



Fresh Amniotic Membrane Transplantation in Ocular Surface Disorder in a Tertiary Teaching Care Hospital, Eastern India

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Abstract

Aim: Evaluating the efficacy of fresh Human Amniotic Membrane (HAM) for various ocular surface reconstructions.

Materials and methods: 54 eyes of 50 patients having ocular surface disorders were evaluated. Out of which 31 patients were male and 19 were females. Indications for AMT included pterygium (18 eyes), bullous keratopathy (4 eyes), climatic droplet keratopathy (3 eyes), Steven Johnson syndrome (14 eyes), chemical burn (3 eyes), corneal ulcer (7 eyes), band keratopathy (2 eyes) and shield ulcers (2 eyes). Fresh amniotic membrane was used in all the cases. The aim of the Amniotic Membrane Transplantation (AMT) was achieving reduction in inflammation, improvement in symptoms and vision, reduction recurrence, promotion of epithelisation in various ocular surface disorders. The analysis of the results was done by defining the outcome as either success or failure.

Result: The age range of the patients was from 18-65 years, out of 50 patients 30 were males and 29 were females. Successful use of AMT was noted in 74.1% (40/54 eyes) cases with very few complication.

Conclusion: Fresh amniotic membrane is found to be a simple, inexpensive, safe and effective method of treatment to reconstruct the ocular surface disorders in many clinically challenging situations in developing countries.

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Keywords: Steven johnson syndrome; Ocular surface disorder; Amniotic membrane; Pterygium.

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Introduction

Amniotic Membrane (AM) comprises of the innermost layer of the foetal membrane. It contains stromal matrix, thick collagen layer, and single layer of epithelium with overlying basement membrane [1]. AM has antiadhesive effects, bacteriostatic effects, promotes epithelisation, and lacks immunogenicity [2]. Due to this property, AM has been used to promote healing of chronic leg ulcers [3], for dressing of burnt skin wounds [4], prevention of tissue adhesives in abdomen, head and pelvic surgeries [5-9]. The normal ocular surface can be damaged by various systemic inflammatory diseases [10], trauma, and primary ocular diseases resulting in its breakdown [11]. These ocular surface disorders are at times refractory to medical treatment and surgical interventions [12]. According to literature Amniotic Membrane Transplantation (AMT) was used successfully to treat these disorders. Its use in Ocular Surface Disorders (OSD) dates back to 1940 when De Roth used it to repair conjunctival defect after symblepharon dissection [13]. Thus gradually amniotic membrane transplantation has been used in patients with symblepharon [14], persistent epithelial defects [15], pterygium [16] and other ocular surface disorders with success [17].

In this report, we have described our experience of transplantation of fresh AM in achieving reduction in inflammation, improvement in symptoms and vision, reducing recurrence, promoting epithelisation in various ocular surface disorders.

Material and methods

Patient selection

This was a prospective non-randomised study conducted in a tertiary health care centre in Odisha. 54 eyes of 50 patients having various ocular surface disorders were included. Severe dry eye, ocular neoplasia, severe chemical burn, children below 15 years and fungal ulcer were excluded from our study. The patient data was recorded and analyzed followed by under proper institutional ethics. Informed consent form was taken from all patients who underwent surgery and all pregnant women before donation of amniotic membrane.

Preparation of amniotic membrane

Placenta was obtain under sterile conditions after elective caesarean section from women who were serologically negative for HIV, Hepatitis B and C viruses and syphilis. The women who had an history of drug intake or alcohol abuse and had multiple sex partner were excluded from the study. The placenta was rinsed several times with normal saline to remove excess clots. The amnion was totally separated from placenta by blunt dissection. The separated membrane was placed in a sterile vial containing normal saline with cock tail of antibiotic at 40 C to be used within 24 hours. This solution used had similar constituents as that advocated by Tseng et al. (Table 1) [18].

Table 1: Contents, concentration of the antibiotic solution used.

