Original Article

Multilayered fresh amniotic membrane transplantation in resistant fungal corneal ulceration

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ABSTRACT

Background: Resistant fungal keratitis is a major cause of corneal blindness, particularly in resource-limited regions where donor tissue is scarce. Amniotic membrane transplantation (AMT) offers potential benefits through its anti-inflammatory, anti-proteolytic, and epithelialization-promoting effects. This study evaluated the efficacy and safety of AMT for treatment-resistant fungal corneal ulcers.

Methods: This prospective, single-arm study enrolled consecutive patients with microbiologically confirmed, treatment-resistant fungal corneal ulcers at Al-Azhar University Hospital, Damietta, between January 2022 and October 2023. All patients underwent standardized single- or double-layer AMT. Baseline and follow-up assessments included best-corrected distance visual acuity (BCDVA, logarithm of the minimum angle of resolution [logMAR]), ulcer size, anterior chamber reaction and depth, and presence of blepharospasm or pain (visual analog scale). Clinical evaluations were performed at baseline, 1 day, and 1, 3, and 6 months postoperatively. Treatment success was defined as complete resolution or significant improvement over 6 months.

Results: A total of 24 patients (mean [standard deviation] age, 59 [7.5] years; 3:1 male-to-female ratio) with resistant fungal corneal ulcers were studied. Most were rural residents (n = 17, 70.8%), and nearly half were farmers (n = 11, 45.8%). Common comorbidities included hypertension and diabetes mellitus. The median baseline ulcer area was 3 mm^2; most ulcers were central (n = 10, 41.7%) or paracentral (n = 8, 33.3%), and 12.5% (n = 3) had perforations. At 6 months, significant improvements were observed: median BCDVA improved from 3.0 logMAR to 2.0 logMAR ($P = 0.001$), ulcers completely closed ($P = 0.001$), and the pain score dropped from 2 to 0 ($P = 0.001$). Anterior chamber reaction and blepharospasm also improved significantly (both $P = 0.001$). Overall, 91.7% (n = 22) achieved complete resolution or marked improvement, and two patients required further surgery. The results showed progressive benefits throughout the follow-up period.

Conclusions: AMT is a safe and effective adjunctive treatment for resistant fungal keratitis, particularly when corneal donors are scarce. The procedure promotes ulcer healing, relieves pain, and improves visual outcomes. Controlled trials are required to confirm these findings and refine patient selection.

KEYWORDS

amniotic membrane, transplantation, clinical efficacy, keratitides, ulcerative keratitis

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How to cite this article: Ezzeldin ER, Tharwat E, Mohammed Mohammed HE, Sayed Ahmed E, Elgazzar AF, Hassan Ahmed RE, Soliman HB, Omran MY, Amer RS, Abdelhameed HM, Abdella WS, Elsayed Abdelkader AM. Multilayered fresh amniotic membrane transplantation in resistant fungal corneal ulceration. Med Hypothesis Discov Innov Ophthalmol. 2025 Summer; 14(2): 1-8. https://doi.org/10.51329/mehdiophthal1518

Received: 25 February 2025; Accepted: 07 June 2025

INTRODUCTION

Infectious keratitis is the leading cause of corneal blindness worldwide, particularly in developing nations. Its annual incidence is 2.5–799 per 100 000 people [1]. Intense medical and/or surgical therapy is typically necessary after patient hospitalization for this painful and potentially blinding ocular emergency [2, 3]. The most common microorganisms causing infectious keratitis vary according to the geographic region, time course of infection, and population-specific risk factors such as agricultural exposure, ocular trauma, and contact lens use. These pathogens may include fungi, bacteria, viruses, parasites, or a combination of organisms (polymicrobial infections) [2, 3].

