

ORIGINAL ARTICLE

Amniotic Membrane Transplantation (AMT) Success Rate in Non-Healing Corneal Ulcers

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ABSTRACT

Introduction: Cornea is the outermost fibrous layer of the eye that is nourished by tears and aqueous humor and lacks blood vessels. One of the main causes of ocular morbidity is corneal ulcers. Non healing corneal ulcers are those that, inspite of appropriate topical medication, do not heal within a period of 2 weeks. These ulcers can result in descemetocoe development and perforation. Nowadays, amniotic membrane transplantation (AMT) is regarded as the preferred course of treatment for these ulcers.

Objective: To determine the amniotic membrane transplantation (AMT) success rate in patients of corneal ulcers that do not heal.

Study Design: Descriptive case series

Settings: Ophthalmology Department, Ward No. 15, Nishtar Hospital Multan.

Duration of Study: 6 months, from February 2023 to July 2023.

Patients & Methodology: This study comprised 86 patients, both male and female, aged 20-70 years, who has been diagnosed with non healing corneal ulcers. Age, gender, height, weight, body mass index(BMI), diabetes and duration of corneal ulcer among the baseline demographics recorded. AMT was positioned stromal side down and the procedure success rate was recorded one month following the transplant.

Results: Mean age was 47.23 ± 13.31 years. Mean BMI was 24.96 ± 4.21 kg/m². Mean duration of corneal ulcer was 7.26 ± 3.05 months. Mean corneal ulcer size before treatment was 5.16 ± 1.34 mm and after treatment was 0.68 ± 1.22 mm. Mean pain score before treatment was 5.93 ± 1.04 and after treatment was 1.24 ± 1.50 . There were 60 (69.77%) males and 26 (30.23%) female patients. Diabetes was diagnosed in 17 (19.77%) patients. Success of AMT was observed in 76 (88.37%) and AMT was unsuccessful in just 10 (11.63%) patients.

Conclusion: By encouraging epithelial repair, reducing discomfort and inflammation without causing any complications, amniotic membrane transplantation can effectively treat severe corneal ulcers that are not responding to medical treatment.

Keywords: Amniotic membrane transplantation, corneal ulcer.

INTRODUCTION

Cornea is the outermost fibrous layer of the eye that is nourished by tears and aqueous humor and lacks blood vessels. Cornea is transparent and it permits light into the eye. Cornea is the most essential structure of the ocular surface.¹

One of the main causes of ocular morbidity in underdeveloped nations is corneal ulcer. Non-healing corneal ulcers are those that, inspite of topical medication, do not appear to be healing within a 2 week period. Such an ulcer may lead to complications like perforation and descemetocoe formation.^{2,3}

Treatment of non-healing corneal ulcers includes removal of the underlying causes.^{4,5} Lubricants that are preservative free are prescribed along with antibiotics that are less toxic to epithelium. Other associated procedures like the use of bandage soft contact lens, conjunctival flap and temporary/permanent tarsorrhaphy can be performed to enhance ulcer healing.^{4,5} Amniotic membrane transplantation (AMT) might be a better alternative due to its biological healing properties in addition to its mechanical properties.^{6,7}

Studies conducted on the outcomes of amniotic membrane transplantation have showed good success rates like a study conducted by Mohan et al. found a success rate of 82.1% after AMT in patients with non-healing corneal ulcers.⁸ Study by Manolova et al. found a success rate of 91.2% after AMT.⁹ A study conducted in Pakistan by Khan et al. reported a success rate of only 74.5%,¹⁰ while another study from Pakistan reported a success rate of 93.3%.¹¹

AMT is an inexpensive, readily available and an effective treatment option for non-healing corneal ulcers, I would like to conduct this study in my setting, to facilitate the patients of this area with an effective and inexpensive treatment option that would help in decreasing the morbidity as well as preserving anatomy of

the globe.

Objective: To determine the amniotic membrane transplantation (AMT) success rate in patients of corneal ulcers that do not heal.

MATERIALS AND METHODS

Study Design: A descriptive case series.

Setting: Ophthalmology Department, Ward No. 15, Nishtar Hospital, Multan.

Study Duration: 6 months, from February 2023 to July 2023

Size of Sample: It calculated using epi-tools calculator. By taking expected frequency of success rate of AMT 91.2%, at margin of error 6% and level of significance 5%, 86 patients were calculated as sample size.

Technique of Sampling: It's a non-probability, consecutive sampling.

Sample Selection:

Inclusion Criteria:

- Patients with diagnosis of corneal ulcers that do not heal.
- Having age 20-70 years
- Both male and female patients.

