

Excision combined with concavity – convex amniotic membrane transplantation in the treatment of intraepithelial epithelioma

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凹凸面生物羊膜移植术治疗上皮内上皮癌的疗效观察

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摘要

目的: 凹凸面生物羊膜移植术治疗上皮内上皮癌的疗效观察。

方法: 针对我科临床诊治为上皮内上皮癌患者 24 例 (24 眼), 均进行肿瘤病灶清除, 随机选择其中 12 例对切除的结膜部分以及巩膜创面行凹凸生物羊膜覆盖, 另外 12 例行普通羊膜覆盖, 术后观察对比角膜伤口的上皮愈合程度, 凹凸面生物羊膜的降解情况, 术后肿瘤复发情况以及其他的并发症。切除的肿瘤均进行组织病理学检查。

结果: 所有患者样本的病理结果均为上皮内上皮癌, 术后 5d 内两组患者术区角结膜上皮已无荧光素钠着染, 14d 左右羊膜完全降解, 两组患者角膜愈合时间无统计学意义, 术后视力提高两组相比较无统计学意义, 随访 1a 期间, 凹凸面羊膜覆盖组较普通羊膜覆盖组复发率低。

结论: 肿瘤切除联合凹凸面生物羊膜移植术是治疗上皮内上皮癌有效的手术方法。

关键词: 凹凸面生物羊膜; 羊膜; 上皮内上皮癌

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Abstract

• **AIM:** To observe the curative effect of excision combined with concavity-convex amniotic membrane transplantation in the treatment of intraepithelial epithelioma.

• **METHODS:** Totally 24 cases of intraepithelial neoplasia (24 eye) diagnosed in our department were studied. The tumors of 12 cases were removed and the wound were covered by the concavity-convex amniotic membrane in

the conjunctiva and part of the resection, and other 12 were covered by the amniotic membrane. The effect of these two surgeries were assessed via observing the epithelial healing, degradation of biological amnion, tear break – up time, tumor recurrence and other complications. The resection of the tumor were analyzed histopathologically.

• **RESULTS:** The pathological results of all the patients were epithelial carcinoma. There were 24 patients that diagnosed –intraepithelial epithelioma pathologically. The conjunctival epithelium healed rapidly and were completed in 5d after operation in both groups. The amniotic membrane was completely degraded in about 14d. Postoperative visual acuity improvement was not statistically significant in two groups. All patients were followed up for more than 1y. The recurrence rates were significantly lower in concavity – convex amniotic membrane group than that in amniotic membrane group.

• **CONCLUSION:** Tumor resection combined with concavity-convex amniotic membrane transplantation is an effective operation method for the treatment of intraepithelial epithelioma.

• **KEYWORDS:** concavity – convex amniotic membrane; amniotic membrane; intraepithelial epithelioma

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INTRODUCTION

Intraepithelial epithelioma, is firstly described by American dermatology doctor Bowen as a precancerous dyskeratosis, which is also known as Bowen's disease^[1-4]. Most scholars advocate removal of tumor by surgical, while simple surgical removal in patients could result in 25% –40% recurrence^[5-8]. Toally 24 cases of intraepithelial cancer patients diagnosed in our department underwent tumor debridement combined with amniotic membrane transplantation or concavity-convex amniotic membrane transplantation. After the operation, the ocular surface regenerated fast and the amniotic membrane was degraded slowly, with little complication and low recurrence rate. It is summarized as follows.

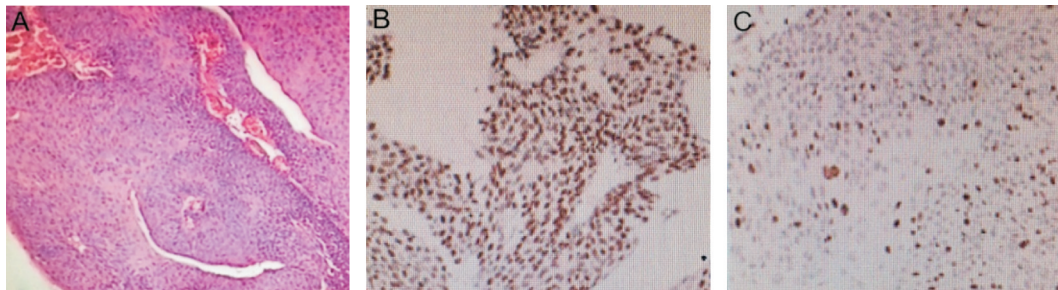


Figure 1 Pathological results A: HE staining; B: P63 remarked immunohistochemical staining, P63(+); C: P53 remarked immunohistochemical staining, P53(+).

