Efficacy and Safety of Amniotic Membrane Transplantation Combined with Closure of Tenon Capsule and Bulbar Conjunctival Space in the Treatment of Primary Pterygium

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Objective. The aim of the study is to evaluate the safety and effectiveness of amniotic membrane transplantation combined with the closure of the tenon capsule and bulbar conjunctival space. Methods. This study retrospectively included 100 patients with primary pterygium who received closed bulbar conjunctiva and tenon capsule space combined with amniotic membrane transplantation in our hospital from January 2020 to June 2021 as the experimental group and 100 patients with routine treatment in the same period as the control group. The postoperative efficacy evaluation and postoperative complications of the two groups were compared, so as to comprehensively evaluate the safety and effectiveness of this method. Results. The results showed that the postoperative complications of the two groups were significantly improved by Fisher’s exact test ($\chi^2 = 14.510$, $P < 0.006 < 0.05$). The comparison results showed that the treatment group showed significant advantages in six indexes compared with the observation group and the difference between the two groups was statistically significant ($P < 0.05$) of in the NRS score, Prabhasawat score, inspection of the ocular surface comprehensive analyzer, corneal fluorescein staining, conjunctival fluorescein staining in the operation area, breakup time of tear film examination of the two groups at 3, 7 and 14 days, and 1, 6 and 12 months after the operation. Conclusions. Amniotic membrane transplantation combined with the closure of the tenon capsule and bulbar conjunctival space is safer than conventional surgery in the treatment of primary pterygium. It has a shorter recovery time, higher safety, and a positive curative effect. It can be considered to popularize this operation in clinic.

1. Introduction

Pterygium is a common chronic ocular surface inflammatory disease. The domestic prevalence rate is about 9.84% [1]. It is estimated that the total number of pterygium in China is more than 100 million. Surgical excision is the only effective treatment for pterygium in clinic [2]. The commonly used surgical methods are based on the excision of pterygium, combined with conjunctival transplantation, amniotic membrane transplantation, and so on. However, at present, there is no combined operation considered to be the best choice [3]. Routine operations will not only affect the local microenvironment stability and scar repair but also easily cause complications such as granuloma and chronic conjunctivitis, resulting in long-term red eye and discomfort. Therefore, the existing surgical methods for pterygium still need to be continuously improved. This study focuses on these aspects.

2. Materials and Methods

2.1. General Information. This study retrospectively included 100 patients with primary pterygium who received closed bulbar conjunctiva and tenon capsule space combined with amniotic membrane transplantation in Yan’an Hospital of Kunming City from January 2020 to June 2021 as the experimental group and 100 patients with routine treatment in the same period as the control group. The postoperative efficacy evaluation and postoperative complications of the two groups were compared, so as to
comprehensively evaluate the safety and effectiveness of this method. Both groups of patients signed informed consent before the operation, and this study has been approved by the medical ethics committee of Yan’an Hospital of Kunming City as seen in Figure 1.

2.2. Inclusion Criteria. The inclusion criteria were as follows: I. age ≥ 18 years. II. pterygium was diagnosed; III. they were treated by closing the space between the bulbar conjunctiva and the tenon capsule, combined with amniotic membrane transplantation.

2.3. Exclusion Criteria. The exclusion criteria were as follows: I. Patients with recurrent or secondary pterygium. II. Patients with ocular surface-related diseases or any history of ocular bleeding. III. History of major ocular trauma or major ocular surgery. IV. Patients with critical diseases such as a malignant tumor or serious infection. V. Pregnant or lactating women. VI. Patients with incomplete follow-up records.

