Amniotic Membrane transplantation for corneal surface reconstruction

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Abstract
Objectives: To evaluate the efficacy of fresh human amniotic membrane for reconstruction in corneal surface diseases.

Material and Methods: 28 eyes of 26 patients with corneal surface disorders were evaluated. Indications for AMT included bullous keratopathy (4 eyes), climatic droplet keratopathy (3 eyes), Steven Johnson syndrome (7 eyes), chemical injury (3 eyes), corneal ulcer (7 eyes), band keratopathy (2 eyes) and shield ulcers (2 eyes). Fresh amniotic membrane was used in all the cases.

Result: The age range was from 18-65 years, out of which 15 were males and 11 were females. Success was noted in 75% (21/28 eyes) with very few complications in an average follow up period of 6 months. The mean epithelisation time varied from 2 – 2.4 weeks.

Conclusion: Fresh amniotic membrane can reduce inflammation, promote epithelisation and decrease in various corneal surface disorders.

Keywords: AMT, AM, Human amniotic membrane, corneal surface disorder, SJS.

Introduction
Foetal membrane consists of two main layers amnion and chorion. Amniotic membrane (AM) is the innermost avascular foetal membrane derived from ectoderm. It thickness varies from 0.02 mm to 0.5 mm and it neither contains nerve or blood vessels. Bourne described amnion as consisting of five layers from within outward, i.e epithelium, basement membrane, compact layer, fibroblast layer and spongy layer.

Since 1910, human amniotic membrane (HAM) has been used for many purposes. It has been used for biological dressing in acute burns, skin ulcers and abdominal wounds. It has also been used as graft for reconstruction of otolarynx and vagina (in the absence of vagina).

In ophthalmology, the first use of HAM was by de Roth in 1940 in treatment of the conjunctival defect after symblepharon caused by chemical injury and phemphigus. Very little regarding amniotic membrane transplantation (AMT) was seen in ophthalmologic literature until 1995, when Kim et al used amniotic membrane for ocular surface reconstruction of severely damaged cornea.
in a rabbit model. He reported that AMT offers 40% chance of restoring corneal clarity and epithelial phenotype in rabbit in which entire corneal and limbal surface had been experimentally destroyed. Since then AMT has been used as a surface or intrastromal graft for different types of ocular surface disorders. In corneal lesion it has been used to treat persistent epithelial defect, neurotropic corneal ulcers, bullous keratopathy, chemical burns, band keratopathy, Steven Johnson syndrome (SJS), and corneal ulcers.

It has been hypothesised that beneficial effects of transplantation of amniotic membrane are due to its role as mechanical barrier, epithelial promoter, anti-inflammatory, antiangiogenic, antibacterial and antiviral activity. These mechanism are attributed to wide range of biological factors like epidermal growth factors, fibroblast growth factors, hepatocyte growth factor and transforming growth factors b1. AM rather than providing substrate acts as a ‘bandage contact lens’ allowing epithelisation to occur under it. Amniotic membrane obtained under sterile condition after elective caesarean section after a full term pregnancy as it may be contaminated by normal vaginal flora during vaginal delivery. For preservation of AM various method has been used. It can also be used fresh (stored at +4°C) or preserved in which most of the clinical trials has been done. An ideal graft used for reconstruction would not only promote healing but would also minimize scaring. It should also be cosmetically acceptable, easily available and relatively easy to perform. HAM clearly satisfies these lines. In this report we share our experience of using fresh amniotic membrane in cornea surface reconstruction.

Materials and Methods
This is a prospective non randomised interventional case series performed in the department of ophthalmology in Odisha, during the period of 2016-17. Informed consent was taken from patient and the donors from whom placenta was collected. Severe dry eye, fungal ulcers and severe chemical burns were excluded from our study.

AMT was performed in 28 eyes of 26 patients with various corneal surface disorders. It was a prospective study over a period of one year. The indications for surgery and goal of treatment are summarised in table 2.

Preparation of amniotic membrane
Placenta was obtained under sterile conditions after elective caesarean section from women who were serologically negative for HIV, Hepatitis B, C and syphilis. The women who had H/o drug intake or alcohol abuse, having multiple sex partners and denied for consent were excluded from the study. The placenta was rinsed several times with normal saline to remove all clots. The amniotic membrane (inner layer) was separated from placenta by blunt dissection through the potential spaces situated between these two tissues with 2 sets of forceps. The separated membrane was placed in a bottle containing normal saline with cock tail regimen of antibiotic at 4°C, as a safeguard against most bacterial and fungal infections. It is to be used within 24 hours of its collection (Table-1). This cocktail regimen of antibiotic solution used was similar to that advocated by Kim et al.

Table -1 Contents and concentrations of antibiotics solution

| Antimicrobial agent | Dose  |
|---------------------|-------|
| Penicillin          | 50 mg/ml |
| Streptomycin        | 50 micro g/ml |
| Neomycin            | 100 mg/ml |
| Amphotericin B      | 2.5 mg/ml |

Surgical procedures
All the surgeries were performed by a single surgeon under peribulbar anaesthesia after extensive pre-operative evaluation. Surgical techniques used depended on the nature of clinical indications.

