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ORIGINAL ARTICLE

Analysis of patients undergoing amniotic membrane transplantation at a tertiary referral hospital

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Abstract

Purpose: In this study, we aimed to determine amniotic membrane transplantation (AMT) indications, early results and demographic analysis of patients who underwent AMT in our clinic according to age and gender.

Methods: The records of 154 patients who underwent AMT at the Ophthalmology Clinic between April 2017 and September 2019 were reviewed retrospectively. Examination findings and demographic data of the patients were examined and recorded. Patients were divided into five groups: 0–10 years (Group 1, n=7), 11–20 years (Group 2, n=9), 21–40 years (Group 3, n=23), 41–60 years (Group 4, n=32), and over 60 years (60–89 years) (Group 5, n=83).

Results: Ninety-five (61.7%) of the patients included in the study were male and 59 (38.3%) were female. The mean age of the patients was 55.72±22.53 (Range: 0–89) years. The most common indications for AMT in all age groups were corneal ulcer (n=47, 30.5%), corneal melting (n=32, 20.8%), and persistent epithelial defect (PED) (n=21, 13.6%). The most common age groups for AMT were Group 5 (n=83, 53.9%), Group 4 (n=32, 20.8%), and Group 3 (n=23, 14.9%). The most common indications for AMT in children and adolescents (0–20 years) were corneal ulcer (n=6, 37.5%) and corneal chemical burns (n=5, 31.2%), while in adults over 21 years of age, AMT indications were corneal ulcer (n=41, 29.7%) and corneal melting (n=29, 21.0%).

Conclusion: The most common indications for AMT in our study were corneal ulcer, corneal melting, and PED. According to the indications, AMT may be a simple and easily applicable surgical method that can be used in the reconstruction of the ocular surface in many corneal and conjunctival pathologies.

Keywords: Amniotic membrane; corneal ulcer; ocular surface reconstruction.

Amniotic membrane (AM) is derived from the innermost layer of the fetal membrane. It is a translucent membrane consisting of three layers: Epithelium, basement membrane, and stroma.[1] In 1910, Davis was the first to report the use of fetal membranes as surgical material during skin transplants.[2]

The use of AM in the reconstruction of conjunctival defects due to burns was first reported in 1940.[3] It has been re-

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ported to be successfully used as an AM patch graft in the treatment of acute ocular burns.[4]

In a study, human AM preserved in 85% glycerol solution at 4°C for 1 year was shown to be as effective as fresh amnion in the treatment of partial-thickness skin burns and in reducing contamination of infected burn wounds in rats.[5]

AM is widely used in many ocular surface reconstructions as it provides an excellent substrate for growth, transport, and adhesion of corneal and conjunctival cells with its natural anti-inflammatory, antifibrotic, and antiangiogenic properties.[6–8]

AM promotes reepithelialization, reduces the formation of scars and new vessels, reduces inflammation, acts as a scaffold for cell growth, contains antimicrobial properties, contains nerve growth factor, and therefore promotes nerve regeneration.[9]

AM can be used as grafts, overlays, or multiple layers. In the graft method, only the defect is coated, while in the covering method AM is applied to the entire cornea. If AM is stored at -80°C, it should be thawed at room temperature before use. The epithelial side is sutured upward and the mesenchymal side is sutured downward to facilitate the adhesion of AM to the ocular surface.[10]

In this study, we aimed to determine the indications for AM transplantation (AMT) in patients undergoing AMT in our clinic and to conduct a demographic analysis of these patients according to age and gender.

Materials and Methods

The records of 154 patients who underwent AMT at the Ophthalmology Clinic between April 2017 and September 2019 were reviewed retrospectively. The protocol was approved by the Ethical Committee of Dicle University Faculty of Medicine (date: 09/01/2020, no: 46), and the study adhered to the tenets of the Declaration of Helsinki. Written informed consent was taken from all participants. All ophthalmologic examination records, including the anterior segment findings, visual acuity, and intraocular pressures of the patients, were examined and the data were recorded. The indications for AMT were examined under eight headings: Corneal ulcer, corneal melting, persistent epithelial defect (PED), pterygium, corneal chemical burn, conjunctival tumor, bullous keratopathy, and corneal perforation. According to their age, patients were divided into five groups: Group 1 (0–10 years), Group 2 (11–20 years), Group 3 (21–40 years), Group 4 (41–60 years), and Group 5 (60–89 years) (over 60 years).