SUBJECTS AND METHODS

The study samples were 24 eyes in 24 cases of intraepithelial epithelioma, aged 55–78y, average 68 ± 6 y old, 15 males, 9 females; preoperative visual acuity: finger counting/30cm–20/25; the course of 1–21mo. The temporal lateral corneal tumors were the most common in the eyelid fissure area, which appear slowing growth of corneal translucent or jelly new creatures. The surface of cornea and conjunctiva were different in different degrees. The tumor will present uneven surface, papillary or cauliflower, pink or white cream, and the surface covered with new biological angiogenesis "hairpin" or "pine" kind. The lesions ranged from 13 cases of $< 1/4$ circle, 5 cases of $1/4 <$ to $< 1/2$, 5 cases of $1/2 <$ to $< 3/4$, 1 case of peripheral $> 3/4$. Intraepithelial epithelioma can begin in any quadrant, as it is illustrated in our cases (Table 1).

Method The operation incision was selected 2 ~ 3mm far from the fluorescein staining boundary. Then we cut bulbar conjunctival and subconjunctival tissue, exposed the tumor, carefully dissected the tumor, and resected the tumor. The operation was close to fluorescein sodium staining boundary to avoid the spread of bighorn conjunctival tissue defect. The resected tumor was sent to perform pathological examination. The tumor invasion and location of the corneal rim were cleaned under microscope. The limbus where the tumor near to the sclera wound was cleaned with micro shear. Then, we measured the exposed range and cut down the tile of concavity–convex amniotic membrane or amniotic membrane to exposed wound. Concave amniotic membrane was resuscitated with sterile saline for 1–2min. After the amniotic membrane was transparent, 8–0 absorbable suture was used to sew up the wound. After the operation, the wound was spread with antibiotic ointment after bandaging. After the first day the eyes were given by antibiotic eye drops and antibiotic ointment.

Postprocessing Observation After the operation, the healing of the wound, the degradation of the amniotic membrane, the condition of the tears, the visual acuity, the recurrence and the complications were observed for more than 1y. The average observation time was 16mo.

Statistical Analysis Statistical analysis were performed using the SPSS software (version 21.0). The *t*-test or Chi-square test was used to establish whether the difference between the two groups was statistically significant. *P* value

less than 0.05 was considered statistically significant.

RESULTS

Pathological Results All of the 24 patients were the epithelial carcinoma (Bowen's disease), which were in accordance with the clinical diagnosis (Figure 1).

Epithelium Healing The repair of the conjunctival epithelium was determined by sodium fluorescein staining, and the corneal epithelium was stained with fluorescein staining and disappeared when it healed well. There were not statistically significance between two groups ($P > 0.05$) (Table 2).

Degradation of Amniotic Membrane Twenty-four cases of patients with amniotic membrane were all good, no shift and off. At 14d or so, the amniotic membrane began to degrade, and the wound was covered with new epithelial cells.

Overflow Tears In the first 3d after operation, the symptoms of the patients were severe, and 3d later the symptoms were relieved. At 7d or so, the symptoms of the patients were disappeared.

Visual Acuity At 5d after corneal epithelial repaired and at 7d after treatment, the visual acuity was recorded. The postoperative visual acuity was not improved in 8 patients, and the other 16 patients improved. Postoperative visual acuity improvement was not statistically significant between two groups ($P > 0.05$) (Table 2).

Complications Corneal epithelium defect happened in 8 patients during the follow-up period, and it healed 3d later. Eight cases of patients had corneal leukoma (Table 1).

Tumor Recurrence All the patients were followed up for 1y, with a mean of 16mo. During the follow-up period, 1 patient was regenerated 3mm × 5mm size cauliflower tumor after 9mo in concavity–convex amniotic membrane group. As well as 4 patients had a relapse in amniotic membrane group. The recurrent tumors were resected in time and combined with the treatment of amniotic membrane transplantation, which had no recurrence in 1y. The recurrence rates of concavity–convex amniotic membrane group were lower than that of amniotic membrane group ($P < 0.05$) (Table 2).

DISCUSSION

Intraepithelial epithelioma (Bowen's disease) was firstly described by the doctor Bowen in the Department of Dermatology as a precancerous lesions in detail^[1-2]. The course is slowly developed epithelial benign tumor, which occurs in middle-aged and elderly men. In two groups of the 24 patients, 66.7% were male^[9-10]. The lesions were located

