

Value of Fresh Amniotic Membrane Graft in Management of Resistant Non Infected Corneal Ulcer

Abdelrahman Gaber Salman

Ophthalmology department -Ain Shams University, Egypt

Abstract

Purpose: To evaluate the benefit of use of widely available fresh amniotic membrane in management of resistant non infected corneal ulcer.

Patients and methods: A Prospective non randomized study was done on 47 cases of resistant non infected corneal ulcer were fresh amniotic membrane Grafts were used after excluding any viral infection by serology.

Results: Age range from 51-78 years, 28 was males and 19 were females. Success rate was 80.8% (38) of cases by anatomical healing of epithelium with improvement of best corrected visual acuity (BCVA) more than two lines in 14 cases (29.8%).

Conclusions: The use of fresh amniotic membrane graft is of significant value as alternative method of treatment for resistant non infected corneal ulcer in absence of eye banks.

Keywords: Fresh amniotic membrane graft; Resistant corneal ulcer

Current medical treatments of non infected resistant corneal ulcer include topical artificial tears, lubricants [1] and experimental trials of fibronectin [2], insulin-like growth factor type I [3] and substance P [3], autologous serum [4] or nerve growth factor [5]. When these medical therapies fail, one may consider patching, scleral contact lens, cyanoacrylate glue [6], conjunctival flap, tarsorrhaphy or penetrating keratoplasty [7]. Recently, amniotic membrane transplantation (AMT) has been successfully used to treat persistent corneal epithelial defects and ulcers from different causes and for corneal and conjunctival surface reconstruction for a variety of ocular surface disorders [8].

In ophthalmology, the first use of AMT was by De Rotth in 1940 [9] who reported partial success in the treatment of conjunctival epithelial defects after Symblepharon. Little else regarding AMT appeared in the ophthalmic literature until 1995 when Kim and Tseng [10] used AMT for ocular surface reconstruction of severely damaged corneas in a rabbit model. Since that experimental study, AMT has been used for different types of ocular lesions as persistent corneal epithelial defects, neurotrophic corneal ulcers, leaking filtering blebs after glaucoma surgery, pterygium surgery, conjunctival surface reconstruction, bullous keratopathy, chemical or thermal burns, ocular surface reconstruction with or without limbal stem cell grafting and in patients with ocular cicatricial pemphigoid or Stevens-Johnson syndrome [11].

For the preservation of amniotic membrane, various methods have been used including fresh (or more appropriately hypothermic) storage (stored at +4°C) and freezing in which most of the clinical experiences with human AMT have been with it, where the fragments of amniotic membrane (AM) tissue are preserved at -80°C. [12] AMNIOGRAFT®, ProKera™ [12] and AmbioDry2™ [13] are examples of AM products which are governed under the FDA human cell and tissue regulatory guidelines. ProKera is a device with amniograft clipped into it. AmbioDry2 is a processed, dehydrated, sterilized AM allograft product derived from the submucosa of the placenta and includes basement membrane and stromal matrix [14].

In our study we evaluated the benefit of use of widely available fresh AM in management of resistant non infected corneal ulcer.

Patients and Methods

Prospective non randomized study was done on 47 cases of resistant non infected corneal ulcer were fresh AM overlay grafts were used after exclusion of viral infection by serology of donor serum.

Patients

In our cases we used a fresh AM because of the absence of preserved AM due to the absence of eye bank in King Saud hospital.

In all cases a written informed consent was taken from patients for operation and from pregnant women for donation of AM and the method of procurement was approved by medical and ethical committees on 1-6-2008.

Inclusion criteria were history of recurrent, deep corneal ulcer resistant to treatment for more than 2 months and negative for bacterial, viral or fungal infection.

Exclusion criteria were history of ocular surface disorder as ocular cicatricial pemphigoid or Steven Johnson syndrome.

Conjunctival swabs and corneal scrapping were done in all cases after stopping all topical antibiotics (gentamycin, chloramphenicol eye drops and ointment and ofloxacin eye drops) for 48 hours and were sent for culture and sensitivity in blood agar, chocolate agar, thioglycate broth and Sabarouds agar. The results of cultures were negative with no growth (sterile ulcer).

Corresponding author: Abdelrahman Gaber Salman, Ophthalmology department -Ain Shams University, Egypt, Tel: 009661551228104, 0020161616730; Fax: 00966663646735; E-mail: Ab_gab@hotmail.com

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Until the result of culture and sensitivity and preparation of the AM we started ofloxacin 0.3%, atropine 1% eye drops and fucithalamic eye ointment with autologous serum eye drops four times daily for four days (20% autologous serum eye drop was prepared by venesection of 100 ml blood by hospital nurses. After blood was sent to the blood bank unit, it was left to clot for 4 hours and centrifuged at 3500 rpm for 5 minutes and then pure serum was separated. Those pure serum bottles were kept frozen at -20°C in the ward. Each bottle was thawed and diluted with BSS solution to be 20% of concentration by pharmacologists [15].