2.4. Scope and Shape Design of Operation Area. The eyelid opener was used to open the eyelid with lidocaine (2 ml, 20 g/L) and adrenaline (0.2 ml, 1 g/L) mixture under the microscope is used to perform infiltration anesthesia subcutaneously on the conjunctiva of the pterygium flesh. After the anesthesia was completed, a “bottom incision” was made at the pterygium flesh at the semilunar fold of the conjunctiva (about 10–12 mm from the corneal limbus) in the direction of the vertical palpebral fissure, and the depth was up to the subcutaneous part of the conjunctiva (the same below), with a length of about 15 mm. Then, we make two “side incisions”, respectively, from the end of the “bottom incision” to the limbus cornea, with the direction biased towards the pupil area while a “top incision” was formed between the incisions on both sides of the limbus cornea, and finally, a “trapezoidal operation area” with a “bottom incision” of 15 mm, a “top incision” of 10 mm and a height of about 10–12 mm is formed.

2.5. Pterygium Excision. We lifted the bulbar conjunctiva on the outside of the incision in the “trapezoidal operation area”, used the micro scissors to separate the conjunctiva and its lower fascia tissue inwards, upwards, and downwards under the conjunctiva, and then swept and bluntly separated the pterygium and sclera in the operation area. Then, we cut the pterygium tissue from the half-moon wrinkle wall, turned the pterygium towards the cornea, and scraped the pterygium tissue on the sclera surface. We lifted the neck of the pterygium, gently tore it off to the head along the direction of its fiber growth, transferred the larger pterygium up and down, and then tore it off until the pterygium tissue was completely stripped.
2.6. Closing of Bulbar Conjunctiva and Tenon Capsule Space Combined with Amniotic Membrane Transplantation. Under the microscope, the treatment group trimmed the biological amniotic membrane to the “trapezoidal amniotic membrane” slightly larger than the operation area with micro-scissors and micro-tweezers. The bottom edge (corresponding to the “bottom incision”) was 16–18 mm, the top edge (corresponding to the “top incision”) was 12–14 mm, and the height was about 14–16 mm. The biological amniotic membrane (State Food and Drug Administration firearms approval no. 3460894, 2010) was used with the base facing downwards. The upper skin is laid up between the scleral surface and conjunctiva in the operation area. At the lacrimal of the caruncle, the tweezers were used to explore the gap between the conjunctiva and the tenon’s capsule. Following, the microscopic tweezers and iris restorer were applied to smoothly extend the bottom edge of “trapezoidal amniotic membrane” into the gap (2–4 mm) for filling. Then, we continuously suture the gap with a 10–0 suture and fix it in the shallow sclera to completely close the gap. The amniotic membrane was attached to the scleral surface by routine intermittent suture in other positions, and the eyes were coated with tobramycin dexamethasone eye ointment after the operation. The control group was treated with routine treatment.

2.7. Postoperative Treatment. The operated eyes were treated with 0.1% fluminolone (tid) and tobramycin eye drops (qd). The gauze was unpacked 24 hours after the operation and the suture was removed 7 days after the operation. Fluminolone (0.1%, tid) and sodium hyaluronate eye drops (0.1%, tid) were used 14 days after the operation. The drug was stopped 1 month after operation.

2.8. Return Visit Time and Return Visit Inspection Index. The follow-up time was 3, 7, and 14 days and 1, 6, and 12 months after the operation; the examination indexes included NRS score, Prabhasawat score, ocular surface comprehensive analyzer, corneal and conjunctival fluorescein staining, and tear film rupture time.

2.9. NRS Score. Numbers 0–10 instead of words were used to express the degree of pain. A straight line was divided into 10 segments and was employed to evaluate the degree of pain in the order of 0–10 points. The writing method is to circle the number describing the most severe pain in the past 24 hours. 0 was painless; 1–3 were mild pain (pain does not affect sleep); 4–6 were moderate pain; and 7–10 were severe pain (unable to sleep or wake up from sleep).

2.10. Outcomes. The health status of the ocular surface microenvironment after operation, cure rate, the 1-year recurrence rate, and postoperative complications are as follows.

(i) Recovery: the operation area is smooth, the conjunctiva was flat, there was no edema and congestion, the corneal epithelium was smooth, and there was no neovascularization and pterygium hyperplasia.