Surgical interventions are necessary in one-quarter to one-third of patients with infectious keratitis during the acute stage to avoid perforation or spread of infection [4, 5]. However, in some cases, donor corneas are not readily available. The effectiveness of penetrating keratoplasty in treating fungal keratitis depends on the surgical technique used, and the risk of recurrent infection persists [6]. The use of amniotic membranes (AMs) in ophthalmology is well established and extensively documented [7-9]. Amniotic membrane transplantation (AMT) promotes epithelialization by expressing anti-angiogenic factors, anti-inflammatory proteins, growth factors, and protease inhibitors. Hence, AMT may be used to alleviate infectious keratitis [10].

AMs facilitate epithelial cell migration and strengthen basal cell connections [11]. They produce basic fibroblast growth factor, hepatocyte growth factor, and transforming growth factor, which enhance corneal epithelial cell development [12]. Transplanted AMs integrate with the corneal stroma in corneal ulcers [13]. They may also prevent polymorphonuclear leukocyte infiltration into the tear film. Tissue inhibitors of matrix metalloproteinase (MMP)-1 and MMP-2 in the AM limit collagen degradation and stromal melting [14].

This study evaluated the effectiveness of AMT in the management of resistant fungal corneal ulcers and assessed the potential risks associated with the procedure.

METHODS

This prospective, interventional, single-arm study included all eligible individuals who consecutively presented with treatment-resistant fungal corneal ulcers at Al-Azhar University Hospital in Damietta between January 2022 and October 2023. This study was conducted in accordance with the principles of the Declaration of Helsinki. Ethical approval was obtained from the Institutional Review Board of the Faculty of Medicine, Al-Azhar University, Damietta (approval number: 0001267-23-08-010). Written informed consent was obtained from all participants before enrollment. This study was registered at ClinicalTrials.gov (Identifier: NCT06070883).

Patients aged ≥18 years presenting with a clinically and microbiologically confirmed fungal corneal ulcer were initially considered for inclusion. Eligible participants had resistant fungal ulcers, confirmed by both clinical evaluation and laboratory culture and sensitivity findings. These ulcers were characterized by a lack of clinical improvement, an increase in ulcer size, deeper stromal involvement, or the development of complications such as hypopyon, despite appropriate antifungal therapy. Patients with or without descemetocele or small perforation were included. Additional criteria required an ulcer size of less than 5 mm at its largest diameter and location within 3 mm of the visual axis. Exclusion criteria included the presence of coexisting ocular or systemic conditions that could confound treatment outcomes, such as autoimmune diseases, total limbal stem cell deficiency, glaucoma, chronic dacryocystitis, or entropion.

All eligible participants underwent a comprehensive clinical evaluation, including a detailed medical and ophthalmic history, best-corrected distance visual acuity (BCDVA) assessment recorded in logarithm of the minimum angle of resolution (logMAR) units, and meticulous slit-lamp biomicroscopic examination (Carl Zeiss Meditec AG, Jena, Germany). Slit-lamp examination was used to assess ulcer size, extent of infiltration, depth of involvement, presence of descemetocele or corneal perforation, corneal opacification grade, anterior chamber (AC) reaction, and AC depth. Digital tonometry and lacrimal syringing tests were performed.

Ulcer size was determined using a slit-lamp beam by measuring the longest horizontal axis of the ulcer and then measuring the longest perpendicular axis. The ulcer area was calculated by multiplying these two dimensions to provide a consistent and reproducible estimate of the ulcer surface area in mm^2. To classify keratitis severity based on the depth of corneal involvement, ulcers were graded as epithelial (limited to the epithelial layer), mid-stromal (involving the mid-stroma), deep-stromal (involving the deep stroma), descemetocele (presence of a descemetocele), or corneal perforation (small, full-thickness perforation). This grading system was developed by the authors specifically for this study.

