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Effects of Amniotic Membrane Transplantation in Ocular Burns: A Meta-Analysis

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Abstract: *Background:* Ocular burns is a serious eye injury with a high rate of blindness, efforts should be made to eliminate the serious complications, prevent from lifelong disability, and improve emergency interventions and treatment. *Objective:* To evaluate the effectiveness of amniotic membrane transplantation (AMT) in ocular burns. *Methods:* The following electronic databases were searched: PubMed, Web of Science, and Cochrane Library. With the keyword "amniotic membrane" and "ocular burn". No limitation of year, language, gender, age, nationality, etc. Animal trials, patients with other ocular surface diseases, and amniotic membrane transplantation combined with other surgeries were excluded. We evaluated the corneal epithelium healing time (CEHT), tear break-up time (TBUT), Schirmer test (ST), corneal neovascularization, the formation of symblepharon, and lid abnormalities after conventional treatment (CT) and AMT. The differences were tested by referring to the Cochrane Handbook. Pooled estimates were determined with RevMan software, version 5.3. *Results:* 5 studies with 310 eyes of 282 participants suffering from ocular burns were included. There was no significant difference between CT and AMT among the following outcomes: CEHT, TBUT, ST, formation of symblepharon, and lid abnormalities, except the extent of corneal neovascularization, which was less in patients treated with AMT (RR 0.81; 95% CI 0.68, 0.96; $I^2 = 40\%$, $p = 0.02$). *Conclusions:* Compared to CT, AMT does not show better advantages in promoting epithelial healing, improving tear film status, and preventing complications such as symblepharon formation and eyelid abnormalities except reducing corneal neovascularization.

Keywords: Ocular Burn, Amniotic Membrane Transplantation, Complications, Meta-analysis

1. Introduction

Ocular burns is a serious eye injury, accounting for 7.7- 18% of all eye injuries, which leads to a high rate of blindness [1]. Chemical and thermal burns are the most common causes in ocular burns. Most of the cases occur in middle-aged men who work in industry, which may definitely cause huge economic impact for the family and society. To eliminate the serious complications and prevent from lifelong disability, emergency interventions and treatments are needed.

Currently, patients experiencing an ocular burns will need a thorough and immediate evaluation and intensive treatment [2]. Conventional treatments (CT) of ocular burns is medical therapy, including topical antibiotics, artificial tears or bandages to promote epithelial healing, ascorbic acid and collagenase inhibitors to reduce ulcer formation, corticosteroids to control inflammation, and symptomatic support therapy for antiglaucoma and analgesia when necessary. However, operations are needed in serious cases which cannot corrected by CT. Various surgical techniques are used in ocular burns, such as amniotic membrane transplantation (AMT), autologous limbal stem cell transplantation, penetrating keratoplasty, and tenonplasty ect.

Amniotic membrane (AM), the innermost layer of the placenta, is a semi-transparent membrane with the thickness of only 0.02–0.05 mm. AM is an ideal substitute for ocular surface reconstruction because it is avascular and contains a variety of cytokines and growth factors, which can promote the formation of the epithelium, maintain normal epithelial phenotype, reduce the inflammatory response, decrease neovascularization and scar formation [3]. However, it is

challenged to maintain the vitality of AM in severe inflammatory environment [4]. Therefore, this meta-analysis is aim to further evaluate the effects of AMT in ocular burns. As far as our information goes, this is the first meta-analysis to compare these two different therapeutic method.

2. Methods

2.1. Search Strategy

The search was performed on May 15, 2020, we searched the following electronic databases: PubMed, Web of Science, and the Cochrane Library. With the keyword "amniotic membrane" and "ocular burn". To improve the retrieval rate, no limitations on year, language, gender, age, nationality were set. In the case of missing records, we manually searched the references of all relevant literatures.

2.2. Inclusion and Exclusion Criteria

2.2.1. Inclusion Criteria

(1) Participants: Patients with ocular burns.
(2) Intervention: AMT combination with CT.
(3) Comparison: Conventional treatment.
(4) Outcomes: Corneal epithelium healing time (CEHT), tear break-up time (TBUT), Schirmer test (ST), corneal neovascularization, the formation of symblepharon, and Lid abnormalities.
(5) Study design: Randomized controlled trial (RCT) and case series (CS).