(ii) Recurrence: the conjunctiva was obviously congested and hypertrophic, and the corneal wound has neovascularization and connective tissue hyperplasia.

2.11. Prabhasawat Score. Grade 1: the appearance of the surgical site is the same as that of the normal bulbar conjunctiva structure. Grade 2: vascular dilatation on the surface of the sclera extending to the limbus of the cornea can be seen at the operation site, but there is no fibrous tissue hyperplasia. Grade 3: on the basis of grade 2, there is obvious fibrous vascular tissue hyperplasia, but it does not exceed the corneal limbus. Grade 4: like scar tissue hyperplasia invading cornea or true pterygium recurrence.

2.12. Inspection of an Ocular Surface Comprehensive Analyzer. The height of the tear meniscus height of the at the inner, middle, and outer measuring points of the patient’s and the lower tear meniscus average height were calculated by the “tear meniscus height calculation mode” of the eye surface comprehensive analyzer. The conjunctival vascular density and hyperemia in the operation area were evaluated by the “eye red analysis model.”

2.13. Corneal and Conjunctival Fluorescein Staining. Corneal fluorescein staining was detected by the corneal quartering method (upper left, upper right, lower left, and lower right quadrants). The fluorescein staining of each quadrant was observed with cobalt blue light under 16X. There was no corneal fluorescein staining was 0 point, there were scattered fluorescein staining spots in the cornea and the number was no more than 5 was 1 point, there were scattered fluorescein staining spots in the cornea and the number had exceeded 5 and there was no fusion was 2 points, and fused fluorescein staining areas or filaments that appeared in the cornea (fluorescent pigment spots could not be counted) were 3 points. The size of the conjunctival staining area in the operation area was measured by Image J (Version 1.52) software.

2.14. Tear Film Rupture Time Examination. After staining with a sodium fluorescein test strip, we asked the patient to blink 3 times, look straight ahead, and observed the formation time from the last blink to the first tear film rupture spot with slit lamp cobalt blue light 16X. We completed three repeated measurements within 2 minutes and took the average value as the final count.

2.15. Statistical Analysis. The statistical analyses were performed using the Statistical Package for the Social Sciences version 20.0 (SPSS Inc., Chicago, IL, USA). Nonnormally distributed metric variables were analyzed by the Kruskal–Wallis test and Mann–Whitney test. \( P \leq 0.05 \) was
3. Results

3.1. Comparison of Preoperative General Conditions between the Two Groups. There was no significant difference in age, gender, height, weight, and BMI between the two groups before operation, all $P > 0.05$, as seen in Table 1.

3.2. Comparison of Postoperative Complications between the Two Groups. The results showed that the postoperative complications of the two groups were significantly improved by Fisher’s exact test $\chi^2 = 14.510$, $P = 0.006 < 0.05$, with a significant statistical difference, as seen in Table 2 and Figure 2.

considered statistically significant. Values were expressed as mean ± standard deviation, unless stated otherwise.

3.3. Comparison of Postoperative Follow-Up Indexes between the Two Groups. The NRS score, Prabhasawat score, inspection of an ocular surface comprehensive analyzer, corneal fluorescein staining, conjunctival fluorescein staining in the operation area, breakup time of tear film examination of the two groups at 3, 7, and 14 days, 1, 6, and 12 months after operation were observed. The comparison results showed that the treatment group showed significant advantages in six indexes compared with the observation group, and the difference between the two groups was statistically significant ($P < 0.05$), as seen in Table 3.
3.3.1. Conjunctival Manifestations before, during, and after Operation. Conjunctival manifestations before, during and after operation are as seen in Figure 3.