In corneal ulcers, debris from base of ulcer was removed with microspatula. Loose epithelium adjacent to edge of ulcer was removed, AM with basement side up was used to cover the defect and...
was sutured with interrupted 10-0 nylon suture on to the cornea. Knots were buried. In case of large defect, AM was used to cover the whole cornea like a bandage contact lens (overlay technique) and was sutured to the episcleral tissue, with 8-0 vicryl suture after performing peritomy. In perforation and corneal thinning entire depth of ulcer was filled with small piece of amniotic membrane trimmed to the size of the defect, a large graft with epithelial side up was then sutured to cover the de-epithelised area with interrupted 10-0 nylon suture and the ends were buried. Superficial keratectomy with AMT was performed in cases of bullous keratopathy, acute chemical burn, band keratopathy, climatic droplet keratopathy and shield ulcer. The number of interrupted sutures depended on the size of the defect. The corneal sutures were removed subsequently as per need.

Post operative regimen
Postoperatively all patients were advised antibiotic eye drop till complete epithelisation. Artificial tear substitute was given frequently to be instilled in the operative eye. Topical steroid which was given was tapered over a period of 6-8 weeks.

Follow-up
The patient was examined the following day and subsequently daily till discharge, then followed up weekly for 1st month and monthly for the next 6 months. Best corrected visual acuity, ocular symptoms (pain and photophobia), suture debris and exposure of knots and any other complications were noted at each visit. Slit lamp examination with fluorescence staining was done to access the extent of epithelisation. Drawing and recording of ulcer size was also done. Outcome was defined as success or failure based on the criteria given in the table-2. AMT done for more than one indication was considered successful if there was success in at least one of the indications with stabilization of the rest.

Table 2 Criteria for success in each category

| Indication for surgery     | Success                                                                 | Failure                                                                 |
|----------------------------|-------------------------------------------------------------------------|-------------------------------------------------------------------------|
|                            | Anterior chamber reformation with cessation of aqueous leakage          | No Anterior chamber reformation and aqueous leakage                      |
| Perforated ulcer           | Relief from pain and irritation                                         | Persistence of pain and irritation                                        |
|                            | No recurrence of disease                                                | Recurrence of disease                                                    |
| Bullous keratopathy        | Healed epithelial defect                                               | No healing                                                              |
| Band keratopathy           | Decrease in pain, irritation and inflammation                           | Persistence of epithelial defect                                          |
| Chemical injury            | Decrease of corneal haze                                                | No reduction in corneal haze                                             |
| Shield ulcer               | Improvement of vision                                                   | Persistence of pain, irritation and photophobia                           |
| Climatic droplet keratopathy | Decrease of corneal haze                                               | Persistence of pain, irritation and photophobia                           |

Results
AMT was done in 28 eyes of 26 patients having different corneal surface disorders, out of which 15 were males and 11 were females. The mean age of presentation was 43.11 years. Maximum numbers of patients were in age group 20-40 years. Out of 28 cases, corneal surface disorder was present in both eyes of 2 patients (4 eyes), in right eyes in 13 patients and in left eyes in 7 patients. The different etiologies included in our study are shown in table 4.

Close observation was done to note re-epithelization of surface, graft retraction or melting, graft infection and recurrence of primary disease. Decrease in corneal haze was noted in 2 patients with climatic droplet keratopathy. There was decrease in pain and irritation in band keratopathy and bullous keratopathy.

Bar chart

| Indication for surgery     | Success                                                                 | Failure                                                                 |
|----------------------------|-------------------------------------------------------------------------|-------------------------------------------------------------------------|
|                            | Anterior chamber reformation with cessation of aqueous leakage          | No Anterior chamber reformation and aqueous leakage                      |
| Perforated ulcer           | Relief from pain and irritation                                         | Persistence of pain and irritation                                        |
|                            | No recurrence of disease                                                | Recurrence of disease                                                    |
| Bullous keratopathy        | Healed epithelial defect                                               | No healing                                                              |
| Band keratopathy           | Decrease in pain, irritation and inflammation                           | Persistence of epithelial defect                                          |
| Chemical injury            | Decrease of corneal haze                                                | No reduction in corneal haze                                             |
| Shield ulcer               | Improvement of vision                                                   | Persistence of pain, irritation and photophobia                           |
| Climatic droplet keratopathy | Decrease of corneal haze                                               | Persistence of pain, irritation and photophobia                           |

Table 3 Criteria for success in each category
Epithelisation time varied from 2- 4.2 weeks. It was delayed in SJS cases.

We encountered very few complications (4/28). In a case of bullous keratopathy the graft dislodgement was due to rubbing of the eye at night on 3rd postoperative day. 2 cases of perforated corneal ulcers developed pain on 8th and 10th post operative day respectively due to graft melting.

Table 3 Age and gender Ratio

| Age group | Male | Female |
|-----------|------|--------|
| 1-20      | 1    | 0      |
| 20-40     | 6    | 4      |
| 40-60     | 7    | 4      |
| >60       | 1    | 3      |

Mean age in males was 41.40 years. Mean age in females was 45.45 years.

