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Our Experience in Amniotic Membrane Transplantation For Ocular SurfaceDisorders - At A Regional Ophthalmic Centre In South India

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Abstract:
Purpose: *Preserved human amniotic membrane (AM) is currently being used for a wide spectrum of ocular surface disorders. Country like India, were there is shortage of cornea and people are below poverty line to buy commercially available AM, hence used as cost effective procedure in our study. AM has a basement membrane, which promotes epithelial cell migration and adhesion, presence of a unique avascular stromal matrix reduces inflammation, neovascularization and fibrosis. The basic tenets of amniotic membrane transplantation (AMT) are to promote re-epithelialization, to reconstruct the ocular surface and to provide symptomatic relief from surface aberrations. However, remarkable improvements have resulted from use of AMT alone or concurrently with limbal stem cell transplantation(LSCT).*
Methodology: *Patients attending tertiary care eye Centre in south India. Subjects for present study were selected by applying the inclusion and exclusion criteria.*
Results: *Forty cases of different ocular surface disorderswere treated with AMT.All cases of chemical symblephara and shield ulcer accepted the graft very well, while 66.7% of traumatic Symblepharon, 85.7% of primary progressive Pterygium, 75% of bullous keratopathy and 57.1% of chemical burns patients accepted the AMT.*
Conclusions: *In our experience of AMT for various ocular surface disorders, there was overall success rate of 75%, comparable to studies by various authors, by various mechanisms. So, AMT becomes an attractive alternative for country like ours where there is shortage of tissues. Hence used either as a substrate or patch graft, to replace ocular surface. Lastly efficacy of AMT is better in ocular surface disorders.*
Keywords:*Amniotic membrane Transplantation, Limbal Stem Cell Deficiency, Ocular Surface Reconstruction.*

I. Introduction

The management of Ocular surface reconstruction techniques have advanced considerably during last few years with advent of AMT and LSCT.[1.2.3 .4]
De Roth (1940) first describedthe use of live placental membranes to repair conjunctival defects.[5] However, the need for repetitive applications made the technique rather unpopular until its revival by Kim and Tseng 1995.
AM is used in generation of a cultivated epithelium, using it as substrate or vehicle to culture the stem cells of limbal origin , cultivated corneal epithelium thus generated on the denuded AM for reconstructing the ocular surface in severe stem cell deficiencies. [6]

Objectives
To assess the efficacy of Amniotic membrane graft for treating the ocular surface disorders as
a) Substrate or
b) Patch (Biological Dressing) or
c) Combination of both.

II. Materials And Methods:

Study Setting: Tertiary eye care Centre and Regional institute of Ophthalmology, Bangalore

Study Subjects: All the patientspresenting ocular surface disorders

Inclusion criteria
a) Sterile corneal ulcers (excluding peripheral ulcers)
b) Symptomatic bullous keratopathy
c) Chemical injury

d) Dry-eye (due to aqueous and mucin deficiency)
e) Primary progressive pterygium with three mm or more infiltration of cornea

Exclusion criteria
a) Infective ulcers
b) Ocular neoplasia
c) Recurrent pterygium
d) Marginal Sterile ulcer

Study Duration: January2012- December 2014.

Study Design: Prospective Interventional Study

Study Samples: Patients with ocular surface disorders, satisfying the above criteria and who gave written consent for the transplantation were included in the study.

Procedure
Technique of Amniotic Membrane Graft Preparation

The amniotic membrane was harvested from consenting sero negative (Negative for Hepatitis B and C virus, Syphilis and HIV) maternal donors during elective caesarian section. Under sterile conditions, the placenta was washed with balanced salt solution to remove clots and debris. The membrane was then bathed in a cocktail of antimicrobials, followed by a second wash with balanced salt solution. Subsequently, the amnion was separated from the chorion and divided into pieces each measuring approximately 2 cm × 2 cm and mounted, stromal side down, onto a nitrocellulose sheet. The membrane was then placed in a plastic container, and stored in saline for subsequent use.This prepared material was obtained from a charitable hospital , banglore where they preserve the membrane and make it commercial available to ophthalmic centres when required.

