

Outcome of application of amniotic membrane graft in ocular surface disorders

Nargis Nizam Ashraf, Muhammad Idrees Adhi

Abstract

Objective: To determine the outcome of application of amniotic graft in ocular surface disorders.

Methods: This cross-sectional study was conducted at Dow University of Health Sciences, Karachi, from January 2010 to December 2012, and comprised patients with ocular surface disorders. Patients' presenting symptoms and signs were recorded. Previously harvested and frozen amniotic graft was applied in different types of ocular surface disorders, such as corneal ulcers, pterygium, keratomalacia, Steven-Johnson syndrome, etc. Following the surgery, patients were assessed for improvement in symptoms and signs related to epithelialisation in corneal ulcers.

Results: Of the 50 patients, 30(60%) were male and 20(40%) female. The overall mean age was 40 ± 19.3 years (range: 9 months to 80 years). Out of the 18(36%) cases of pterygium, there was recurrence in 5(27.7%) cases. There were 26(52%) patients of corneal ulcers, of whom re-epithelialisation occurred in 21(80.7%) patients.

Conclusion: Amniotic membrane grafting was found to be a safe procedure for ocular surface disorders.

Keywords: Amniotic graft, Corneal ulcers, Ocular surface disorders, Re-epithelialisation, Pterygium. (JPMA 67: 1045; 2017)

Introduction

Ocular surface includes cornea, sclera and associated conjunctiva. Of the many disorders affecting these structures, corneal ulcers and pterygium are particularly difficult to treat. If corneal ulcers are not covered promptly, they may result in endophthalmitis and visual loss. Pterygia often recur after excision. There have been many different methods of treating these conditions with variable results. These include surface stabilisation procedures such as occlusion of puncta, tarsoraphy, conjunctival flap, anterior stromal puncture and phototherapeutic keratectomy, ocular surface transplantation such as conjunctival autograft and limbal stem cell transplantation. Since the increased use of amniotic grafts in ophthalmology, this trend has changed with better outcomes in managing the diseases of ocular surface.¹

Amniotic membrane has many beneficial characteristics; it promotes re-epithelialisation, reduces formation of scar and new vessels, reduces inflammation, doesn't promote immune rejection, acts as a scaffold for growth of cells over it, it contains nerve growth factor and therefore promotes nerve regeneration, and has antimicrobial properties.² It helps stabilise tear film and improve corneal sensation, it decreases epithelial cell death and promotes cloning in them. Also, it is transparent, elastic and endures freezing at high temperatures.³

.....
Department of Ophthalmology, Unit-1, Dow University of Health Sciences, Karachi.

Correspondence: Nargis Nizam Ashraf. Email: nargis.ashraf99@hotmail.com

It is essential for re-epithelialisation to occur that the amniotic graft is applied in a secure manner. The standard technique is to apply the graft with interrupted sutures. Modified continuous blanket suture is also being used for fixing amniotic graft.⁴ The inlay or overlay method can be used to apply the graft. In the inlay method only the defect is covered, whereas in the overlay method graft is applied on the whole cornea.⁵ Then there is the sandwich method which comprises both inlay and overlay grafting.⁶

When amniotic membrane was used in the initial phases of aggressive eye infections, it showed better results. There was decreased scarring and better healing.⁷ It has these properties because of the presence of collagen types 3, 4, 5 and 7. Other substances found are fibronectin, laminin and growth factors.⁸ Other attributes of the amniotic membrane are that it reduces fibrosis, is antimicrobial and doesn't induce immune reactions.⁹

It has been observed that de-epithelialised amniotic membrane is better for differentiation and growth of cells, hence better wound healing.¹⁰ Hyper dry amniotic membrane which is applied with tissue adhesive is much easier to apply than with the sutures.¹¹

Amniotic grafts are being used in conjunction with irradiated acellular corneal grafts to help reduce rejection. This is because of T-cell suppression properties of amniotic membrane.¹²

The current study was planned to observe the short-term outcome manifested by improvement of symptoms and long-term outcome assessed by re-epithelialisation in corneal ulcers and the absence of recurrence in cases of

pterygium.

Patients and Methods

This cross-sectional study was conducted at Ophthalmology Unit 1, Dow University of Health Sciences (DUHS) and Civil Hospital, Karachi (CHK), from January 2010 to December 2012, and comprised patients with ocular surface disorders who were enrolled using non-probability purposive sampling technique.