Corneal opacification was clinically graded according to the visibility of iris details as follows: grade 0 indicated a clear cornea with full visibility of iris details, grade 1 represented mild opacification with slight blurring of iris details, grade 2 was defined as moderate opacification with partial obscuration of iris details, and grade 3 indicated severe opacification with no visible iris details [15]. The AC reaction was graded using the standardized uveitis nomenclature (SUN) criteria based on the number of cells observed in a 1 mm × 1 mm slit beam: grade 0 (no cells), grade 0.5+ (1–5 cells), grade 1+ (6–15 cells), grade 2+ (16–25 cells), grade 3+ (26–50 cells), and grade 4+ (> 50 cells) [16]. AC depth was evaluated using slit-lamp biomicroscopy by focusing on the cornea and crystalline lens. If corneal opacity impaired visibility, adjustments were made to the slit-lamp beam angle, and alternative illumination techniques, such as retroillumination, were used to enhance visualization. The AC depth was recorded as normal or flat.

Pain was assessed using the Visual Analog Scale (VAS), a validated psychometric instrument comprising a 10-cm horizontal line, on which 0 represents no pain and 10 represents the worst imaginable pain. Patients were instructed to mark a point along the line that best represented their subjective level of ocular pain [17].

Corneal scrapings were obtained from the base and edges of the ulcer. Fungal keratitis was diagnosed based on the presence of fungal hyphae on microscopic examination, with confirmatory culture. Fungal culture positivity was defined as growth of fungal colonies on blood agar plates. Initial medical management included a 1-week course of topical fortified ceftazidime (50 mg/mL; Amoun Pharmaceutical Co., Cairo, Egypt) and a 5% natamycin suspension (Natacyn; Alcon Laboratories, Inc., TX, USA). Systemic fluconazole (0.2 g/day IV; Pfizer Ltd., USA) was administered to patients presenting with descemetocele, hypopyon, impending corneal perforation, or actual corneal perforation.

For AM preparation, human placentas were obtained following elective cesarean deliveries under sterile conditions. All donors underwent comprehensive serological screening to exclude transmissible pathogens, including human immunodeficiency virus, hepatitis B virus, and *Treponema pallidum*. Residual blood clots were removed from the placental tissue by rinsing with sterile saline containing 50 μg/mL penicillin, 50 μg/mL gentamicin, 100 μg/mL neomycin, and 2.5 μg/mL amphotericin B. This process was conducted within a laminar flow hood to maintain aseptic conditions. The AM was then carefully separated from the underlying chorion using blunt dissection, spread flat with the epithelial (basement membrane) side facing upward, and mounted onto nitrocellulose paper. For preservation, the prepared AM was stored at −70°C in sterile vials containing a cryoprotective solution of Dulbecco's Modified Eagle Medium (DMEM; Gibco, CA, USA) and glycerol in a 7:3 (v/v) ratio [18].

All eligible eyes underwent AMT under strict aseptic conditions in the operating room. In cooperative patients, topical anesthesia was achieved using 0.4% benoxinate hydrochloride (Benox®, EIPICO, Egypt). For less cooperative patients, a peribulbar injection of a 3 mL anesthetic mixture containing lidocaine 2% (Alexandria Pharmaceutical Co., Alexandria, Egypt) and bupivacaine (Marcaine, Sunny Pharmaceutical, Egypt) was administered.

After cleansing the ocular surface, necrotic tissue from the ulcer base was carefully debrided and cultured on MacConkey agar (Thermo Scientific Oxoid MacConkey Agar No. 3, Catalog No. CM0115) for microbiological and antifungal sensitivity tests. Any loose epithelium or indurated ulcer margins were excised to create a clean recipient bed. The AM was trimmed to match the size and shape of the corneal ulcer and was placed over the defect, with the epithelial (basement membrane) side facing upward. It was then secured to the cornea using either interrupted or continuous 10-0 nylon sutures (Ethilon®, El-Helaly Trading Co., Cairo, Egypt).

For ulcers involving more than three-quarters of the corneal stromal depth or those associated with a descemetocele, a double-layer AMT was performed. In this technique, two AM layers were applied with both epithelial surfaces facing upward. The layers were sutured in place using continuous 10-0 nylon sutures after trimming to conform to the ulcer margins.

