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The Effect of Amniotic Membrane Extracted Eye Drop on Repairing The Corneal Epithelial in Patients after Trans-Epithelial Photorefractive Keratectomy: A Randomized Controlled Trial

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Abstract

Objective: Recent studies imply extensive applications for the human amniotic membrane (hAM) and its extract in medicine and ophthalmology. The content of hAM meets many requirements in eye surgeries, such as refractive surgery as the most important and commonly used method for treating the dramatically increasing refractive errors. However, they are associated with complications such as corneal haziness and corneal ulcer. This study was designed to evaluate the impact of amniotic membrane extracted eye drop (AMEED) on Trans-PRK surgery complications.

Materials and Methods: This randomized controlled trial was performed during two years (July 1, 2019-September 1, 2020). Thirty-two patients (64 eyes), including 17 females and 15 males, aged 20 to 50 years (mean age of 29.59 ± 6.51) with spherical equivalent between -5 to -1.5 underwent Trans Epithelial Photorefractive Keratectomy (Trans-PRK) surgery. One eye was selected per case (case group) and the other eye was considered as control. Randomization was done using the random allocation rule. The case group was treated with AMEED, and the artificial tear drop every 4 hours. The control eyes received artificial tear drops instilled every 4 hours. The evaluation continued for three days after the Trans-PRK surgery.

Results: A significant decrease in CED size was found in the AMEED group on the second day after surgery (P=0.046). Also, this group had a substantial reduction in pain, hyperemia, and haziness.

Conclusion: This study showed that AMEED drop can increase the healing rate of corneal epithelial lesions after Trans-PRK surgery and reduce the early and late complications of Trans-PRK surgery. Researchers and Ophthalmologists should consider AMEED as a selection in patients with persistent corneal epithelial defects and patients who have difficulty in corneal epithelial healing. We understood AMEED has a different effect on the cornea after surgery; therefore, the researcher must know AMEED's exact ingredients and help expand AMEED uses (registration number: TCTR20230306001).

Keywords: Amnion, Photorefractive Keratectomy, Refractive Surgical Procedures, Wound Healing

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Introduction

Recent studies have shown that the human amniotic membrane (hAM) and its extract have many potentials in medicine and ophthalmology applications because of their unique content (1, 2). The hAM includes growth factors, collagens (I, III, IV, and V), lamina, and cytokines with anti-inflammatory (3), anti-scaring, anti-apoptotic, anti-angiogenic (4), and anti-fibrotic properties (1). In the literature, a standard method has been described that produces an amniotic membrane extract for making the amniotic membrane extracted eye drop (AMEED) with the same properties as the amniotic membrane. According to the relevant reports, AMEED increases *in vitro* limbal stem cells and corneal epithelial repair in rabbits (5) and horses (6) after injury. No complications have been reported for AMEED yet (5, 7).

On the other hand, refractive error is the most common cause of vision problems globally. According to a statement issued by the World Health Organization, myopia as a refractive disorder will affect half of the world's population by 2050 (8, 9). One way to eliminate the refractive conditions is surgery, which can be performed through various methods, including photorefractive keratectomy (PRK), Trans-PRK, laser subepithelial keratomileusis (LASEK), and laser in situ keratomileuses (LASIK). All these methods can also be used to correct the corneal curvature. In the Trans-PRK modality, which is a less invasive method compared to PRK, the patients suffer from two types of post-surgery complications: early and late complications. Early complications include CEDs, eye pain, conjunctival

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injection or hyperemia, tearing, and photophobia. Late complications include visual acuity decrease and corneal haziness, which reaches its peak within 1 to 2 months after surgery and then gradually disappears within 6 to 12 months, which can delay reaching the patient's final vision (10, 11).

Accordingly, a triple-blinded historical randomized controlled trial was designed to evaluate the impact of AMEED on the CEDs, conjunctival injection, eye pain, corneal haziness, and visual acuity after Trans-PRK surgery.