AM Preparation and Surgical Method

Placenta was collected from healthy women after elective caesarean section. It was thoroughly cleaned with normal saline solution and transported to the cornea bank of our hospital in normal saline with gentamicin. The amnion was separated from the chorion membrane and then washed with normal saline solution containing gentamicin and amphotericin B. The AM was prepared by blunt dissection, then flattened on a nitrocellulose paper carrier, typically epithelial side up, and cut into multiple pieces of different sizes. It was stored in a vial containing Dulbecco's modified Eagle medium (DMEM; Gibco, Karlsruhe, Germany) and glycerol at −80°C.[11] The mother was examined for HIV, HbsAg, and VDRL. Serology was repeated 3–6 months after labor to discard any infected material that could have been in the window period during the initial screening.[12] Amnion was removed from glycerol and kept in normal saline for 30 min at room temperature before surgery. AMT was performed in the operating room under local anesthesia or general anesthesia.

Statistics

IBM SPSS Statistics software, version 22 (IBM Corp, Chicago, Illinois, USA) was used for data analysis. Continuous variables are reported as mean ± standard deviation, and categorical variables are reported as frequency and percentage.

Results

We found that 95 (61.7%) of the patients were male and 59 (38.3%) were female. The mean age of the patients was 55.72±22.53 years. The mean age of the patients was 55.72±22.53 (0–89) years (Table 1).

Among all age groups, the most common indications for AMT were corneal ulcer (n=47, 30.5%), corneal melting (n=32, 20.8%), and PED (n=21, 13.6%) (Table 2). The most common age groups for AMT were Group 5 (n=83, 53.9%), Group 4 (n=32, 20.8%), and Group 3 (n=23, 14.9%). The most common indications for AMT in children and adolescents (0–20 years) were corneal ulcer (n=6 patients, 37.5%) and corneal chemical burns (n=5, 31.2%), while in adults older than 21 years, the indications were corneal ulcer (n=41, 29.7%) and corneal melting (n=29, 21.0%) (Table 3).

Table 1. Demographic data of patients

Characteristics	
Age (year, mean±standard deviation)	55.7±22.5
Gender (male/female, n [%])	95 (61.7)/59 (38.3)
Affected eye (right/left, n [%])	79 (51.3)/75 (48.7)

Table 2. Amniotic membrane transplantation indications

Indications	n	%
Corneal ulcer	47	30.5
Corneal melting	32	20.8
Persistent epithelial defect	21	13.6
Pterygium	15	9.7
Corneal perforation	14	9.1
Bullous keratopathy	11	7.1
Corneal chemical burn	10	6.5
Conjunctival tumor	4	2.6
Total	154	100

In our clinic, 47 (30.5%) patients underwent AMT for corneal ulcer. Seven patients underwent repeat AMT after an average of 1.5 months. Thirty-two (20.8%) patients with corneal melting for infectious or non-infectious reasons underwent AMT. In 28 patients, AM was applied as a covering, and in four patients, the AMT was filled and covered with several layers. Twenty-one (13.6%) patients underwent AMT for PED. Of these patients, eight had penetrating keratoplasty, four had keratitis, six had neurotrophic ulcer, and three had shield epithelial defects. Of the 21 patients, three underwent AMT again. Ten (6.5%) patients underwent AMT for chemical burns. Of the ten patients, eight had mild to moderate chemical burns, while two patients had severe chemical burns. Of the ten patients, four underwent AMT twice and ix patients had AMT once. Four (2.6%) patients underwent AMT due to large conjunctival defects after conjunctival mass excision. Fourteen (9.1%) patients underwent AMT for corneal perforation. Fifteen (9.7%) patients underwent AMT for pterygium. Four patients had recurrent pterygium. AMT was applied alone to six of 15 patients, and AMT and conjunctival grafts were applied together in nine patients. Eleven (7.1%) patients with bullous keratoplasty underwent AMT.

