation and vascularisation and mean epithelialisation time of 3.3 weeks. Success was observed in all four cases of partial LSCD and 11 eyes of total LSCD. Surgical failure was observed in five severe cases. Significant improvement was observed in all cases after surgery except for 2 eyes that maintained pre-operative visual acuity and was concluded that AMT seems to be an effective adjuvant for ocular surface reconstruction in chemical burns with partial LSCD. When performed in conjunction with LSCD, it is also effective in most cases of total LSCD.

According to Annie Joseph (2001) (41) four eyes of three patients who suffered severe chemical and thermal burns were studied. The aim of AMT was to prevent symblepharon formation, promote conjunctival regeneration, inhibit corneal melting by promoting epithelialisation and to protect the ocular surface while associated lid burns were treated.

Three of the four eyes developed symblepharon and progressive corneal melt requiring urgent tectonic keratoplasty. All four eyes had persistent epithelial defects less than 25% of conjunctival regeneration occurred in three eyes. Two eyes auto eviscerated, one patient underwent lid sparing exenteration for a painful blind eye and one eye became phthysical

Table 16: Comparison between present study and earlier study--Symblepharon

Present study		**Panda et. Al**
No. of Cases	02	24
Success	01	20 **(80%) successful** **4 (20%) partially successful**
Failure	01	

Since the sample size in our study is very small the study cannot be strictly compared.

According to Panda et al(42), the study showed that AMT was helpful in traumatic symblepharon with a success rate of 80% and 4% had partial success, who maintained smooth ocular surface and free movements. But in our study AMT was successful in one case of symblepharon due to trauma with a wooden stick and in 2nd case the symblepharon was due to chemical injury etiology. The recurrence in this eye

was probably due to greater subconjunctival fibroblastic response that led to eventual recurrence of symblepharon

Table 17: Comparison in case of PED

	Present study	**Pinnita et. al**
No. of Cases	02	10
Success	01	8**(80%) successful** **2 (20%) partially successful**
Failure	01	

In the first case of PED , with etiology of Dry eye (keratoconjunctivitis sicca) was treated using amniotic membrane after failure of usage of medications like lubricatives, Bandage contact Lens, and Punctal occlusion. An overlay technique of AM grafting with Basement membrane side up was done and re-epithelialisation was seen after 4 weeks of surgery, no recurrence of the epithelial defects were noted in the subsequent follow ups upto 3 months. However in another case of PED secondary to immunological disease was treated with AM, in the due course of 3 months of follow up epithelial defects persisted amounting to failure.

In a study done by Pinnita prabhaswat et al(43) to evaluate the efficacy of amniotic membrane transplantation (AMT) in persistent corneal epithelial defect with or without stromal thinning and corneal perforation. They observed that 8 patients(without stromal thinning) had successful re-epithelialisation amounting to 80 % success.

Cicatrising conjunctivitis :

In the present study 4 eyes of 3 cases of cicatrising conjunctivitis were included. One case had AcuteTEN (erythema multiforme major) which failed after forniceal reconstruction and AMT , however resurgery was done which later showed success. In the other two patients who presented with acute stage of Steven Johnson syndrome, both the patients did not show any form of recurrence of symblepharon and fornices were well maintained , with no recurrence of epithelial erosions 16 weeks after surgery where as other patient having ocular cicatricial pemphigoid had recurrence of symblepharon , recurrence of epithelial erosions and increased vascularity. The present study showed a total success of 75 % in this study Better results were obtained when treated in acute stages.

Immunosuppresives like prednisolone 1mg/kg body weight were started prior to surgery in 2 cases of Steven Johnson and one case of Acute TEN . In case of ocular cicatricial pemphigoid cyclophosphamide of 1mg/kg body weight was added to reduce the dosage of steroids (OCP cases requires very high dosage of steroids) i

The major pathogenesis in Acute cicatricial conjunctivitis is due to raw surfaces of the conjunctiva which were subsequently covered by Amniotic membrane promoting the healing of symblepharon and epithelial defects

In chronic stage, the major problem lies in the constant conjunctival scarring ,fibrosis and consecutive limbal stem cell deficiency. Therefore the Amniotic membrane alone will not be helpful and has to be adjuncted with Limbal stem cell transplant.

Therefore the present study shows success in Acute stages of Cicatrising conjunctivitis of steven johnsonson and TEN.

John et al. first reported the beneficial effects of AMT in the acute stage of toxic epidermal necrosis.[44]

Honavar et al. evaluated the role of AMT as a preliminary step in the sequential management of SJS.[45]Creditable improvements in the ocular surface were measured in terms of greater patient comfort, reduced surface inflammation, decrease in the severity of vascularization and absence of recurrent corneal erosions. In 9/10 patients, there was a significant improvement in the ocular surface with deepening of fornices.

Barabinoet al. noted a slight deterioration in the clinical effects with time owing to the ongoing surface inflammation](46)and deep-seated conjunctival[47] injury.

Comparison between present study and earlier study for Ocular surface squamous neoplasia (OSSN)

Since a single case of OSSN was included in the study, it cannot be strictly compared, but it was observed in the present study that AMT used for OSSN was successful with no recurrence observed till 6 months

In a study done Anita gupta, Carol Karp et al (35) Amniotic membrane as a treatement was successful for conjunctival surface reconstruction after excision of benign as well as malignant tumours

In another study done by Sanghamitra burman et al(36) statesAmniotic membrane transplantation has been reported to be successful in conjunctival surface reconstruction after excision of benign as well as malignant tumors such as conjunctival melanomas, lymphomas and OSSN. When used as a graft to cover the conjunctival wound it provides a substrate for the migration of conjunctival epithelial

cells. Surface lesions are particularly challenging when they arise multifocally or extend over large areas and warrant an extensive conjunctivectomy. The advantages of AMT over conjunctival autografts and mucous membrane grafts in this scenario, include superior postoperative cosmesis, absence of donor site morbidity complicating the harvest of mucosal and conjunctival autografts (CAG) and the ability to clinically monitor local recurrence of tumor beneath the transparent AMG. [(37)]Combined therapeutic approaches consisting of extensive tumor removal, cryotherapy, topical mitomycin C and AM allograft can be effective in the management of diffuse conjunctival melanomas arising from primary acquired melanosis (PAM).[,(38)]