Table 4 Etiological groups included in study

| Etiology                               | Number of eyes |
|----------------------------------------|----------------|
| Bullous Keratopathy                     | 4              |
| Band Keratopathy                        | 2              |
| Climatic droplet keratopathy            | 3              |
| Chemical injury                         | 3              |
| Shield ulcer                            | 2              |
| Corneal ulcer                           | 7              |
| SJS                                     | 7              |

Table 5 Mean epithelisation time (in weeks)

| Type of corneal surface disease | Mean epithelisation time |
|---------------------------------|--------------------------|
| Bullous Keratopathy             | 3.4                      |
| Band Keratopathy                | 2                        |
| Climatic droplet keratopathy    | 2.8                      |
| Chemical injury                 | 3.1                      |
| Shield ulcer                    | 2.4                      |
| Corneal ulcer                   | 3.4                      |
| SJS                             | 4.2                      |

Table 6 Surgical outcome of AMT in each indication

| Type of corneal surface disease | Number of eyes | Procedure                        | Result   | Complication          |
|---------------------------------|----------------|----------------------------------|----------|------------------------|
| Bullous Keratopathy             | 4              | Superficial keratectomy +SL AMT  | 100%     | -                      |
| Band Keratopathy                | 2              | Superficial keratectomy +SL AMT  | 50%      | Graft dislodgement     |
| Climatic droplet keratopathy    | 3              | Superficial keratectomy +SL AMT  | 66.66%   | Graft retraction       |
| Chemical injury                 | 3              | SL AMT                           | 66.66%   | -                      |
| Shield ulcer                    | 2              | Removal of plaque + AMT          | 100%     | -                      |
| Corneal ulcer                   | 7              | Debridement + SL AMT or ML AMT   | 71%      | 2 graft melts          |
| SJS                             | 7              | SL AMT                           | 71%      | 29%                    |

Note: SL- Single Layer, ML- Multi layer

Discussion

Our study shows that fresh human amniotic membrane is useful for corneal surface disorders. Using this technique we have achieved overall success in 75% cases. The usefulness of this procedure however differs in different indications. Hence the individual success rate thus varies. (Table 6).

Steven- Johnson Syndrome and chemical burns result in limbal stem cell deficiency. This results in corneal vascularization. It is very difficult to treat these disorders, and conventional PKP also fails in many cases. However Tseng et al. stated in a study way back in 1998 that amniotic membrane transplantation can be done with partial limbal stem cell deficiency which yielded good results. Thus amniotic membrane transplantation alone was seen to reduce symptoms like photophobia, discomfort, redness and irritation and improve corneal smoothness and visual acuity. In our series, 2 cases with chemical injury out of 3 had good epithelial healing with a MET of 3.1 weeks and simultaneously showed decrease in symptoms (66%). While 5 out of 7 cases of SJS showed improvement in photophobia, redness and irritation (79%). These improvements in findings may be due to increased goblet cell density due to amniotic membrane grafting. Since AMT will not be effective in cases of total and diffuse stem cell deficiency, we have excluded these cases from our study.

In bullous keratopathy, the corneal epithelium becomes unhealthy and has irregular surface due to endothelial dysfunction. This causes ocular surface break down and pain. This condition generally requires a PKP to reduce pain and
improve vision. AMT is found to be effective and can be used as an alternative to conjunctival graft in relieving pain and preventing recurrent corneal erosion in bullous keratopathy. The exact mechanism is unclear. In our series, all 4 cases of bullous keratopathy that received AMT showed improvement from pain and irritation (100%). This result is same as was obtained by Tseng and Pires et al.

Out of 7 cases of corneal ulcer which had received single or multiple layer AMT, 5 cases showed improvement in healing process with a MET of 3.4 weeks (71%). There was good restoration of corneal surface and reduction in inflammation. This is attributed to fact that AM contains growth factors and it acts like basement membrane and facilitates the migration of epithelial cells. One case of perforated corneal ulcer showed a formed anterior chamber without any further aqueous leakage. However graft failure was observed in 2 cases of active infective keratitis due to graft melt.

In our series main aim of AMT in band keratopathy was to reduce pain and irritation and in climatic droplet keratopathy was to improve vision. 2 cases of band shaped keratopathy due to trauma were taken, 1 of the patients accepted the graft with symptomatic improvement and a mean epithelization time of 2 weeks. The other patient graft failed due to dislodgement by mechanical rubbing of eye by the patient (success rate of 50%). All 3 cases of climatic droplet keratopathy showed postoperative relief from watering and improvement of vision. The mean epithelisation time was 2.8 weeks. These results were almost similar to the results obtained from the previous studies. There was one late graft failure due to retraction bringing down the success rate to 67%. Both the patients with shield ulcer however showed successful rep epithelisation and symptomatic relief.

Conclusion
In summary, amniotic membrane transplantation seems to be an useful method in reducing symptoms of pain, photophobia and lacrimation caused by corneal surface disorders. Further it is useful in improving vision by reducing stromal haze and inducing epithelisation. Thus fresh AM can be used to heal various corneal surface disorders and maintain globe integrity. Further studies in larger scales are required to elaborate this action of AMT.

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