Exclusion Criteria:

- Patients diagnosed of having Steven Johnson syndrome (through medical records).
- Patients having perforated eye secondary to corneal ulcer.

Data Collection Procedure: After approval from research evaluation unit of Nishtar Hospital, Multan, patients who were present in department of Ophthalmology with diagnosis of non-healing corneal ulcers fulfilling the inclusion criteria were considered for this study. 86 patients were included. Prior to their inclusion in the present study, all patients were provided written informed consent. Information was recorded about the patients' age, sex, diabetes and duration of corneal ulcer. Amniotic membrane transplantation was done by consultant ophthalmologist having at-least 3 years of post-fellowship experience. Following an elective cesarean delivery in individuals who tested negative for

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HIV, hepatitis B, hepatitis C and syphilis, AM was extracted under sterile settings. Before taking AM, the female patients were asked to sign an informed consent that they wished to give their AM. After that, the AM was sliced into pieces of 2 by 2 cm and adhered on nitrocellulose backing paper. The grafts were then stored in 50 µg/ml penicillin, 50 µg/ml streptomycin, 100 µg/ml neomycin and 2.5 µg/ml amphotericin B with balanced salt solution at +4°C for two weeks.¹² Operative swabs were used for scraping the ulcer's base while the patient was under local anesthesia. Overlay AM grafts were completed. Using interrupted 6-0 Vicryl sutures and episcleral bites around the limbus, a single layer of AM was sutured. The AM spanned the entire corneal surface. The stromal side of the membrane was positioned downward. One month after the transplantation, procedural success rate was noted. A predesigned Proforma was used to collect all study data.

Data Analysis: SPSS v20.0 was used to analyse the data. For quantitative characteristics such as age, weight, height, BMI and ulcer duration, mean & standard deviations were computed. Success rate and gender were examples of qualitative characteristics that were displayed as percentages and frequencies. Stratification was used to adjust for effect modifiers like gender, age, diabetes (yes/no), ulcer duration and BMI. To find out how these confounding factors affected the AMT success rate, the post-stratification chi-square test was used. A P-value of less than 0.05 was considered a significant difference.

RESULTS

Mean age was 47.23 ± 13.31 years. The minimum age was twenty while the maximum age was seventy years. (Table 1).

There were more males as compared to females included in this study. There were 60 (69.77%) males and 26 (30.23%) female patients (Figure 1).

Mean height was 167.20 ± 8.07 cm. Mean weight of patients was 69.02 ± 10.97 kgs. Mean BMI was 24.96 ± 4.21 kg/m². Minimum BMI was 18.10 kg/m² and maximum BMI was 39.10 kg/m² (Table 2).

Mean duration of corneal ulcer was 7.26 ± 3.05 months. Minimum duration was two months and maximum duration was fifteen months (Table 3).

Mean corneal ulcer size before treatment was 5.16 ± 1.34 mm. Minimum size was 02 mm and maximum size was 08 mm (Table 4).

Mean corneal ulcer size after treatment was 0.68 ± 1.22 mm. Minimum size was 0.00 mm and maximum size was 05 mm (Table 5).

Mean pain score before treatment was 5.93 ± 1.04 . Minimum score was 04 and maximum score was 8.00 (Table 6).

Mean pain score after treatment was 1.24 ± 1.50 . Minimum score was zero and the maximum score was 6 (Table 7).

Diabetes was diagnosed in 17 (19.77%) patients while remaining 69 (80.23%) patients were not having diabetes (Figure 2).

On frequency of success rate of AMT, success of AMT was observed in 76 (88.37%) and AMT was unsuccessful in just 10 (11.63%) patients (Figure 3).

Patients who were between 20-45 years of age, stratification of age was performed. Success rate of AMT was observed in 37, while in patients having age 46-70 years, success rate was observed in 39 patients. With a P-value of 0.605, this change was statistically insignificant. (Table 8).

Table 1: Age related descriptive statistics

Age in years	
Mean	47.23
Standard deviation	13.31
Min.	20
Max.	70

Gender stratification was also conducted in males, success of AMT was found in 52 versus in females AMT was successful in

24. With a p-value of 0.454, this difference was also statistically insignificant. (Table 9).

On the basis of BMI and ulcer duration, stratification was also performed. No association was found with success rate of AMT.

Table 2: Height, weight & BMI related descriptive statistics

	Hgt. (cm)	Wg. (Kg)	BMI (Kg/m ²)
Mean	167.20	69.02	24.96
S.D.	8.07	10.97	4.21
Minimum	143	47	18.10
Maximum	182	97	39.10

Table 3: Ulcer duration related Descriptive statistics

Ulcer duration in Months	
Mean	7.26
S.D.	3.05
Min.	2
Max.	15

Table 4: Descriptive statistics of corneal ulcer size before treatment.