Patients with impending perforation or perforated corneal ulcers, pterygium, keratomalacia, Steven-Johnson syndrome, Bowen's disease, exposed lateral rectus, etc. were included.

Patients for whom corneal or scleral graft was available, or for whom tarsorrhaphy was the better option and those who refused this treatment were excluded.

Informed consent was obtained from all patients after admission. Clinical assessment of patients included a detailed history of presentation and complete physical examination, including thorough ophthalmological examination. Ophthalmological parameters recorded were pain, evidence of inflammation, such as conjunctival redness and irritation, visual acuity, corneal surface for site, size and shape of the ulcer, location, size and extent of corneal encroachment of pterygium. They were investigated routinely for haematological and biochemical profile for assessment for fitness for surgery.

The recipient eye was prepared according to the type of operation to be performed. Scar tissue was dissected and excised in order to prepare the bed to receive the amniotic membrane.

Amniotic membrane harvested from patients undergoing elective Caesarean section and who had previously been screened for hepatitis B and C and for human immunodeficiency virus (HIV) was used for the study. This membrane had been treated by washing with gentamicin, penicillin and amphotericin B, and after being cut in small pieces, it was placed on sofratulle, stored in small test tubes in dimethyl sulfoxide (DMSO) and had been stored at -20°C for one month and -80°C for 6 months. Details of preservation and storage methods are given elsewhere.¹³

Samples were defrosted before use.

In cases of corneal ulcers, the area was first debrided and then amniotic graft was applied with epithelial side up and sutured interrupted with 8/0 vicryl.

In case of perforated corneal ulcers, 2-3 layers of graft were applied after folding them on each other (Figure-1).

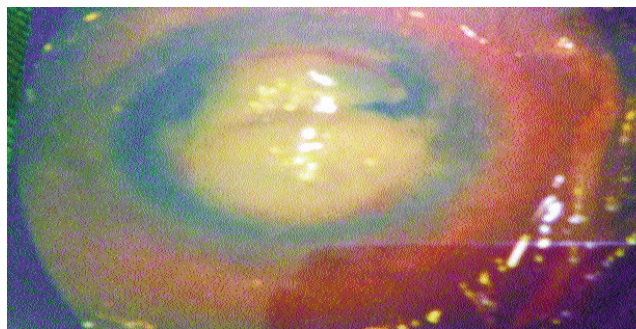


Figure-1: Amniotic graft in a case of corneal perforation.



Figure-2: Amniotic graft after pterygium excision.

In case of pterygium, a single layer was applied after the pterygium had been excised (Figure-2).

Patients were reassessed the day after surgery, after one week, 2 weeks, one month and then monthly for 6 months. They were assessed for relief of pain, reduction in features of inflammation like conjunctival redness and irritation and improvement of vision.

Relative descriptive statistics, frequency and percentage were computed for qualitative variables like outcome, sex, complications, etc. Quantitative variables like age, duration, etc. were presented as mean \pm standard deviation.

Fisher's exact test was used to check significance. $P < 0.05$ was considered as significant.

Results

Of the 50 patients, 30(60%) were male and 20(40%) were female. Follow-up record was available for 43(86%) patients and 7(14%) were lost to follow up, including 3(42.9%) females and 4(57.1%) males. The overall mean age was 40 ± 19.3 years (range: 9 months to 80 years). There were 14(28%) cases of impending perforation.

Table-1: Descriptive Statistics of personal characteristics of patients.

	All patients at baselines	All patients who completed the study
Age	40.4±19.7	40.0±19.3
Gender		
Female	20 (40%)	17 (39.5%)
Male	30 (60%)	26 (60.5%)
Eye		
Left	22 (44%)	18 (41.9%)
Right	28 (56%)	25 (58.1%)

Primary and recurrent pterygium were found in 9(18%) and 8(16%) patients, respectively. There were 7(14%) patients of infected corneal ulcer and 9(18%) had perforated corneal ulcers. Also, 1(2%) had keratomalacia, 1(2%) patient requiring grafting had Bowen's disease and in 1(2%) patient there was exposed lateral rectus.