Table 1 Clinical data in 24 cases of intraepithelial epithelioma

| Case No. | Age (a) | Sex | Duration of lesion (mo) | Location | Visual acuity | | Complications | Corneal epithelial healing (d) |
|----------|---------|-----|-------------------------|---|-----------------------|--------|-----------------|--------------------------------|
| | | | | | Initial | Final | | |
| 1 | 69 | M | 6 | a quarter of nasal cornea and 4mm wide ring of surrounding conjunctiva Oculus Dexter (OD) | 20/100 | 20/63 | - | 4 |
| 2 | 65 | M | 4 | a quarter of nasal cornea and 5mm wide ring of surrounding conjunctiva OD | 20/32 | 20/25 | - | 5 |
| 3 | 59 | F | 5 | a quarter of temporal cornea and 3mm wide ring of surrounding conjunctiva Oculus Sinister(OS) | 20/50 | 20/40 | - | 4 |
| 4 | 68 | M | 10 | a quarter of temporal cornea and 4mm wide ring of surrounding conjunctiva OS | 20/25 | 20/25 | - | 5 |
| 5 | 75 | M | 7 | three-quarters of nasal cornea and 4mm wide ring of surrounding conjunctiva OD | finger counting /30cm | 20/200 | Corneal leukoma | 7 |
| 6 | 72 | M | 10 | two-quarters of nasal cornea and 3mm wide ring of surrounding conjunctiva OD | 20/200 | 20/100 | Corneal leukoma | 5 |
| 7 | 61 | F | 9 | a quarter of temporal cornea and 3mm wide ring of surrounding conjunctiva OS | 20/32 | 20/32 | - | 4 |
| 8 | 71 | M | 8 | three-quarters of temporal cornea and 4mm wide ring of surrounding conjunctiva OS | 20/100 | 20/100 | Corneal leukoma | 5 |
| 9 | 64 | M | 7 | three-quarters of temporal cornea and 3mm wide ring of surrounding conjunctiva OS | 20/40 | 20/40 | - | 4 |
| 10 | 73 | F | 19 | two-quarters of nasal cornea and 3mm wide ring of surrounding conjunctiva OD | 20/63 | 20/40 | - | 4 |
| 11 | 74 | F | 21 | three-quarters of temporal cornea and 4mm wide ring of surrounding conjunctiva OS | 20/200 | 20/100 | Corneal leukoma | 5 |
| 12 | 55 | M | 6 | 360° involvement of cornea and 3-4mm wide ring of surrounding conjunctiva OD | 20/200 | 20/100 | - | 6 |
| 13 | 65 | F | 11 | three-quarters of temporal cornea and 4mm wide ring of surrounding conjunctiva OS | 20/200 | 20/100 | - | 7 |
| 14 | 62 | M | 12 | a quarters of temporal cornea and 3mm wide ring of surrounding conjunctiva OS | 20/200 | 20/100 | - | 8 |
| 15 | 78 | F | 19 | two-quarters of nasal cornea and 3mm wide ring of surrounding conjunctiva OD | 20/40 | 20/32 | - | 4 |
| 16 | 64 | M | 7 | two-quarters of nasal cornea and 4mm wide ring of surrounding conjunctiva OD | 20/200 | 20/100 | Corneal leukoma | 4 |
| 17 | 73 | M | 10 | a quarter of nasal cornea and 5mm wide ring of surrounding conjunctiva OD | 20/25 | 20/25 | - | 7 |
| 18 | 72 | F | 14 | a quarter of nasal cornea and 4mm wide ring of surrounding conjunctiva OD | 20/200 | 20/63 | - | 5 |
| 19 | 77 | M | 15 | a quarter of temporal cornea and 4mm wide ring of surrounding conjunctiva OS | 20/32 | 20/32 | - | 6 |
| 20 | 74 | M | 6 | a quarter of temporal cornea and 3mm wide ring of surrounding conjunctiva OS | finger counting /30cm | 20/200 | - | 8 |
| 21 | 76 | F | 14 | a quarter of temporal cornea and 4mm wide ring of surrounding conjunctiva OS | 20/63 | 20/63 | - | 5 |
| 22 | 61 | M | 16 | three-quarters of temporal cornea and 4mm wide ring of surrounding conjunctiva OS | 20/32 | 20/25 | Corneal leukoma | 7 |
| 23 | 58 | F | 11 | a quarter of temporal cornea and 3mm wide ring of surrounding conjunctiva OS | 20/200 | 20/100 | Corneal leukoma | 4 |
| 24 | 71 | M | 5 | Two-quarters of nasal cornea and 3mm wide ring of surrounding conjunctiva OD | 20/100 | 20/100 | Corneal leukoma | 8 |

Patients 7, 15, 19, 21, 22 underwent treatment followed a recurrence. Patients 1 to 12 were treated with concavity-convex amniotic membrane, others were treated with amniotic membrane.