Technique of Amniotic Membrane Graft Transplantation

Informed consent was obtained from each patient before surgery. Preoperatively, detailed medical history was obtained including the presence or otherwise of diabetes or collagen vascular diseases. Complete ophthalmic examination including visual acuity, intraocular pressure, slit lamp examination and fundoscopy were performed. 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 dexamethasone was given and lids were closed.

The patient was prepared for surgery. A peribulbar anaesthesia was given. The amniotic membrane obtained from Lion's eye hospital 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 was performed, and the membrane was placed on the cornea to cover the defect and excess of AM was trimmed and was sutured with interrupted 10-0 nylon suture on the cornea and 8-0 vicryl suture onto sclera whenever required. Patients were prescribed steroid antibiotic drops and tear substitutes. Patients were examined on postoperative day 1, and of 1st week, 2nd week 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.

III. Results

TABLE 1: Gender wise distribution of Study Subjects

Gender	Frequency (%)
Female	16 (40)
Male	24 (60)
Total	40 (100)

TABLE 2: distribution of study subjects according to ocular surface disorders

Disorders	Frequency (%)
Bullous Keratopathy	4 (10)
Chemical burns	7 (17.5)
Chemical burns with symblepharon	2 (5)
Persistent epithelial defect secondary to Mooren ulcer	1 (2.5)
Primary progressive Pterygium	21 (52.5)
Shield ulcer	2 (5)
Traumatic symblepharon	3 (7.5)
Total	40 (100)

TABLE 3: outcome of amt in study subjects

Ocular Surface Disorders	Outcome			Total
	Success	Re-surgery	Failure	
Bullous Keratopathy	3	0	1	4
Chemical burns	3	1	3	7
Chemical burns with Symblepharon	2	0	0	2
Persistent epithelial defect secondary to Mooren ulcer	0	1	0	1
Primary progressive Pterygium	18	0	3	21
Shield ulcer	2	0	0	2
Traumatic Symblepharon	2	1	0	3

Out of four cases of Bullous Keratopathy receiving AMT, only one showed recurrence, while other three patients were symptomatically better.75% of success and 25% of failure of AMT was seen.

Among 7 cases, 4 patients belonged to Grade II and 3 patients to Grade III to Hugh's classification, graftwas taken up by 4 patients (57.1%) successfully, but 2 patients had superficial vascularisation and 1 patient had superficial and deep vascularisation who did not take up the graft, so regrafting was done, that also was not taken up by the patient. So there was 42.9% failure rate so patients were advised keratoplasty.

Patient with Mooren's ulcer even on regraft did not take up the graft, nor there was pain or vision. Majority of the cases of Primary Progressive Pterygium that is 18(85.7%) accepted the AMT remaining 3(14.3%) patients did not accept the graft.Both the cases of shield ulcers treated with AMT accepted the grafts successfully.On the whole 80% of the Symblepharon cases accepted the AMT, 100% success was in the chemical Symblepharon cases, while there was failure rate (33.3%) seen in the AMT done for Symblepharon cases due to trauma.

IV. 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. [7]

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 [.8] 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.[9-11] 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.

TABLE 4: comparison between present study and earlier study for bullous keratopathy

	Present Study	Edgar M. Espana et al. (2003) [12]	Pires Renato1999[13]
Success	3(75%)	15(83.4%)	45(90%)
Failure	1(25%)	3(16.6%)	5(10%)
Total No. of Cases	4(100%)	18(100%)	50(100%)

Since sample size in our study is very small the study cannot bestrictly compared but it was observed in our study that AMT when used for aphakic bullous keratopathy was successful in 75% of cases, i.e. patients were symptomatically relieved of their pain as compared to 83.4% of Espana et al.The study conducted by Espana included 18 eyes with bullous keratopathy presenting with intractable pain or discomfort and poor visual potential. Pain relief was obtained in 15 patients (88%). One eye had enucleation for persistent pain. Corneal epithelial healing was complete in all except one eye. [12]

