ABSTRACT

Objective: Severe corneal ulcer requires immediate intensive topical antibiotics, although potentially toxic to corneal epithelium and may contribute for prolonged corneal epithelial defect. Fresh-amniotic membrane transplantation (F-AMT) could act as an adjuvant therapy that counterbalances the epithelial toxicity effect and exerting antimicrobial actions. This study evaluates the clinical improvement and factors associated with the success rate of F-AMT using suturing technique.

Methods: This was a retrospective cohort study of 28 patients (28 eyes) conducted at Dr Sardjito General Hospital, Yogyakarta. Inclusion criteria were patients with severe corneal ulcer unresponsive to conventional medical treatment who underwent multilayer F-AMT using suturing technique. Clinical improvement such as hypopyon, size of the corneal defect, and antibiotic medication were evaluated.

Results: Corneal epithelial healing was effectively restored at 25 days post F-AMT. There were statistically significant difference in patient’s blepharospasm (p= 0.00) and pain scale postoperative (p=0.00). There was no significant difference in patients visual acuity postoperative. Factors such as the size of the corneal defect, hypopyon, leucocyte count and neutrophil count were not correlated to epithelial healing. Failure was noted in 7 patients (25%) because of a persistent corneal defect. No patient developed major immediate postoperative complications.

Conclusion: F-AMT could be an optional adjuvant therapy with excellent results to treat non-healing severe corneal ulcer unresponsive to medical treatment by promoting epithelial healing.

Keywords: fresh amniotic membrane transplantation, corneal ulcer, non-healing, epithelial healing, clinical improvement.

Based on Jones criteria severe corneal ulcer fall into category of ulcer diameter more than 5 mm and a depth of more than 50% of the cornea, was located within 3 mm from the visual axis, or had a moderate to severe anterior chamber reaction. Microbial infection directly responsible to corneal damage. Furthermore corneal destruction may be caused by the associated inflammatory response. Aggressive topical broad-spectrum antibiotic as initial therapy is most critical in the course of severe corneal ulcers. However topical antibiotic is potentially toxic to the corneal epithelium and may contribute to a prolonged corneal epithelial defect.

Amniotic membrane is the innermost layer of the fetal membranes. It has a stromal matrix, a thick collagen layer, and an overlying basement membrane with a single layer of epithelium.
membrane has unique properties including anti-adhesive effects, bacteriostatic properties, wound protection, pain reduction, and epithelialisation effects. Another characteristic of the amniotic membrane is the lack of immunogenicity.\textsuperscript{3} Moreover, amniotic membrane has been used as a surgical material for several decades.\textsuperscript{6}  

F-AMT as an adjuvant treatment for acute severe corneal ulcer counterbalances the epithelial toxicity of fortified antibiotic eye drops while exerting as-yet unclear antimicrobial actions and acting as a long-term drug delivery system. AMT can also directly promote rapid epithelialization and reduce stromal inflammation and ulceration in experimental bacterial keratitis.\textsuperscript{7} Therefore, F-AMT may also allow earlier use of topical steroids, which may prevent excessive scar formation. Although the benefits of early performed AMT are to be considered in terms of reducing the duration of hospitalization and improving the patient compliance to treatment.\textsuperscript{4}  

From the previous study suggest F-AMT can be resorbed more quickly than that dried amnion.\textsuperscript{8} Kruse et al. also reported rapid healing of the epithelial defect with an increase in stromal thickness after multilayer amniotic membrane transplantation (AMT) for deep corneal ulcers.\textsuperscript{9} Recently, Tseng and colleagues reported their success in using amniotic membrane (single and multilayer as a graft and patch) in persistent and progressive neurotrophic corneal ulcers.\textsuperscript{10} Aim of this study is to evaluate the efficacy and factors associated with the success rate of F-AMT using suturing technique.

**SUBJECT AND METHODS**

The study design is a retrospective cohort study conducted at the department of ophthalmology, Dr Sardjito General Hospital in Yogyakarta from January until December 2015. After providing written informed consent for hospital based review study, this study consist of 28 patients (28 eyes).

**Inclusion criteria**
1. Patient with severe corneal ulcer (based on Jones criteria)
2. Having a non-healing corneal ulcer, unresponsive to medical treatment.
3. Patients are willing to sign informed consent and follow the inspection schedule.