At the end of the procedure, topical gatifloxacin ointment (Zymaxid®, Nile Pharmaceuticals and Chemical Industries Co., Cairo, Egypt) was instilled, and the eye was patched for 6 h. Postoperative antimicrobial therapy was resumed thereafter using topical moxifloxacin (VIGAMOX®, Alcon Laboratories) administered every 6 h for the first 2 days, followed by twice-daily dosing for the remainder of the treatment period.

Postoperative evaluations were conducted on day 1 and subsequently at 1, 3, and 6 months. Each visit included a comprehensive clinical assessment that involved BCDVA testing, slit-lamp examination with documentation of ulcer size and AC reaction, evaluation of ulcer healing status, presence of blepharospasm, and pain assessment using the VAS. Treatment success was defined as complete resolution or significant improvement in the clinical signs and symptoms associated with persistent fungal keratitis at the 6-month follow-up visit.

Statistical analyses were conducted using IBM SPSS Statistics for Windows (version 26.0; IBM Corp., Armonk, NY, USA). The normality of data distribution was assessed using the Kolmogorov–Smirnov test. Qualitative variables are summarized as frequencies and percentages, whereas quantitative variables are presented as means with standard deviations (SDs) or medians with interquartile ranges (IQRs), depending on the data distributions. The Friedman and Wilcoxon signed-rank tests were used for paired comparisons of quantitative variables. Paired categorical variables were analyzed using the Cochran's Q and McNemar's tests, as appropriate. A *P*-value of <0.05 was considered statistically significant.

RESULTS

A total of 24 patients with a mean (SD) age of 59 (7.5) years (range, 42–73 years) (Table 1) and a diagnosis of resistant fungal corneal ulcers were included. Full demographic details, baseline ocular findings, and postoperative outcomes are summarized in Tables 1 and 2. The male-to-female ratio was 3:1. Nearly half of the patients were farmers (n = 11, 45.8%) and most resided in rural areas (n = 17, 70.8%). The most common systemic comorbidities were hypertension and diabetes mellitus (Table 1).

Table 1. Demographic and baseline clinical characteristics of the study participants

Variables	Value
Age (y), Mean ± SD (Range)	59 ± 7.5 (42 to 73)
Sex (Male / Female), n (%)	18 (75) / 6 (25)
Occupation, n (%)	
Farmer	11 (45.8)
House wife	6 (25.0)
Carpenter	7 (29.2)
Residency (Rural / Urban**), n (%)**	17 (70.8) / 7 (29.2)
Comorbidities, n (%)	
HTN	9 (37.5)
DM	7 (29.2)
Cardiac diseases	3 (12.5)
Renal diseases	1 (4.2)
No comorbidities	4 (16.7)
Ulcer size (mm²), Median (IQR) (Range)	3 (1) (1 to 3)
Ulcer site, n (%)	
Central	10 (41.7)
Paracentral	8 (33.3)
Peripheral	6 (25.0)
Ulcer depth, n (%)	
Less than 1/3	6 (25.0)
1/3 to 1/2	15 (62.5)
Perforation	3 (12.5)
Corneal opacification (Presence/ Absence), n (%)	17 (70.8) / 7 (29.2)
Grades of corneal opacification	
Grade 0	7 (29.2)
Grade 1	9 (37.5)
Grade 2	7 (29.2)
Grade 3	1 (4.2)
AC reaction (Grade), Median (IQR) (Range)	2 (1) (1 to 5)
AC depth (Normal / Flat), n (%)	21 (87.5) / 3 (12.5)
Grades of keratitis, n (%)	
Epithelial	3 (12.5)
Mid-stromal	10 (41.7)
Deep-stromal	7 (29.2)
Descemetocele	1 (4.2)
Corneal perforation	3 (12.5)
Hypopyon (Presence / Absence), n (%)	2 (8.3) / 22 (91.7)
Surgical trauma (Presence / Absence), n (%)	2 (8.3) / 22 (91.7)
Contact lens use (Yes / No), n (%)	2 (8.3) / 22 (91.7)
Surface disorder, n (%)	
Trachoma	4 (16.7)
Dry eye	1 (4.2)
No surface disorder	19 (79.2)

Abbreviations: y, years; SD, standard deviation; n, number; %, percentage; HTN, hypertension; DM, diabetes mellitus; mm², square millimeters; IQR, interquartile range.