CONCLUSION

As conventional methods in the management of ocular surface disorders have a limited success, AMT remains one of the most effective alternative in maintaining ocular surface integrity. When medical treatment fails and the defect or ulcer persists, conventional surgical treatments become indicated and should include punctal occlusion, application of bandage contact lens or tissue adhesive, lamellar or full thickness corneal transplantation, tarsorrhaphy, and/or conjunctival flap. AMT has now become a powerful surgical tool in the armamentarium of ophthalmology.

Insult to ocular surface had led to delayed epithelialisation of ocular surface, persistent inflammation and progressive tissue melting. Healing may occur with neo-vascularisation and conjunctivalisation. Conjunctival involvement too may lead to scarring, symblepharon formation and tear film deficiency. Until Kim and Tseng showed that amniotic membrane transplantation facilitated corneal surface reconstruction.

Rapid healing and reduction of ocular surface inflammation following AMT can be explained by the following several mechanisms of action.

The amniotic membrane provides a new basement membrane, which is an important substrate for supporting adhesion and growth of epithelial progenitor cells, including the stem cells.

Amniotic membrane also exerts an anti-inflammatory effect.

Stromal matrix of the amniotic membrane has a direct anti-scarring effect as evidenced by its suppression of TGF-β signaling and myofibroblast differentiation.

The combination of the above three actions may help to re-establish a micro-environmental niche that is conducive for the growth of epithelial progenitor cells. Amniotic membrane may promote nerve regeneration by maintaining nerve growth factor (NGF) signaling.

Thus AMT, having following advantages namely easy availability, relative ease of surgery and devoid of risk of allograft rejection, very useful technique which not only supplements other treatment modalities but also supplant them.

Observations in the present study divided into various groups showed

In Group of pterygium cases the study showed very good acceptance of the graft with minimal recurrences. Thus demonstrating the role of AMT in treating primary progressive pterygium.

The present study states success of AMT in grade II (hughs classification) cases of chemical burns in terms of re-epithelialisation, increased patient comfort, decreased inflammation and vascularity. However in cases of grade III and IV, AM alone is not successful and thus may require Limbal stem cell transplantation for better results

In case of OSSN , a single case was included in this study which showed no recurrence after excision of the growth and AMT

However a good sample size is needed to prove the efficacy of AM in cases pertaining to surface neoplasias

In patients presenting with persistent epithelial defects, the results showed no recurrence of epithelial defects arising secondary to keratoconjunctivitis sicca. However amniotic membrane should be tried after the failure of medical therapy, BCL, Punctal occlusions etc

However PED's secondary to auto immune disease showed recurrence in late stages because of continued inflammatory process. Immunosuppresants play an important role in decreasing the inflammation and maintaining the integrity of ocular surface after AMT. Due to smaller sample size it cannot be strictly proven .

Patients presented in acute stages of cicatrizing conjunctivitis like Steven Johnson, Toxic epidermonecrolysis ets showed good results after AMT in terms of decreased inflammation, vascularity and increased patient comfort. However immunosuppressant's play a crucial role and should be started prior to surgery and continued thereafter for few months and tapered slowly to maintain ocular surface integrity.

But recurrence occurs in few cases in later stages due to continuous inflammatory response in the body. As it was a short term follow up, a longer follow up is required to know the efficacy.

SUMMARY

Human amniotic membrane transplantation (AMT) is currently being used for a wide spectrum of ocular surface disorders. The presence of a unique avascular stromal matrix of the amniotic membrane reduces inflammation, neo-vascularization and fibrosis. The basic tenets of amniotic membrane transplantation are to promote re-epithelialisation, to reconstruct the ocular surface and to provide symptomatic relief from surface aberrations. AMT is a useful technique for reconstruction of surface defects resulting from removal of ocular surface disorders. AMT has effectively restored a stable corneal epithelium in eyes with, PEDs and corneal ulcers.

The above study has conclusively shown that AMT is a very useful technique for primary pterygium, acute stages of cicatrizing conjunctivitis and acute stages of chemical burns . AMT has also shown better results in cases of traumatic symblepharon , OSSN and persistent epithelial defects secondary to Keratoconjunctivitis sicca. But AMT was found to be less effective for chemical burns of grade 3 and 4 (partial and total LSCD), persistent epithelial defects secondary to autoimmune cause and late stages of cicatrizing conjunctivitis like ocular cicatricial pemphigoid etc.

However due to smaller sample size statistical signifgicance could not be obtained in certain surface disorders.

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ANNEXURE –I : PROFORMA

: **SCREENING FORM** :

ID No:

Name: (First Name) (Middle Name)

Age:

Sex :

Address :

Occupation ;

Religion : 1- Hindu 2 - Muslim 3 - Christian 4 - Sikh 5 Others(Specify)

Date of Admission:

Date of Discharge :

Diagnosis:

Proposed Surgery:

Is the patient eligible for Study ? 1 - Yes 2 - No 1 -

Has informed consent been taken ? Yes 2 - No

Final Result Information :

1. Ineligible
2. Eligible, Refusal
3. Eligible, Participating

ID No:

Doctor's Name ;

Doctor's Signature :_ Date:

I.D. No: ☐ ☐ ☐

CHIEF COMPLAINTS :

Pain ☐ Redness ☐ Fleshy mass ☐ Trauma ☐

HISTORY OF PRESENT ILLNESS:

1. Pain

Severity:- Mild ☐ 1-Yes2-No Duration

Moderate ☐ 1-Yes2-No

Severe ☐ 1-Yes 2-No

2. History of Redness: - ☐ 1-Yes 2-No Duration ☐ 1 days 2-wks 3-mnths

3. History of Watering: - ☐ 1 -Yes 2-No Duration ☐ 1 -days 2-wks 3-mnths

4. History of Photophobia: ☐ 1-Yes 2-No Duration ☐ 1 -days 2-wks 3-mnths

5. History of Discharge: - 1-Yes 2-No Duration ☐ 1 days 2-wks 3-mnths

Nature of Discharge ☐ 1-Mucoid 2-purulent 3-Mucopurulent

6. History of Trauma: ☐ 1-Yes 2-No Duration ☐ l -days 2-wks 3-mnths

Nature of injury ☐ 1-wodden 2-vegetative matter 3-iron/steel

7 .History of Fleshy mass: ☐ 1-Yes 2-No

☐ 1-Progressive 2-Non-Progressive

8. History of Chemical injury: - ☐ 1-Yes 2- Duration ☐ 1-days 2-wks 3-mnths

Nature of injury ☐ 1-acid 2-alkali

9. First Aid

☐ 1-Received 2-Not Received Duration ☐ 1-days 2-wks 3-mnths Nature of first aid received ☐ 1-thorough eye wash 2-medications used

PAST HISTORY:

Diabetes : ☐ Duration:_____months / years

Hypertension: ☐ Duration:_____months / Years

Any other medical disorders :_____

General physical examination

\Vital signs :
Pulse rate (Per minute): ☐☐☐

Pallor: ☐

Blood Pressure : (mm of Hg ☐☐☐ ☐☐☐

Oedema: ☐

☐

Temperature : °C"

Cardiovascular system : ☐ 1-Normal; 2-Abnormal; If abnormal specify :_____

Respiratory system : ☐ 1-Normal ; 2-Abnormal; If abnormal specify :_____

Nervous System : ☐ Normal: 2-Abnormal; If Abnormal specify :_____

Lymphadenopathy: ☐

Ocular Examination

☐ (1-Errect; 2 Tilted)

Head Posture: ☐ (1-Symmetrical; 2-Asymmetrical) ☐

Facial Symmetry: Visual ☐ (1-Parallel ; 2-Deviated)

Axes:

Extra Ocular Movements: Right Eye Left Eye Binocular

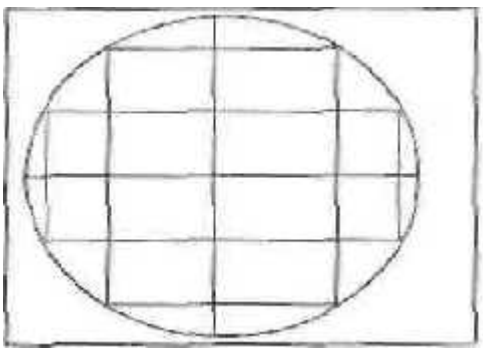
Visual Acuity:

Distant:

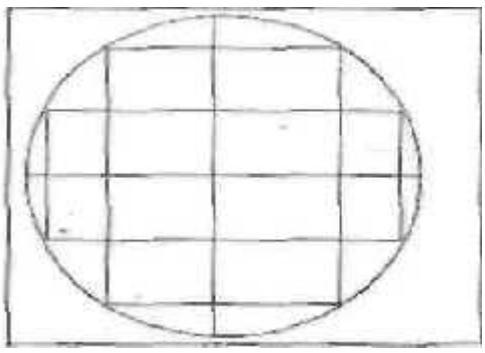
Pinhole:

Near:

	(RIGHT EYE)	(LEFT EYE)
Adnexa		
Conjunctiva		
Cornea	•	-
Sclera		
Anterior chamber		
Iris		
Pupil		
Lens		



Right Eye



Left Eye

PROVISIONAL DIAGNOSIS :_____

FUNDUS: Right Eye Left Eye

1 WNL ☐ ☐

2. Findings (Specify)

INVESTIGATION:

1. Random Blood Sugar---------- mg%

2. - Urine Albumin ☐ (1-Present; 2-Absent)

Sugar ☐ (1-Present; 2-Absent)

Microscopy ☐ (1-Pus cells ; 2-No pus-cells)

3. Lacrimal Sac patency: (1 - Patent; 2 - Blocked)

Right Eye ☐ Left Eye ☐

4. Intra ocular Pressure : ☐☐ Right Eye ☐☐ Left Eye

(mm of Hg)

5. Any other:

FINAL DIAGNOSIS ;

FOLLOW UP PLAN

	1st Week	2-3 Weeks	1-Month	3- Months
1) Conjunctiva 1. Normal 2. Conjunctival congestion 3. Ciliary congestion 4. Others				
2) Amniotic Membrane: 1. Intact 2. Partial disintegration 3. Complete disintegration 4. Receding				
3) Corneal Clarity 1. Clear 2. Partial clear 3. Opaque 4. Status Quo				
4) Re Epithelialisation 1. Partial 2. Complete 3. No Response				
5) Vascularisation 1. Present a. Superficial b. Deep 2.Not present				
6) Sutures 1. Intact 2. Lose				
7) Recurrence of Pterygium 1.Early 2. late 3.No recurrence				
8**) Bandage Contact Lens** 1. Present 2. Not Present 3. Removed				
9) Symblepharon Recurence 1. Early 2. Advanced				
10) Others				
11) Complication if any				

ANNEXURE II - INFORMED CONSENT DOCUMENT

I.D. No ☐ ☐ ☐

Mr.

/Mrs./Ms_____

you are invited to participate in our research study titled **"TO STUDY THE EFFICACY OF AMNIOTIC MEMBRANE TRANSPLANTATION IN OCULAR SURFACE DISORDERS"** conducted by Dr._____ Post-Graduate student in M.S. Ophthalmology under the guidance of Dr. _____ M.S, D.O.M.S Professor, Department of Ophthalmology, J N Medical College, Belgaum.

