

Amniotic membrane transplantation for infectious corneal ulcer treatment: a cohort retrospective study

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ABSTRACT

BACKGROUND Corneal ulcer is one of the most common causes of corneal blindness. This study aimed to describe the outcomes of amniotic membrane transplantation (AMT) in patients with infectious corneal ulcer.

METHODS A retrospective cohort study based on medical records of patients who underwent an adjuvant AMT procedure over a 2-year period (2015–2017) was conducted at Cipto Mangunkusumo Hospital Jakarta. Uncorrected visual acuity (UCVA) was measured with the Snellen chart. Treatment success was marked by complete healing (disappearance of corneal infiltrates, epithelial defect closure, and corneal scar formation). Healing time was the duration from AMT surgery to complete healing.

RESULTS 50 cases of infectious corneal ulcer, 12 of which had perforation were included. Gram-positive cocci (18%), gram-negative rods (14%), fungi (4%), and *Acanthamoeba* (2%) were isolated from culture specimens. Successful results were observed in 90% of cases (20/21 moderate cases and 25/29 severe cases). Healing time was 21 (14–63) days in moderate cases and 28 (14–90) days in severe cases. Baseline UCVA improved from 2.48 (0.22–2.80) logMAR to 1.30 (0–2.80) logMAR within 3–4 weeks postoperatively and to 0.94 (0–2.80) logMAR at the last follow-up. Post-AMT complications included recurrent perforation in 4 eyes, persistent epithelial defects in 2 eyes, amniotic membrane infection in 1 eye, and membrane retraction in 1 eye.

CONCLUSIONS AMT was successfully used to treat moderate and severe infectious corneal ulcer, particularly in nonresponsive and some perforated cases. AMT provides biochemical and mechanical support for corneal wound healing with good visual outcomes.

KEYWORDS amniotic membrane, corneal perforation, infectious keratitis

Infectious corneal ulcer that accounts for 5% of blindness worldwide, is the most common cause of corneal blindness in developing countries.¹ Darsini et al² reported 158 new cases of infectious corneal ulcer in 2013 at Cipto Mangunkusumo Hospital. Proliferation of microorganisms is followed by the secretion of proteolytic enzymes and toxins. It triggers inflammatory response through activation of polymorphonuclear leukocytes, lymphocytes, and cytokines, causing destruction and necrosis of the corneal stroma. This

lead to vision impairment due to permanent corneal opacification and complications, such as perforation, glaucoma, and endophthalmitis.³

Adjuvant therapy such as corneal glue, patch grafts, conjunctival flaps, amniotic membrane transplantation (AMT), and keratoplasty is prescribed in cases that are chronic, unresponsive, or resistant to medical therapy to help rehabilitate corneal integrity. Amniotic membrane (AM) is the deepest layer of the placenta consisting of epithelium, basement

membrane, and avascular stroma. In addition to its antimicrobial properties, the AM has the potential to inhibit inflammation, angiogenesis, and protease activities. This action assists in accelerating epithelialization and reducing cicatrix.⁴ AMT has been widely used to repair various ocular surface conditions, such as autoimmune persistent corneal epithelial defects, vernal keratoconjunctivitis, limbal stem cell deficiency (LSCD), bullous keratopathy, chemical trauma, conjunctival defects after tumors or pterygium excision, fornix reconstruction, and release of symblepharon.^{5,6} AMT is widely available at low cost. It offers relatively simple surgical technique with low intra- and postoperative complications resulting in reduced failure rates of elective keratoplasty, and minimal without immune reaction.