Table 2. Longitudinal changes in clinical and patient-reported outcomes over the 6-month follow-up period

Variables	Preoperative	1-month postop	3-month postop	6-month postop	*P*-value	*P*-value
BCDVA (logMAR), Median (IQR)	3 (0.0)	3 (1.0)	2 (1.0)	2 (1.4)	**0.001** [a]	$P_1 = 0.1$ [c] **$P_2 = 0.001$** [c] **$P_3 = 0.001$** [c] **$P_4 = 0.001$** [c] **$P_5 = 0.001$** [c] $P_6 = 0.1$ [c]
VAS score, Median (IQR)	2 (1.0)	1 (0.0)	0.5 (1.0)	0 (1.0)	**0.001** [a]	**$P_1 = 0.001$** [c] **$P_2 = 0.001$** [c] **$P_3 = 0.001$** [c] **$P_4 = 0.008$** [c] **$P_5 = 0.001$** [c] **$P_6 = 0.04$** [c]
Ulcer size (mm^2), Median (IQR)	3 (1.0)	1 (1.0)	1 (1.0)	0 (0.0)	**0.001** [a]	**$P_1 = 0.001$** [c] **$P_2 = 0.001$** [c] **$P_3 = 0.001$** [c] **$P_4 = 0.001$** [c] **$P_5 = 0.001$** [c] **$P_6 = 0.001$** [c]
AC reaction, Median (IQR)	2 (1.0)	1 (1.0)	1 (1.0)	0 (0.0)	**0.001** [a]	**$P_1 = 0.001$** [c] **$P_2 = 0.001$** [c] **$P_3 = 0.001$** [c] **$P_4 = 0.001$** [c] **$P_5 = 0.001$** [c] **$P_6 = 0.002$** [c]
Presence of Blepharospasm, n (%)	15 (62.5)	3 (12.5)	0 (0.0)	0 (0.0)	**0.001** [b]	**$P_1 = 0.001$** [d] **$P_2 = 0.001$** [d] **$P_3 = 0.001$** [d] **$P_4 = 0.001$** [d] $P_5 = 0.2$ [d] $P_6 = 0.2$ [d]

Abbreviations: postop, postoperative visit; BCDVA, best-corrected distance visual acuity; logMAR, logarithm of the minimum angle of resolution; IQR, interquartile range; VAS, visual analog scale; mm^2, square millimeters; AC, anterior chamber. Note: *P*-values < 0.05 are shown in bold; *P*-values correspond to the following statistical tests: Friedman test ([a]), Cochran's Q test ([b]), Wilcoxon signed-rank test ([c]), and McNemar test ([d]); Pairwise comparisons were conducted between the following time points: P_1, preoperative vs. 1-month postoperative; P_2, preoperative vs. 3-month postoperative; P_3, preoperative vs. 6-month postoperative; P_4, 1-month vs. 3-month postoperative; P_5, 1-month vs. 6-month postoperative; and P_6, 5-month vs. 6-month postoperative.

At baseline, the median (IQR) ulcer area was 3 (1) mm^2. The most frequent ulcer location was central (n = 10, 41.7%), followed by paracentral (n = 8, 33.3%) and peripheral (n = 6, 25%). Regarding ulcer depth, 62.5% (n = 15) extended to the mid-stroma, 25% (n = 6) involved less than one-third of stromal thickness, and 12.5% (n = 3) presented with small frank perforations. Corneal opacification was documented in 70.8% (n = 17) of cases, and most were graded as mild or moderate (grades 1–2). The median (IQR) AC reaction was 2+ (1), and the AC depth was normal in 87.5% of patients (n = 21). Keratitis was classified as epithelial (n = 3, 12.5%), mid-stromal (n = 10, 41.7%), deep-stromal (n = 7, 29.2%), descemetocele (n = 1, 4.2%), or perforation (n = 3, 12.5%). Hypopyon was present in 8.3% (n = 2) of the patients. Additional ocular risk factors included prior surgical trauma (n = 2, 8.3%), contact lens use (n = 2, 8.3%), and ocular surface disease (n = 5, 20.9%) (Table 1).