According to Pires Renato (1999), AMT was performed at five centers on 50 consecutive eyes of 50 patients with sympatomatic bullous keratopathy and poor vision. During the mean follow-up period of 33.8 weeks after AMT, 43 of 48 eyes with intolerable pain preoperatively required amniotic membrane transplantation, one required a conjunctival flap for pain relief, and one had reduced pain. Epithelial defects in 45 of 50 eyes created and covered by AMT healed rapidly within three weeks. Only four eyes showed recurrent surface breakdown. Epithelial edema or bullae recurred in a smaller area in five eyes and pseudopterygium developed in one eye. [13]

TABLE 5: comparison between present study and earlier study for primary progressive pterygium

	Present study	Pinnita Prabhasawat[14]
No. of Cases	21	46
Success	18 (85.7%)	41 (89.1%)
Failure	3 (14.3 %)	5 (10.9%)

There is a very good correlation in the success rate of present study (85.7%) and the study of Pinnita et al.(89.1%), thus demonstrating the supremacy of AMT in primary progressive Pterygium..[14] According to Ma Hui-Kang David, et al. (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. {15]

According to Pinnita Prabhasawat et al. (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 conjunctival autograft consisted of 78 eyes with primary and 44 eyes with recurrent pterygia (the recurrence rate was 2.6%, 9.1% and 4.9%).

TABLE 6: comparison between present study and earlier study for shield ulcers

	Present study	M.S.Sridhar, et. al.[16]
No. of Cases	02	04
Success	02	04
Failure	00	00

In both the studies, Shield ulcers treated with AMT healed successfully.

TABLE 7: comparison between present study and earlier study for chemical burns

	Present study	Jose Alvaro, et. al.(2003)[17]	Annie Joseph (2001)
No. of Cases	07	20	4 → 3 (chemical) 1 (thermal)
Success	04 (57.1 %)	15 (75%)	0
Failure	03 (42.9%)	05 (25%)	4 (100%)

Since the sample size in our study is very small the study cannot be strictly compared. In our study, it was observed that among seven cases, four took the graft successfully of the remaining three, two had superficial vascularisation, while one had both superficial and deep vascularisation.

According to Jose Alvaro Perira Gomes et al. (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. [17]

According to Annie Joseph (2001) 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 pthysical.

TABLE 8: comparison between present study and earlier study – traumatic symblepharon

	Present study	Panda et.al[18]
No. of Cases	03	24
Success	02 (66.7%)	20 (80%) successful ; 4 (20%) partially successful
Failure	01 (33.3%)	0

Since the sample size in our study is very small the study cannot be strictly compared. According to Panda et al, 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 66.7% of the cases of traumatic Symblepharon with 33.3% failure. [18]and in patients with chemical burns with Symblepharon it was observed that those who had moderate to severe dry eye showed normal Schirmers after AMT, while in patients with persistent epithelial defect due to Mooren's did not take up the graft, where regrafting was done and there was failure of the regrafts also.

V. Conclusion

As conventional methods in the management of ocular surface disorders have a limited success,

AMT remains one of the most challenging entities facing the clinician today. When medical treatments fail and the defect or ulcer persists, conventional surgical treatments become indicated and should include punctual 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 mayConjunctival 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 mechanisms of action.

Firstly, 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.

Secondly, the amniotic membrane exerts an anti-inflammatory effect.

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

Fourthly, 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.

Fifthly, the amniotic membrane may promote nerve regeneration by maintaining nerve growth factor (NGF) signaling.

Thus AMT, having following advantage 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.

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 ocular surface disorders. AMT has effectively restored a stable corneal epithelium in eyes with, PEDs and corneal ulcers.

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.

The above study has conclusively shown that AMT is a very useful technique for primary Pterygium, shield ulcer of VKC and chemical Symblepharon. However it is found to be less effective for bullous keratopathy, chemical burns, traumatic Symblepharon and persistent epithelial defect secondary to Mooren's ulcer.

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