Preparation of fresh AM

AM was obtained under sterile conditions after the elective caesarean delivery on patients who were seronegative (HIV, hepatitis B, C viruses and syphilis) and cases with negative results were included. Serological tests were carried out by enzyme linked immune sorbent assay (ELISA). Under a lamellar flow hood, the placenta was first washed free of blood clots with sterile saline. The inner amniotic membrane was separated from the rest of the chorion by blunt dissection (through the potential spaces between these two tissues) and rinsed in sterile saline (2 liters) and later in 4%, 8% and 10% dimethylsulphoxide (DMSO) phosphate buffered saline (PBS) for 5 minutes each, successively. The membrane was then flattened onto a nitrocellulose paper, with the epithelium / basement membrane surface up.

The amniotic membrane was then cut into 2 x 2 cm pieces and mounted on nitrocellulose backing paper. The grafts were then stored in 50 µg/ml penicillin, 50 µg/ml streptomycin, 100 µg/ml neomycin and 2.5 µg/ml amphotericin B with balanced salt solution at +4°C for two weeks [16].

AM transplantation

Under local anesthesia, the base of the ulcer was debrided with



Figure 1: AMT first day post-operative.



Figure 2: AMT two weeks post-operative.

surgical sponges. Overlay total AM grafts were done. A single layer of the AM was secured with interrupted 6-0 Vicryl sutures with episcleral bites around the limbus. The whole corneal surface was covered by the AM. The membrane was placed with stromal side down.

Postoperative care and evaluation

After operation the patient started on Pred Fort, Allergan (Prednisolone acetate 1%) eye drops 5 times a day, fucithalamic eye ointment twice a day, oflox, allergen, (Ofloxacin 0.3%) eye drops every 2 hours and Cefuroxime 750mg IV twice a day. Figures 1 & 2 have shown the first and two weeks postoperative appearance of the eye, respectively.

Patients post discharge medication Before epithelialisation were on the following medication: Augmentin tablet 375mg 3 times a day for three days, Oflox, Allergan (Ofloxacin 0.3%) eye drops and Pred Fort ,Allergan ,(Prednisolone acetate 1%) eye drops four times a day. After epithelialisation was completed, the ofloxacin was discontinued but the predfort was tapered off then to be tapered over four weeks, and fucithalamic eye ointment at bed time for four weeks. Fluorescein staining was used to detect epithelial defects and documentary photographs were taken if necessary.

Data were recorded and analysed using SPSS (Statistical Package for Social Sciences V.13).

Results

Age range was 51-78 years, 28 were males and 19 were females. Success rate was 80.8% (38) of cases by anatomical healing of epithelium by negative flourescein stain.

With improvement of best corrected visual acuity (BCVA) more than two lines in 14 cases (29.8%).

Before AM transplantation

On examination preoperative BCVA was: no light perception in 3 cases, PL to HM in 20 cases, 1/60 to 3/60 in 12 cases and more than 3/60 in 12 cases. Cornea was vascularised in 30 cases (11 total and 19 partial) whereas ulcer was central in 15, deep to Descemet membrane in 10 and non adherent in 41 cases. All these ulcers had been persistent for more than 2 months and 9 (19.1%) eyes had shown progressive thinning of the ulcer bed.

30 of 47 (63.8%) corneal ulcers were neurotrophic corneal ulcers with decreased corneal sensation. These neurotrophic corneal ulcers were developed following diabetes mellitus (16 eyes), keratoplasty (2 eyes), herpes zoster ophthalmicus (6 eyes), herpes simplex keratitis (3eye) radiation (2 eyes) and removal of acoustic neuroma with neuroparalysis (1 eye).

Besides the neurotrophic state, 15 eyes (31.9%) had suffered from mechanical trauma caused by additional lid problems such as trichiasis or entropion with misdirected eyelash and 2 eyes (4.2%) had lagophthalmos.

Other ocular abnormalities included cataract in 9 patients, pseudophakia in eleven patients and glaucoma in six eyes. Sixteen patients had diabetes mellitus and twelve had hypertension.

After AM transplantation

Thirty eight eyes of AMT achieved rapid epithelialisation in 17 days (SD 8) range 14-34 days. 5 eyes of them showed delayed healing and needed tarsorrhaphy.



