

AMNIOTIC MEMBRANE TRANSPLANTATION FOR OCULAR SURFACE RECONSTRUCTION

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SUMMARY – The purpose of this study is to report our clinical experience and the effect of human amniotic membrane transplantation on severe ocular surface diseases. From 1998 to 2000 at Sestre milosrdnice University Hospital amniotic membrane transplantation was performed consecutively in 27 eyes: 16 eyes had persistent corneal ulceration and 11 bullous keratopathy. In the group with persistent corneal ulceration the average time of epithelisation was 23.1 days and epithelial defect recurred after the AMT in 75%, while in the eyes suffering from bullous keratopathy epithelisation took place in 19.6 days in 72.7%. AMT can be considered an effective alternative for treating severe ocular surface disease.

Key words: *amniotic membrane transplantation, persistent corneal ulceration, bullous keratopathy*

Introduction

Management of ocular surface disturbances still remain a therapeutical problem despite various conservative and surgical treatments. Some of them previously had no effective management and led to loss of vision.

One of such conditions is persistent corneal ulcer developed as a result of the breakdown of corneal epithelial surface. Exogenous and endogenous factors could lead to corneal ulcer such as infection, trauma, radiation, anterior segment surgery, chemicals, diabetes mellitus, recurrent corneal erosions and tumors.¹⁻³ A severe complication caused by corneal ulcer is imminent perforation. In order to avoid complications including perforation many medical and surgical treatments have been developed. Current medical treatments include topical artificial tears and lubricants. Nowadays new materials have been studied such as fibronectin,⁴ substance P and insulin like growth factor type I.⁵ After an unsuccessful medical treatments surgical therapy may be considered such as cyanoacrylate glue,⁶

conjunctival flaps,^{7,8} tarsorrhaphy,⁹ lamellar or penetrating keratoplasty.^{1,3,10}

Bullous keratopathy is a condition characterized by corneal stromal edema with or without epithelial bullae. Breakdown of endothelial function lead to increased hydration and intraepithelial edema that could lead to recurrent or persistent erosion. Patients with bullous keratopathy may suffer from ocular pain and reduced vision.^{11,12} Bullous keratopathy caused by various intraocular surgical procedures and nonsurgical causes can be treated with bandage contact lens,¹³ anterior stromal punctures,¹⁴ epikerato-phakia,¹⁵ conjunctival flap,¹⁶ excimer laser phototherapeutic keratectomy¹⁷ and annular keratotomy.¹⁸

The aim of this study was to determine the effect of human amniotic membrane transplantation on severe ocular surface diseases, persistent corneal ulcer and bullous keratopathy.

Material and Methods

From 1998 to 2000 at Sestre milosrdnice University Hospital - University Department of Ophthalmology amniotic membrane surgery was performed consecutively in 27 eyes: 12 female and 15 male ages between 49 and 78

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years. An informed consent was obtained from each patient before surgery. Based on the underlying causes of ocular diseases they were divided into 2 groups: 16 eyes had persistent corneal ulceration and 11 bullous keratopathy. All surgical procedures were performed by the same surgeon.

Amniotic membrane with an attached placenta was obtained shortly after Cesarean section. By serological testing human immunodeficiency virus (HIV), hepatitis virus type B and C and syphilis were excluded. The placenta was cleaned by washing with BSS containing penicillin (50 mg/ml), streptomycin (50 mg/ml), neomycin (100 mg/ml), and amphotericin B (2.5 mg/ml). The amniotic membrane was separated from the chorion and was flattened over a nitrocellulose filter paper with epithelial surface up, and the stromal matrix down in contact with the paper. Prepared amnion was placed in a plastic container and stored at -80°C .