4. Discussion

Pterygium is a common chronic ocular surface inflammatory disease, and the prevalence rate in China is about 9.84% [4]. Based on this calculation, it is estimated that the total number of patients with pterygium in China is more than 100 million [5]. Clinically, the clinical manifestations of the disease are mainly characterized by the proliferation and degeneration of the conjunctiva in the palpebral fissure area and the invasion of the lower fibrous vascular tissue across the limbus to the cornea in a “triangle” [6–8]. Although the pathogenesis of pterygium is not clear, long-term ultraviolet radiation, destruction of the limbal stem cell barrier, and degeneration and proliferation of conjunctiva are considered to be the three factors that promote the occurrence of primary pterygium [9, 10]. Surgical excision is the only effective treatment in the clinic and the commonly used methods are based on the excision of pterygium, combined with conjunctival transplantation, amniotic membrane transplantation, and so on [11–13].

However, no combined surgery is considered to be the best choice nowadays. Conjunctival transplantation usually takes the conjunctival tissue at the upper limbus, which has the advantage of direct and complete component transplantation, and prevents the proliferation and migration of the diseased conjunctiva through healthy conjunctival tissue [14–16]. The transplantation will bring regional damage to the upper conjunctiva, which will not only affect the local microenvironment stability and form tissue scar repair but also have a great adverse impact on glaucoma surgery. The scope of conjunctival transplantation is limited. For large pterygium, the complete resection of diseased tissue could not be guaranteed. Amniotic membrane transplantation is another common combination of pterygium resection and as a kind of basement membrane for tissue growth, amniotic membrane is transplanted into the operation area after pterygium resection, which can help the normal conjunctival epithelial cells around it to proliferate and migrate, reform healthy conjunctival tissue, and avoid damaging the healthy conjunctival tissue of patients [17–19]. The postoperative recurrence rate is close to that of conjunctival transplantation. If some pathological tissue remains after operation, it may still use amniotic membrane for proliferation and migration, and then form recurrent pterygium [20–22]. In conclusion, incomplete resection of degenerative conjunctiva and its underlying tissues, slow recovery of limbal stem cells in the damaged area, chronic conjunctival inflammation, and persistent congestion in the operation area are the reasons for the increase of postoperative recurrence rate [23, 24]. At the same time, the recovery of pterygium after operation is the reconstruction process of ocular surface microenvironment stability. The conjunctiva in the nasal blepharoplasty area is the common site of pterygium. The normal morphology of the tear meniscus and nasal conjunctival semilunar fold below this area is an important factor to maintain the microenvironment stability [25]. If there is residual fascia tissue and the half-moon fold incision is not well aligned during the operation, it will affect the proliferation and migration of normal conjunctival epithelial cells around this area, cause abnormal tear distribution and dynamics in this area, and finally form complications such as granuloma and chronic conjunctivitis, resulting in long-term red eye and discomfort [26, 27]. Therefore, the existing surgical methods for pterygium still need to be continuously improved. Alsarhani et al. [2] thought the double-sliding conjunctival flap surgery appeared to be a useful method, with a better success rate and lower pterygial recurrence in pterygium surgery. In particular, when pterygium is larger or a recurrent type, this technique can be easily cover the bare sclera, as compared to any transposition conjunctival flap operation [2].

Tenon capsule is a thin and dense fibrous membrane between orbital fat body and eyeball, and the gap between tenon capsule and bulbar conjunctiva is considered to be an important part of pterygium pathological tissue proliferation. Barraquer first proposed that closing the space between bulbar conjunctiva and tenon capsule can help to reduce the recurrence rate of pterygium after operation [28–31]. This method can effectively organize the proliferation of degenerative fascia tissue under conjunctiva, reduce the expression of inflammatory factors and vascular endothelial growth factor, and reduce the invasiveness of pterygium. Since then, Solomon and Gayatri Devi have emphasized the importance of this technology in their respective experiments [32–34]. At present, the technique of sealing bulbar conjunctiva and tenon capsule is common in the combined conjunctival flap transplantation. Compared with the traditional conjunctival transplantation, this method can further reduce the postoperative recurrence rate, but it does not improve the upper conjunctival injury caused by conjunctival transplantation and the limited range of operation area [35, 36]. In this experiment, we used the method of closing.
Table 3: The difference of postoperative complications between the two groups.