Infectious corneal ulcer cases at Cipto Mangunkusumo Hospital vary in severity. Treatment of cases with minimal or no improvement is focused on intensive fortified antimicrobial therapy and supportive medications while investigating the definitive etiology. In such cases, there is an anticipated delay initiating the appropriate therapy, and the ulcer has reached a more severe stage with ongoing corneal destruction. The AM is an adjuvant therapeutic option that does not only supports corneal integrity but also promotes healing. It is used for cases that require corneal donors or emergency keratoplasty. To help ophthalmologists in considering AMT to treat infectious corneal ulcer as early as possible before complications progress, this study was aimed to evaluate the clinical characteristics treatment outcome, and complications of AMT surgery for infectious corneal ulcers at Cipto Mangunkusumo Hospital.

METHODS

A cohort retrospective cohort study was conducted in the Infection and Immunology Division of the Department of Ophthalmology, Faculty of Medicine Universitas Indonesia-Cipto Mangunkusumo Hospital. Inclusion criteria were data from all infectious corneal ulcer patients who underwent AMT between July 2015 and June 2017. Diagnosis was made through Gram/KOH swabs, microorganism culture results, or when the infection improved after administration of antimicrobial agent. The indications for the AMT procedure included ulcers with >50% corneal depth, descemetocoele, perforation, and had no clinical

improvement despite intensive use of medication. The exclusion criterion was history of other surgeries for corneal ulcers prior to AMT.

Data were collected from medical records, including: duration of onset (time from the first appearance of symptoms associated with the corneal ulcer to the first visit), predisposing risk factors (trauma, contact lens and topical steroid use, ocular surface and eyelid function disorder, and diabetes mellitus), pathogens (bacteria, fungi, and parasites), clinical characteristics of the ulcer (location, depth, size of ulcer, and hypopyon), healing time, complications, and subsequent surgery if needed. The visual acuity was also retrieved from medical records and was converted from data measured by a Snellen chart and converted to logarithm of the minimum angle of resolution (logMAR) unit. The uncorrected visual acuity (UCVA) was measured at baseline, 3–4 weeks and 3 months postoperatively, and the final visit as best-corrected visual acuity (BCVA). The severity of the ulcer before surgery was classified as: mild (<2 mm in diameter, and/or <1/3 stromal depth, no hypopyon, no perforation); moderate (2–6 mm in diameter, and/or 1/3–2/3 stromal depth, and/or any hypopyon, no perforation); and severe (>6 mm in diameter, and/or >2/3 stromal depth, any hypopyon, an impending perforation or a perforation). Response to treatment is defined successful when the eye showed new forming epithel tissue, reduced inflammation, shrank infiltrate, and formation of adequate anterior chamber. The therapeutic response was considered failed if there was no epithelialization, ulcer or perforation recurred, and alternative procedure should be taken to close the perforation.

Surgical technique

The patients received intensive antimicrobial and/or antifungal and supportive therapy (cycloplegic, ocular hypotensive agents, and lubrication) prior to surgery. Surgery was performed under topical or general anesthesia. First, the ulcer base and surrounding corneal tissue were scraped to clear necrotic tissue. Freeze-dried AM was cut according to the shape and size of the ulcer and placed in one to several layers to fill the base of the ulcer or corneal perforation according to its depth. The AM edge was sutured to 1/3 of the stromal thickness of the healthy cornea with three or four interrupted 10/0 nylon sutures. A bandage contact lens was

placed on the cornea to facilitate epithelialization. Additional measures were performed as needed, including irrigation and aspiration of the hypopyon, an intracameral injection with antibiotics or antifungals, and reformation of the anterior chamber in cases of perforation and a flat anterior chamber. Antimicrobial and supportive therapy were administered after surgery. The sutures and bandage contact lens were removed in week 2–4.

Data were analyzed using the SPSS version 20.0 (IBM Corp., USA) for MacOS. Quantitative variables are expressed as mean (standard deviation) or median (range) based on normality of the data, while qualitative variables are described as proportions. The Mann–Whitney and Fisher’s test were used to compare unpaired categorical variables. The Wilcoxon test was used to compare paired categorical variables. A p -value <0.05 was considered significant. All information contained in the medical records was kept confidential.