Respected sir/ madam, we request you to enroll yourself to participate in our study as, you are eligible for participating in this study. During the study you will be asked some questions in detail regarding your present complaint and you are supposed to answer to the best of your knowledge.

Your participation in research is voluntary, your decision whether or not to participate in the study will not affect your relationship with J N Medical college. If you decide to participate you are free to withdraw at any time.

Purpose of the Study :

The purpose of research is to compare the effectiveness of square edge intraocular lens in prevention of posterior capsule opacification with that of conventional round edge intraocular lens.

Procedure Involved:

If you agree to participate in this study, you will be asked to give detailed history of the disease you have and you will have to undergo necessary investigations and have to under go surgery. You would be asked to follow up on specified dates when your progress would be monitored, documented and if necessary photographed.

Risks and Benefits :

As such there are no major risks involved, however some discomfort may occur during the process of investigations and the risks involved with the anaesthetic procedure and with cataract surgical procedure for which all precautions will be taken. As such minimal risk is involved in the operative procedure mentioned above. If you agree to enroll in the study you will be helpful in choosing better intraocular lens in terms of prevention of posterior capsule opacification . Your participation may benefit you and others with cataract in future, by helping us to learn more about the posterior capsule opacification and it's prevention. No financial incentives are promised to you for being a part of study.

Alternatives:

Your decision whether or not to participate in this study will not affect the quality of treatment you receive and if you are not willing to participate, Further you may withdraw from the study at any time.

Costs for participating in this research :

There will not be any extra cost incurred by you. The participant will have to pay for the investigations which are the part of the existing management protocol for this ailment. There is not commitment for any reimbursement or any other compensation for the participant.

Privacy and Confidentiality :

The only people to know that you are a research subject are members of the research team. No information about you or information provided by you during the research will be disclosed to others without your written permission, except :

1. In emergency to protect your rights and welfare.
2. If required by law

Authorization to Publish Results :

When the results of the research are published or discussed, in a conference, no information will be displayed that would disclose your identity. Any information that is obtained in connection with this study and that can be identified with you will remain confidential .

Compensation :

In the event of injury related to the study, treatment will be made available through KLE Prabhakar Kore Hospital and M R C, Belgaum. There is no compensation or payment for such medical treatment by law. The doctors and the staff will provide facilities and medical attention to you.

Questions:

If you have any questions about the research you may please contact:

1) Chief investigator, Dr. _____ P.G. Department of Ophthalmology, J N Medical College, Belgaum . Contact No: _____.
2) Guide, Dr. _____, Professor, Department of Ophthalmology, J N Medical College, Belgaum . Ph: _____.
3) Dr. _____, Principal, J N Medical College Belgaum and Chairman of Institutional Ethics Committee. Ph: _____.

Consent Statement

I.D. No : ☐ ☐ ☐

I Mr./Ms./Mrs._____Voluntarily agree for the participation as a subject of study. By signing this consent form I am not giving up any of my legal rights, I may withdraw from the study anytime. I am signing the consent form after having read or been read for me in vernacular language, including the risks and the benefits and having all my questions answered.