2.2.2. Exclusion Criteria

(1) Patients with other ocular surface diseases.
(2) Other interventions: AMT combined with other surgeries, such as conjunctival flap, autologous limbal stem cell transplantation, and penetrating keratoplasty, etc.
(3) Animal trials.

2.3. Risk of Bias Assessment

Two reviewers (Hua Wang and Jun-jie Tang.) individually assessed the risk of bias in each study in accordance with the Cochrane Handbook using the following parameters: adequacy of sequence generation; allocation concealment; blinding of participants, personnel, and outcome assessors; incomplete outcome data; and selective outcome reporting.

2.4. Data Extraction

To ensure the quality and accuracy of the whole process, data extraction was performed by two reviewers (Hua Wang and Jun-jie Tang.) independently by reading the full text of all included literature. The data from qualified studies were extracted, including author's name, publication year, country, type of study, sample size, gender ratio, follow-up time, age, whether complications such as CEHT, TBUT, ST, corneal neovascularization, the formation of symblepharon and lid abnormalities occur. Any disagreements regarding inclusion and exclusion of studies were resolved through discussion.

2.5. Heterogeneity Assessment

As described in the Cochrane Handbook for Systematic Reviews of Interventions. Heterogeneity was determined by examining forest sample plots and testing with chi2 to determine the percentage of variation that was not due to sampling error. When $I^2 < 50\%$, fixed-effects model was used; on the contrary, when $I^2 > 50\%$, random-effects model was used to pool the data. If significant heterogeneity existed, sensitivity analysis was performed. A P value < 0.05 was considered statistically significant using a 2-sided test.

2.6. Statistical Analysis

RevMan software (version 5.3) was used to perform the data analysis. The relative risk (RR) with 95% confidence interval (CI) were used in dichotomous data such as corneal neovascularization, the formation of symblepharon, and lid abnormalities. The standard mean differences (SMD) with 95% CI were used to analyze the continuous outcomes including CEHT, TBUT, and ST.

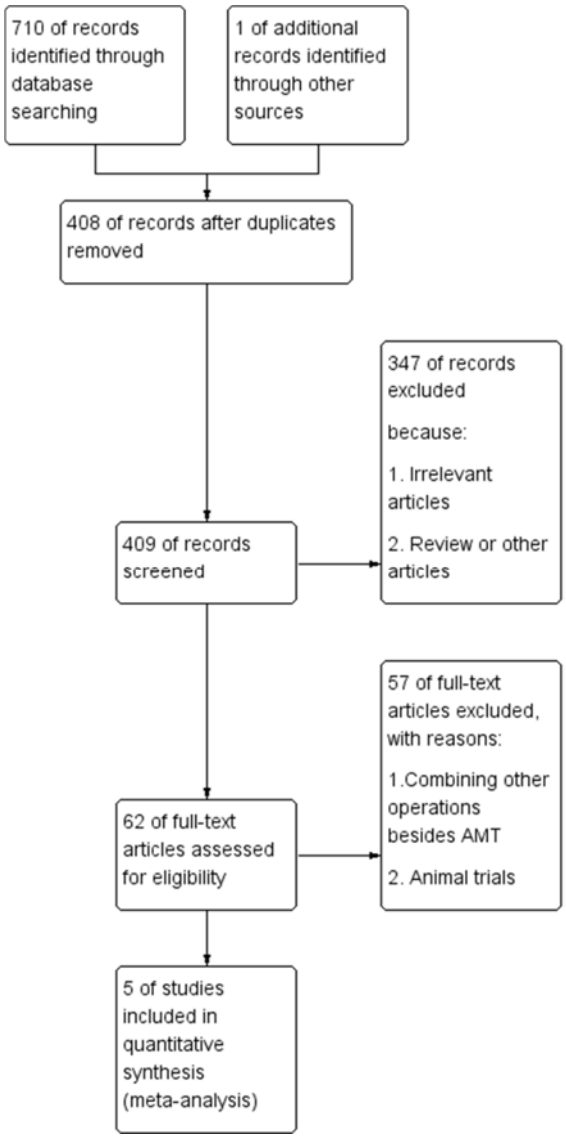

Figure 1. *Flow diagram of literature research.*

3. Results

3.1. Results of the Search

The selection process of articles was shown in a flow diagram (Figure 1). According to the search strategies, 710 articles were retrieved through multiple databases before May 15, 2020. Furthermore, 1 additional study was included by manually searching the references of relevant article [5]. 302 duplications were removed. Subsequently, after reviewing the abstracts of the remaining 409 studies, 347 of records were excluded because of the irrelevant articles. The full text of 62 remained articles were assessed for eligibility, multiple treatments and animal trials were excluded, and eventually, 5 eligible studies [5-9] with 310 eyes were included.