RESULTS

There were 56 cases of infectious corneal ulcer underwent AMT procedures between July 1st, 2015 and June 30th, 2017. Six cases with a history of previous other surgery were excluded. Thus, 50 cases were included in the study. The demographic characteristics of the patients and clinical characteristic of the ulcers are presented in Table 1. The most common local predisposing factors for corneal ulcer were nonsurgical trauma

(22 eyes with metal cutting fragments, foreign body particles, and nail or wire scratches), followed by contact lens use (14 eyes), eyelid function disorders (5 eyes with lagophthalmos, entropion, or chronic blepharitis), ocular surface disorders (5 eyes with history of Stevens–Johnson syndrome [SJS]), irradiation-induced dry eye (1 eye), allergic conjunctivitis (1 eye), and surgical trauma (2 eyes

Table 1. Demographic and clinical characteristics

Variable	n (%) (N = 50)
Male sex	22 (44)
Age (years), mean (SD)	38.1 (16.9)
Ulcer location	
Central	35 (70)
Paracentral	11 (22)
Peripheral	4 (8)
Ulcer size (mm)	
<2	19 (38)
2–6	23 (46)
>6	8 (16)
Ulcer depth	
<1/3	2 (4)
1/3–2/3	28 (56)
>2/3	8 (16)
Perforation	12 (24)
Ulcer grading	
Moderate	21 (42)
Severe	29 (58)

SD=standard deviation

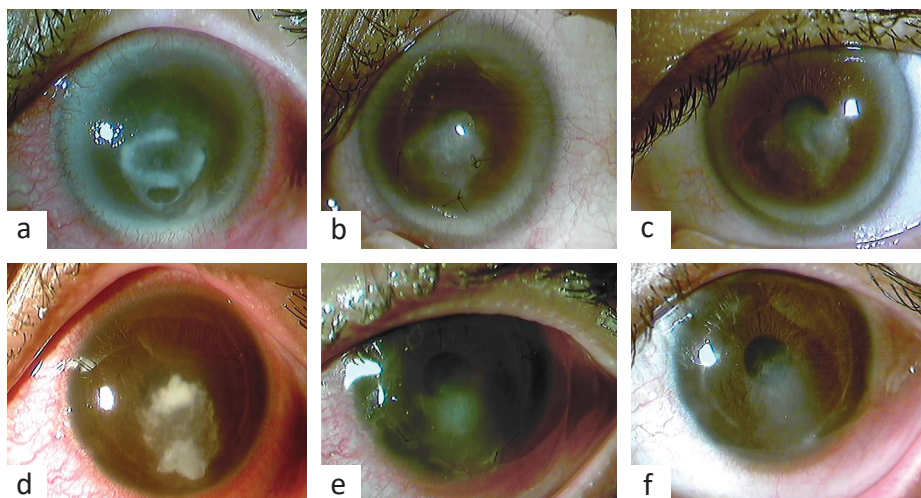


Figure 1. Contact lens induced corneal ulcer in: (a) preoperative; (b) post-AMT; and (c) post-suture removal, and corneal ulcer caused by *Fusarium sp.* in: (d) preoperative; (e) post-AMT; (f) post-suture removal

Table 2. Etiology of the ulcers based on culture result

Results	n (%)
Positive culture results	28 (68)
Bacteria (n = 16)	
Gram-positive coccus	
<i>Staphylococcus aureus</i>	1 (4)
<i>Staphylococcus epidermidis</i>	4 (14)
<i>Staphylococcus saprophyticus</i>	3 (11)
<i>Enterococcus faecium</i>	1 (4)
Gram-negative rods	
<i>Pseudomonas aeruginosa</i>	3 (11)
<i>Enterobacter aerogenes</i>	1 (4)
<i>Stenotrophomonas maltophilia</i>	2 (7)
<i>Staphylococcus epidermidis</i> + <i>Pseudomonas aeruginosa</i>	1 (4)
Fungi (n = 2)	
<i>Fusarium</i> sp.	1 (4)
<i>Paecilomyces</i>	1 (4)
<i>Acanthamoeba</i>	1 (4)
No microbial growth	9 (32)