Corneal Ulcer Size before treatment (mm)	
Mean	5.16
S.D.	1.34
Minimum	02
Maximum	08

Table 5: Descriptive statistics of corneal ulcer size after treatment.

Corneal Ulcer Size after treatment (mm)	
Mean	0.68
S.D.	1.22
Minimum	0.00
Maximum	5.00

Table 6: Descriptive statistics of pain score prior to treatment.

Pain Score prior to treatment	
Mean	5.93
S.D.	1.04
Minimum	04
Maximum	08

Table 7: Descriptive statistics of pain score after treatment.

Pain Score after treatment	
Mean	1.24
S.D.	1.50
Minimum	0.00
Maximum	6.00

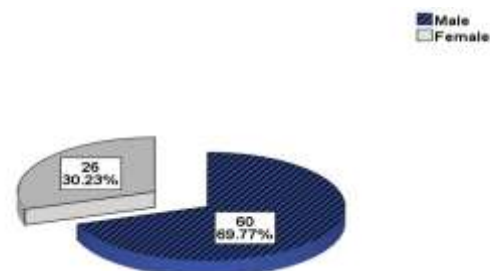


Figure 1: Frequency of gender.

Table 8: Age stratification to ascertain the relationship b/w age and AMT success rate

Age Group	AMT		P-value
	Yes	No	
20-45 Years	37	04	0.605
46-70 Years	39	06	

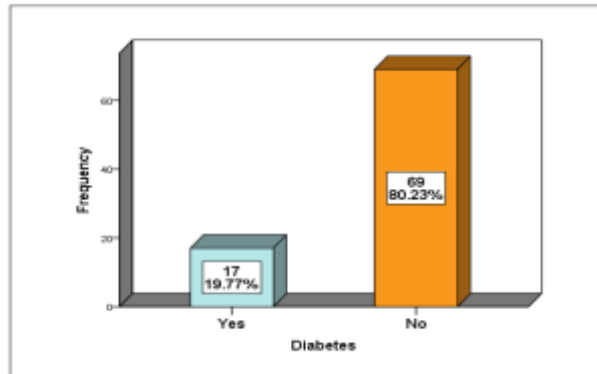


Figure 2: Frequency of diabetes

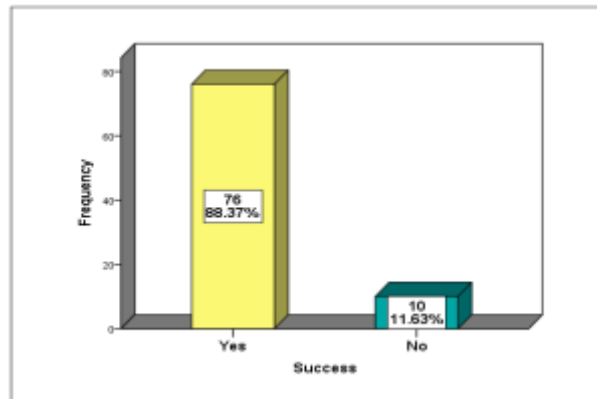


Figure 3: Frequency of success rate of AMT

Table 9: Stratification of gender to determine the association of gender with success rate of amniotic membrane transplantation (AMT).

Gender	Amniotic Membrane Transplantation (AMT)		P-value
	Yes	No	
Male	52	08	0.454
Female	24	02	

Table 10: BMI stratification to ascertain the relationship b/w BMI & AMT success rate

BMI	Amniotic Membrane Transplantation (AMT)		P-value
	Yes	No	
≤29.99 Kg/m ²	40	06	0.661
≥25.00 Kg/m ²	36	04	

Table 11: Diabetic stratification to ascertain the relationship between diabetes & success rate of AMT

Diabetes	Amniotic Membrane Transplantation (AMT)		P-value
	Yes	No	
Yes	15	02	0.984
No	61	08	

Table 12: Stratification of ulcer duration to ascertain the relationship b/w ulcer duration & AMT success rate

Ulcer Duration	AMT		P-value
	Yes	No	
2-6 Months	15	02	0.984
7-15 Months	61	08	

DISCUSSION

According to histology, AM is composed of a single epithelial layer, thick basement membrane and a non-vascular stroma. A number of mechanisms have been put forth to explain the AM's biological

actions in modulating adult wound healing toward the fetal direction with anti-inflammation, antiscarring and antiangiogenesis.¹³