3.2. Characteristics of the Included Studies

The included studies were published from 2005 to 2020 in India and the USA. These studies included 310 eyes of 282 patients with ocular burns. Most of them were middle-aged men, four were randomized controlled trials and one was a case series. The duration of follow-up ranged from 3 to 18 months. The study by Tandon et al had the largest sample number (n = 100). Sharma et al had the most complete results in 2015 and 2016, including all the required outcome variables. All the relevant information was exacted and is summarized in Table 1.

Table 1. *Characteristics of Included Studies.*

Study	Year	Country	Design	Eye/Patient	Gender (man/female)		Age Mean±SD		Follow-up (mo)	Response variable
					CT	AMT	CT	AMT		
Tamhane	2005	India	RCT	44/37	17/7	14/6	16±10	18±12	18	2.3.4.5
Tandon	2011	India	RCT	100/100	44/6	43/7	NM		3	4.5.6
Sharma	2015	India	CS	55/55	NM		19.6±13.7	29±15.9	3	1.2.3.4.5.6
Sharma	2016	India	RCT	51/30	NM		21.9±13.9	18.1±11.3	3	1.2.3.4.5.6
Eslani	2019	USA	RCT	60/60	28/2	28/2	27 ± 7	24 ± 6	13	1.4.5

NM=no mention; 1=complete epithelialization time; 2=tear break up time; 3=schirmer test; 4=symblepharon; 5=neovascularisation; 6=Lid abnormalities.

3.3. Quality of the Evidence

The risk of bias in the included studies is summarized in Table 2. There was no clear disagreement between the 2 reviewers. One of the five articles did not mention the use of random allocation, and two did not mention the use of allocation concealment. The rest show low risk.

Table 2. *Risk of Bias in Included Studies.*

Study* (yr)	Random Sequence Generation (Selection Bias)	Allocation Concealment (Selection Bias)	Masked Participants and Personnel (Performance Bias)	Masked Outcome Assessment (Detection Bias)	Incomplete Outcome Data (Attrition Bias)	Selective Reporting (Reporting Bias)
Tamhane (2005)	Low	Unclear	Low	Low	Low	Low
Tandon (2011)	Low	Low	Low	Low	Low	Low
Sharma (2015)	High	Unclear	Low	Low	Low	Low
Sharma (2016)	Low	Low	Low	Low	Low	Low
Eslani (2019)	Low	Low	Low	Low	Low	Low

*First author.

3.4. Corneal Epithelium Healing Time

Based on the standard that we defined, the CEHT could be obtained in 3 included studies [5, 8, 9]. CEHT ranged from 22 to 75.8 days and 56.7 to 72.6 days, in 63 eyes treated with AMT and 65 eyes received only CT respectively. There was no significant difference between AMT and CT in CEHT (SMD -0.96; 95% CI -2.31, 0.38; I^2 = 91%; P = 0.16) (Figure 2A). We tried to eliminate study to reduce heterogeneity (After the studies were excluded according to the publication year, the heterogeneity was: I^2 = 96%, I^2 = 60%, I^2 = 91%). Subgroup analysis of age was performed to determine whether the result were influenced by age (SMD -0.49; 95% CI -0.87, 1.85; I^2 = 92%; P = 0.48) (Figure 2B). We considered that the CHET might be related to the degree of injury, but there were only two studies which divided patients into different groups according to severity.

3.5. Tear Break-up Time

Three studies evaluated the tear film status using the TBUT at 3 months follow-up [5, 6, 8]. Mean TBUT ranged from 4.18 to 10.7 s in 52 eyes treated with AMT and 5.09 to 10.3 s in 58 eyes without AMT. Meta-analysis showed that TBUT in AMT was similar to CT (SMD 0.22; 95% CI -0.40, 0.85; I^2 = 62%; p = 0.48) (Figure 3A). After removing the retrospective study of Sharma et al., heterogeneity dropped from I^2 = 62% to I^2 = 0%, but difference were still insignificant (P = 0.71) (Figure 3B).

