A STUDY ON AMNIOTIC MEMBRANE TRANSPLANTATION IN OCULAR SURFACE DISORDERS

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ABSTRACT

Purpose*:* To evaluate the outcome of preserved amniotic membrane transplantation (AMT) forocular surface reconstruction in ocular surface disorder.

Methods*:* A prospective study on 25 consecutive eyes with ocular surface diseases was performed. In all, 6 eyes had lime burns and 4 eyes had acid burns,5 eyes had corneal ulcer,5 eyes had persistent epithelial defect,4 eyes had pterygium,1 eye had symblepheron. AMT was performed within 3 weeks of injury. Post-operative assessment for ocular surface epithelization, corneal vascularization, symblepharon formation, and visual outcome at the time of complete epithelization was done.

Results*:* Patients were followed up for 10.14±4.41 months. All patients had immediate relief of pain postoperatively. Of 25 eyes, 24 eyes (96%) showed epithelialization within 1–4 weeks (15.33±9.91 days). The final visual acuity improved in 23 of 25 eyes (92%). Eyes with burns of grade II and III showed more visual improvement than those with grade IV burns. None of the eyes showed perforation. Symblepharon was seen in 1 of 25 eyes (4%). Of 25 eyes, 12 (48%) experienced limbal stem cell deficiency and 32% (8/25eyes)showed superficial corneal vascularization for >6 clock hrs.

Conclusions*:* Amniotic membrane transplantation is a good and viable option in ocular surface disease and reconstruction its properties and its function as a substrate for cultivating limbal epithelial corneal cells.

Keywords: Amniotic membrane, Ocular Surface disease, chemical burn, epithelization,limbal stem cell deficiency

INTRODUCTION

Ocular surface burns are one of the true ophthalmic emergencies. The ocular surface comprises the cornea, conjunctiva, Eyelid, lacrimal gland and any disorder in these structure Can be classified as ocular surface disorder (OSD). OSD including Dry eye disease,persistent epithelial defect (PED), blephritis, meibomian gland dysfunction, allergic eye disease, chemical and thermal burn, pterygium, corneal ulcer, CDK(climatic droplet keratopathy)[1]. While adults are prone to occupational or household chemical or thermal burns, paediatric age group is at risk of corneal blindness while playing with lime packets or firecrackers.[1] Acid injuries have been considered less destructive compared to alkali burns, however, strong acids like hydrochloric, nitric, and sulfuric acids can be as dangerous as alkali injuries.[2] Although lime has poor penetration, it may cause a prolonged and severe damage as a retained particulate matter.[3] Irrespective

of the nature of ocular surface burns, the aim of management in the acute phase includes removing the offending agent if any, promoting
ocular surface epithelization[4], controlling the inflammation and intra ocular pressure (IOP), support of reparative process by avoiding further epithelial and stromal breakdown, preventing infection, and other complications.[5] Since its first use in ophthalmic surgery by De Roth, Amniotic membrane (AM) is used for a variety of ocular surface conditions requiring either ocular surface healing or reconstruction, as in acute and chronic stages of ocular surface burns.[6-9]
Restoration of vision after ocular surface burns is possible with the restoration of ocular surface later in stages with newer simple procedures like simple limbal stem cell transplant if one can achieve eye integrity with less or no ocular surface comorbidities[10].Primary & intensive management or a timely referral in the acute phase to maintain eye integrity and reduce ocular surface comorbidities needs to be reemphasized among the ophthalmic community. Hence, this study aims to evaluate the outcomes of early intervention with amniotic membrane transplantation (AMT) in ocular surface disease at a tertiary eye care center in western India. Amniotic Membrane helps in

1. Proliferation and differentiation of stromal fibroblasts.
2. Promoting epithelial cell migration, adhesion and differentiation.
3. Supporting the growth of the epithelial progenitor cells by prolonging their life span and maintaining their clonogenicity.
4. Suppressing the expression of certain inflammatory cytokines that originate from the ocular surface epithelium, including interleukin 1α (IL-1α), IL-1β, IL-2, IL-8, interferon γ, tumor necrosis facrtor-α and platelet derived growth factor.
5. Reducing vascularization and scarring.