| Time  | Characteristics                              | Treatment group (N=100) (n (%)/mean ± SD) | Observation group (N=100) (n (%)/mean ± SD) | $t/\chi^2$ | P   |
|-------|---------------------------------------------|------------------------------------------|---------------------------------------------|------------|-----|
| 3 days| NRS score                                   | 38 (38.0) 6.659 0.036                    | 31 (31.0)                                  |            |     |
|       | Prabhasawat score                           | 0 (0.00) 120.000 <0.05                   | 0 (0.00)                                   |            |     |
|       | Inspection of an ocular surface comprehensive analyzer | 1.40 ± 0.18 13.630 <0.01 | 1.82 ± 0.25                               |            |     |
|       | Corneal fluorescein staining                 | 3.60 ± 0.88 5.856 <0.01                  | 4.50 ± 1.26                                |            |     |
|       | Conjunctival fluorescein staining in the operation area | 0.49 ± 0.12 5.206 <0.01                   | 0.59 ± 0.15                                |            |     |
|       | Breakup time of the tear film (s)           | 3.72 ± 1.05 7.849 <0.01                  | 2.56 ± 1.04                                |            |     |
|       | Repair time of the corneal wound epithelium |                                             | 8.629 0.003                               |            |     |
|       | $\triangle$ Corneal astigmatism (°)         | 0.33 ± 0.22 2.903 0.004                   | 0.45 ± 0.35                                |            |     |
| 7 days| NRS score                                   | 42 (42.0) <0.05                          | 20 (20.0)                                  |            |     |
|       | Prabhasawat score                           | 9 (9.0) <0.05                            | 2 (2.0)                                    |            |     |
|       | Inspection of the ocular surface comprehensive analyzer | 1.26 ± 0.11 11.670 <0.01                | 1.45 ± 0.12                                |            |     |
|       | Corneal fluorescein staining                 | 1.40 ± 0.6 6.143 <0.01                   | 1.90 ± 0.55                                |            |     |
|       | Conjunctival fluorescein staining in the operation area | 0.13 ± 0.03 22.880 <0.01                  | 0.48 ± 0.15                                |            |     |
|       | Breakup time of the tear film (s)           | 4.55 ± 1.14 8.459 <0.01                  | 3.21 ± 1.1                                 |            |     |
| 14 days| NRS score                                   | 100 (100.00) 127.900 <0.05                | 64 (64.0)                                  |            |     |
|       | Prabhasawat score                           | 63 (63.0) 36.210 <0.05                   | 21 (21.0)                                  |            |     |
|       | Inspection of the ocular surface comprehensive analyzer | 1.11 ± 0.15 14.110 <0.01                | 1.39 ± 0.13                                |            |     |
|       | Corneal fluorescein staining                 | 0.24 ± 0.43 15.420 <0.01                  | 1.20 ± 0.45                                |            |     |
|       | Breakup time of the tear film (s)           | 5.33 ± 1.24 6.104 0.01                   | 4.35 ± 1.02                                |            |     |
| 1 month| NRS score                                   | 100 (100.00) 33.470 <0.05                 | 64 (64.0)                                  |            |     |
|       | Prabhasawat score                           | 78 (78.0) 56.230 <0.05                   | 25 (25.0)                                  |            |     |
|       | Inspection of the ocular surface comprehensive analyzer | 0.97 ± 0.18 11.950 <0.01                | 1.25 ± 0.15                                |            |     |
|       | Corneal fluorescein staining                 | 0.04 ± 0.2 22.760 <0.01                   | 0.70 ± 0.21                                |            |     |
|       | Breakup time of the tear film (s)           | 7.01 ± 1.53 12.890 <0.01                  | 4.32 ± 1.42                                |            |     |
the space between bulbar conjunctiva and tenon capsule combined with amniotic membrane transplantation for the first time to treat primary pterygium. The space between bulbar conjunctiva and tenon capsule was closed by amniotic membrane packing and continuous suture, so as to reduce the expression of inflammatory factors and vascular endothelial growth factor in the operation area and avoid the proliferation of granulation tissue after operation. Amniotic membrane transplantation instead of conjunctival transplantation can remove pterygium and surrounding suspicious lesion areas to the greatest extent without damaging the healthy tissues in other areas. As an ideal basement membrane, the amniotic membrane can help the rapid proliferation and migration of conjunctival epithelial cells around the operation area, so as to disturb the homeostasis of ocular surface microenvironment to the minimum and avoid complications and adverse reactions after conjunctival transplantation. We will evaluate the health status of the patient’s surface microenvironment before and after operation by NRS, Prabhasawat scoring method, ocular surface comprehensive analyzer, corneal fluorescein staining, tear film rupture time, etc. At the same time, we will establish a