Subject Name :_____

Signature or the Left Thumb Print of Subject:_____

Witness Name: _____

Signature of Witness : _____

Investigators Name :_____

Signature of Investigator :_____

Date :_____

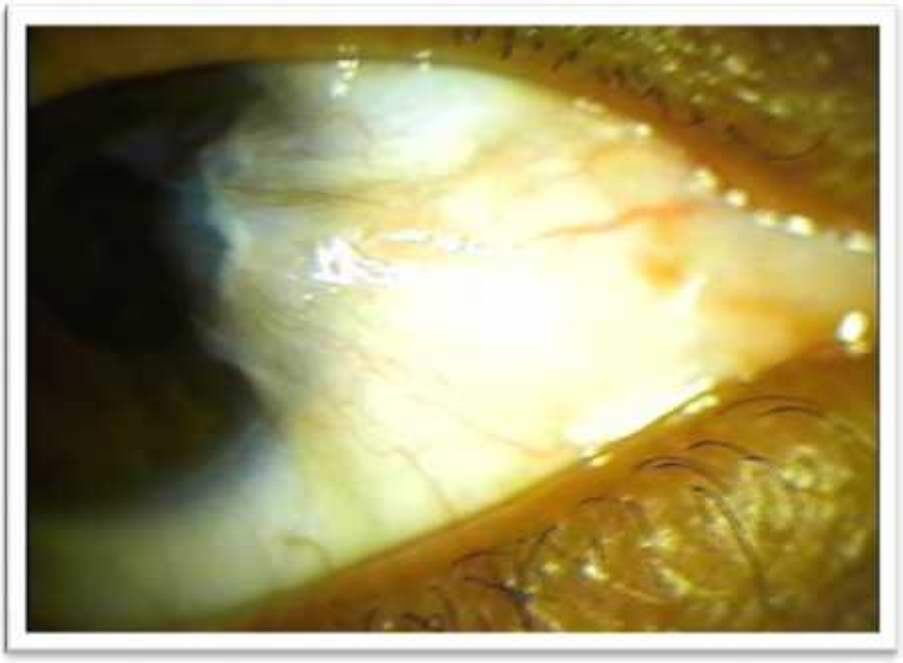
Place :_____

ANNEXURE – III – PHOTOGRAPHS

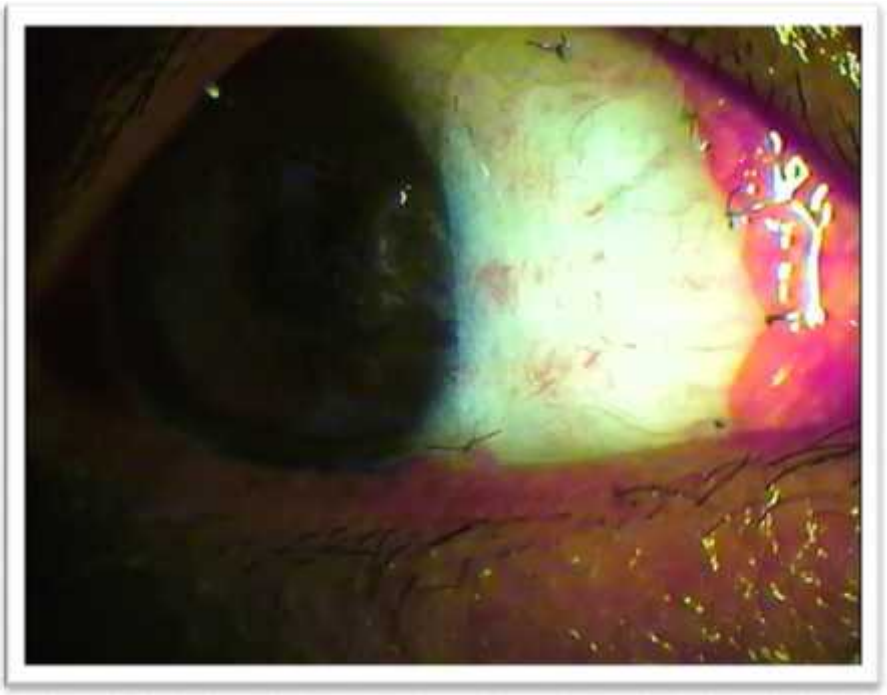
Photograph 1: Amniotic membrane being peeled from nitrocellulose sheet

Photograph 2: Amniotic membrane stored in Dulbecco's Modified Eagle's Medium (DMEM)

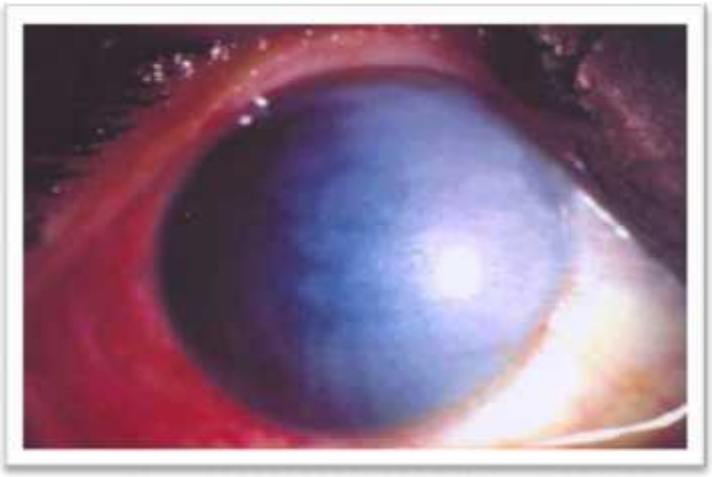
Photograph 3: Amniotic Membrane



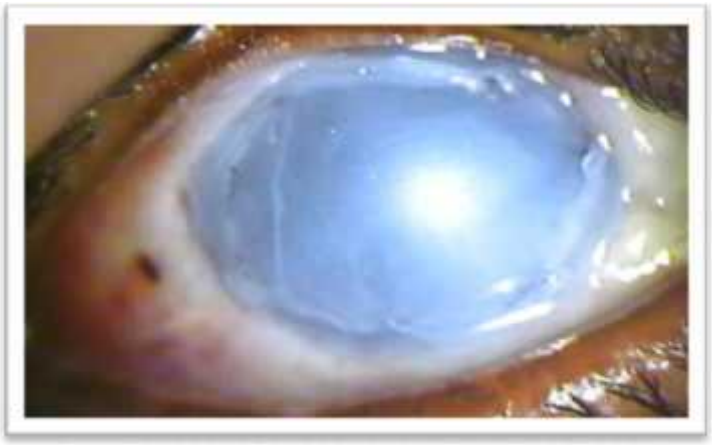
Photograph 4: Grade II Pterygium



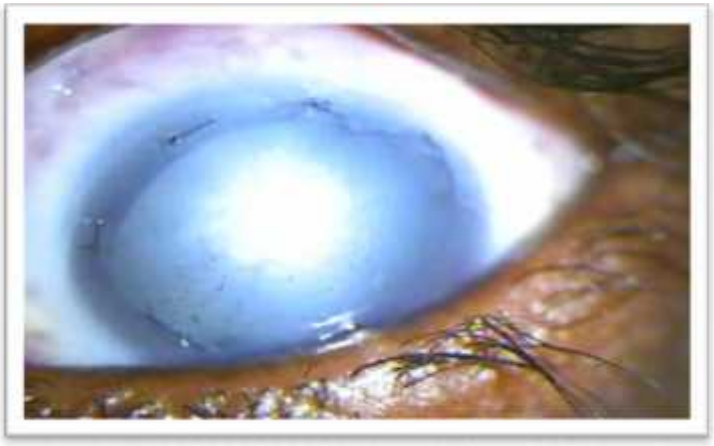
Photograph 5: Pterygium excision and AMT