While pain, features of inflammation, irritation and visual impairment were the predominant features of presentation, their distribution varied with the pathology. All the patients with corneal ulcers and perforation had pain and irritability and other features of inflammation with impairment of vision while those with pterygium were free of pain as expected. The predominant presentation in patients with pterygium was that of irritation and impairment of vision where the pterygium had encroached on the cornea.

All patients underwent surgery according to the above protocol. There was an immediate improvement in the features of inflammation in all cases of corneal ulcers and perforation. Pain settled immediately and there was resolution of redness and irritation. In 26(52%) cases of corneal ulcers, there was re-epithelialisation in 21(80.7%) while 3(11.5%) required a second grafting procedure. On long-term follow-up, the epithelialisation appeared to be stable.

There was no significant improvement of vision in patients with corneal ulcers as the epithelialised graft was still hazy. The patients were later advised keratoplasty. Following keratoplasty, vision returned to normal in all cases.

Table-2: Outcome of all patients included in the study between different gender.

Gender	Outcome						
	Re-epithelialisation	No recurrence	Recurrence of pterygium	Healing of defect	Retinal detachment	Lost for follow-up	Ulcer not healed
F	11	1	3	0	0	3	2
M	14	5	4	1	1	4	1

Table-3: Distribution of pathology.

Statistics		Valid	43
DIAGNOSIS		Missing	7
DIAGNOSIS		Frequency	Percent
Valid	Bowens disease	1	2.1
	Corneal Ulcer	4	8.5
	Exposed lat rectus	1	2.1
	Impending perforation	14	28
	Keratomalacia	2	4.2
	Mooren ulcer	1	2.1
	Perforated corneal ulcer	9	19.1
	Primary Pterygium	9	18
	Recurrent Pterygium	8	16
	Steven Johnson synd	2	4.2
	Total	43	94

Out of the 18(36%) patients of pterygium, there was an immediate improvement in irritation; but as expected there was no improvement of vision in cases where it was impaired. In the long-term follow-up, 12(70.6%) of the 17(34%) patients remained free of recurrence but 5(29.4%) had recurrence of pterygium; of them, 3(60%) had primary and 2(40%) had recurrent pterygium.

There was no case of infection or rejection of the graft.

Out of 14(28%) cases of impending perforation, re-epithelialisation occurred in 11(78.6%) cases, 1(7.1%) patient lost to follow-up, whereas in 2(14.3%) patients it did not heal. In the patient with exposed lateral rectus, healing was achieved.

Healing of defect occurred in males and also retinal detachment. Ulcer was not healed in 2(4%) females and 1(2%) male. The frequency of re-epithelialisation was more among males. Recurrence of pterygium was more in females.

Discussion

Several studies have been conducted on the use of amniotic membrane in ocular surface disorders, and this is

now a well-established method of achieving re-epithelialisation in corneal ulcers and prevention of recurrence in cases of pterygium.^{8,14-16}

Nubile et al.¹⁷ in a prospective, non-comparative, interventional study evaluated the results of integration of amniotic membrane into corneal stroma. Out of the 22 patients with non-infectious corneal ulcers, successful incorporation was found in 20(90.9%). Our results of successful re-epithelialisation in 21 out of 26 patients (80.7%) are comparable although our study patients were more heterogeneous and also included ulcers with infectious aetiology.

Thatte² reported results of a series of 65 patients with different aetiologies who underwent amniotic membrane grafting. Out of the 65 patients, 28(43%) had primary (21 patients) and recurrent (7 patients). Corneal ulcers, thinning and perforations accounted for 21(32.3%) patients. As in our patients, in this series as well there was an early relief of symptoms and reduction in signs of inflammation. Recurrence of pterygium was seen in 3(4.61%) patients. In our study, the recurrence rate (27.7%) was much higher. However, the numbers in both the studies were too small to draw statistically significant conclusions. Two patients had graft rejection in Thatte's series. Graft rejection was not seen in any of our cases.

In the series of Hamza et al.⁸ of 30 patients of ocular surface disorder managed with amniotic membrane grafting ocular surface defect was present in both eyes in 9 cases whereas in 21 cases these defects were monocular. Twenty-seven out of 30 patients had some degree of pain, out of which 13 had severe pain. One month after the grafting, 25 out of 30 patients were completely free of pain and the remaining five had mild to moderate pain. These results are similar to ours where most patients were free of pain within 15 days of surgery. As in our series, in their cases too there was minimal improvement of vision after surgery and 23 out of 30 patients had best corrected visual acuity of 6/60.