Table 3. Surgical outcomes and healing time

Ulcer grading	Outcome, n (%)		<i>p</i> *	Healing time (days), median (range)	<i>p</i> †
	Success	Failure			
Moderate (n = 21)	20 (95)	1 (5)	0.38	21 (14–63)	0.27
Severe (n = 29)	25 (86)	4 (14)		28 (14–90)	
Total	45 (90)	5 (10)		24.5 (14–90)	

*Fisher's exact test; †Mann-Whitney test
Failed treatments were excluded for the healing time calculation

Table 4. Visual acuity before and after AMT

	Time	N	Median (logMAR)	<i>p</i> *
UCVA	Preoperative	48	2.48 (0.22–2.80)	1.00
	1 week	48	2.08 (0.30–2.80)	0.14
	3–4 weeks	39	1.30 (0–2.80)	<0.001
	3 months	29	0.70 (0.07–2.80)	<0.001
	Last visit	48	0.94 (0–2.80)	<0.001
BCVA	Last visit	15	0.43 (0.43)*	

AMT=amniotic membrane transplantation; UCVA=uncorrected visual acuity; BCVA=best corrected visual acuity

*Wilcoxon test, presented in mean (standard deviation). Two eyes were excluded from the baseline visual acuity calculation (non-functional eye and eye with long standing retinal detachment). A 0 logMAR means a value of 6/6 from Snellen chart, whereas 2.80 logMAR means perception of light at 1 m. Each letter score increase is equivalent to 0.02 logMAR unit decrease

Table 5. Surgical outcomes based on risk factors and clinical characteristics

Surgical outcomes	Outcome, n (%)	
	Success	Failure
Risk factor* (n = 40)		
Surgical trauma	2 (100)	0 (0)
Non-surgical trauma	19 (86)	3 (14)
Contact lens use	14 (100)	0 (0)
Eyelid surface disorder	5 (100)	0 (0)
Ocular surface disorder	5 (100)	0 (0)
Topical steroid use	4 (80)	1 (20)
Diabetes mellitus	2 (100)	0 (0)
Etiology (n = 19)		
Bacteria	16 (100)	0 (0)
Fungi	1 (50)	1 (50)
<i>Acanthamoeba</i>	1 (100)	0 (0)
Perforation (n = 50)		
No perforation	35 (92)	3 (8)
Perforation ≤1–5 mm	6 (86)	1 (14)
Perforation >1–5 mm	4 (80)	1 (20)
Duration of onset (weeks), (n = 50)		
≤2	26 (93)	2 (7)
>2	19 (86)	3 (14)

*One eye had multiple predisposing factors

with phacoemulsification and pterygium avulsion). Five eyes had history of topical steroid use and two subjects were known to have diabetes. The median ulcer size was 3.1 (0.7–9) mm. Seventeen (34%) ulcers were accompanied by hypopyon. Twelve eyes with perforations have median of 1.5 (0.9–5.5) mm in diameter, descemetocoele was observed in six eyes. No endophthalmitis was recorded. The median duration of onset was 14 (2–120) days. Twenty-two cases developed symptoms over 2 weeks, and 15 (68.2%) were classified as severe. The median time from the first visit to surgery was 1 (1–90) days. The median follow-up time was 11.4 (3–94.4) weeks.

Microorganisms were found in cultures from 25 eyes, dominated by Gram-positive cocci (Table 2). The most common etiology was *Staphylococcus epidermidis*. The success of AMT based on ulcer grading is shown in Table 3. Success occurred in 45 eyes with a median healing time of 24.5 days. No significant differences were observed in the proportion of successful cases or healing time between the moderate and severe ulcer groups.