Because of its strength, elasticity, semitransparency, healing property and resemblance with ocular tissue,
amniotic membrane appears to be a suitable tissue for the management of ocular surface disorder.
Preserved human amnion has been successfully used as a biological bandage.The amniotic membrane (AM) is the innermost layer of the fetal membranes of the placenta. It is avascular and has an epithelial layer with a sub-adjacent avascular stromal layer. The amniotic membrane is one of the thickest membranes in the human body. Histologically the amnion is 0.02-0.5 mm five layered membrane:
1. the cuboidal epithelial layer
2. the basement membrane
3. compact layer
4. fibroblast layer
5. intermediate(spongy) layer

OPHTHALMIC INDICATIONS:
Conjuctival surface reconstitution:
1. Pterygium surgery
2. Chemical burn
3. Cicatrizing conjunctivitis
4. Ocular surface squamous neoplasia (OSSN)
5. Ocular cicatrizing pamphigoid or Stevens- johnson syndrome

Corneal surface reconstruction:
1. Persistent epithelial defect (PED)
2. Bullous keratopathy
3. Shield ulcer of vernal keratoconjuctivitis
4. Ulcerative keratitis

Amniotic membrane is available in 5 forms
1. Freshly prepared amniotic membrane
2. Cryopreserved amniotic membrane
3. Freeze dried amniotic membrane
4. Proker
5. Amniotic membrane extract (AMX)

Principles of surgery
Inlay or graft technique:
When the AMG is tailored to the size of the defect and is meant to act as a scaffold for the epithelial cells, which then merges with host tissue, it is referred to as a graft.
Overlay or patch technique:
When the AM is used akin to a biological

Materials and methods

A prospective analysis of case records was performed, amniotic membrane transplantation was performed on 25 patients. The age ranged between 6–53 years. The male: female ratio was 10:4 with a mean age of 20.60±11.93 years for the males and 16.25±8.42 years for females.

Inclusion Criteria

1. Chemical injury grade II and onwards.
2. Persistent corneal epithelial defect
3. Thermal injury
4. Non-infective corneal ulcer
5. Primary pterygium

Exclusion Criteria:

1. The cases of grade I ocular surface burn
2. Minimal corneal erosions occurring for first time which are healing spontaneously.
3. Lids abnormalities like entropion, trichiasis and meibomitis.

Data were recorded for age, gender, time of presentation to the study centre since injury, type of injury (accidental/assault), nature of injury (acid/alkali/ thermal), site of injury (occupational/household), laterality, primary consultation and treatment taken if any. Other factors considered were interventions done at the study centre, visual acuity and clinical findings (corneal findings: presence of particulate agent, e.g., lime, epithelial defect, haze, melting, infection; conjunctival findings: presence of particulate agent, e.g., lime, epithelial defect area, blanching, subconjunctival haemorrhage, necrosis, symblepharon). All patients received a detailed ophthalmic examination, including visual acuity, slit lamp, tonometry, and fundus examination (wherever possible). The Schirmer test with and without anaesthesia was used to evaluate tear function. A detailed assessment of limbal ischaemia and conjunctival involvement in the form of necrosis, lime deposits, and ischaemia was made. Cases with lid involvement were excluded from the study.

Late outcomes were measured for corneal vascularization (clock hours) and symblepharon development (graded as mild, moderate, and severe for horizontal fornices and vertical bulbar involvement, higher grade considered in case of difference in grading for both for convenience).[15] Visual acuity of more than two lines -from the presentation on the Snellen chart was considered as improvement on complete epithelization. Each outcome was measured for the age groups (≤14 years of age as paediatric and >14 years of age as adult), each type of agents (acid, alkali, and thermal), each grade of Injury (grades III, IV, V, and VI as per Dua's classification), time of AMT performed (in ≤7 days and in >7-14 days post insult.

Treatment strategy:For chemical injury patients thorough irrigation with normal saline or balanced salt solution (BSS) and removal of the particulate matter if any under local anaesthesia on presentation was performed in cases presenting in immediate phase. Estimation of injury and grading was done as per Dua's classification.[11,12,13] Medications used were topical antibiotic eye drops (four times/day) and antibiotic eye ointments (once at night), artificial tear substitutes (hourly basis), topical steroid preparations (four times/day to hourly frequency as per inflammation severity and duration of presentation), Vitamin C eye drops preparations (hourly basis), Cycloplegic eye drops, and IOP-lowering agents if indicated as per the recommended frequency. Systemic doxycycline (if not C/I), analgesic/anti-inflammatory medications, and Vitamin C supplementation as per the age and weight of the patient were added to the treatment.[2,3] All the patients underwent AM transplant procedure within 1-3 week of ocular surface diseases under local anesthesia. Amniotic membrane graft was placed over ocular surface disease followed by bandage contact lens (BCL) over it. The follow-up schedule was on the first post-operative day and as per requirement in individual case thereafter. Post-operatively steroid was used for 4-6 times a day and tapered as per the response over 4-6 weeks.