Antimicrobial agent	Dose
Penicillin	50 mg/ml
Neomycin	100 mg/ml
Streptomycin	50 microg/ml
Amphotericin B	2.5 mg/ml

Surgical procedure

All the amniotic membrane grafting was performed under peribulbar block and by single surgeon after pre-operative evaluation. The technique of AMT used was determined based on the nature of the clinical condition. In corneal ulcers, debris from base of ulcer was cleaned. Epithelium near the edge of ulcer was removed if found to be loose, AM with basement side up was used to cover the defect and was sutured with 10-0 nylon suture on to the cornea (Figure 1a and b). In case of large defect AM was used to cover the whole cornea and was sutured to the episcleral tissue after performing peritomy. In pterygium, AMT was done after excision of pterygium, the amnion keeping the epithelial side up was placed to fully cover the conjunctival defect and sutured using 8-0 vicryl suture (Figure 2a and b). In symblepharon however, release of symblepharon was done and fornices were freed. Bare sclera thus prepared was covered with amniotic membrane keeping epithelial side up and then sutured with 8-0 vicryl suture with episcleral tissue to edge of the conjunctiva present along the bare sclera (Figure 3a and b). Fornix reconstruction was done in required cases. In perforation and corneal thinning, the whole depth of the ulcer were filled with small pieces of amniotic membrane trimmed to fill in the defect; a large graft with epithelial side up was then sutured to cover the de-epithelised area with 10-0 nylon suture. Superficial keratectomy with AMT was performed in cases of bullous keratopathy, acute chemical burn (Figure 4a and b), band keratopathy, climatic droplet keratopathy, and shield ulcer.

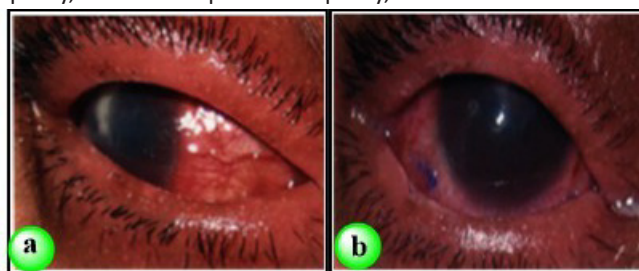


Figure 1: (a) 5 mm x 3 mm non healing neurotrophic ulcer with stromal infiltrate in supero and infero temporal quadrant. (b) Healed ulcer with nebulo macular scar.

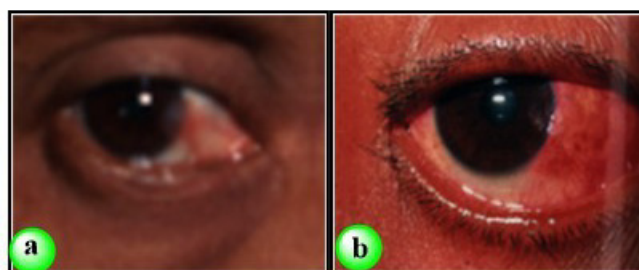


Figure 2: (a) Pterygium extending up to 2 mm onto cornea. (b) Excision and AMG graft (post op 2 week).

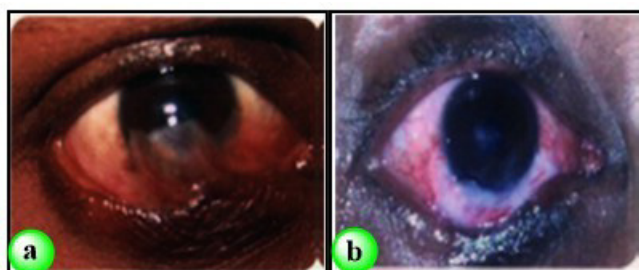


Figure 3: (a) Symblepharon involving inferior fornix and inferior part of cornea. (b) Post pterygium excision with AMT (4 week).

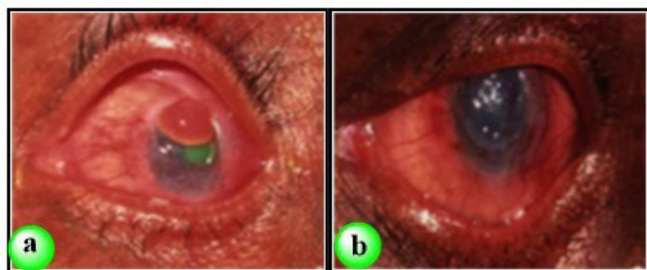


Figure 4: (a) Persistent Epithelial Defect (PED) with overhanging pyogenic granuloma after chemical injury. (b) Healed PED after excision of pyogenic granuloma (Overlaid technique).