An increase in the median UCVA by as much as 1.54 logMAR was observed at the end of follow-up compared with baseline. Significant improvements in UCVA also occurred at 3–4 weeks and 3 months postoperatively. The mean BCVA at the end of the follow-up was 0.43 logMAR (Table 4). Table 5 describes the success of therapy based on risk factors and clinical characteristics. The proportion of successful cases of AMT surgery was high (80–100%) regardless of risk factors, etiology, perforation status, or duration of onset. Effectiveness decreased when the onset exceeded 2 weeks or when any perforation of more than 1.5 mm existed. Most moderate cases (12 eyes), severe cases (22 eyes), and bacterial ulcers (12 eyes) were decided to receive AMT at the first visit. There were 9 eyes (6 moderate and 3 severe cases) in which the AMT was decided more than 2 weeks after the first visit, including all cases with positive fungal and *Acanthamoeba* culture results.

Postoperative complications related to AM in this study were persistent epithelial defects in two eyes; AMT was repeated in one eye and achieved success, while the other eye underwent a tectonic keratoplasty (failed). Failure also occurred because of recurrent perforations in four eyes, each of which was treated with corneal glue, periosteal graft, and tectonic keratoplasty with good results, while one patient was scheduled for tectonic keratoplasty but did not continue therapy. An AM infection in one eye improved with intensive antimicrobial therapy. One eye experienced AM retraction 2.5 weeks after the procedure, and AMT was repeated with good outcome. The ulcer-related complications were neovascularization (8 eyes), anterior synechiae (4 eyes), secondary glaucoma (2 eyes), and high astigmatism (2 eyes).

DISCUSSION

In this study on infectious corneal ulcer, treatment with AMT yielded remarkable healing rate. The surgical technique used in this study was a multilayered AM method, where the AM functions as a basement membrane and the substrate facilitates migration and adhesion of epithelial cells, as well as cellular differentiation, and a stable corneal surface is formed. Moreover, the AM acts as a biological bandage to protect epithelial growth, inhibit the activity of polymorphonuclear cells, lymphocytes, macrophages, and inflammatory cytokines in the

stroma and tears, induce apoptosis of inflammatory cells, and release growth factors.^{7,8} Mechanical and biochemical processes promote faster healing of the cornea and minimize the formation of scar tissue, resulting in better final visual acuity. Treatment with AMT was associated with a substantial visual improvement in the current study. These properties are the advantages of AMT over other adjuvant procedure options.

The success of AMT is affected by perforation size, ulcer grading, microbial virulence, and limbal stem cell damage.⁹ Ulcer diameter in this study was 3.1 (0.7–9) mm with a 90% success rate. This is similar to studies by Abdulhalim et al¹⁰ and Chen et al¹¹ that showed success rates of 90% and 83%, with ulcer sizes of 5.45 ± 1.73 mm and 5.43 ± 2.61 mm, respectively. The size of the corneal perforation in the present study was 1.5 mm with an 80% success rate. This is consistent with the study of Rodríguez-Ares et al,⁹ which reported that AMT is effective for ≤ 1.5 mm perforations. The success rates of AMT for infectious corneal ulcer with varying pathogens and severity gradings are reported to be 73.5–100%.^{7,8,12–15}

Current study showed that the AMT procedure for infectious corneal ulcer was highly successful in some cases including perforation and nonhealing cases despite adequate antimicrobial therapy. AMT improved the healing of moderate and severe corneal ulcers (Table 3). Moderate ulcers healed 7 days faster than severe ulcers, although the difference was not significant. This is consistent with Gicquel et al¹⁴ in which bacterial corneal ulcers healed in 25.5 ± 9.7 days after AMT. Hapsari et al¹⁶ reported that it takes 36 days post-AMT to achieve cicatrization in all eyes with bacterial corneal ulcers. All ulcers with positive bacterial culture result healed completely, indicating that bacteria are generally more easily eradicated and more responsive to therapy. Altay et al¹⁵ showed a 100% success rate of AMT for bacterial corneal ulcers without recurrence or subsequent perforation.