Materials and Methods

Ethical considerations

The study was considered by the Baqiyatallah Ethics Committee (IR.BMSU.BAQ.REC.1399.032) and Thai clinical trials registry (TCTR20230306001) all patients who agreed to undergo trans-PRK surgery signed an informed consent to be entered into this clinical trial. The study was performed following the principles of the Helsinki Declaration.

Intervention

This randomized controlled trial was carried out in the Baqiyatallah Hospital between 2019 and 2020 on patients aged 20 to 50 years who attended the ophthalmology clinic for refractory problems. They were included in the study if they had a range of spherical equivalent between -5 to -1.5, measured by an auto-refractometer and the approval of trial lenses. The patients with a history of using topical ophthalmic drugs in the past three months, eye surgery at any time, and use of drugs that affect the tear condition (blood pressure medication, rheumatism medication, antipsychotic medication, etc.) were excluded from the study. Additionally, the patients with a history of any eye diseases other than refractive disorders, diabetes history, drug allergy, rheumatic disease, or any other systemic disease, and anisometric eyes were excluded from the study. During the Trans-PRK surgery (SCHWIND AMARIS 1050RS) on each patient, one eye was selected as the case, and the fellow eye was considered as the control using a random allocation rule for randomization. Contact lenses and routine medications were used in patients after Trans-PRK surgery. The case group was treated with AMEED (Lifecell Pharmaceuticals, Inc. Iran) every four hours and artificial tear drop every four hours (conventional treatment with AMEED) up to three days post-surgery. In the control eyes (fellow eyes), artificial teardrop (conventional treatment) was instilled every four hours within the same duration. The cornea was examined daily in both groups until the complete epithelial repair. The patients' eyes were stained with fluorescein, and a photo of the stained part (the same part of the epithelial defect) was taken with a photo slit device. The size of the defect in the picture was determined by ImageJ version 1.2 software, and then it was augmented by manual refinement. Patients after corneal epithelial healing were re-examined in the first, third, and sixth months after surgery.

It should be noted that both groups of patients have received routine and standard treatments. Regular medications included chloramphenicol 0.5% ophthalmic drop (Sina Darou Laboratories, Inc. Iran), Betamethasone (Darou Pakhsh Pharmaceutical Manufacturing Company, Iran), artificial tears (Iranian Parenteral & Pharmaceutical Co. Iran), Fluorometholone (Sina Darou Laboratories, Inc. Iran), and Liposic ophthalmic gel (Bausch and Lomb, Inc. UK) for both groups.

All measurements were performed using a Haag-Streit Slit lamp (BQ 900, Haag-Streit, Koeniz, Switzerland) with a standard zoom of sixteen. We evaluated the conjunctival injection grading using the Efron system (Fig.1) (12) and measured the corneal haziness according to the standard evaluation (Table 1) (13). The pain measurement was also based on a 0-10 Likert scale.

Statistical analysis

The collected data were analyzed by SPSS software version 16 (IBM, Chicago). We used Mann-Whitney U and Kendall correlation tests. $P<0.05$ was considered statistically significant, and data was depicted by mean ± standard deviation (95% confidential interval).