All surgeries were performed with the patient receiving retrobulbar anesthesia (2% lidocain). After anesthesia in eyes with persistent sterile corneal ulceration the base

of the ulcer was debrided and poorly adherent epithelium adjacent to the edge of the ulcer was removed up to the area where the epithelium became adherent. The amniotic membrane was removed from the storage medium peeled from the nitrocellulose filter paper. Then it was placed on the surface of the cornea to cover the ulcerated area with the epithelial surface up. Deep ulcers were filled with more than one layer of amniotic membrane. The AM was secured to the edge of the defect by interrupted 10.0 nylon sutures. After the transplantation, a bandage contact lens was applied in 5 eyes and partial tarsorrhaphy was added in 2 eyes. In the postoperative period topical Maxitrol (neomycin sulphate, ploymyxin B sulphate and dexamethason) was administrated 4 times a day.

In the bullous keratopathy group the loose epithelium was removed with a 0.12 forceps up to the area where it became adherent and the surgical technique was the same as described previously. Postoperative medications included Maxitrol (neomycin sulphate, ploymyxin B sulphate and dexamethason) and was administrated 4 times a day.

Table 1. Demographic and clinical data of corneal persistent ulcer before amniotic membrane transplantation

PERSISTENT CORNEAL ULCERATION	
Age	65.4 (49-71)
Number of eyes (Male/Female)	16 (9/7)
Causes of ulcer	a) Neurotrophic keratopathy Diabetes mellitus (n=5) Post keratoplasty (n=2) Removal of acoustic neurinoma (n=1) Herpes zoster ophthalmicus (n=2) b) Keratoconjunctivitis sicca (n=1) c) Postinfection (n=5)
Duration (weeks)	32.3 (11-53)
Associated diagnosis (ocular/systemic)	Glaucoma surgery (n=2) Cataract surgery ECCE (n=4) PHACO (n=1) Penetrating keratoplasty (n=2) Vitrectomy (n=1) Lid surgery (n=1)
Previous treatment	Lubrication (n=16) Punctal occlusion (n=6) Patching and bandage contact lens (n=7) Partial tarsorrhaphy (n=1)

Patients were followed up before epithelisation once a week and after epithelisation once a month.

Results

Amniotic membrane transplantation (AMT) for persistent corneal ulceration

Amniotic membrane was transplanted in 16 patients (16 eyes) and the results are summarized in Table 1. The mean age of patients was 65.4 years (ages between 49 and 71), 7 females (43.7%) and 9 males (56.3%). The mean follow up after AMT was 8.9 months (range: 5-14 months). ATM was performed for persistent sterile corneal ulcer caused by neurotrophic keratopathy (n=10), keratoconjunctivitis sicca (n=1) and after severe infection (n=5). Some pre-existing ocular diseases were noted in the eyes: glaucoma (n=4), proliferative diabetic retinopathy (n=1), nonproliferative diabetic retinopathy (n=1), Fuchs's dystrophy (n=1), keratoconjunctivitis sicca (n=4), entropion (n=1). According to the ocular diseases 69% eyes underwent one or more previous surgery (glaucoma surgery, cataract operation –ECCE and PHACO, perforative

keratoplasty, vitrectomy, lid surgery). The duration of persistent corneal ulcer varied from 11 to 53 weeks with an average of 32.3 weeks, so all patients were initially treated with tears substitutes and lubricants and during some time with prophylactic antibiotics. Previous management also included for exposure problems punctal occlusion in 6 eyes, patching and bandage contact lens in 7 eyes and partial tarsorrhaphy had been performed in 1 eye. One eye had suffered from mechanical trauma caused by entropion, and one eye had had additional lagophthalmos. The epithelial defect recurred after the AMT in 75% (Table 3). Nine eyes (56.3%) were healed after the first AMT, while in 3 eyes AMT was repeated. The average time of epithelisation was 23.1 days. The visual acuity was improved in 6 eyes during the follow up of 8.9 months. In 8 eyes the visual acuity was unchanged and was worsened in 2 eyes (due to proliferative diabetic retinopathy and glaucoma with developing cataract).