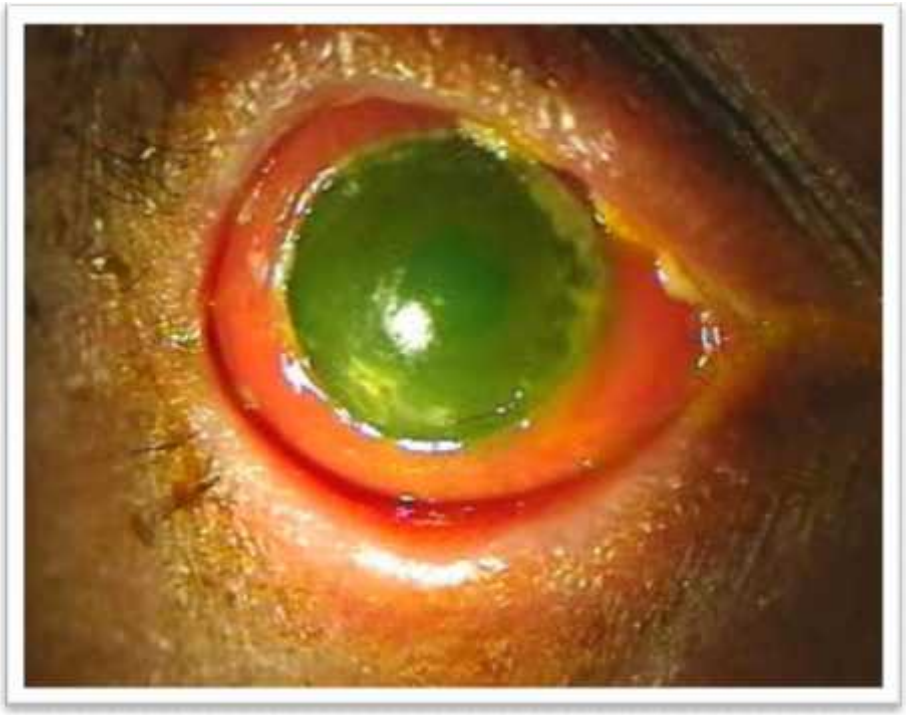
Photograph 6: Chemical burns – Pre-operative photograph



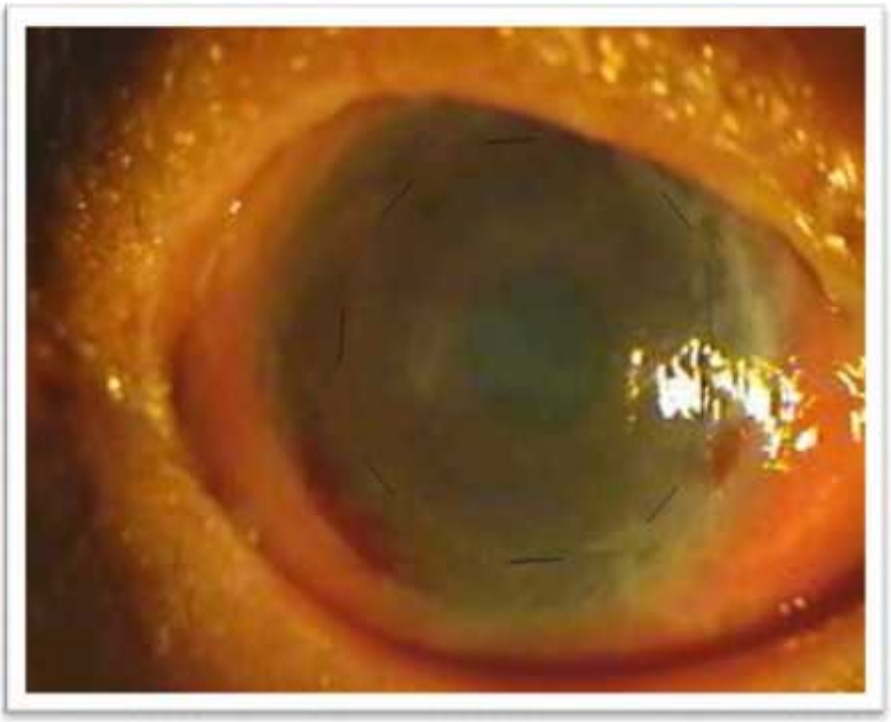
Photograph 7: 1 day post AMT showing well placed graft



Photograph 8: 1week post AMT showing early disintegration of graft in grade 3 burns