In the 6 (12.7%) eyes with uncontrolled diabetes mellitus, the ulcers were not healed (positive fluorescein stain) on day 14 and partial tarsorrhaphy was added and the healing was completed on day 34 by anatomical healing of epithelium (negative fluorescein stain).

In 9 (19.2%) eyes there were impending perforation (2), severe uncontrolled diabetes mellitus (4), post lagophthalmos (2) and post acoustic tumour (1), the ulcer did not heal and required referral for higher center for penetrating keratoplasty.

The healed corneal surface was accompanied by reduced inflammation but without disappearance of vascularization.

Discussion

Several characteristics explain why the AM can be useful to promote epithelial healing, the epithelium produces various growth factors and the basement membrane facilitates migration of epithelial cells, reinforces adhesion of basal epithelial cells and may promote epithelial differentiation [17]. It acts as a biological bandage contact lens to prevent mechanical trauma from lids, prevents exposure and reduces surface dryness.

The AM can be obtained either from elective caesarean or normal vaginal deliveries. In our cases we used placenta obtained after elective caesarean for a seronegative for hepatitis B, C virus women to avoid the greater risk of infection from normal vaginal delivery as it is recommended by Philip et al. [18] that amnion for use in ocular surface procedures should be better retrieved from placentas following elective caesarean deliveries because of greater risk of contamination from pathogenic bacteria on placentas from vaginal deliveries [18].

We used a non-preserved AMT in resistant non infected corneal ulcer which showed good results in promoting epithelial healing, reducing inflammation and increase comfort, as compared to Ucakhan et al. [19] that evaluated safety and efficacy of non-preserved AMT with or without limbal autograft transplantation in acute and chronic chemical eye injuries. They found that AMT promoted epithelial healing, reduced inflammation, increased comfort and decreased severity of vascularization, but in our patients there were no decrease in severity of vascularization.

Kruse et al. [20] noted that ocular surface inflammation was markedly reduced following AMT. This finding may be explained by other recent studies showing that the stromal matrix of the amniotic membrane excludes inflammatory cells, contains various forms of protease inhibitors and suppresses transforming growth factor β (TGF- β) signaling and proliferation and myofibroblast differentiation of normal human corneal and limbal fibroblasts [8].

When AM used as a patch, AM is invariably dissolved. When used as a graft, AM promotes epithelialisation over it and is frequently preserved and may become quite transparent over time [21].

Persistent exposure as seen in cases with severe entropion, may be a limiting factor for delayed epithelialisation seen in 6 eyes with poor eyelid blinking associated with severe entropion. In these cases it is advised to do early partial tarsorrhaphy.

Because the neurotrophic state invariably leads to aqueous tear deficiency, it is advised that punctual occlusion be performed before tarsorrhaphy. Prolonged exposure also explains why the membrane eventually dissolved partially or totally. Although recurrent breakdowns have been reported [20].

Philip et al. [18] reported a clinical and in vitro comparison between fresh and frozen amniotic membrane grafts [18]. They found that in both cases the cornea reepithelialised and visual acuity improved, showing no differences in healing process between the two. In our cases we found some clinical and practical difficulties as: a theoretical risk of disease transmission, the need to find a suitable donor sufficiently far in advance of surgery to allow processing and testing and coordination with the admission to hospital of the recipient, as compared to Philip et al. [18]. There were few studies done for the advantages and disadvantages of fresh AMT because most of the clinical experiences with human AMT have been with tissue preserved AMT. In our study we concluded that all these difficulties are not sufficient reasons to prevent the use of fresh AM. AM graft can be used either as a single or multiple layers.

There are two techniques for using amniotic membrane graft, either using the inlay technique which involves placement of the AM graft into the corneal ulcer secured into place by interrupted sutures without extending beyond the edge of the epithelial defect, or using the overlay technique in which the entire corneal surface including the limbus is covered with the AM graft. We used the overlay technique and this was comparable to Letko et al. [21] who compared inlay versus overlay AM grafting techniques in ability to heal persistent corneal epithelial defects and he found no difference in terms of healing time and recurrence rate [21].

Our study reported the success of using single layer method for the management of resistant non infected corneal ulcer. We started with single layer as it was technically easier and we were planning to use multilayer in case of failure of single layer but fortunately the single layer was successful in (80.8%) and this was comparative with Hanada et al. [22] that reported complete healing in 16.5 ± 8.0 days in 8/11 eyes using multiple layers of amniotic membrane to treat deep corneal ulcers with descemetocoele [22].

In conclusion, although the use of fresh tissue may be associated with a higher risk of blood-borne diseases, still the non-preserved AM is important for ocular surface reconstruction in hospitals where there is no banked frozen tissue and in that where cost and availability may prohibit the use of the preserved tissue.

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