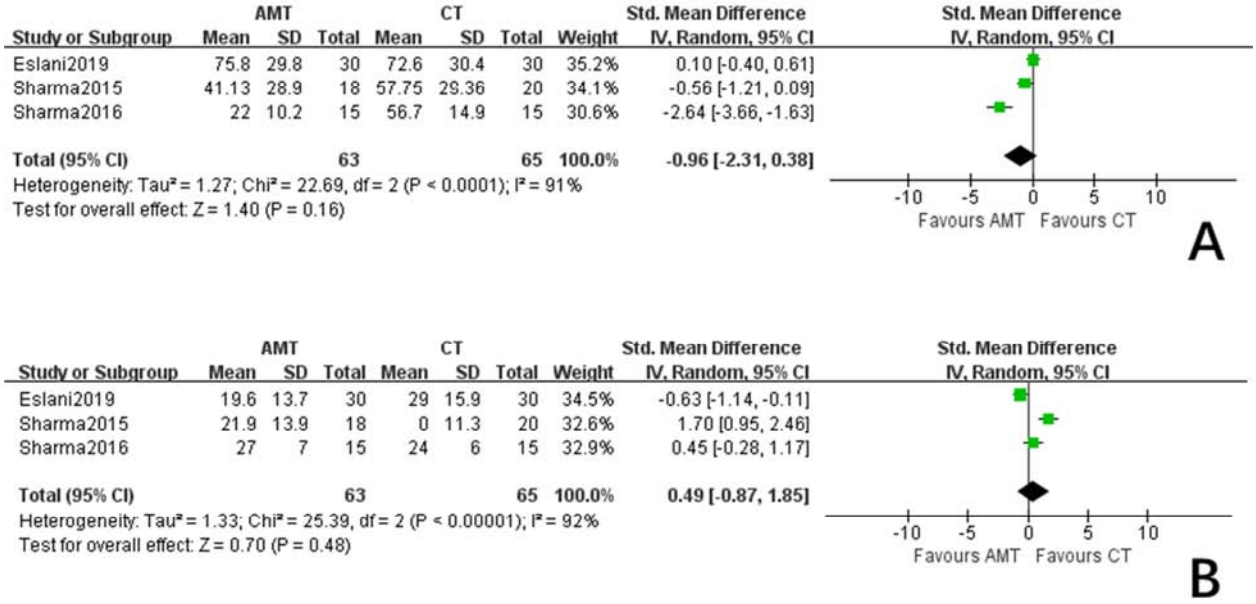

Figure 2. *Forest plot for CEHT and subgroup analysis of age.*

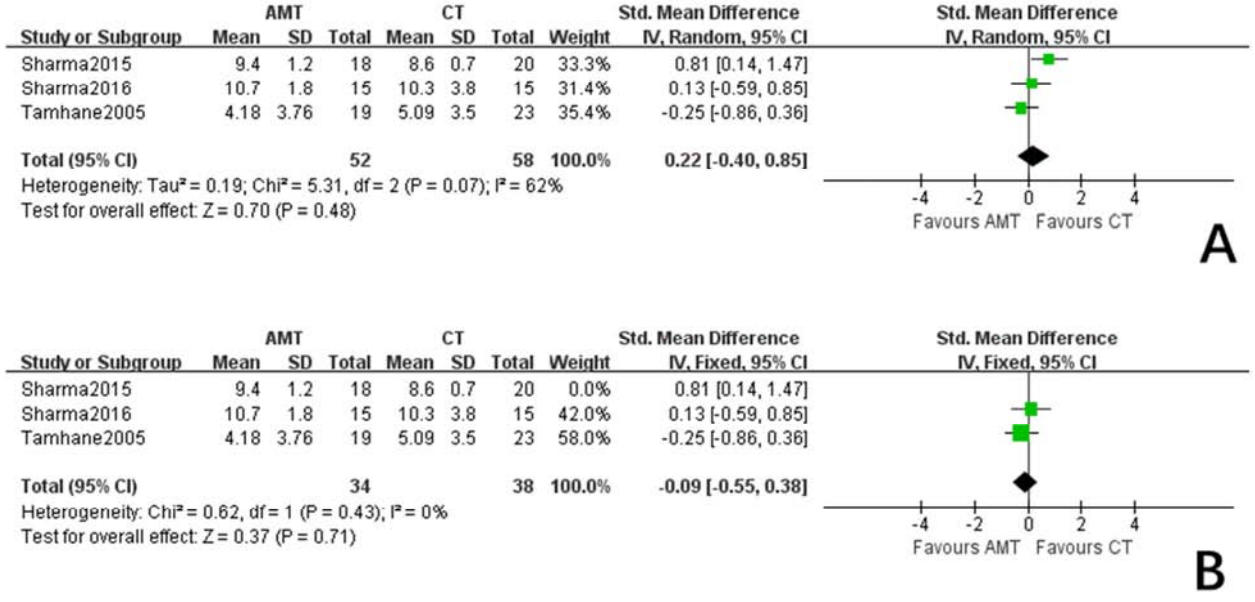

Figure 3. *Forest plot for TBUT.*

3.6. Schirmer Test

Schirmer test at 3 months follow-up was performed in three included studies to evaluate tear film status [5, 6, 8]. Mean ST was similar between two treatments and the heterogeneity was high. (SMD 0.07; 95% CI -0.72, 0.87; I^2 =76%; p=0.86) (Figure 4A). After removing the RCT of Sharma et al, heterogeneity dropped from I^2 =76% to I^2 =0%. The results showed there were still no significant difference (P = 0.14) (Figure 4B).

3.7. Neovascularization

All studies recorded the occurrence of neovascularization at 3 months follow-up [5-9]. Neovascularization occurred in 60% (79/132) of AMT and 73% (101/138) of CT (RR 0.81; 95% CI 0.68, 0.96; I^2 =40%; p=0.02) (Figure 5A). Compared with CT alone, combined AMT can reduce the formation of postoperative corneal neovascularization.

3.8. Symblepharon

Five included studies evaluated the postoperative symblepharon at 3 months follow-up [5-9]. Symblepharon occurred in 37% (49/132) of AMT and 46% (64/138) of CT (RR 0.81; 95%CI 0.61, 1.07; I^2=0%; p=0.14) (Figure 5B). The results were no significant.

3.9. Lid Abnormalities

Three studies recorded the postoperative lid abnormalities

at 3 months follow-up [5, 7, 8]. Lid abnormalities occurred in 8% (7/83) of AMT and 12% (10/85) of CT (RR 0.74; 95% CI 0.32, 1.71; $I^2 = 0\%$; $p = 0.49$) (Figure 5C). The results were no significant.