Discussion

In our study, the most common indications for AMT were corneal ulcer, corneal melting, and PED. AMT was most commonly used in patients over 60 years and 40–60 years of age. In our study, the most common indications in children and adolescents were corneal ulcer and corneal chemical burns, while in adults over 21 years of age, the indications were corneal ulcer and corneal melting.

There are several methods for AM storage, including cryopreservation, lyophilization, and dry form storage. Lyophilization led to a greater reduction in the amounts of growth factors compared to cryopreservation, but no significant difference was found in the concentration of various growth factors between fresh frozen and lyophilized AM. While freeze-protected AM has been shown to have better preserved basement membrane components, lyophilized, and air-dried. AM has the advantage of allowing room temperature storage and longer expiration time.[12] AMs used in our clinic are stored by the cryopreservation method.

AM can be used in the presence of ulcers that are refractory to medical treatment, and descemetocele or perforation. AM provides rapid recovery and less fibrosis in patients who do not respond to other treatment.[12] After 6 months of follow-up in our clinic, patients treated with AMT and medical therapy showed a marked reduction in inflammation and improvement in symptoms. Although AMT has been used effectively in microbial keratitis, the regression time of keratitis following AMT treatment is not known.[13]

Corneal melting describes a reduction in corneal thickness that can cause perforation in severe cases. Melting can be triggered by various factors such as immune-mediated diseases, ocular surface diseases, trauma, surgery, and infection.[14] In our clinic, significant improvement was observed in the examined area in 26 of 32 patients, but six patients did

Table 3. Amniotic membrane transplantation indications according to age groups

Indications	Age Groups					Total n (%)
	Group 1 n (%)	Group 2 n (%)	Group 3 n (%)	Group 4 n (%)	Group 5 n (%)	
Corneal ulcer	2 (1.3)	4 (2.6)	8 (5.2)	6 (3.9)	27 (17.5)	47 (30.5)
Corneal melting	2 (1.3)	1 (0.7)	3 (1.9)	7 (4.4)	19 (12.5)	32 (20.8)
Persistent epithelial defect	–	–	–	6 (3.9)	15 (9.7)	21 (13.6)
Pterygium	–	–	6 (3.9)	5 (3.2)	4 (2.6)	15 (9.7)
Corneal perforation	1 (0.7)	–	1 (0.7)	2 (1.3)	10 (6.4)	14 (9.1)
Bullous keratopathy	-	1 (0.7)	1 (0.7)	2 (1.3)	7 (4.4)	11 (7.1)
Corneal chemical burn	2 (1.3)	3 (1.9)	4 (2.6)	1 (0.7)	–	10 (6.5)
Conjunctival tumor	–	–	–	1 (0.7)	3 (1.9)	4 (2.6)
Total						154 (100)

not show enough improvement. The lack of improvement in six patients was thought to be because the melting area was >3 mm and the depth of the thinning area was greater. AMT was found to be highly effective in patients with <2 mm of thinning area and in patients without severe thinning.

Corneal melting treatment has changed dramatically in recent years. Some of the new topical drugs used for this purpose are lubricants such as hyaluronic acid, autologous serum, and fibronectin; and epithelialization stimulants and growth factors.[15] The use of a soft contact lens may also be a useful therapy. In addition, systemic drugs such as tetracycline derivatives, immunosuppressants and biological agents can be used for this purpose. Surgical approaches such as tarsorrhaphy, conjunctival flaps, lamellar corneal transplantation, and AMT are used when clinical treatment is inadequate.[14]

PED is managed by the treatment of the underlying disease process to control inflammation and protect the surface. AM has several properties that increase epithelialization and reduce inflammation.[16] In our clinic, it was observed that the epithelium of 21 patients who underwent AMT for PED was completely healed after an average of 6 months of follow-up. Two of the patients who needed AMT again had neurotrophic keratitis and one had non-healing epithelial defects due to herpetic keratitis. Multiple AM layers increase stromal thickness in deep and perforated non-infectious ulcers, providing collagen, and growth factors for epithelial healing.[16]