The penetration of a topical antifungal medication through the cornea, anterior chamber, and vitreous is generally very low. Fungal filaments infiltrate deep in the corneal stroma. Thus, a long duration is needed for topical drug administration to achieve healing. Chen et al¹¹ reported the occurrence of AM melting in 25% of fungal corneal ulcer cases that required keratoplasty. Thatte et al¹⁷ reported that all failed cases were caused by fulminant fungi.

Significant visual improvement was achieved by 3 weeks, with the sustained improvement through 3 months after surgery (Table 4). Combined antibiotic and AMT resulted in significantly higher final UCVA by 0.26 logMAR and lower cicatrix density than treatment with antibiotic alone.¹⁸ Tabatabaei et al¹³ showed a 2.5 times greater increase in UCVA and BCVA in the AMT group than in the antibiotic group of bacterial ulcers.

In the present study, a high success rate was achieved in all predisposing risk factor groups (Table 5). Rodríguez-Ares et al⁹ concluded that the success of therapy is not affected by comorbidities (diabetes, partial LSCD, or severe dry eye). About 14% of the failures occurred in the group of eyes with a history of nonsurgical trauma (metal cutting fragments and foreign body particles). These failed cases first presented with either two-thirds corneal depth involvement or perforated eye. Deep trauma or abrasions of the corneal epithelium affecting more than 50% of the thickness and disrupting the basement membrane structure enabling fluid to migrate into the stromal layer, which impairs the adhesion between the epithelial cells and basement membrane. This process leads to persistent epithelial defects. Once this occurs, the regeneration of keratocytes and stromal matrix is delayed and a longer healing time is required.¹⁹ Paolin et al⁷ reported a 70–100% success rate from AMT in corneal ulcers caused by trauma.

There were two eyes with history of SJS underwent AMT. One eye showed a successful result and another eye developed a recurrent erosion, thus requiring retransplantation. This complication was attributed to LSCD and the inflammation that underlies SJS disease. Kang et al²⁰ reported infectious keratitis in 35% of eyes with LSCD associated with SSJ, in which moderate to severe grade was a significant risk factor. Stem cells are needed to repopulate the corneal epithelium and prevent conjunctival epithelial invasion that may induce inflammation, neovascularization, and recurrent erosion. The LSCD of both eyes in this study was graded as mild to moderate with some portion of healthy limbal tissue. Thus, a good final outcome could be achieved.

Failure treatment response was observed in two out of nine eyes, in which decision to receive AMT was taken more than 2 weeks after initial visit. This observation indicates that an early AMT procedure within 2 weeks of the initial therapy will likely help reduce inflammation, accelerate healing, and inhibit

the progression of corneal thinning in unresponsive ulcers. Thus, a higher success rate is expected. In the case of fungi and *Acanthamoeba*, the decision to undergo AMT was determined later than in bacterial cases. This might be due to faster progression from bacterial ulcers to corneal melting and perforation than fungal and *Acanthamoeba* ulcers.

Some limitations of this study should be noted, including its retrospective nature. Some of the microbial examinations did not detect any microorganisms and a few data were not available. In these cases, the diagnosis of infectious ulcer was established by clinical findings, the treatment response, and the judgment of an immunology consultant. In addition, this study did not include the results of AMT treatment for infectious corneal ulcer cases that have received prior surgical therapy, and no viral case was described in this study. Thus, the results of this study cannot address these aforementioned cases. There was also a high loss to follow-up rate.

In conclusion, the AMT procedure was successfully used to treat moderate and severe infectious corneal ulcers, particularly in cases that were nonresponsive to medical therapy and some perforated cases. The decision to perform the AMT procedure should be made less than 2 weeks since the first visit along with intensive medications to accelerate healing, reduce the risk of further corneal thinning or perforation, and achieve better final visual acuity.

Conflict of Interest

The authors affirm no conflict of interest in this study.

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