



TO EVALUATE EFFICACY OF HUMAN AMNIOTIC MEMBRANE TRANSPLANTATION FOR OCULAR SURFACE RECONSTRUCTION IN TERMS OF VISUAL IMPROVEMENT AND SURFACE REGULARIZATION IN WESTERN RAJASTHAN.

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ABSTRACT

INTRODUCTION: Present study was conducted to assess the efficacy and success rate of amniotic membrane transplantation on the basis of improvement in symptoms and visual acuity, achievement of corneal transparency and corneal thickness and healing of the ulcer by re-epithelization. **METHODOLOGY:** A study was conducted on 52 diagnosed ocular surface disorder patients who underwent amniotic membrane transplantation. A detailed postoperative evaluation of the patient along with follow up was done to study the success rate and efficacy of amniotic membrane transplantation. **RESULTS:** A total of 52 eyes of 52 patients underwent surgery over a period of 12 months including 44 males and 08 females. The average age of these patients was 46.6 years ranging from 9 years to 74 years. Success, partial success and failure were obtained in 38 eyes (73.08%), 13 eyes (25%) and 1 eye (1.92%) respectively. In non-healing corneal ulcer 12 out of 15 patients were rated as success and improved vision of approximately 2 Snellen's lines. Success rate was 73.83% in eyes with persistent epithelial defect and 75% in eyes with descemetocoele. Cases with pterygium and symblepharon achieved success rate of 70% and 62.50% respectively. **CONCLUSIONS:** This study concludes that amniotic membrane transplantation is a safe, simple, inexpensive and effective for different ocular surface disorders with a satisfactory success rate.

KEYWORDS : Stem cells, Amniotic membrane graft, Ocular surface diseases.

INTRODUCTION

The limbus is a 1.5-2 mm band of narrow tissue which is straddling the cornea and the conjunctiva and it is the site of the corneal epithelial stem cells.

Stem cells are responsible for the ultimate cellular replacement and tissue regeneration in all self-renewing tissue¹. Chemical / thermal injuries, contact lens induced keratopathy, Stevens-Johnson syndrome, neurotrophic keratopathy multiple surgeries or cryotherapies to the limbal region, or toxic effects from lens-cleaning solutions, pterygium, psuedopterygium and peripheral corneal ulcerative keratitis may result in loss of stem cells². The ocular surface breaks down and if the normal epithelialization process fails the corneal defects may become chronic. Conjunctival epithelium may replace corneal epithelium resulting in loss of corneal transparency. Chronic inflammation may then occur which may lead to loss of visual acuity.

In significant conjunctival and corneal damage, various biological tissues have been attempted to be used to reconstruct the ocular surface or as donor tissue to repair or to decrease the inflammation. Amniotic membrane transplantation (AMT) has been successfully used for reconstruction of a variety of ocular surface disorders. If compared with other biologic tissues which are used as reconstructive grafts, the amniotic membrane has more advantages; It is thinner and it never becomes necrotic thus better tolerated by the patient³. It is a substrate of conjunctiva not a substitute, where the conjunctival cells migrate and regenerate thus forming new and healthy tissue. The regenerating epithelium is shielded by the AM as it acts as a biological bandage from the frictional forces generated by the blinking movements of the eyelids⁴. Regenerating corneal epithelium facilitates for epithelial cell migration^{5,6}; basal epithelial cell adhesion reinforcement^{7,9}; promotes epithelial cell differentiation¹⁰⁻¹²; and prevents apoptosis^{13,14}. AM has anti-fibrotic and anti-inflammatory properties.

Fetal hyaluronic acid, an important constituent of the AM stromal matrix helps to suppress transforming growth factor beta (TGF β) thus signaling the reduced expression of transforming growth factor beta (TGF β). The proliferation of limbal, corneal and conjunctival fibroblasts is inhibited. Anti-inflammatory effect of AM also inhibits the expression of pro inflammatory cytokines released from the damaged ocular surface and also by showing that in the matrix of AM inflammatory cells get trapped and undergo apoptosis. In addition to the anti-inflammatory properties AM also has anti-angiogenic and

antimicrobial properties.