This study analysed the efficacy of F-AMT in relieving pain, blepharospasm, corneal epithelial healing and improvement of visual acuity. Other associated factors such as hypopyon, size of the corneal defect, and antibiotic medication were evaluated.

**Exclusion Criteria**
1. Suspicion of corneal ulcer caused by non-infection, virus
2. Lesions on the cornea are covered by the epithelium
3. Patients who can not participate in therapy and follow-up evaluation
4. Patients are pregnant and breastfeeding
5. The patient refuses to be the subject of the study.

*Fig 1. Pre operative, during surgery and ten days post surgery. Fresh amnion membrane covers the entire cornea*
Post-operative Evaluation and Follow up

Patients were examined for size of the corneal defect, hypopyon, leucocyte count and neutrophil on each visit. Surgical success was defined as complete epithelialisation of the ocular surface as demonstrated by biomicroscopy and negative fluorescein staining without recurrence within one month. We defined surgical success as complete epithelialisation of the corneal defect as demonstrated by negative fluorescein staining without recurrence within one month.

RESULT

Terdapat 46 mata yang dilakukan uji A total of 28 patients (28 eyes) with result mean age were 47.68 years (SD ± 15.64) and majority of patients is male (82%). Patients had different occupations; dominantly was a labor in 17 patients (60.7%). This incidence is likely due to exposure to work accidents, lack of knowledge, delays coming to the specialists eye, using alternative medicine and alternative therapies that develops in the countryside. Possible causative area for risk factor dominantly happened in working area in 10 patients (35.7%). Error! Reference source not found. displays the patient's demographic data.

Table 1. Demographic data

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Mean ± SD, n%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>47.68 ± 15.64</td>
</tr>
<tr>
<td>Sex</td>
<td></td>
</tr>
<tr>
<td>Men</td>
<td>23 (8.21)</td>
</tr>
<tr>
<td>Women</td>
<td>5 (17.9)</td>
</tr>
</tbody>
</table>

Tabel 2 displays postoperative clinical outcome. Analysis of Wilcoxon and McNemar test displayed that there was a statistically significant difference in patients' blepharospasm (p=0.000) and pain scale postoperative (p=0.003) but there was no statistically significant in VA improving (p=0.118).

Table 2. Clinical results

<table>
<thead>
<tr>
<th>Variable (n=28)</th>
<th>Pre</th>
<th>Post</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>LogMAR VA (mean±SD)</td>
<td>2.38±0.46</td>
<td>2.23±0.59</td>
<td>0.118</td>
</tr>
<tr>
<td>VAS score (mean±SD)</td>
<td>3.10±1.34</td>
<td>1.03±1.26</td>
<td>0.000</td>
</tr>
<tr>
<td>Blepharospasm</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>27 (96.4)</td>
<td>10 (35.7)</td>
<td>0.000</td>
</tr>
<tr>
<td>No</td>
<td>1 (3.6)</td>
<td>18 (64.3)</td>
<td></td>
</tr>
<tr>
<td>VAS score</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No (0)</td>
<td>1 (3.6)</td>
<td>13 (46.4)</td>
<td>0.003</td>
</tr>
</tbody>
</table>

Table 1 displays postoperative clinical outcome. Analysis of Wilcoxon and McNemar test displayed that there was a statistically significant difference in patients' blepharospasm (p=0.000) and pain scale postoperative (p=0.003) but there was no statistically significant in VA improving (p=0.118).
Analysis of Spearman correlation (Table 1) showed factors such as age, sex, occupation, hypopyon, blepharospasm before surgery, VAS, leucocyte count, and neutrophil count were not correlated to epithelial healing (p>0.05). Moreover, this study found there were 7 cases of f-AMT failure.