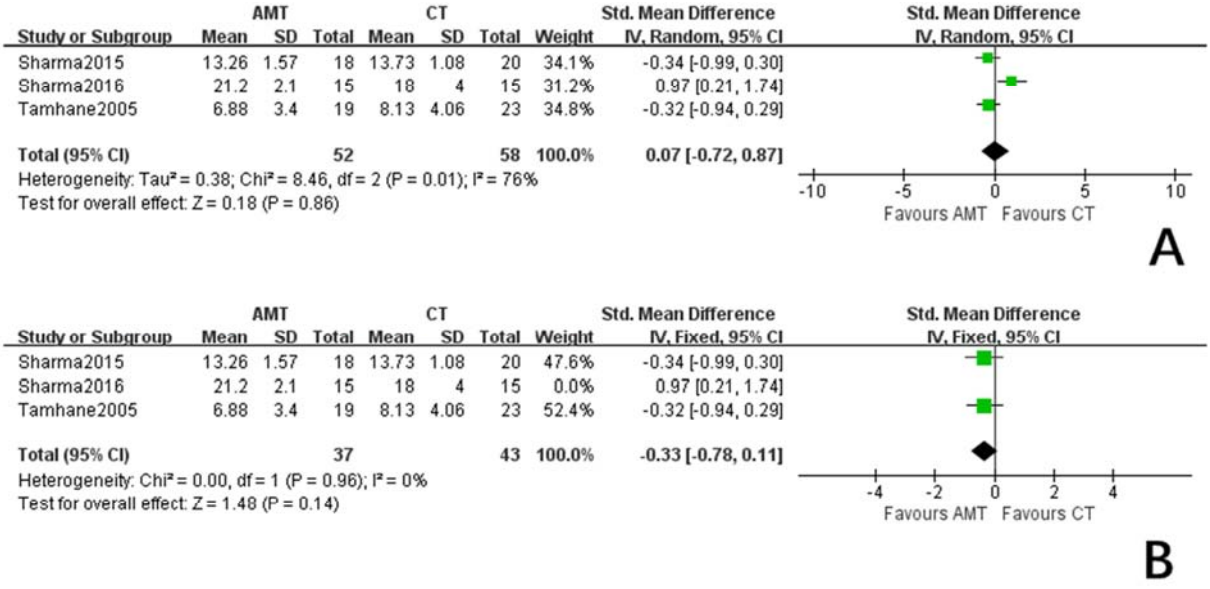

Figure 4. *Forest plot for ST.*

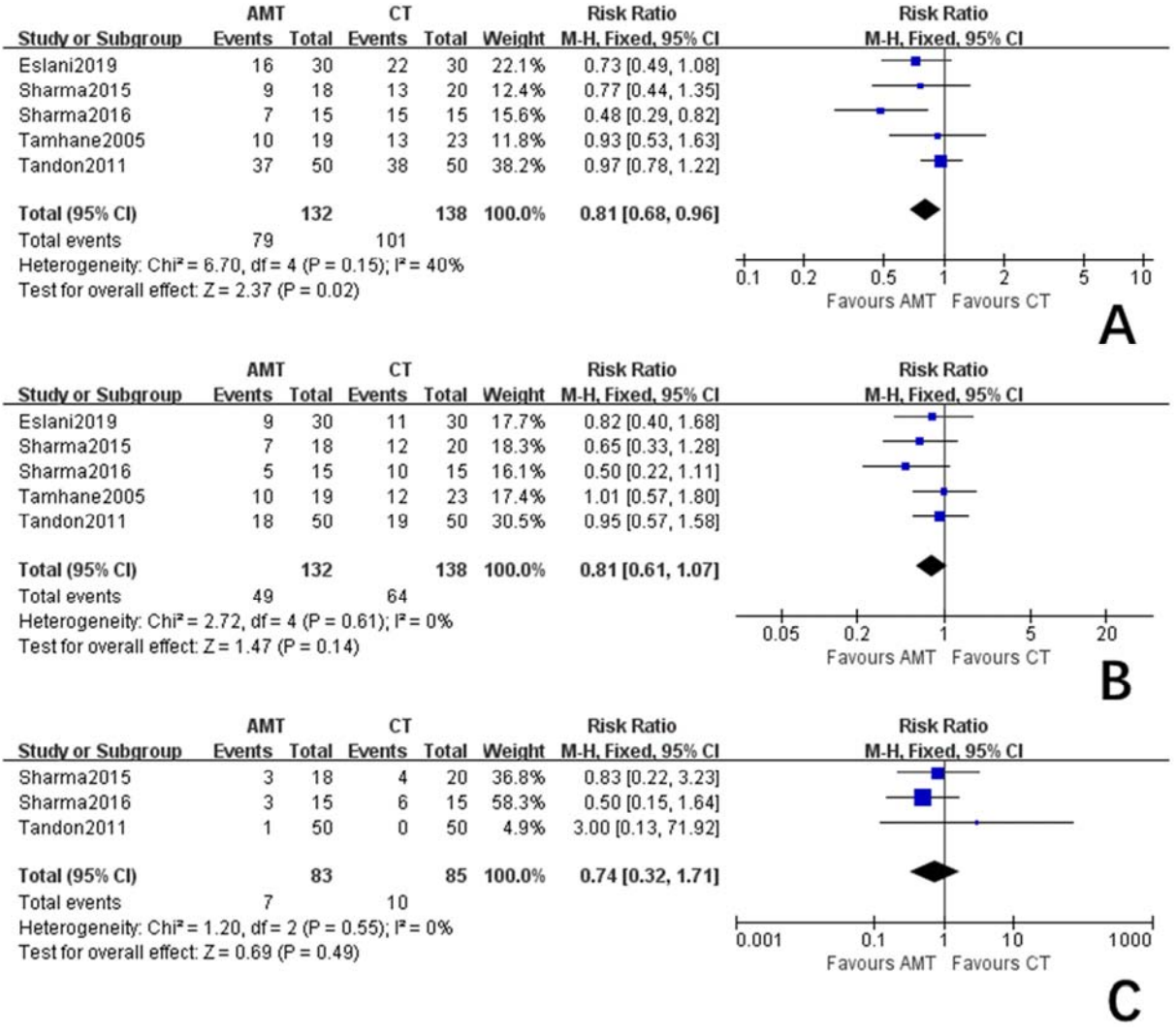

Figure 5. *Forest plot for Neovascularization, symblepharon, and Lid abnormalities.*

4. Discussion

Results of this meta-analysis showed that amniotic membrane transplantation had no obvious benefits in promoting epithelial healing, stabilizing tear film, and preventing complications such as symblepharon formation, lid abnormalities except reducing the formation of corneal neovascularization. This was consistent with the conclusion of Sharma et al in 2016 [8]. Hao et al [10] shows that amniotic membrane epithelial and mesenchymal cells can produce various antiangiogenic proteins. Some of these proteins appear to be abundantly deposited within the stroma of the amniotic membrane. These findings may explain the clinical observation, consistent with our conclusions, that amniotic membrane can mitigate ocular surface neovascularization. This meta-analysis collected data from four RCTs and one case series including 310 eyes with ocular burns. Among this studies, four were conducted in India while the fifth was performed in USA. Assessments of the gender and age distribution showed that ocular burns mainly occurred in the working-age population and was more frequently appeared in males; therefore, this meta-analysis definitely had a significant socioeconomic impact.