METHODS AND MATERIALS

The present clinical study was undertaken to review the characteristics of amniotic membrane that make it potentially useful to treat ocular surface abnormalities and to discuss the current indications, the surgical technique, and the outcome of AMT.

A hospital based prospective study was conducted between October 2017 to September 2018 in the department of Ophthalmology, Mathura Das Mathur Hospital, Dr. Sampurnanand Medical College, Jodhpur with due permission from the Institutional Ethical Committee and Review Board and after taking written informed consent from patients and also from parents when appropriate.

Sample size is calculated to be 52 subjects at α error 0.05 and study power 80% (20% allowable error) assuming 75% efficacy of amniotic membrane transplantation¹⁵.

The patients included in the study were diagnosed with persistent corneal epithelial defect, thermal burns, chemical injury, non-infective Corneal ulcers, mechanical trauma and primary pterygium and Infected ulcers rendered sterile with antibiotics. The patients with minimal corneal erosions, minimal chemical/ thermal burns, minimal mucocutaneous lesions which were healing spontaneously or with medications, patients with entropion, ectropion, trichiasis were excluded from the study. Fresh Amniotic membrane was harvested from consenting seronegative (hepatitis B and C virus, syphilis and human immunodeficiency virus) maternal donors during elective caesarian section in Mathura das mathur hospital, in department of obstetrics and gynaecology. The placental membrane was washed under sterile conditions in a balanced salt solution (BSS) to remove clots and debris. In the cocktail of antimicrobial medium, the membrane was bathed and stored at 4°C. In 24 hrs. of harvest the membrane was used.

The AM is placed basement/epithelial membrane side up and is trimmed to fit the size of the underlying stromal or epithelial defect. It is sutured to the episcleral and conjunctiva using 8-0 vicryl and to the cornea using non absorbable 10-0 nylon sutures. The basement membrane of the amnion acts as an excellent substrate for growth, therefore it is preferred to keep the basement/epithelial membrane side up. basement of the progenitor epithelial cells by prolonging their lifespan, maintaining clonogenicity and preventing apoptosis¹⁶.

Efficacy was monitored on the basis of improvement in symptoms and visual acuity, achievement of corneal transparency and corneal thickness, healing of the ulcer by re-epithelization and formation of anterior chamber.

Accordingly, patient's outcome was described as successful, intermediate and failure (Table 1).

Table 1: Outcome definitions

| OUTCOME | DEFINITION OF OUTCOME |
|-----------------|---|
| Successful | Healed and stable surface |
| | Corneal thickness achieved >50% of original |
| | Residual opacity <50% of preoperative corneal opacity |
| | Complete symptomatic relief |
| Partial success | Healed corneal surface |
| | Corneal thickness <50% of original, or |
| | Requiring any subsequent intervention after surgery (hypopyon wash, air injection in AC, re-grafting) |
| Failure | Not healed corneal surface, or |
| | Progressive thinning |
| | No improvement in symptoms. |

The outcome of success for symblepharon was defined on the follow up of 6 months as being free of scar or inflammation, restoration of a stable depth fornix, and no motility restriction. Partial success was defined as focal recurrence of scar tissue without inflammation, whereas failure was defined as the return to inflamed and scarred tissue in the area of surgery and obliteration of the fornix at the last follow up period.

Recurrence of pterygium was defined as any fibrovascular growth of conjunctival tissue extending more than 1.5 mm across the limbus.

POSTOPERATIVE CARE AND EVALUATION

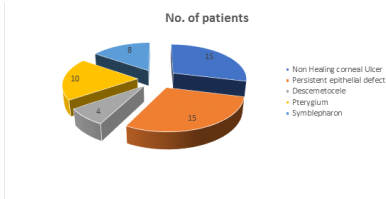
The patient was followed weekly and was routinely treated with topical 0.3% ofloxacin twice a day and 1% prednisolone acetate, three times a day before epithelialisation. Following healing, after epithelialisation was completed, the topical ofloxacin was discontinued but the prednisolone was tapered off. Fluorescein staining was used to detect epithelial defects.