When properly processed and preserved, preferably using the cryopreservation method, this semi-transparent and resilient tissue has been successfully used as a surgical graft for a wide range of ophthalmic indications.^{14,15} In particular, more than 800 scientific papers detailing the use of AMT for a range of ocular reasons have been published in the past 15 years. The majority of the studies testify to the clinical efficacy of AMT in gearing adult wound healing toward regeneration with minimal inflammation and scarring, suggesting that AM, like fetal tissue, carries a similar feature that may not only promote healing but also facilitate regeneration.^{16,17}

Amniotic membrane transplantation has been used for the treatment of various unresponsive ocular surface diseases to topical medication during the last two decades.¹⁸ It is clearly demonstrated that AMT has a positive effect on the healing process of the damaged eye and its application is indispensable for the reduction of prominent subjective symptoms like pain, photophobia, tearing and hyperemia.¹⁹ Still a steady rise in thickness of cornea and re-epithelialisation is the most important advantage of AMT.

Espana et al. reported that AMT provided pain relief in 88% and epithelial healing in majority of the patients who presented with bullous keratopathy and less visual potential. They concluded that AMT is a safe and durable alternative to conjunctival flaps when corneal transplantation isn't an option.²⁰

Either a routine vaginal delivery or an elective C-section can yield the AM. In our cases, we used placenta obtained after elective caesarean for a seronegative hepatitis B, C virus women, to avoid the greater risk of infection from normal vaginal delivery as it is recommended by Philip et al. that amnion for use in ocular surface procedures should be better retrieved from placentas following elective caesarean deliveries because of greater risk of contamination from pathogenic bacteria on placentas from vaginal deliveries.²¹

In a refractory, non-infected corneal ulcer, we applied a preserved AMT, which demonstrated promising outcomes in enhancing comfort, decreasing inflammation & encouraging epithelial repair. Fresh and refrigerated AM grafts were compared clinically and in vitro by Philip et al. They found that in both cases the cornea re-epithelialised and visual acuity improved, showing no differences in healing process between the two.²¹

In both acute and chronic chemical eye injuries, Ucakhan et al. assessed the safety & effectiveness of non-preserved AMT with or without limbal autograft transplantation. They found that AMT promoted epithelial healing, reduced inflammation, increased comfort and decreased severity of vascularization, but in our patients there were no decreased in severity of vascularization.²²

Kruse et al. noted that ocular surface inflammation was markedly reduced following AMT.²³ This finding may be explained by other recent studies showing that the stromal matrix of the amniotic membrane excludes inflammatory cells, contains various forms of protease inhibitors and suppresses transforming growth factor β (TGF- β) signaling and proliferation and myofibroblast differentiation of normal human corneal and limbal fibroblasts.²⁴

Amniotic membrane graft can be applied in two ways: either by the inlay technique which involves inserting the AM graft into the corneal ulcer & holding it there with interrupted 10/0 nylon sutures, or by the overlay technique, which covers all of the corneal surface, including the limbus with the AM graft. We used the overlay technique and this was comparable to Letko et al. who compared inlay versus overlay AM grafting techniques in ability to heal persistent corneal epithelial defects and he found no difference in terms of healing time and recurrence rate.^{25,26}

The study under discussion highlights the success of single layer approach in treating non-infected corneal ulcers. In our study the success rate of 88.37% was found. Our success rate using single layer technique is comparable to that of double layer

technique of AMT. Hanada et al. that reported complete healing in 16.5 ± 8.0 days in 8/11 eyes using multiple layers of amniotic membrane to treat deep corneal ulcers with descemetocoele.²⁶

A study conducted by Mohan et al. on clinical outcomes of AMT in patients with non-healing corneal ulcers reported a success rate of 82.1%.⁸ Another Study conducted by Manolova et al. on the success rate of AMT found a success rate of 91.2% after AMT.⁹ Khan et al. conducted a study in Pakistani population on the success rate of AMT in non-healing corneal ulcers & reported a success rate of only 74.5%,¹⁰ while another study from Pakistan reported a success rate of 93.3%.¹¹

On the basis of present study results and existing literature, we can conclude that treatment of non-healing corneal ulcers using AMT is an acceptable option and is associated with very high success rate, at the cost of no or minimum number of complications.

CONCLUSION

AMT can effectively treat non-healing corneal ulcers that are resistant to topical medication. These results are consistent with international and local studies reinforcing AMT as a natural graft with potent antiinflammatory, antiscarring and antiangiogenic properties. Its ability to provide a scaffold for epithelial growth while simultaneously supporting the wound healing environment makes it suitable for persistent epithelial defects and corneal ulcers resistant to topical medication. Given its high efficacy, low complication rate and cost effectiveness, AMT should be considered as a frontline surgical option in the treatment of non-healing corneal ulcers, particularly in developing countries. Continued research with longer follow-up and large samples will further validate these findings.

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