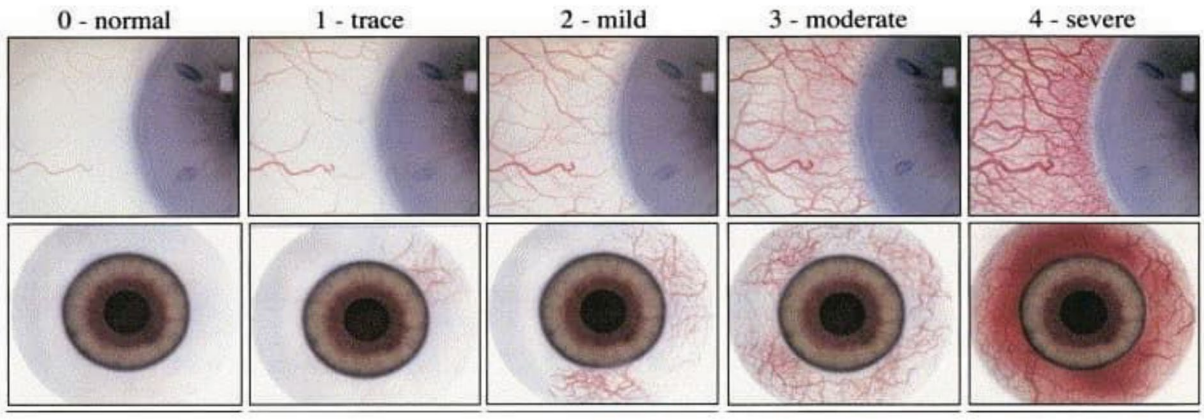

Fig.1: Conjunctival Injection Grading in Efron system. Efron system was used for injection grading in this study.

Table 1: Corneal haziness grading according to standard evaluation

Grade	Characteristics
Grade 0	No corneal haze
Grade 1	Iris details visible
Grade 2	Pupillary margin visible, iris details not visible
Grade 3	Pupillary margin not visible
Grade 4	Corneal totally opaque

The standard evaluation was used for corneal haziness grading in this study.

Results

This study evaluated 32 patients (64 eyes), including 17 females and 15 males, with a mean age of 29.59 ± 13.02 years at the time of Trans-PRK surgery. After three days post-surgery, all CEDs disappeared from the cornea in both groups. On the second day after surgery, corneal epithelial defect in AMEED and control groups was 2.42 mm^2 and 3.84 mm^2, respectively, showing a significant difference (P=0.046, Table 2).

Table 2: CED Size in myopic patients after TRANS-PRK surgery in AMEED and control group (fellow eye)

Groups	AMEDD	Control
Size on surgery day (mm^2)	46.82 ± 3.70	46.52 ± 6.02
Size first day after surgery (mm^2)	14.77 ± 12.64	17.34 ± 12.36
Size second day after surgery (mm^2)	2.42 ± 6.28	3.84 ± 4.76*
Size third day after surgery (mm^2)	0.18 ± 2.08	0.08 ± 0.98

Data are presented as mean ± SD. *; P<0.05, Mann-Whitney U.

Moreover, the conjunctival injection was markedly more reduced in the AMEED group on the second (P=0.019) and third days (P=0.004) after surgery (Table 3). Three months after surgery, the haziness was significantly lower in the AMEED group (P=0.040, Table 4). As expected, the corneal haziness in each follow-up had a significant negative correlation with the visual acuity. Spearman correlation test between visual acuity and haziness for first, third, and sixth month post-surgery in all patients was R1=-0.49 (P<0.001), R3=-0.57 (P<0.001), and R6=-0.75 (P<0.001), respectively.

Table 3: Injection in myopic patients after TRANS-PRK surgery in AMEED and control group (fellow eye)

Groups	AMEED	Control
Pain first day after surgery	3.65 ± 3.02	3.87 ± 4.16
Pain second day after surgery	1.15 ± 2.10	1.43 ± 2.58
Pain third day after surgery	0.03 ± 0.34	0.31 ± 0.84*
Injection first day after surgery	2.09 ± 1.70	2.40 ± 1.66
Injection second day after surgery	0.96 ± 1.28	1.37 ± 1.30*
Injection third day after surgery	0.09 ± 0.58	0.40 ± 0.98*

Data are presented as mean ± SD. *P<0.05, Mann-Whitney U.

Table 4: Haziness and visual acuity (Late complication) in myopic patients after TRANS-PRK surgery in AMEED and control group (fellow eye)

Groups	AMEED	Control
Haziness after 1 month	0.40 ± 0.88	0.46 ± 1.00
Haziness after 3 months	0.12 ± 0.66	0.34 ± 0.96*
Haziness after 6 months	0.06 ± 0.48	0.06 ± 0.48
Visual acuity baseline	0.77 ± 0.60	0.76 ± 0.64
Visual acuity after 1 month	0.02 ± 0.06	0.02 ± 0.06
Visual acuity after 3 months	0.00 ± 0.02	0.00 ± 0.02
Visual acuity after 6 months	0.0000 ± 00	0.0000 ± 00

Data are presented as mean ± SD. *P<0.05, Mann-Whitney U.