COMPLICATIONS

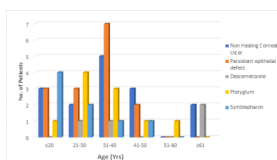
Immediate complications which are developing within 1 month of surgery are detachment of amniotic membrane, lid abscess and conjunctivitis. In the patient with bullous keratopathy the epithelial defect healed rapidly in 1week despite of detachment of amniotic membrane so that the patient became pain-free. From a patient with Steven-Johnson syndrome as a result of bacterial infection and the detachment of membrane and its dissolution in that area, the eye developed conjunctivitis. This complication was successfully treated with antibiotics.

Late complications that is corneal ulcer developed after 1 month of surgery and the epithelial surface had healed completely.

OBSERVATIONS



The various ocular surface disorder for which AMT was done is shown in above pie chart. The maximum number of cases were those of non-healing corneal ulcer and persistent epithelial defect.



The age distribution varied over a wide range . The youngest patient in the study was a 9-year-old boy with a symblepharon secondary to traumatic injury to the eye while the oldest patient was 74 years old male with corneal ulcer. The average age of patients in this study was 46.6 year.

TABLE 1: EXTENT IN AREA OF AMT

| Indication | Extent in area of AMT | | | | Total |
|------------------------------|-----------------------|---|------------------|------------|-------|
| | Whole cornea | Cornea & conjunctiva (5-7 mm around limbus) | Conjunctiva only | Lid to lid | |
| Non Healing Corneal Ulcer | 15 | 0 | 0 | 0 | 15 |
| Persistent epithelial defect | 0 | 7 | 0 | 8 | 15 |
| Descemetocoele | 4 | 0 | 0 | 0 | 4 |
| Pterygium | 0 | 0 | 10 | 0 | 10 |
| Symblepharon | 0 | 0 | 6 | 2 | 8 |
| Total | 19 | 7 | 16 | 10 | 52 |

Table 2: IMPROVEMENT IN VISUAL ACUITY

| Indications | Pre op visual acuity | | | | | | Total |
|------------------------------|----------------------|------------|-----------|-------------|------------|-------------|-------|
| | 6/12 or better | 6/18-6/24 | 6/36-6/60 | <6/60 | NFFC | HM | |
| Non-healing Corneal ulcer | 0 | 0 | 0 | 1 | 6 | 8 | 15 |
| Persistent epithelial defect | 0 | 0 | 1 | 9 | 2 | 3 | 15 |
| Descemetocoele | 0 | 0 | 0 | 0 | 0 | 4 | 4 |
| Pterygium | 7 | 2 | 1 | 0 | 0 | 0 | 10 |
| Symblepharon | 2 | 4 | 2 | 0 | 0 | 0 | 8 |
| Total | 9 (17.31%) | 6 (11.54%) | 4 (7.69%) | 10 (19.23%) | 8 (15.38%) | 15 (28.85%) | 52 |

TABLE 3: POST-OP VISUAL ACUITY-

| Indication | Post op visual acuity | | | | | | Total |
|------------------------------|-----------------------|-----------|-------------|-----------|-----------|-----------|-------|
| | 6/12 or better | 6/18-6/24 | 6/36-6/60 | <60/60 | NFFC | HM | |
| Non-Healing corneal ulcer | 1 | 4 | 10 | 0 | 0 | 0 | 15 |
| Persistent epithelial defect | 4 | 5 | 3 | 1 | 1 | 1 | 15 |
| Descemetocoele | 0 | 0 | 2 | 2 | 0 | 0 | 4 |
| Pterygium | 9 | 0 | 1 | 0 | 0 | 0 | 10 |
| Symblepharon | 3 | 4 | 1 | 0 | 0 | 0 | 8 |
| Total | 17 (32.69%) | 13 (25%) | 17 (32.69%) | 3 (5.77%) | 1 (1.92%) | 1 (1.92%) | 52 |

TABLE 4: RESULTS

| Indication | Results | | | | | | Total |
|------------------------------|---------|-------|-----------------|-------|---------|-------|-------|
| | Success | | Partial Success | | Failure | | |
| | N | % | N | % | N | % | |
| Non-Healing Corneal ulcer | 12 | 80.00 | 3 | 20.00 | 0 | 0.00 | 15 |
| Persistent epithelial defect | 11 | 73.33 | 4 | 26.67 | 0 | 0.00 | 15 |
| Descemetocoele | 3 | 75.00 | 1 | 25.00 | 0 | 0.00 | 4 |
| Pterygium | 7 | 70.00 | 2 | 20.00 | 1 | 10.00 | 10 |

| | | | | | | | |
|--------------|----|-------|----|-------|---|------|----|
| Symblepharon | 5 | 62.50 | 3 | 37.50 | 0 | 0.00 | 8 |
| Total | 38 | 73.08 | 13 | 25.00 | 1 | 1.92 | 52 |