All ocular burns treatments aim to restore eye surface structure and function. By conventional treatment, most patients with mild to moderate ocular burns can maintain the stability of the ocular surface function, for most patients with severe eye burns, however, continue to develop severe limbal stem cell deficiency, usually complicated with corneal neovascularization, corneal perforation and other serious complications, so autologous limbal stem cell transplantation, and penetrating keratoplasty etc surgical techniques are necessary. Amniotic membrane was first used as a biofilm along with the chorionic membrane to promote the healing of skin burns by Davis in 1910 [11]. In ophthalmology, it was first used for the treatment of conjunctival defects by de Ro¨tth in 1940 [12], and it was first used for chemical burns by Sorsby et al in 1946 [13]. The increased use of the amniotic membrane in ocular diseases was due to its high levels of growth factors and anti-inflammatory properties, which promote rapid epithelialization and reduce eye surface inflammation and scarring [14]. But the role of amniotic membrane in treating ocular burns is controversial. Several studies were selected to identify the effect of AMT in ocular burns [5-9, 13, 15-26] (Table 3).

Table 3. *Several studies on AMT for the treatment of ocular burns.*

study	year	country	study design	injury grades	AMT eyes	control eyes	age	gender man/female	follow-up (mo)	conclusion
Sorsby	1946	UK	CS	NM	30	0	14-64	NM	NM	AMT was beneficial
Sorsby	1947	UK	CS	NM	28	0	6-64	NM	NM	AMT was beneficial
Meller	2000	USA	CS	II-IV (R-H)	13	0	38.2±10.6	10/1	4	AMT was beneficial
Sridhar	2000	India	CS	NM	2	0	17-20	2/0	4-6	AMT was beneficial
Joseph	2001	UK	CS	IV (R-H)	4	0	40-65	4/0	NM	AMT was not beneficial
Kobayashi	2003	Japan	CS	II-III (R-H)	5	0	24-46	5/0	14-24	AMT was beneficial
Arora	2005	India	CS	II-IV (R-H)	15	0	6-53	10/5	10.14±4.4	AMT was beneficial In mild burns
Tejwani	2007	India	CS	II-VI (Dua)	24	0	NM	NM	6	AMT was beneficial
Kheirkhah	2008	USA	CS	I-III (R-H)	5	0	3-53	3/2	7-29	AMT was beneficial in mild and moderate burns
Gheorghe	2016	Romania	CS	NM	28	0	NM	NM	1	AMT was beneficial
Westekemper	2017	Germany	CS	I-IV (R-H)	72	0	37.3 ±11.6	48/6	36.4	AMT was beneficial
López-García	2006	Spain	CS	III-IV (Dua)	12	12	NM	13/5	9	AMT was beneficial
Prabhasawat	2007	Thailand	CS	II-IV (R-H)	13	8	36.9 ± 11.7	NM	8.0±6.8	AMT was beneficial in moderate burns
Sharma	2015	India	CS	III-V (Dua)	18	20	control: 19.6±13.7 AMT: 29±15.9	NM	3	AMT was beneficial
Tamhane	2005	India	RCT	II-IV (R-H)	24	24	control: 16±10 AMT: 18±12	31/13	18	AMT was beneficial in moderate burns
Tandon	2011	India	RCT	II-IV (R-H)	50	50	3-60	87/13	3	AMT was beneficial in moderate burns
Sharma	2016	India	RCT	III-V (Dua)	15	15	control: 21.9±13.9 AMT: 18.1±11.3	NM	3	AMT was beneficial
Eslani	2019	USA	RCT	IV (R-H)	30	30	25 ± 7	56/4	20.3±2.5	AMT was not beneficial in severe burns