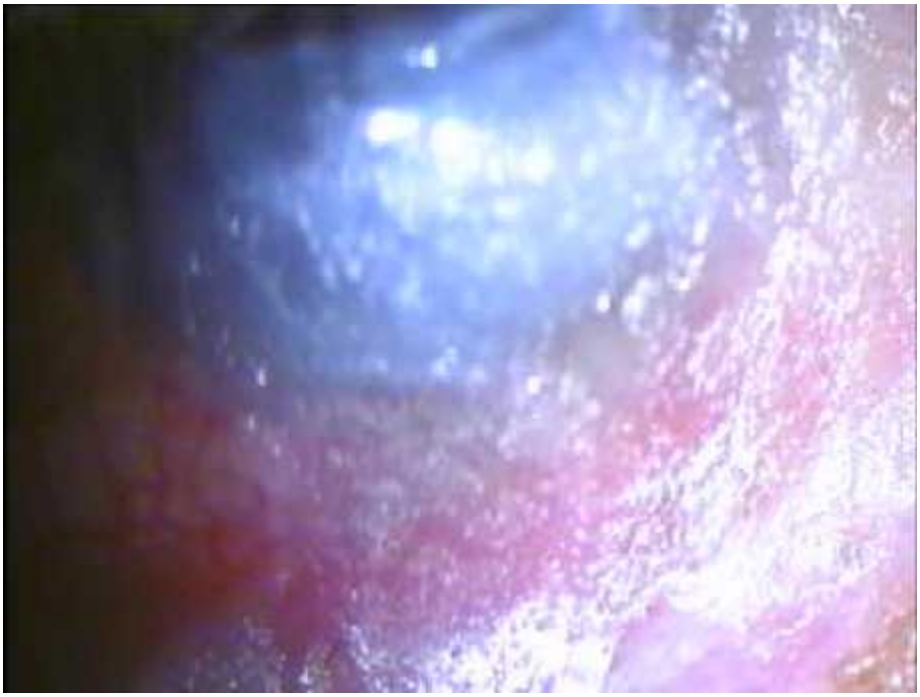


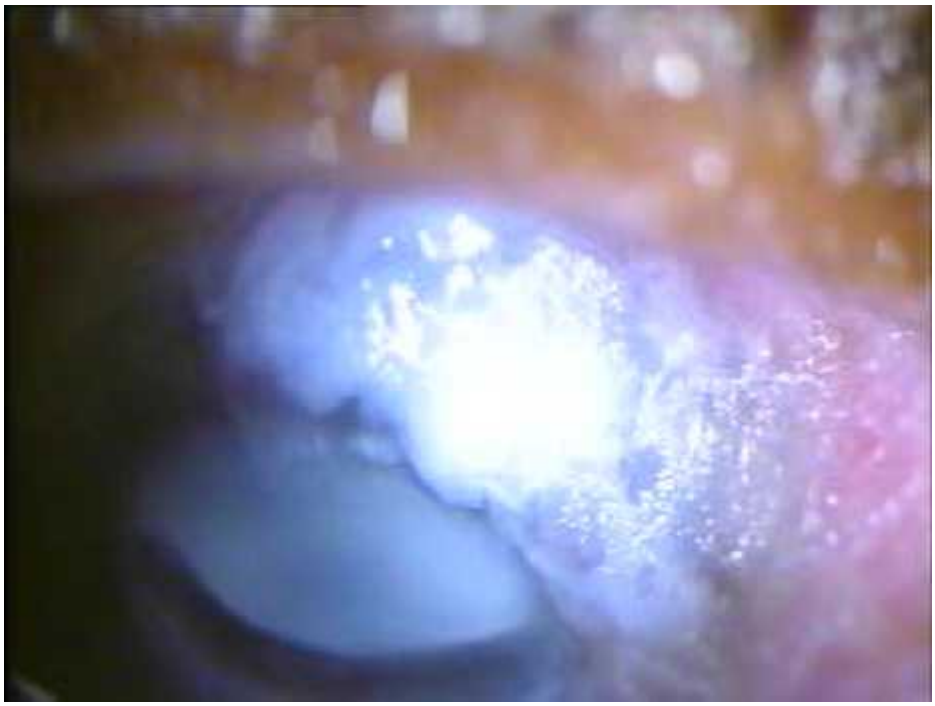
Photograph 9: Cicatrising Conjunctivitis (Steven Johnson)

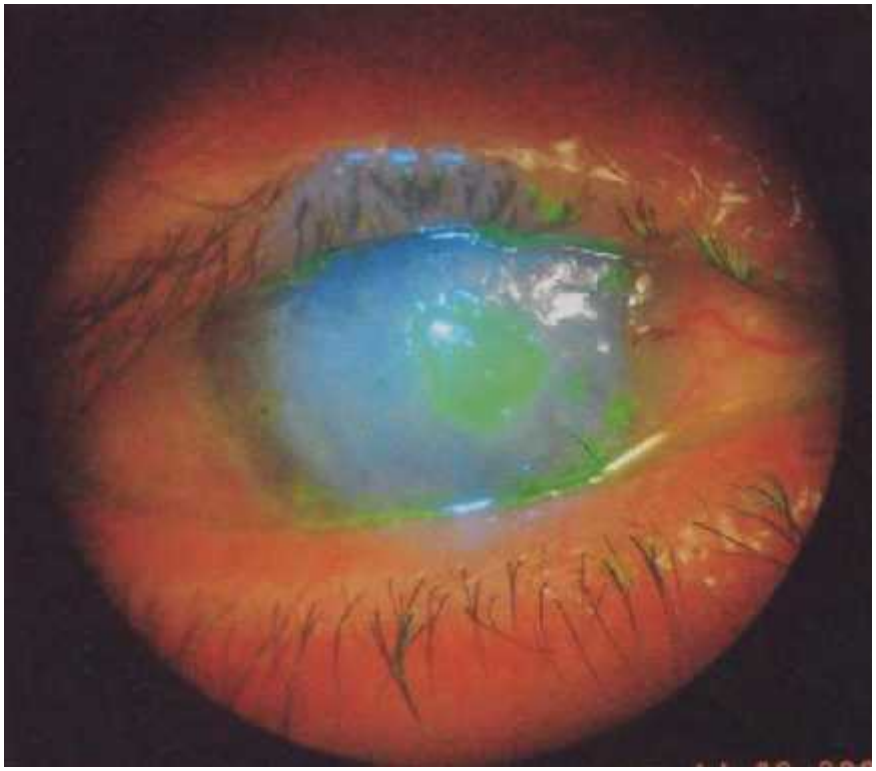


Photograph 10: Post AMT

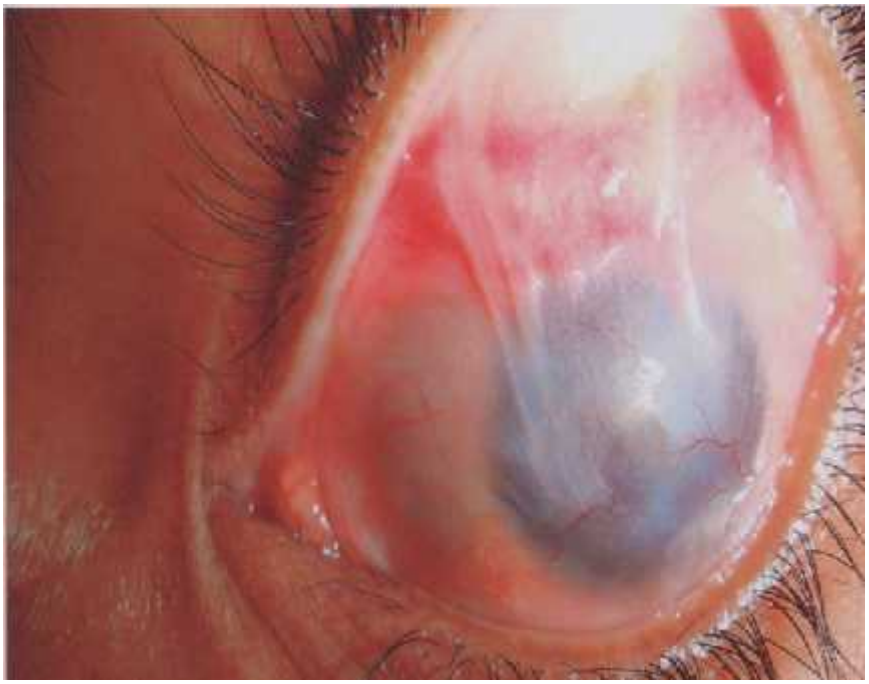
Photograph 11 & 12: Cicatrising Conjunctivitis (OCP)