<table>
<thead>
<tr>
<th>Variable (n=28)</th>
<th>r</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>-0.107</td>
<td>0.587</td>
</tr>
<tr>
<td>Sex</td>
<td>-0.162</td>
<td>0.412</td>
</tr>
<tr>
<td>Occupation</td>
<td>0.99</td>
<td>0.616</td>
</tr>
<tr>
<td>Hypopyon</td>
<td>-0.167</td>
<td>0.397</td>
</tr>
<tr>
<td>Blepharospasm</td>
<td>0.111</td>
<td>0.574</td>
</tr>
<tr>
<td>Visual Analog Score</td>
<td>-0.117</td>
<td>0.554</td>
</tr>
<tr>
<td>Leucocyte</td>
<td>-0.051</td>
<td>0.796</td>
</tr>
<tr>
<td>Neutrophil</td>
<td>-0.117</td>
<td>0.552</td>
</tr>
</tbody>
</table>

DISCUSSION

Amniotic membrane (AM) has several characteristic that could be useful to promote epithelial healing.11,12 Lee and Tseng used the amniotic membrane to treat persistent epithelial defects, and epithelialisation occurred in 10 of 11 consecutive patients.13,14 The basement membrane of the AM closely resembles that of the conjunctiva and cornea, especially with regards to its collagen composition. Thus it serves as a substrate on which epithelial cells can grow easily. Four main effects on the regenerating corneal epithelium have been described: (1) facilitation of epithelial cell migration; (2) reinforcement of basal epithelial cell adhesion; (3) promotion of epithelial cell differentiation; and (4) Prevention of apoptosis. These properties render it suitable for use in cases of nonhealing or persistent epithelial defects of the ocular surface, especially that of the cornea.13, 15 In this study, the amniotic membrane promoted rapid corneal healing in 21 of 28 patients with severe corneal ulcer unresponsive to medical treatment. After F-AMT corneal epithelial healing was effectively restored at day-25. This result is consistent with the previous study by Yang et al. that surgical cases healed in 24.4±13 days for keratoconjunctivitis.16,17 Result of this study showed that appropriate surgical treatment could produce significant improvements of the corneal surface even at severe condition despite hypopyon, leucocyte count and neutrophil count of the patients. In addition, no patient developed major immediate postoperative complications. It is well established that fresh amniotic membrane has many properties that make it favourable for transplant surgeries: an avascular stromal matrix, antiinflammatory, and anti-scarring properties, and the ability to enhance epithelialization, and so on.5,15 However, there were 7 cases noted with failure in this study because of the persistent corneal defect which is not correlated with pre surgery condition such as age, sex, occupation, hypopyon, blepharospasm before surgery, VAS, leucocyte count, and neutrophil count. Persistent corneal defect may happened in this study because other abnormalities (such as diffuse limbal stem cell deficiency) that we did not count before. All of these cases underwent re-AMT and tectonic keratoplasty. Therefore, these results suggest that in patients with associated ocular surface abnormalities (such as diffuse limbal stem cell deficiency) F-AMT may be only useful as a temporary measure before other reconstructive surgical procedures. Moreover, this result is in line with other study.15,21

A literature review reveals conflicting reports about the anti-microbial properties of AM. Burn patients treated with AM have been shown to have decreased bacterial counts and control of infections.15,18,19 Antibacterial effects have been demonstrated against both gram-
positive cocci including streptococci and Staphylococcus aureus as well as gram-negative bacilli including Escherichia coli and Pseudomonas aeruginosa. These antibacterial effects have been attributed to the presence of several anti-microbial factors in the amniotic fluid, including bactericidal, beta-lactamase, transferrin and 7S immunoglobulin. Other investigators, however, believe that the AM does not per se contain any chemical antimicrobial substances, but rather constitutes an effective physical barrier against infection because of its ability to adhere strictly to the underlying surface.15,16

Multilayer AM has been used to treat non-traumatic micro-perforations and descemetoceles with up to 72.7% to 82.3% success rate being reported.20,21 AM in this situation provides tectonic support, collagen substitution for corneal stroma and anti-inflammatory and antifibrotic actions which halt progressive tissue degradation. Depending on the underlying severity and extent of the disease process it may be used as a permanent surgical therapy or as a temporizing measure until a more definitive surgical procedure can be performed.15,21

F-AMT using suturing technique can successfully treat severe corneal ulcer unresponsive to medical treatment by promoting epithelial healing, relieve the pain and inflammation without any complication. Medical treatment, including topical antibiotic, still needed. The authors declare neither there is any conflict of interest nor financial disclosure.

Limitation of this study is a relatively small number of patients, confounding factors such as different causative agent, limbal stem cell deficiency, and type of medication before. A larger, randomised, controlled study may be needed.

CONFLICT OF INTEREST
Author declare that there is no conflict of interest in this study

Reference