Post-operative regimen

Post-operative care consisted of instillation of antibiotic eye drop till complete epithelisation. Artificial tear substitute was instilled frequently in the operating eye. Topical steroid eye drops was given in a tapering manner over a period of 6-8 weeks. The patients were examined the following day and subsequently daily till discharge. They were followed up weekly for the 1st month and monthly for the next 6 months. Patients were observed for symptoms, signs and complications. Close follow up was done to observe the reduction in pain, redness and photophobia. Slit lamp examination with fluorescent staining was done to assess the epithelisation, drawing and recording of ulcer size was done for corneal ulcers. Outcome was defined in each category as success or failure based on the criteria as tabulated (**Table 2**). AMT done for more than one indication was considered successful if there was success in at least one indication with stabilization of the rest.

Table 2: Criteria for success or failure of grafting.

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

Results

Anniotic membrane transplantation was done in 54 eyes of 50 patients with different ocular surface disorders, of which 30 (60%) were males and 20 (40%) were females as tabulated in **Table 3**. The mean age of patients at the time of surgery was 41.45 years. Mean age of males 41.26 years and mean age of females 41.65 years. The maximum number of eyes was in the age group 20-40 years. Ocular surface disorder was found in both eyes of 4 cases, 23 eyes had defect in right eyes while in 27 eyes left eye was involved out of 50 cases. The various etiologies for the ocular surface disorders are shown in **Table 4**. Most of the patients had pterygium and SJS. Shields ulcer and band keratopathy were less commonly opted for AMT in our study close observation was done to note postoperative inflammation, infection, graft retraction and rejection, reformation of anterior chamber in corneal perforations and recurrence of primary disease. Reduction in inflammation was noted in corneal ulcers, chemical injury and shield ulcers. There was also decreased conjunctival and circumciliary congestion along with epithelial and stromal healing. Corneal haze was reduced and vision was improved in 2 cases of climatic droplet keratopathy. All cases of epithelial defect had rapid re-epithelisation. We encountered a few complications. One case of pterygium developed stitch granuloma, which resolved after removal of sutures and application of topical antibiotics. Out of 4 recurrent cases of pterygium which were taken for AM one case developed recurrence within 4 months of follow up. It was posted for re sur-

gery. Out of 3 perforated corneal ulcer 2 cases developed pain on 8th and 10th postoperative day respectively due to graft melt. Displacement of the graft in a patient of band keratopathy was seen due to accidental rubbing.

Table 3: Age and gender comparison.

Age group	Male	Female
1-20 years	4	2
20-40 years	10	7
40-60 years	14	7
60 years	2	4

Table 4: Etiology of the ocular surface disorders.

Ocular surface disorders	Cases	Eyes involved
Pterygium(Primary and Recurrent)	18	18
Steven Johnson Syndrome	10	14
Corneal ulcer	7	7
Bullous keratopathy	4	4
Band keratopathy	2	2
Climatic droplet keratopathy	3	3
Chemical injury	4	4
Shield ulcer	2	2

Discussion

There are extensive reports of use of AM in various ocular surface disorders [19-21]. There are many AM properties, which help promote its use in OSD [22]. It serves as a basement membrane promoting epithelial cell migration, adhesion of epithelial cells and preventing apoptosis. Absence of HLA-A and B or DR prevents immunological rejections and suppresses lymphocyte action thus reducing graft rejections. Their inherent antimicrobial property reduces post-operative infections. Its high tensile strength helps cover large ocular surface like a bandage contact lens, it also allows epithelisation under its cover. Presence of Nerve growth factors is effective to treat neurotropic corneal ulcer. Due to its anti-angiogenic effect, on its transplantation blood vessel proliferation is reduced. Its anti-scarring and anti-inflammatory actions help reduce inflammation in acute burn. Thus, overall it provides a proliferative sheet covering and preventing infections.

This study comprises various etiological groups with indication of AMT. Results was observed in form of success rate of AMT and details are mentioned in **Table 5**. AM can be used as patch or as graft. When used as patch it gets dissolved but when used as a graft it promotes epithelisation over it and is thus preserved. It may though become transparent over time.

In our case series, all the cases were primary pterygium. The success rate of AMT was found to be 94.44%. Only one case developed recurrence at the end of 4 months (5.55%). Pravasawat et al. reported the recurrence rate with primary pterygium to be 10.9% with conjunctival limbal autograft and AMT [23]. Solomon et al. further found that double layered AMT along with intraoperative subconjunctival Triamcinolone reduces the recurrence rate of primary pterygium to 3% [24].

