www.jmscr.igmpublication.org
Impact Factor (SJIF): 6.379
Index Copernicus Value: 79.54
ISSN (e)-2347-176x ISSN (p) 2455-0450
crossrefDOI: https://dx.doi.org/10.18535/jmscr/v6i11.08

IGM Publication
Journal Of Medical Science And Clinical Research
An Official Publication Of IGM Publication

Amniotic Membrane transplantation for corneal surface reconstruction

Authors
Suchismita Mishra, Jayashree Dora, Ramesh Chandra Mohapatra, Pragati Jain, Swati Samant*
Department of Ophthalmology, IMS and SUM Hospital, Siksha O Anusandhan University, K8, Kalinga Nagar, Bhubaneswar-751003
Corresponding Author
Dr Swati Samant, MS
Associate Professor, Department of Ophthalmology, IMS and SUM Hospital
Email: *swatisamantdhar@gmail.com*

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 in75% (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[1, 2]. Bourne described amnion as consisting of five layers from within outward, i.e epithelium, basement membrane, compact layer, fibroblast layer and spongy layer.[1]

Since 1910, human amniotic membrane (HAM) has been used for many purposes. It has been used for biological dressing in acute burns[3], skin ulcers[4] and abdominal wounds[5]. It has also been used as graft for reconstruction of otolarynx and vagina (in the absence of vagina)[6].

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[7]. 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[8]. 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[9], neurotropic corneal ulcers[10], bullous keratopathy[11], chemical burns[12], band keratopathy[13], Steven Johnson syndrome (SJS) [14] and corneal ulcers[15].

It has been hypothesised that beneficial effects of transplantation of amniotic membrane are due to its role as mechanical barrier, epithelial promoter, antiinflammatory, antiangiogenic, antibacterial and antiviral activity.[16]

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.[17]AM rather than providing substrate ,acts as a" bandage contact lens" allowing epithelisation to occur under it.[18]

Amniotic membrane obtained under sterile condition after elective caeserean 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 ^{0} 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 4^0 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*[19].

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 microsponge. 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-o 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 interrup-ted10-0 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 month. Best corrected visual aquity, 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
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 pain and irritation	Persistence of pain and irritation
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

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.

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 10thpost 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 Success	Result Failure	Complication
Bullous Keratopathy	4	Superficial keratectomy +SL AMT	100%	0%	-
Band Keratopathy	2	Superficial keratectomy +SL AMT	50%	50%	Graft dislodgement
Climatic droplet keratopathy	3	Superficial keratectomy +SL AMT	66.66%	33.33%	Graft retraction
Chemical injury	3	SL AMT	66.66%	33.33%	-
Shield ulcer	2	Removal of plaque + AMT	100%	0	
Corneal ulcer	7	Debridement + SL AMT or ML AMT	71%	29%	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[20]. 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 [21].Since AMT will not be effective in cases of total and diffuse stem cell deficiency [20], 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[22, 23]. 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[24].

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[25-27] .One cases 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[28-30]. 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 repithelisation 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.

References

1. Bourne GL. The microscopic anatomy of the human amnion and chorion .Am J Obstet Gynecol 1960;79:1070-3.
2. Danforth DN, Hull RW. The microscopic anatomy of foetal membranes with particular reference to detailed structure of the amnion.Am J Obstet gynecol 1958;75:536-50.
3. Bose B. Burn wound dressing with human amniotic membrane. Ann R CollSurg Engl.1979 Nov;61(6):444-7.
4. Somerville PG.The possible use of amniotic membrane in chronic leg ulcers. Phlebologie.1982 Jan-Mar;35(1):223-9.
5. Silverton JS, Trelford JD, Roussere JT, Wolfe BM, Conti S. The use of amniotic membrane in acute massive full-thickness loss of the abdominal wall from clostridialmyonecrosis. Ann Plast Surg. 1979 Dec;3(6):558-66.
6. Dhall K. Amnion graft for treatment of congenital absence of the vagina.Br J ObstetGynaecol. 1984 Mar;91(3):279-82.
7. De Roth A .Plastic repair of conjunctival defects with membranes. *Arch Ophthalmol* 1940;23:522-5
8. Kim JC, Tseng SC. Transplantation of preserved human amniotic membrane for surface reconstruction in severely damaged rabbit corneas. Cornea. 1995Sep; 14(5):473-84.
9. Lee S and Tseng SCG. Amniotic membrane transplantation for persistent epithelial defect with ulceration. Am J Ophthalmol.1997;123:303-312.