Prabhasawat initially mentioned advantages of applying amniotic graft in cases of pterygium. After that there have been many reports of applying amniotic graft in cases of pterygium with no recurrence.¹⁸

When treating ocular surface disease it is important to keep in mind the "Big Four", i.e. tear film, blinking, corneal sensation and stem cells.¹⁶

Modifications in the use of amniotic membrane have been introduced. Canturk et al. combined amniotic graft with conjunctival autograft for chemical burn. This combination gave a better outcome than either method

used on its own.¹⁴ Fascial and mucosal grafts are also applied as an alternative but they have their limitations. Healing of the donor area may take time and surgical time is prolonged.¹⁹

Amniotic membrane has been used in cases of symblepharon with 71% successful results. This is useful in chemical injuries where the membrane is applied to the fornices and therefore prevents adhesions.²⁰ It is also being used in high-risk cases of penetrating keratoplasty (PKP) where it is applied along with the corneal graft. Basically, graft rejection is prevented by the anti-inflammatory properties of the amniotic membrane.²¹ Kruse et al. also noted that after applying amniotic membrane there was much less inflammation on the ocular surface.⁵

When histologically analysed at first, epithelialisation occurs over the defect followed by incorporation of the amniotic graft into the stroma. This occurs in about 2 weeks as was observed on confocal microscopy.

In cases of penetrating keratoplasty with high risk in addition to amniotic graft, limbal stem cells are now transplanted over. In one study the amniotic graft was placed over the corneal graft and the donor limbal tissue over the amniotic membrane.²² In cases of chemical injury, there were 50% chances of corneal graft being successful on its own. This was improved to 70% with limbal stem cell transplant in conjunction with PKP.²³

Amniotic membrane grafting has also been done in cases of conjunctival and orbital malformations. As their excision involves wide areas, reconstruction is required.²⁴

Keratoprosthesis²⁵ is an alternative to corneal grafting following amniotic membrane grafting.

In our study the graft was sutured to the conjunctiva using 8/0 vicryl. Quicker methods of applying the graft such as with cyanoacrylate glue are making its use more popular.²⁶

Conclusion

Amniotic membrane transplantation is a safe procedure and can be performed in any setting where facilities for storage of amniotic membrane are available. In cases of corneal ulcers and perforation, this procedure is successful in achieving adequate epithelialisation and healing of the ulcer with resolution of inflammation. In cases of pterygium, the rate of recurrence is unacceptably high for it to be used as a sole procedure. The study confirms the observation of previous investigators who consider amnion transplantation an efficient therapeutic method for a multitude of eye diseases.

Acknowledgements

We are grateful to Dr. Muneera Shakir from whom we learnt the method of procurement of amniotic membrane.

Disclaimer: This is a dissertation-based article by the same authors.

Conflict of Interest: None.

Source of Funding: None.