Amniotic membrane transplantation (AMT) for symptomatic bullous keratopathy

ATM was performed in 11 eyes suffering from ocular surface pain associated with poor visual potential (Table

Table 2. Bullous keratopathy demographic and clinical data before amniotic membrane transplantation

BULLOUS KERATOPATHY	
Age (years)	65.9 (51-78)
Number of eyes (Male/Female)	11 (6/5)
Causes	Pseudophakia (n=6) PHACO (n=2) ECCE (n=4) Aphakia (n=2) Failed graft (n=2)
Duration (weeks)	41.3 (18-73)
Associated diagnosis (ocular/systemic)	Glaucoma (n=2) Diabetes mellitus (n=3) Nonproliferative diabetic retinopathy (n=2)
Previous surgery	Trabeculectomy (n=2) Second implantation IOL (n=1) Vitrectomy (n=1) Anterior vitrectomy (n=1)
Previous treatment	Artificial tears, lubricants, prophylactic antibiotics (n=5) Soft contact lens (n=4)

Table 3. Outcome after amniotic membrane transplantation

	Associated treatment	Epithelisation (days)	Healing (%)	Follow up (months)	Postop.visual acuity (better/same/worse)
PERSISTENT CORNEAL ULCUS	Soft contact lens (n=5)	23.1	12/16	8.9	6/8/2
	Partial tarsoraphy (n=2)	(7-39)	(75%)	(5-14)	
BULLOUS KERATOPATHY	Soft contact lens (n=9)	19.6	8/11	7.8	1/10/0
		(8-35)	(72.7%)	(2-15)	

2). The average age was 65.9 years (range 51-78 years) and most of them were pseudophakic eyes (n=6) caused by ECCE (n=4) and PHACO (n=2). Three eyes (3/6) underwent additional ocular surgery (trabeculectomy, the secondary implantation of IOL and vitrectomy). In aphakic bullous keratopathy both (n=2) were caused by ECCE and one of them underwent partial anterior vitrectomy and secondary developed glaucoma. In the group of failed corneal grafts both were pseudophakic (ECCE) and one of them had trabeculectomy. Additional ocular diseases included glaucoma in 2 eyes, nonproliferative diabetic retinopathy in 2 eyes. All eyes had been treated with artificial tear drops, lubricants, 5 eyes with prophylactic antibiotics and bandage contact lens was used in 4 eyes. The duration of bullous keratopathy varied from 18 to 73 weeks (mean 41.3 weeks). Epithelisation took place in 19.6 days (8-35 days) in 8 eyes (72.7%) (Table 3). AMT was repeated in 3 eyes with residual pain. Amniotic membrane transplant failed in one eye and after repeated AMT the eye became pain free. In the second eye pain was reduced and unchanged in the third eye. Visual activity was improved in 1 eye from finger counting to 0.05, and in other eyes remained unchanged.

Discussion

In this study we have reevaluated the efficacy and safety of amniotic membrane transplantation in different ocular surface disorders: persistent ocular ulceration and symptomatic bullous keratopathy. The AMT has been used since 1910.g. by Davis¹⁹ in general surgery for skin transplantation. In ophthalmology the first AMT was reported by De Roth²⁰ in the reconstruction of conjunctival defects in 1940.g. Kim and Tseng²¹ reported new dimension and contribution of amniotic membrane transplantation used in ocular surface reconstruction in rabbits after epithelial

removal and limbal lamellar keratectomy. Recently, amniotic membrane has been used for ocular reconstruction following chemical or thermal burns,²² advanced ocular cicatricial pemphigoid and Steven-Johnson syndrome (23), for pterygium excision,²⁴ conjunctival surface reconstruction,²⁵ sterile corneal ulceration²⁶ and symptomatic bullous keratopathy.²⁷