DISCUSSION

In this study amniotic membrane transplantation was performed in patients with either non-healing corneal ulcer, persistent epithelial defect, descemetocoele, pterygium or symblepharon.

A total of 52 eyes of 52 patients underwent surgery over a period of 12 months, maximum number of cases were of non-healing corneal ulcers (15 cases) 28.85% followed by persistent epithelial defect, 15 cases (28.85%) and pterygium 10 cases (19.23%). Other indications included, descemetocoele, 04 cases (7.69%), and symblepharon 8 cases (15.38%).

The study group included 44 males and 08 females. The average age of these patients was 46.6 years ranging from 9 years to 74 years.

Depending upon the size of lesion amniotic membrane graft of size 4mm to the size including whole of the cornea and 5-7 mm from the limbus were applied. In deep corneal ulcers (4 cases) multiple layers of amniotic membrane were applied. Multiple layers were also applied in 4 cases with descemetocoele and 5 cases of symblepharon to give strength to the graft.

Although it was not uncommon to encounter minor problems such as detachment of membrane (12 cases), conjunctivitis (4 cases), corneal ulcer (2 cases), symblepharon (6 cases), we did not note any sign of graft rejection and only 2 patients became clinically worse than before the surgery in terms of visual acuity or inflammation. Two cases developed corneal ulcer after complete healing of the lesion. This was due to rubbing of their eyes accidentally which was treated successfully on the same line of treatment for corneal ulcer. Membrane was reapplied in cases where membrane detached within 1-2 days. The membrane did not detach further and lesion healed completely. Conjunctivitis was treated with topical antibiotics. Based on the criteria given in the table-7, success, partial success and failure were obtained in 38 eyes (73.08%), 13 eyes (25%) and 1 eye (1.92%) respectively. Details of the success rate in each group are shown in table-18.

In non-healing corneal ulcer 12 out of 15 patients were rated as success and improved vision of approximately 2 Snellen's lines. Success rate was 73.83% in eyes with persistent epithelial defect and 75% in eyes with descemetocoele. Cases with pterygium and symblepharon achieved success rate of 70% and 62.50% respectively.

AMT has come up as a very effective managing technique for refractory ulcers. It aids in permanent healing of the refractory infective keratitis and prepares the cornea for definitive reconstructive procedure if required. We have found that AMT represents a viable method of treatment to promote healing and prevent progressive melting of refractory infectious keratitis^[17]. Besides being cost-effective it's easy to perform, with a short learning curve. Thus, it might be considered a first line surgical technique when maximal medical treatment has failed.

In chemical burn cases immediate AMT is quiet effective for treating acute chemical burn up to grade 3 burn. Use of amniotic membrane alone in acute chemical burns with extensive limbal ischaemia and conjunctival (grade 4 burn) involvement was not very successful in establishing the ocular surface. It mainly plays an adjunctive role in limbal stem cell deficiency in severe chemical burns with near total limbal ischaemia.

We prefer AMT for primary pterygium because of faster healing time, less discomfort and acceptable recurrence rate, and believe that amniotic membrane transplantation is an appropriate treatment modality for the surgical management of primary pterygium. This may be particularly advantageous for patients with glaucoma who require intact conjunctiva for future glaucoma procedures.

AMT alone is safe and effective method for symblepharon. Considering the potential adverse effects associated with limbal excision, also AMT is an effective method of fornix reconstruction for the repair of symblepharon in a variety of ocular surface disorders.

CONCLUSIONS:

This study has shown that the amniotic membrane can be used

effectively to treat various ocular surface disorders.

From the study we can conclude that amniotic membrane transplantation is a safe, simple, inexpensive and effective for different ocular surface disorders with a satisfactory success rate.

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