The early post-operative assessment was done for (1) Symptomatic improvement (ocular pain, watering, and photophobia) and (2) Epithelization of cornea and conjunctiva [Fig. 1].

Complete epithelization of ocular surface was considered as maintenance of intact epithelization without recurrence of defect in subsequent follow-up period with or without ocular surface scarring.

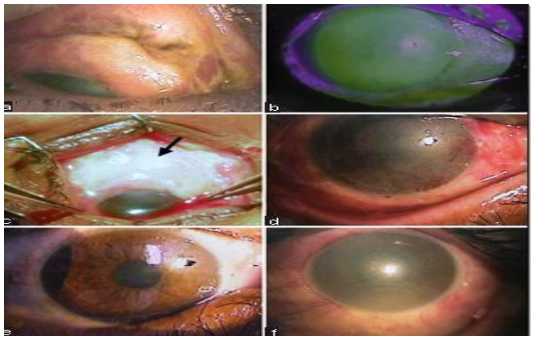
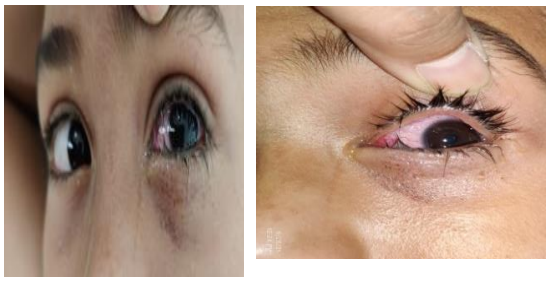


Figure 1: Assessment and management of ocular surface chemical burn.

Case 1. Dua·s grade 4 chemical burn (a-e) examination of upper lid eversion(a),

(b)flouroscein stain,(c) debridement of necrotic tissue and removal of retained lime (black arrow) from superior fornix,

(d) AM placed with bandage contact lens on post op day 1, (e) complete epithelization 3weeks post operatively.



(A) **(B)**

Figure no 2- Assessment and management of ocular surface burn after placement of preserved amniotic membrane

(A)Pre op Clinical pic after 8 hrs of Ocular surface injury

(B)Post op Clinical pic after 7 day of preserved amniotic membrane

(AM) transplacement with bandage contact lens (BCL) on it

Table 1: Demographic and preoperative details

Patient details.		No.of Pateints	Total No of pateints
Age	≤14 years	10(40%)	25
	>14 years	15(60%)	
Gender	Male	15(60%)	25
	Female	10(40%)	
Laterality	Unilateral	18(72%)	25
	Bilateral	7(28%)	
Location	Work	10(40%)	25
	Home/nearby	15(60%)	
Visit	Primary	20(80%)	25
	Referral	2(20%)	
Etiology			
1.chemical Injury	Acid	4(16%)	25
	Alkali	6(24%)	
2Corneal ulcer		5(20%)	
3Persistent Epithelial defect		5(20%)	
4 Pterygium		4(16%)	
5Symblepheron		1(4%)	

Table no-2 observation table Epithelial healing time (days)

Indication	≤14 days	15-21 days	22-28 days	≥1month	Total
Chemical injury	3	4	2	1	10
Corneal ulcer	2	2	1	0	05
Persistent epithelial defect	1	2	2	0	05
Pterygium	3	0	1	0	04
Symblepheron	0	1	0	0	01
Total	9(36%)	9(36%)	6(24%)	1(4%)	25

Table no 3- Improvement in visual acuity Pre op visual acuity

Indication	6/12or better	6/18-6/24	6/36-6/60	<6/60	NFFC	H M	TO TA L
Chemical injury	3	3	2	2	0	0	10
Corneal ulcer	1	2	2	0	0	0	5
Persistent epithelial defect	2	2	1	0	0	0	5
Pterygium	3	1	0	0	0	0	4
Symblepher on	1	0	0	0	0	0	1
Total	10(40%)	8(32%)	5(20%)	2(8%)	0	0	25

Table no 4: post op visual acuity

Indication	6/12or better	6/18-6/24	6/36-6/60	<6/60	NFFC	HM	TOTAL
Chemical injury	3	3	2	2	0	0	10
Corneal ulcer	1	2	2	0	0	0	5
Persistent epithelial defect	2	2	1	0	0	0	5
Pterygium	3	1	0	0	0	0	4
Symblepheron	1	0	0	0	0	0	1
Total	10(40%)	8(32%)	5(20%)	2(8%)	0	0	25

Improvement in visual acuity seen in 23/25 eyes (92%).