At the 6-month follow-up, there was a statistically significant improvement in visual and clinical outcomes (Table 2). Median (IQR) BCDVA improved from 3.0 (0.0) logMAR preoperatively to 2.0 (1.4) logMAR postoperatively ($P = 0.001$). The median (IQR) ulcer size decreased from 3 (1.0) mm^2 to complete closure (0 [0.0] mm^2; $P = 0.001$). The median (IQR) VAS pain score decreased significantly from 2 (1.0) to 0 (1.0) ($P = 0.001$). AC reaction and blepharospasm had also significantly improved at the final follow-up (both $P = 0.001$) (Table 2).

Pairwise comparisons at the interim visits confirmed significant progressive improvements across most parameters (most $P < 0.05$) (Table 2). The overall treatment success rate was 91.7% (n = 22). Two patients required additional surgical intervention because of persistent infection.

DISCUSSION

In this prospective, interventional study, AMT demonstrated high efficacy in managing treatment-resistant fungal corneal ulcers. At 6 months, 91.7% of the eyes achieved complete resolution or marked clinical improvement, with significant gains in BCDVA, ulcer closure, and alleviation of AC reaction, blepharospasm, and ocular pain. Ulcers were mostly mid-stromal

and frequently affected older rural male patients with systemic comorbidities. These findings support AMT as a valuable adjunctive therapy in cases of resistant fungal keratitis, particularly when donor corneas are scarce or conventional medical therapy alone is insufficient.

The demographic profile observed in our study aligns with that of previously published epidemiological data on corneal ulcers, which consistently indicate a higher prevalence among male patients and a similar age distribution [19-22]. This sex disparity is plausibly attributed to greater environmental hazard exposure and trauma-related risk factors commonly associated with occupational and outdoor activities more frequently undertaken by men [20-22].

The factors conferring risk of developing microbial keratitis differ greatly according to geographic and socioeconomic contexts. In developing nations, 48.6% to 65.4% of corneal ulcers are attributed to non-surgical ocular trauma [23, 24]. In our series, 91.7% (n = 22) of the patients developed fungal keratitis due to non-surgical causes; nearly half were farmers (n = 11, 45.8%) and most resided in rural areas (n = 17, 70.8%). Basak et al. [25] identified ocular trauma in 82.9% (n = 994) of suppurative keratitis cases, with vegetative matter accounting for 59.6% (n = 715).

A consistent early finding in our study was a marked reduction in ocular inflammation after AMT, as evidenced by significant decreases in AC reaction and ulcer size. The median BCDVA was initially 3 logMAR and remained unchanged until the third postoperative month, after which it significantly improved to 2 logMAR at 6 months. This delayed visual gain may reflect temporary visual obscuration caused by the AM covering the cornea. Comparable delayed improvement in visual acuity was noted by Ferreira De Souza et al. [26], who reported postoperative visual acuity of ≤ 20/400 in 60% of eyes initially, then reaching 69% at 3 months and 78% at 6 months [26]. Similar findings have been reported by other investigators [19, 27-29].

Pain relief was another notable outcome; most patients reported no postoperative pain, which is consistent with the results of Altay et al. [14]. This analgesic effect may be mechanical, as the AM functions similarly to a bandage contact lens before re-epithelialization, thereby reducing nociceptor exposure [30-32]. In terms of ulcer depth, keratitis was classified as epithelial (n = 3, 12.5%), mid-stromal (n = 10, 41.7%), deep-stromal (n = 7, 29.2%), descemetocele (n = 1, 4.2%), or perforation (n = 3, 12.5%). Altay et al. [14] reported neither perforations nor stromal infiltration, although they did observe one descemetocele [14].