AM is used in pterygium surgery because it can suppress fibroblasts. After changing the surgical technique, recurrence rates in primary and recurrent pterygiums were reported as 3% and 9.5%, respectively.[17] AM is a good alternative to conjunctival autograft in patients who have undergone multiple conjunctival surgeries or need preservation of the conjunctiva due to possible glaucoma surgery.[12] In our clinic, 15 patients underwent AMT for pterygium. There was no recurrence in AMT and conjunctival graft patients during the 6-month follow-up, but recurrence was observed in three of the six AMT-only patients.

Chemical burns, severe ocular surface inflammation, and epithelial destruction in the acute phase can progress to tissue dissolution. The purpose of using AMT is to reduce inflammation, increases epithelialization, and prevents tissue necrosis, thus reducing vision loss by reducing scar development. In ten patients who underwent AMT due to chemical burns in our clinic, a significant decrease in inflammation and a significant improvement in the corneal epithelium were observed. In mild-to-moderate chemical injuries, AMT restores corneal and conjunctival surfaces and prevents symblepharon formation in severe burns.[18] Patients with severe chemical burns were treated with AMT for a 2nd time and limbal stem cell transplantation was recommended.[19]

Conjunctival autograft and mucosal grafts have been used in ocular surface reconstruction although they exhibit poor cosmetic appearance, increased risk of infection, limited availability, and scar formation in donor sites.[20] AMT promotes epithelial wound healing and exerts potent anti-inflammatory and anti-scarring effects on the ocular surface. These valuable properties make the AM an ideal tissue for reconstruction of ocular surface tumors.[21,22] In our clinic, four patients underwent AMT due to large conjunctival defects after conjunctival mass excision. One patient underwent AMT for the 2nd time due to a large tissue defect. After 6 months of follow-up, conjunctival defects were completely closed in all patients. AMT contributes to healthy ocular formation by providing more tissue for conjunctival reconstruction, especially in common conjunctival melanoma.[23] In 21 ocular surface squamous neoplasia patients, Palamar et al.[24] found that AMT for ocular surface reconstruction was an effective long-term procedure even for tumors larger than 10 mm.

The friction of the nerves exposed in the edematous cornea and the development of bullae are the main causes of pain in bullous keratopathy.[12] AMT was used to relieve pain in eyes with symptomatic bullous keratopathy. Pain relief ranged from 88% to 90% between 4 weeks and 45 months.[25] In our clinic, 11 patients with bullous keratoplasty underwent AMT to reduce pain and inflammation before penetrating keratoplasty. Pain and inflammation decreased significantly after AMT.

AM has been reported to be effective in wound healing by allowing differentiation and growth of cells.[26] In our clinic, 14 patients underwent AMT for corneal perforation. These patients had leakage despite corneal primary suturing. While 11 of 14 patients underwent AMT with intraoperative primary suturing, three patients were treated because of leakage detected during post-operative follow-up. After AMT, leakage disappeared completely in all patients.

In this study, the reason why men constitute the majority of the surgical population may be that men are more involved in agriculture and animal husbandry activities and outdoor activities in our region. Furthermore, male patients may have more access to health centers and thus be diagnosed more quickly. Likewise, the reason for the high number of elderly patients was thought to be late admission to the hospital after trauma due to the low sociocultural level of our region and possible immune weakness in this age

group. In our study, corneal ulcer was frequently detected as a result of increased exposure to trauma due to regional factors, ocular surface deterioration due to susceptibility to dry eye due to the warm climate of our region, and late admission to health centers due to low sociocultural level.

Conclusion

We found that AMT can be a simple and easily performed surgical method for the reconstruction of ocular surfaces in many corneal and conjunctival pathologies such as corneal ulcer, corneal melting, and pterygium. However, there is a need for long-term results and multi-center studies about this surgical procedure for these indications.

Ethics Committee Approval: This study was approved by Dicle University Faculty of Medicine Ethics Committee (09.01.2020 date; number 46).

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