NM=no mention; CS=case series; RCT=randomized controlled trial; R-H: Roper-Hall classification; AMT= amniotic membrane transplantation.

Many studies have reported the beneficial effect of amniotic membrane in the treatment of ocular surface burns. However, Joseph et al [18] reported that conjunctiva loss and eyelid abnormalities occurred in four patients with grade IV burns after treating with AMT which indicating that the amniotic membrane was not available in all kinds of ocular surface reconstruction. Although many studies have reported the good results of AMT [13, 15-17, 19-24], the drawbacks must be noted when interpreting these results, as none of these reports had a control group. Despite Lopez-Garcia et al [25] established a control group in 2006 and confirmed that AMT improved corneal re-epithelialization earlier than medical

therapy, this experiment was conducted using impression cytology. The re-epithelialization model defined in this study was cellular and may be different from the classic concept of overlap corneal surface with the normal corneal phenotype of the epithelial cells. Sharma et al demonstrated that umbilical cord serum and AMT, as adjunctive standard drugs for acute chemical injury, were equally effective [5, 8]. Since umbilical cord serum is as effective as AMT, whether AMT is necessary? Besides, in their study, there were no patients with grades VI burns (Dua's classification), very few patients with grades V burns, and most are mild to moderate in their study. In addition, the initial epithelial defect and visual acuity were unclear in the amniotic membrane group: for patients with the binocular disease, the severe one was randomly assigned and the other eye was assigned to another group, which might also be responsible for the discrepancy. Based on their research, that AMT was beneficial only applied to mild to moderate patients, this was consistent with others conclusions [7, 9, 26].

Although AMT can reduce patients' pain scores, it had no obvious effect on improving complications such as symblepharon and lid abnormalities [8]. Our results demonstrate this again. There was not statistically significant in CEHT, TBUT, ST, symblepharon, and lid abnormalities between AT and CT. Although AMT can reduce the incidence of corneal neovascularization after surgery, however, considering the cost of surgery, such as anesthesia accidents, surgery costs, etc. For children who could not cooperate with the operation, was general anesthesia necessary? Moreover, there was a risk of infection in AMT [27], and at least two serological tests should be performed before surgery to prevent and reduce window period infection. Last but not least, amniotic membranes required expensive processing and preservation measures that were currently not available in most hospitals.

5. Conclusion

Compared to conventional medical therapies, AMT combined with conventional treatments can effectively reduce the formation of postoperative corneal neovascularization in ocular burns, but there was no difference in corneal epithelium healing time, the state of the tear film, symblepharon and lid abnormalities.

Limitations

Considering the inclusion and exclusion criteria, we only analyzed 310 eyes from 5 references. The limited sample size, differences in the experimental population and methods may influence the results. Lacking of original data, subgroup analysis of damage degree in CEHT was unclear. Besides, this meta-analysis only selected objective criteria, such as epithelial healing time, tear film state, and postoperative complications such as symblepharon. Subjective indicators, such as pain score, were needed to be further clarified in future analysis to better evaluate the effects of AMT in ocular burns.

Declaration of Interest

The authors declare that they have no competing interests.

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