10. Khokhar S, Natung T, Sony P, Sharma N, Agarwal N, Vajpayee RB. Amniotic membrane transplantation in refractory neurotrophic corneal ulcers: a randomized, controlled clinical trial. Cornea. 2005 Aug;24(6):654-60.
11. Pires RTF, Tseng SCG, Prabhasawat P, PuangricharernV, Maskin SL,Kim JC and Tan DTH. Amniotic membrane transplantation in symptomatic bullous keratopathy. Arch Ophthalmol. 1999;117: 1291-1297.
12. Tsubota K, Satake Y, Kaido M, Shinozaki N, Shimmura S, Bissen-Miyajima H, Shimazaki J. Treatment of severe ocular-surface disorders with corneal epithelial stem-cell transplantation. N Engl J Med. 1999 Jun 3;340(22):1697-703.
13. Anderson DF, Prabhasawat P, Alfonso E, Tseng SC. Amniotic membrane transplantation after the primary surgical management of band keratopathy.Cornea. 2001 May;20(4):354-61.
14. Honavar SG, Bansal AK, Sangwan VS, Rao GN. Amniotic membrane transplantation for ocular surface reconstruction in Stevens-Johnson syndrome. Ophthalmology.2000 May;107(5):975-9.
15. Mohan S, Budhiraja I, Saxena A, Khan P, Sachan. Role of multilayer amniotic membrane transplantation for the treatment of resistant corneal ulcers in North India. Int Ophthalmol. 2014 Jun;34(3):485- 91.
16. Dua HS, Gomes JA, King AJ, Maharajan VS. The amniotic membrane in ophthalmology. Surv Ophthalmol. 2004 Jan-Feb;49(1):51-77. Review.
17. Allen CL, Clare G, Stewart EA, Branch MJ, McIntosh OD, Dadhwal M, Dua HS,Hopkinson A. Augmented dried versus cryopreserved amniotic membrane as an ocular surface dressing. PLoS One. 2013 Oct 30;8(10):e78441.
18. Azuara-Blanco A, Pillai CT, Dua HS. Amniotic membrane transplantation for ocular surface reconstruction. Br J Ophthalmol. 1999 Apr;83(4):399-402.
19. Kim JC,Tseng SC. Transplantation of preserved human amniotic membrane for surface reconstruction in severely damaged rabbit cornea.Cornea.1995:21:169-72.
20. Tseng SCG, Prabhasawat P, Barton K, Gray T and Meller D (1998). Amniotic membrane transplantation with or without limbal allografts for corneal surface reconstruction in patients with limbal stem cell deficiency. Arch Ophthalmol 116: 431–441.
21. Ocular surface reconstructed by preserved human amniotic membrane. Arch Ophthalmol 115: 1360–1367.
22. Lee S and Tseng SCG (1997) Amniotic membrane transplantation for persistent epithelial defects with ulceration. Am J Ophthalmol123: 303–312.
23. Espana EM, Grueterich M, Sandoval H, Solomon A, Alfonso E, Karp CL, *et al*. Amniotic membrane transplantation for bullous keratopathy in eyes with poor visual potential. J Cataract Refract Surg 2003;29:279-84.
24. Pires RTF, Tseng SCG, Prabhasawat P, Puangsricharern V, Maskin SL, Kim JC and Tan DTH (1999) Amniotic membrane transplantation for symptomatic bullous keratopathy. ArchOphthalmol 117: 1291–1297.
25. Lee SH, Tseng SC. Amniotic membrane transplantation for persistent epithelial defects with ulceration. Am J Ophthalmol. 1997 Mar;123(3):303-12.
26. Tseng SC, Prabhasawat P, Lee SH. Amniotic membrane transplantation for conjunctival surface reconstruction. Am J Ophthalmol. 1997 Dec;124(6):765-74.
27. Shimazaki J, Shinozaki N, Tsubota K. Transplantation of amniotic membrane and limbal autograft for patients with recurrent

pterygium associated with symblepharon. Br J Ophthalmol. 1998 Mar;82(3):235-40.

28. Solomon A, Meller D, Prabhasawat P, John T, Espana EM, Steuhl KP, Tseng SC. Amniotic membrane grafts for nontraumatic corneal perforations, descemetoceles, and deep ulcers. Ophthalmology. 2002 Apr;109(4):694-703.
29. Chen JH, Ma DH, Tsai RJ. Amniotic membrane transplantation for pseudomonal keratitis with impending perforation. Chang Gung Med J. 2002 Mar;25(3):144-52.
30. Nubile M,Carpineto P, Lanzini M, Ciancaglini M,Zuppardi E, Mastropasqua L. Multilayer amniotic membrane transplantation for bacterial keratitia ith corneal perforation after hyperopic photorefractive keratectomy: case report and literature review.J Cataract Refract Surg 2007;33:1636-40.