The amniotic membrane is composed of monolayer epithelial cells, a basement membrane, and an avascular stromal matrix.²⁸ The membrane permits rapid epithelization due to various actions: it facilitates migration of epithelial cells,²⁹ reinforces adhesion of basal epithelial cells,³⁰ promotes epithelial differentiation³¹ and prevents epithelial apoptosis.³² The stromal side of the membrane also contains a component that suppresses TGF- β signalling, and the proliferation and myofibroblast differentiation of normal human corneal and limbal fibroblasts³³ and normal conjunctival and pterygium body fibroblasts.³⁴ Furthermore, amniotic membrane produces basic fibroblast growth factor, hepatocyte growth factor and transforming growth factor β ,³⁵ stromal matrix excludes inflammatory cells³⁶ and contains several forms of protease inhibitors.³⁷ These actions are the reason why stromal inflammation is reduced after AMT and corneal neovascularisation is mitigated.

AMT can be considered as effective management for treating persistent sterile corneal ulceration. Most of eyes (10/16) developed corneal ulcer caused by neurotrophic keratopathy and by damage to the corneal sensory nerve. Mostly, the nerve was affected by ischaemia due to diabetes mellitus, infection by herpes zoster, surgical removal of acoustic neurinoma and after keratoplasty. In five eyes corneal problems had developed after severe microbial infections and in one eye after severe keratoconjunctivitis. During a mean follow up of 23.1 days epithelisation took place in 12 eyes (75%). For three eyes, an additional AMT was necessary. This result is consistent with previ-

ous reports in which ATM was used to treat corneal ulcers. Lee et al²⁶ performed AMT in 11 eyes with corneal ulceration of different causes and 10 of 11 eyes were healed. Kruse et al³⁸ reported corneal ulcerations treated by multilayer AMT and 9 of 11 remained stable for 1 year. According to previous report (Kruse et al) we noted that ocular surface inflammation was reduced after AMT. These findings support recent studies showing that the stromal matrix of the amniotic membrane excludes inflammatory cells,³⁶ 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.³²

Patients with symptomatic bullous keratopathy suffer from ocular pain associated with reduced vision. As a result of histopathological changes in cornea poor epithelial adhesion may persist and lead to recurrent or persistent erosion. We performed AMT in 11 eyes that were refractory to conventional treatment. Eight eyes (72.7%) became pain free during the following period of 19.6 days (8-35 days), which is in accordance with the results of other studies.²⁷

Pain relief after AMT is associated with restoration of corneal epithelial integrity and can be contributed to therapeutic amniotic membrane effect whose properties have been mentioned. Four of the eyes with good visual potential underwent corneal transplantation.

Conclusion

The AMT can be helpful in the treatment of severe ocular surface disease. It may be considered as alternative method for ocular surface reconstruction that are refractory to conventional treatment. Persistent corneal ulceration and bullous keratopathy can be effectively treated by amniotic membrane transplantation.

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Sažetak

REKONSTRUKCIJA POVRŠINE OKA AMNIJSKOM MEMBRANOM

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Cilj ovog rada bio je prikazati naše kliničko iskustvo i rezultate prilikom transplantacije amnijske membrane kod teških bolesti prednjeg segmenta oka. U razdoblju od 1998 do 2000.g. na Klinici za očne bolesti KB «Sestre milosrdnice» transplantirali smo amnijsku membranu u 27 očiju: 16 očiju s perzistentnim ulkusom rožnice i 11 očiju s buloznom keratopatijom. U grupi s perzistentnim ulkusom rožnice prosječno vrijeme epitelizacije rožnice iznosilo je 23.1 dan, te defekt epitela zacijelio je nakon transplantacije amnijske membrane u 75%, dok kod očiju s buloznom keratopatijom došlo je do epitelizacije tijekom prosječno 19.6 dana u 72.7%. Transplantacija amnijske membrane predstavlja efikasno alternativno liječenje teških oboljela prednjeg ožnog segmenta

Ključne riječi: transplantacija amnijske membrane, perzistentni ulkus rožnice, bulozna keratopatija