References

1. AryaSK, BhalaS, MalikA, SoodS. Role of amniotic membrane transplantation in ocular surface disorders. *Nep J Ophthalmol* 2010; 145-53.
2. Thatte S. Amniotic membrane transplantation: an option for ocular surface disorders. *Oman J Ophthalmol* 4 2011; 67- 72.
3. Sangwan V S, Burman S, Tejwani S, Mahesh S P, Murthy R . Amniotic membrane transplantation: a review of current indications in the management of ophthalmic disorders. *Indian J Ophthalmol* 2007; 251-60.
4. Zhang Z D, Ma H X, Chen D, Lei M, Liu J B, Lu F and Qu J. A novel technique of modified continuous blanket suture for amniotic membrane fixation in severe ocular surface diseases. *JAMA Ophthalmol* 2013; 941-7.
5. Salman AG. Value of fresh amniotic membrane graft in management of resistant non-infected corneal ulcer. *J Clinic Experiment Ophthalmol* 2010; 1:1000108.
6. Thomassen H, Pauklin M, Steuhl K P, Meller D. Comparison of cryopreserved and air-dried human amniotic membrane for ophthalmologic applications. *Graefes Arch ClinExpOphthalmol* 2009; 1691-700.
7. Davidian M. Amniotic membrane for ocular inflammation. *Ophthalm Management* 2013; 17: 30-3.
8. Hamza MS, Rizwanullah M, Hashmi A H, Sahaf I A. Amniotic membrane transplantation in ocular surface disorders. *Pak J Ophthalmol* 2011; 27: 138-41.
9. Vayghan G P, Peirovi H, Niknejad H. Inducing of angiogenesis is the net effect of the amniotic membrane without epithelial cells. *Iran J Med Hypotheses Ideas* 2011; 5: 16.
10. Riau AK, Buerman RW, Lim LS, Mehta JS. Preservation, sterilization and de-epithelialisation of human amniotic membrane for use in ocular surface reconstruction. *Biomaterials* 2010; 31: 216-25.
11. Kitagawa K, Yanagisawa S, Watanabe K, Yonoki T, Hayashi A, Okabe M et al. A hyperdry amniotic membrane patch using a tissue adhesive for corneal perforations and bleb leaks. *Am J Ophthalmol* 2009; 148: 383- 9.
12. Wee S W, Choi S U, Kim J C. Deep anterior lamellar keratoplasty using irradiated acellular cornea with amniotic membrane transplantation for intractable ocular surface diseases. *Korean J Ophth* 2015; 79-85.
13. Ashraf N N, Siyal N A, Sultan S, Adhi M I. Comparison of efficacy of storage of amniotic membrane at - 20 and - 80 degrees centigrade. *JColl Physicians Surg Pak* 2015; 25: 264-7.
14. Sinha R, Tinwala S I, Shekhar H, Titiyal JS. Amniotic membrane transplantation in ocular surface disorders. *J Clin Ophthalmol Res.* 2013 ; 1: 64-9.
15. Mastrota K M. Amniotic membrane in ocular surface disease. *Optometry Times*, 2013 December.
16. Kenyon K R. Ocular surface disease: surgical strategies. *Rev Ophthalmol.* 2010 Jul 15.
17. Nubile M, Dua H S, Lanzini M, Ciancaglini M, Calienno R, Said DG, et al. In vivo analysis of stromal integration of multilayer amniotic membrane transplantation in corneal ulcers. *Am J Ophthalmol* 2011; 151: 809-22.
18. Memerezhadeh F, Fahd A K, Shamie N, Chuck RS. Comparison of de-epithelialized amniotic membrane transplantation and conjunctival autograft after primary pterygium excision. *Eye* 2008; 22: 107-12.
19. Okabe M, Kitagawa K, Yoshida T, Suzuki T, Waki H and Koike C, et al. Hyperdry human amniotic membrane is useful material for tissue engineering: Physical, morphological properties, and safety as the new biological materials. *Journal of biomedical materials research. J Biotechnol Biomater* 2016; 6: 251. doi 10.4172/2155-952x.1000251.
20. Rahman I, Said DG, Maharajan VS, Dua HS. Amniotic membrane in ophthalmology indications and limitations. *Eye* 2009; 23: 1954-61.
21. Nguyen P, Rue K, Heur M, Samuel C Y. Ocular surface rehabilitation: application of human amniotic membrane in high-risk penetrating keratoplasties. *Saudi J Ophthalmol* 2014; 28: 198-202.
22. Capozzi P, Petroni S, Buzzonetti L. Combined HLA matched limbal stem cell allograft with amniotic membrane transplantation as a prophylactic surgical procedure to prevent corneal graft rejection after penetrating keratoplasty: case report. *Ann Ist Super Sanita* 2014; 50: 298-300.
23. Subramaniam S V, Kunjal S, Fatima A, Gaddipati S, Emuganti G K, Sangwan S. Coculture of autologous limbal and conjunctival epithelial cells, to treat severe ocular surface disorders: Long-term survival analysis. *Indian J Ophthal.* 2013; 61: 202-7.
24. Manisha M, Milton W, Aaron F. Amniotic membrane grafting in the management of conjunctival vascular malformations. *Ophthalmic plastic Reconstruct Surg* 2009; 25: 371-5.
25. Sharma N, Ghatak U. Advances in surgical management of ocular surface disease. *JIMSA* 2010; 23: 115-20.
26. Amniotic membrane embraced as versatile treatment for ocular surface disease. *Ocular surgery news* 2011 Nov 25.