Our recurrence rate was 8.3% (n = 2), possibly reflecting diminished anti-inflammatory activity after AM dissolution [11]. This highlights the need for ongoing topical antifungal and anti-inflammatory therapy [33]. Our ulcer healing success rate of 91.7% (n = 22) compares favorably with those reported by Altay et al. (85.72%) [14], Rodriguez-Ares et al. (73% in perforations) [27], and Mohan et al. [19], who reported 82.1%, 78.5%, and 75% success rates at 1, 3, and 6 months, respectively [19]. Other series reported varied outcomes: Lamas-Francis et al. [34] achieved a 62.8% success rate in culture-proven infectious keratitis, whereas Schuerch et al. [35] observed a 70% success rate with a single AMT.

Our study supports the value of multilayer AMT for deep non-healing ulcers, likely due to the membrane's growth factors and pro-epithelialization properties [36, 37]. Dekaris et al. [29] reported a 72% success rate with multilayer AMTs versus 64% with monolayer AMTs. Similarly, Prabhasawat et al. [38] observed an 82.1% success rate for multilayer AMTs. However, Letko et al. [39] detected no significant difference between onlay and inlay AMTs, perhaps because multilayer grafts were not used.

Recurrence and poor prognosis are more likely in cases of total limbal stem cell deficiency or autoimmune diseases [28]; therefore, we excluded such cases. Infection remains a key risk factor for recurrence, as noted by Chen et al. [40] and Solomon et al. [41], who reported five recurrences in 34 eyes. Prabhasawat et al. [38] documented complications, including neurotrophic keratopathy and perforation, in 17.9% of cases [38]. In the current study, two patients required additional surgical intervention because of persistent infection.

In our cohort, two patients (8.3%) had a hypopyon >2.5 mm that resolved within a week. Importantly, our findings suggest that AMT does not impede the penetration of topical antifungal or antibacterial agents. Kim et al. [42] demonstrated higher mean tear ofloxacin levels in AMT-treated rabbit eyes than in control eyes, highlighting the AM as a potential drug delivery platform.

The strengths of this study include its prospective design, standardized surgical technique, and comprehensive follow-up. However, the absence of a control group and the relatively small sample size limit generalizability. Further randomized controlled trials comparing AMT with PK or alternative adjunctive therapies could clarify the relative efficacy and long-term impact of AMT on visual rehabilitation. Further research should explore optimal patient selection, timing of intervention, and cost-effectiveness, particularly in resource-limited settings. Expanding evidence in these areas may help to refine treatment algorithms for resistant fungal keratitis and reduce corneal blindness in high-risk populations.

CONCLUSIONS

AMT proved to be an effective adjunctive therapy for resistant fungal corneal ulcers, achieving high rates of clinical resolution and significant improvements in BCDVA, ulcer healing, and patient comfort. Its application is particularly valuable for mid-stromal ulcers among older, high-risk populations in rural areas, where donor corneas are often scarce. Although encouraging, these findings should be interpreted in light of the study's single-arm design and modest sample size. Larger controlled studies are warranted to validate efficacy, define optimal indications, and compare long-term outcomes with those of conventional surgical options. Nevertheless, AMT remains a promising and accessible intervention for mitigating the vision-threatening sequelae of severe fungal keratitis.

ETHICAL DECLARATIONS

Ethical approval: This study was conducted in accordance with the principles of the Declaration of Helsinki. Ethical approval was obtained from the Institutional Review Board of the Faculty of Medicine, Al-Azhar University, Damietta (approval number: 0001267-23-08-010). Written informed consent was obtained from all participants before enrollment. This study was registered at ClinicalTrials.gov (Identifier: NCT06070883).

Conflict of interest: None.

FUNDING

None.

ACKNOWLEDGMENTS

None.

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