Photograph 13: Fluorescent staining of the epithelial defect secondary to Kerato Conjuctivitis Sicca



Photograph 14: Symblepharon.

ANNEXURE – IV: MASTER CHART

KEY TO MASTER CHART

Sex M- Male

F- Female

Eye Affected : RE – Right eye

LE – Left eye

Diagnosis: SJS: Steven Johnson syndrome

TEN: Toxic epidermonecrolysis

OCP: Ocular cicatricial pemphigoid

PED: Persistent epithelial defect

KCS: Keratoconjunctivitis sicca

Limbal Sq C C: Limbal squamous cell carcinoma

AMT: Amniotic membrane transplantation

Outcome:

S: Success

F: Failure

Follow up

SV: Superficial vascularisation

DV: Deep vascularisation

CM: Corneal melting

Sym: Symblepharon

MASTER CHART

Pterygium											
Sl No	**IP No.**	**Age**	**Sex**	**Laterality**		**Etiology**	**Grading**	**Treatment**	**Recurrance**		**Complication**
				Eye	**Side**				**Early**	**Late**	
1	381218	65	F	LE	N	Pterygium	II	Excision + AMT			
2	381217	35	F	RE	N	Pterygium	II	Excision + AMT			
3	380840	30	F	LE	N	Pterygium	II	Excision + AMT			
4	353212	45	F	LE	N	Pterygium	II	Excision + AMT		+	Vascularisation
5	351819	70	M	RE	N&T	Pterygium	II	Excision + AMT			
6	351821	55	M	RE	N	Pterygium	II	Excision + AMT			
7	351824	60	M	LE	N	Pterygium	II	Excision + AMT			
8	337419	34	M	RE	N	Pterygium	II	Excision + AMT	+		Vascularisation
9	337413	65	M	LE	N	Pterygium	III	Excision + AMT			
10	1084023	58	F	RE	N	Pterygium	II	Excision + AMT			

Pterygium											
Sl No	**IP No.**	**Age**	**Sex**	**Laterality**		**Etiology**	**Grading**	**Treatment**	**Recurrance**		**Complication**
				Eye	**Side**				**Early**	**Late**	
11	361868	36	M	RE	N&T	Pterygium	II	Excision + AMT			
12	355351	70	F	LE	N	Pterygium	III	Excision + AMT			
13	318522	40	F	LE	N	Pterygium	II	Excision + AMT			
14	351825	55	F	RE	N	Pterygium	II	Excision + AMT			
15	351828	60	F	LE	N	Pterygium	II	Excision + AMT			

Chemical Burns											
Sl No	**IP No**	**Age**	**Sex**	**Etiology**	**EYE**	**Hugh's Grading**	**Treatment**	**Outcome**		**Resurgery**	**COMPLICATIONS**
								S	**F**		
1	354147	40	M	Ammonia	RE	IV	AMT		F	Done	SV&DV,SYM,CM
2	354147	40	M	Ammonia	LE	III	AMT		F	_	SV&DV,SYM
3	330987	26	M	Caustic Soda	LE	II	AMT	S		_	_
4	328951	58	M	Caustic Soda	RE	III	AMT		F	_	SV&DV
5	328951	58	M	Caustic Soda	LE	III	AMT		F	_	SV&DV
6	354918	32	M	Caustic Soda	RE	II	AMT	S	_	_	_

Cicatrising Conjunctivitis									
Sl no	**IP NO**	**Age**	**Sex**	**Eye**	**Etiology**	**Treatment**	**Resurgery**	**Outcome**	**Complications**
1	318688	32	F	LE	TEN	SYM release with AMT	Done	S	SV
2	399578	22	F	LE	SJS	AMT	_	S	SV
3	399578	22	F	RE	SJS	AMT	_	S	
4	355859	12	F	LE	OCP	AMT + Symblepherectomy	_	F	SV ,DV,SYM,CM

SYMBLEPHARON											
Sl No	**IP No**	**Age**	**Sex**	**Etiology**	**EYE**	**Grading**	**Ocular Motility**	**Treatment**	**Resurgery**	**Outcome**	
										S	**F**
1	323390	45	M	Traumatic	LE	Partial	Restricted	Excision and AMT	_	S	_
2	309104	49	M	Chemical	LE	Partial	Restricted	Excision and AMT	Done	_	F

PED								
Sl No	**IP No**	**Age**	**Sex**	**Eye**	**Etiology**	**Treatment**	**Outcome**	**Recurrence of epithelial defects**
1	349776	23	M	LE	PED Sec to KCS	AMT	**S**	**No**
2	318520	59	M	RE	PED Sec to Autoimmune disease	AMT	F	Yes

Ocular surface squamous neoplasia								
Sl No	**IP No**	**Age**	**Sex**	**Eye**	**Etiology**	**Treatment**	**Outcome**	**Recurrence**
1	1249626	60	F	RE	Limbal Sq.C.C	Excision + AMT	S	No