| Time          | Characteristics                                         | Treatment group (N=100) (n (%)/mean ± SD) | Observation group (N=100) (n (%)/mean ± SD) | t/χ²   | P  |
|---------------|--------------------------------------------------------|-----------------------------------------|---------------------------------------------|--------|----|
| NRS score     | 0                                                      | 100 (100.00)                            | 75 (75.0)                                   | 28.570 | <0.05 |
|               | 1                                                      | 0 (0.00)                                | 21 (21.0)                                   |        |    |
|               | 2                                                      | 0 (0.00)                                | 4 (4.0)                                     |        |    |
| Prabhasawat score | 6 months                                            | 89 (89.0)                               | 65 (65.0)                                   | 16.260 | <0.05 |
|               | 1                                                      | 11 (11.0)                               | 35 (25.0)                                   |        |    |
| Inspection of the ocular surface comprehensive analyzer | 0.83 ± 0.13                                | 1.02 ± 0.12                              | 10.740                                      | <0.01  |
| Corneal fluorescein staining | 0.11 ± 0.35                               | 0.56 ± 0.32                              | 9.489                                       | <0.01  |
| Breakup time of the tear film (s)   | 7.77 ± 1.35                              | 4.35 ± 1.42                              | 17.460                                      | <0.01  |
| NRS score     | 0                                                      | 100 (100.00)                            | 80 (80.0)                                   | 22.220 | <0.05 |
|               | 1                                                      | 0 (0.00)                                | 19 (19.0)                                   |        |    |
|               | 2                                                      | 0 (0.00)                                | 1 (1.0)                                     |        |    |
| Prabhasawat score | 12 months                                           | 93 (93.0)                               | 72 (72.0)                                   | 11.090 | <0.05 |
|               | 2                                                      | 7 (7.0)                                 | 23 (23.0)                                   |        |    |
| Inspection of the ocular surface comprehensive analyzer | 0.82 ± 0.11                                | 0.98 ± 0.09                              | 11.260                                      | <0.01  |
| Corneal fluorescein staining | 0.14 ± 0.4                                 | 0.55 ± 0.35                              | 7.714                                       | <0.01  |
| Breakup time of the tear film (s)   | 7.79 ± 1.23                              | 5.02 ± 1.19                              | 16.190                                      | <0.01  |

SD, standard deviation; △ Change of corneal astigmatism before and after operation; NRS, numerical rating scale.

Figure 3: Conjunctival manifestations before, during, and after the operation. The ocular manifestations of pterygium before operation show obvious conjunctival neovascularization and hyperemia (a). The scope of operation was determined during operation (b). Postoperative reexamination shown that ocular conjunctival congestion was significantly improved and neovascularization was significantly reduced (c).
one-year follow-up file to observe the recurrence rate of the patient one year after operation in order to comprehensively evaluate the safety and effectiveness of this method. The successful implementation of this trial is expected to provide a new surgical method for the clinical treatment of pterygium, which can remove the focus more completely, not damage the healthy tissues, recover quickly, and have a low recurrence rate.

Data Availability
The simulation experiment data used to support the findings of this study are available from the corresponding author upon request.

Conflicts of Interest
The authors declare that there are no conflicts of interest regarding the publication of this paper.

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