Discussion

Refractive errors, especially myopia, are dramatically increasing worldwide (14, 15). Refractive surgery is a popular method of solving this problem. PRK and TRANS-PRK surgery are surface ablation methods for epithelial removal. Trans-PRK is a less invasive method and can control the size of the corneal epithelial defect (16-18). As long as CEDs exist on the cornea's surface, they can be the beginning of many other problems (19). The most notable consequence is corneal ulcers due to epithelial progression in inflammatory processes (20). Therefore, one of the most critical measurements to prevent postoperative complications after refractive surgery is the faster resolution of epithelial defects. The triple-blind placebo-controlled RCT used in this study assessed the effects of AMEED in postoperative TRANS-PRK surgery complications compared to the control group (fellow eyes) and showed faster epithelial healing without clinically relevant side effects.

The present study's findings showed that on the surgery day and the first day after surgery, the CED size was similar in both groups. On the second day after surgery, the CED size was significantly smaller in the AMEED group than in the controls. On the third day after surgery in both groups, CED disappeared. Considering that literature suggests time as one of the most influential factors, in the present study, in the AMEED-received eyes, CEDs healed faster; however, on the third day, all CEDs disappeared due to 24 hours crossing. This rapid healing rate could be because of the mostly young age of subjects (mean age was 29.59 years) and the absence of any ocular disease other than a refractive error in all cases. In a review by Murri et al. (7), the efficacy of AMEED for the acceleration of the corneal epithelial defect was investigated, and it was shown that it is potentially valuable for the ocular surface disorder. Another study by Sabater-Cruz et al. (21) used AMEED for the wound healing delay group and dry eye disease group for the long term. The

study demonstrated that AMEED could heal persistent epithelial defects, dry eye disease, and corneal ulcers without any adverse effects. Also, a survey by Kordić et al. (22) depicted that AMEED was successfully applied for persistent epithelial defect in two patients (two drops per hour), which is consistent with the present result.

In the studied patients, the reported pain as a post-surgical early complication on the first and second day after surgery was similar between AMEED and control eyes. On the third day after the operation, the pain significantly reduced in AMEED groups. The conjunctival injection was markedly less on the second and third days after surgery. Similarly, a case series study on the chemical burn showed that amniotic membrane extract significantly reduced pain and inflammation (23). These results indicate that AMEED has an ameliorative effect on the post-surgical early complications.

Furthermore, a significant difference was observed in the haziness as a late complication three months after surgery between the AMEED and control groups. Accordingly, we hypothesized that time has a vital role in haziness results since that in the first-month follow-up, AMEED and control groups were quite similar in haziness, while after six months, haziness was recovered entirely. We concluded that in the first month the time has not been enough to make differences.

Therefore, for a more reliable evaluation, performing the same procedure in a population of older adults with short interval consideration is suggested. Moreover, considering less than 24 hours intervals of the last CED sizing examination time would probably reveal different results. A permeable study demonstrated the dose-dependent efficacy of AMEED (5). Therefore, applying AMEED at a higher dose (with intervals less than every 4 hours) is expected to confer more difference between test and control eyes.

Conclusion

This study shows that AMEED can increase the healing rate of corneal epithelial lesions after Trans-PRK surgery and reduce the early and late complications of Trans-PRK surgery. Further studies can approve this medication for reducing Trans-PRK complications. Conducting other studies for evaluation of AMEED for treatment of patients with persistent corneal epithelial defects and patients who have difficulty in corneal epithelial healing is also suggested. Considering the variety of ingredients present in this extract, finding the active ingredient that delivers the greatest effect on improving epithelial defects will be of interest.

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Authors' Contributions

A.R., Kh.J., A.Ch.; Concept and design. A.R., A.Ch., H.A., A.A.A., M.N.; Data acquisition and drafting the manuscript. A.R., A.Ch., H.A., A.A.A., M.N., K.J.; Data analysis and final approval of the manuscript. K.J.; Critical revising of the manuscript. All authors read and approved the final manuscript.

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