Table 2 Comparison of the therapeutic indexes in the two groups

| Operation method | Cases (n) | Postoperative visual acuity improved | Corneal epithelial healing time (a) | Recurrence rates |
|------------------------------------|-----------|--------------------------------------|-------------------------------------|-------------------|
| concavity-convex amniotic membrane | 12 | 0.07±0.09 ^a | 4.92±0.9 ^a | 0.08 ^b |
| Amniotic membrane | 12 | 0.08±0.09 ^a | 5.50±1.62 ^a | 0.33 ^b |

^aP>0.05, ^bP<0.05.

at the junction of the conjunctiva. The cause is now thought to be related to UV irradiation^[11], but the main reason is not yet clear. The tumor is currently advocated for surgical removal, but simple resection resulted in 25% to 40% recurrence rate^[2]. In the two groups of patients, the conjunctiva were stained with fluorescein sodium before the resection of tumor. According to the color canceration area and parakeratosis conjunctival epithelium area, the tumors were removed completely. None of the two groups had a recurrence from peripheral tumor resection, and the location of tumor attachment is easy to relapse. It is widely reported to inhibit the proliferation of tumor cells using mitomycin C^[12], and local frozen which were used as adjuvant therapy to reduce tumor recurrence^[13]. Currently research found using mitomycin C can cause corneal ulcer, scleral ischemia, scleral thinning and other complications. As there are toxic effects of mitomycin C on corneal endothelial cell coverage, and mitomycin C has no apparent advantage in reducing intraepithelial cancer recurrence, most scholars suggest not to use it in the surgical treatment^[14-15]. The freezing treatment mainly caused cell protein denaturation, cell rupture, and then promote tumor cell apoptosis to kill tumor cells^[16-17]. As a result, the patients were easier to have a high degree of edema and congestion, out of the fissure area. Part of the organization is frozen too much, appear corneal edema and then the corneal opacity, and even lower intraocular pressure and other complications. In recent years, it has been reported that to treat large area of epithelial carcinoma with radiotherapy alone, while it must strictly control the radiation dose and follow up on time. Yet it extremely easy to cause cyclitis, and the pathological changes in the lens and retina^[18-19]. The patients in this group were covered with the concave and convex amniotic membrane. Compared with the traditional amniotic membrane group it has the following advantages^[20-24]: 1) making it more easily to identify the basement membrane surface and upper leather; 2) the biological structure is complete, and the fresh amniotic membrane is basically the same; 3) the biological active ingredient is preserved, the relative degradation time is prolonged, and it is more advantageous to the wound healing; 4) the key to the operation of this group is to remove the lesions completely, and to remove the tumor completely. The edge of the corner is completely removed, especially in the basal part of the tumor in order to form a better service. In the two groups, despite the tumor invasion were relatively superficial, there is still a large area of conjunctival wound and corneal epithelial defect due to the removal of large area. If using the direct suture, it is extremely easy to cause the conjunctival sac narrow and even the eyelid ball adhesion. So we covered the cornea and conjunctiva with the concave and convex amniotic membrane or amniotic membrane. As the concave and convex amniotic membrane is rich in many kinds of growth factors, it can reduce the early postoperative edema and inflammatory reaction. Meanwhile, to a certain extent, it promoted the repair of conjunctival epithelium and wound,

especially for large area of conjunctival defect and corneal epithelial defect. The concave surface of the biological membrane was dissolved in 14d at most. The concave convex amniotic membrane played a part of role of conjunctiva during this period of time. This is particularly important for maintaining the micro environment of the ocular surface, and laying a certain foundation for the health of the patients in the late stage of the ocular surface. In 14d after surgery, all patients were reconstructed with their ocular surface. Patient foreign body sensation was significantly improved. In vision recovery, patients were improved after 5d of corneal epithelial repair and 7d after the removal of amniotic membrane suture. There was no change in visual acuity of 9 patients, and the visual acuity of 15 patients was improved by different degrees. The reason might be that the patients with preoperative tumor invasion and extent caused by corneal refractive error, after tumor resection and alleviate ametropia. At the pupil area after tumor invasion, epithelial opacity is a direct impact on patients with vision. The visual acuity was significantly increased, while part of patients with visual improvement was not obvious. Patients with cataract and other factors would affect the visual acuity, such as age - related macular degeneration etc. Twenty-four patients were followed up and among them 2 patients appeared partial epithelial deletion of the cornea. The reason might be the mass invasion and expansion after the restoration of corneal epithelium, and corneal contact occurred epithelial damage. Eight cases appeared corneal white spot, which might result from the tumor invasion and the damage caused by the matrix layer after the injury. No recurrence was found during the follow-up of 19 cases. Five cases of patients with recurrent was considered to the reason of its tumor invasion and range, the lesions involved deeper tissue and residual tumor cells in intraepithelial epithelioma. After the application of concave and convex amniotic membrane covering surgical treatment, the curative effect was satisfactory with a further coverage with convex and concave surfaces of biological amnion compared with amniotic membrane. Through the two groups of cases, we can remove the mass of the tumor combined with the amniotic membrane transplantation, which can be as much as possible to achieve little damage, to retain the original tissue structure, to reduce the incidence of postoperative recurrence and complications. It is a relatively effective treatment for epithelial cancer.

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