Outcome of application of amniotic membrane graft in ocular surface disorders

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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. Pterygiums 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

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 be 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 sofrafelt, 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.

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).

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 ± 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.
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.

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.\textsuperscript{8,14-16}

Nibile et al.\textsuperscript{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.

Thatte\textsuperscript{2} 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.\textsuperscript{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.\textsuperscript{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.\textsuperscript{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.\textsuperscript{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.\textsuperscript{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.\textsuperscript{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.\textsuperscript{21}
Kruse et al. also noted that after applying amniotic
membrane there was much less inflammation on the
ocular surface.\textsuperscript{3}

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.\textsuperscript{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.\textsuperscript{23}

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

Keratoprosthesis\textsuperscript{25} 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.\textsuperscript{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.
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