In our study, patients with corneal ulcer showed improvement in healing process, reduction of inflammation and restoration of corneal surface. The success rate of amniotic membrane grafting in 7 eyes of corneal ulcer (refractory to conventional treatment) was 71%. AMT was also considered a useful adjunct for managing deep ulcer which showed poor wound healing de-

spite treatment, impending perforation, and small perforations to enhance tectonic strength. Hanada et al. performed AMT in 11 cases of corneal ulcer and got a success rate of 72.7% [25].

Pain and photophobia were reduced in bullous keratopathy. According to Renato et al., the success of AMT in bullous keratopathy was 90% as against our study where it was 75% [26]. Also AMT used in Climatic droplet keratopathy was successful in improving vision and reducing corneal haze, Rao et al. had an overall success rate of 80% with superficial keratectomy and AMT in corneal degeneration [27]. AMT used for Band keratopathy after surgical removal of calcific deposition in our study yielded a success rate of 50%. Anderson et al. found an overall success rate of 93.3% with the same procedure [28]. Owing to our small sample size, our success rate couldn't be strictly compared.

The cases selected for AMT in chemical injury in our study showed remarkable symptomatic relief and rapid epithelisation with a success rate of 75%. Tejwani et al. performed AMT in 72 eyes with a success rate of 72.9% in chronic cases and 87.5% in acute cases [29].

Post-operative complications in our series were not significant. 1-graft retractions, 1 graft loss and 2 cases of AM melting were observed during the 6 months follow up period. However, postoperative microbial infection was not seen even though we used fresh amniotic membrane in our study.

Philip et al. published a clinical and in vitro comparison between frozen and fresh amniotic membrane grafts and found no significant difference between two [30]. In our case, we found out that there were practical difficulties such as risk of disease transmission and need of suitable donor before surgery. Most of the studies are about their clinical experience with human AMT that have been done using tissue preserved AMT. There are few studies showing the difference between the fresh and preserved AM. Though there are difficulties, they are not sufficient reasons to prevent the use of fresh amniotic membrane in developing countries like ours where there is difficulty in getting preserved AM due to cost factors.

Table 5: Surgical outcome of AMT in different ocular surface disorder.

Indication of AMT	Eyes	Procedure	Graft	Patch	Both	Success	Failure
Pterygium	18	Excision+AMT	18	0	0	94.44%	5.56%
SJS	14	Symblepharon release+AMT or AMT	7	3	4	57%	43%
Corneal ulcer	7	Debridement+SLAMT/ML AMT	2	0	5	71%	29%
Bullous keratopathy	4	Superficial keratectomy +SL AMT	4	0	0	75%	24%
Band keratopathy	3	Superficial keratectomy +SL AMT	1	2	0	50%	50%
Climatic droplet keratopathy	3	Superficial keratectomy +SL AMT	2	1	0	67%	33%
Chemical injury	4	Symblepharon release+AMT or AMT	3	1	0	75%	25%
Shield ulcer	2	Removal of plaque+AMT	0	2	0	100%	0%

Conclusion

In conclusion, although fresh AM may be associated with risk of transmission of blood-borne disease, still fresh AM is simple, inexpensive and reasonably effective for ocular surface reconstruction in setups where availability of banked frozen tissue is an issue and where cost of its procurement may prohibit the use of the preserved tissue.

Core tip: This was a prospective non-randomised study conducted in a tertiary health care centre in Odisha. 54 eyes of 50 patients having various ocular surface disorders were included. Fresh amniotic membrane is found to be a simple, inexpensive, safe and effective method of treatment to reconstruct the ocular surface disorders in many clinically challenging situations in developing countries.

Declaration

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Author contributions: Mishra S, Samant S, Dhar SK drafted the manuscript, analysis, data collection; and Navyasree C and Pati BS and Mohapatra RC assisted with data analysis, design of the study and data collection; all authors read and approved the final manuscript.

Institutional review board ethics: The study was reviewed, approved and fully under the institutional ethics of Institute of Medical Sciences & SUM Hospital and SUM Ultimate Medicare, Siksha 'O' Anusandhan Deemed to be University, Bhubaneswar, India.

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