Statistical analysis:

The pre-operative and postoperative data were analyzed by w^2 in the MS EXCEL spreadsheet and analysis was done using Statistical Package for Social Sciences (SPSS) version 21.0. A *P* value of <0.05 was considered statistically significant.

Results

The demographic and clinical characteristics are summarized in Table 1.

Notably, the pain was dramatically relieved after the AMT in all patients. Mostly eyes were completely free from any pain or discomfort, watering and photophobia after AMT. None of the patients developed corneal perforation.For a follow up period of 10.14±4.4 months.

Of 25 parients, 12 (48%) experienced limbal stem cell deficiency and showed superficial corneal vascularization for >6 clock hrs was seen in 53.33% (8/25 patients). The severity of corneal vascularization was related to the severity of chemical burn (*P* ¼ 0.038, w^2 test). At the end of 1st and 3rd month post AMT, all patients had stable tear films with none having a value less than 5 mm on Schirmer's test.

Discussion

Amniotic membrane, the outermost portion of foetal membranes possesses anti-inflammatory,[13] anti scarring,[14] stem cell proliferating,[15] and epithelialization promoting effects on the ocular surface. It has been found useful in the treatment of persistent epithelial defect (PED) with ulceration,[16] primary, and recurrent pterygium with symblepharon,[17] for conjunctival surface reconstruction[18] and in combination with limbal grafts in the reconstruction of ocular surface in advanced ocular cicatricial pemphigoid and Steven–Johnson syndrome[19] as well as chemical and thermal burns.[20] Reports regarding the role of

AMT in acute ocular burns success are highly variable.

In a similar study Meller *et al*[9] he treated 13 eyes of acute burns with AMT within 2 weeks after the injury. A total of seven eyes had grade II–III burns and six eyes had grade IV burns. Epithelial defects of all but two patients healed in 2–5 weeks. Only one patient developed a symblepharon. All eyes with grade IV burns experienced limbal stem cell deficiency. Out of five patients with total limbal ischaemia, three required limbal stem cell transplantation, one required large penetrating keratoplasty and conjunctival flap for a corneal perforation, and the last patient had a persistent epithelial defect at the end of 4 months after which he was lost to follow up. AMT alone could not maintain the ocular surface for burns with total limbal ischaemia.

Similarly, Dua *et al*[10] reported that in extremely severe burns AMT does not establish the ocular surface or preserve the integrity of the globe.

AMT promoted epithelialization and none of the eyes had persistent epithelial defects, and improvement in visual acuity was noted in all eyes. None of the eyes developed ulceration or perforation. Although AMT was not totally effective in preventing symblepharon and corneal vascularization, their severity was mild to moderate. The utility of AM in grade IV was found to be highly limited. Symblepharon and corneal vascularization were noted in all eyes. In all, three eyes developed ankyloblepharon and two eyes went into phthisis. It thereby suggested that in severe burns with extensive conjunctival damage it does not completely restore the conjunctival surface and with associated extensive limbal stem cell damage it does not prevent

the sequelae of limbal stem cell deficiency. Although stable, a less inflamed external ocular surface was achieved in most of the cases and this may aid in obtaining more successful results when limbal stem cell transplantation is resorted to at a later date.

Different delivery techniques like AM extracts, Pro-Kera, and modified ocular surface ring (MOSR) for AMT in ocular surface burns have been studied, but their widespread use is limited due to varied extent of ocular surface burns in the early phase, affordability and availability of AM device or AM itself for repeated need in acute phase especially in the Indian scenario.

Conclusions*:* Amniotic membrane transplantation with preserved amniotic membrane increases patient comfort and reduces inflammation. In mild burns, AMT alone restores corneal and conjunctival surfaces. In moderate to severe burns, it probably reduces conjunctival scarring sequelae, but does not prevent the sequelae of limbal
stem cell deficiency that requires further limbal stem cell transplantation. In the acute stage, amniotic membrane transplantation probably has a protective role against the progressive melting and perforation, persistent epithelial defect; limbal stem cell transplantation.

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