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Original Article

A retrospective clinical study of Xinjiang Uygur patients with corneal allograft rejection

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Abstract: Background: To explore the causes of corneal allograft rejection in Xinjiang Uygur patients and the factors that affect rejection through a retrospective clinical analysis. Methods: A retrospective analysis of 126 Uygur cases from January 2010 to November 2014 in which corneal transplantation had been performed at the Xinjiang Urumqi ENT hospital. Of the treated patients, 85 eyes belonged to male patients and 41 eyes belonged to female patients. Patients were aged 10-77 years (mean age 46.14 ± 8.20 years). Surgical methods included penetrating keratoplasty (75 eyes) and lamellar keratoplasty (38 eyes). Follow-up time ranged from 0.5 to 3 years and a total of seven pre-operative keratopathies were observed: walleye, corneal ulcer, bullous keratopathy, corneal degeneration. Eye changes included 72 cases of limbal vascularization and 15 cases of high intraocular pressure. Allograft rejection was observed in 25 eyes. Results: The pre-operative keratopathies associated with the highest incidences of allograft rejection were: viral corneal ulcer, bullous keratopathy, adhesive walleye, and fungal corneal ulcers. The rate of allograft rejection using avascular corneal tissue was 10%, while the rate was 36% with severly-vascularized cornea. The earliest time of rejection was 20 days after surgery, while the latest was 16.4 months after surgery. Heavy corneal vascularization is associated with more rapid post-operative rejection. The rate of allograft rejection was higher after combined surgery when compared to penetrating keratoplasty or lamellar keratoplasty alone, while the rate was higher with penetrating keratoplasty than with lamellar keratoplasty. With increasing graft diameter, there was an increase in post-operative allograft rejection. Allograft rejection was significantly increased when graft diameter was above 7.75 mm. Conclusion: The major cause of corneal allograft rejection is viral corneal ulcers. High corneal vascularization, combined surgical methods, large diameter graft transplantation are all risk factors for allograft rejection.

Keywords: Corneal transplantation, allograft rejection, vascularization, graft

Introduction

Corneal transplant surgery is the most effective means of treatment in patients with corneal blindness. Internationally, the success rate of conventional corneal transplantation is now over 90% [1]. Currently, the main reason for the failure of corneal transplant surgery is allograft rejection after surgery [2]. Corneal allograft rejection is am inflammatory reaction caused by the antigenicity of the donor cornea and is a delayed hypersensitivity reaction. It is a currently unresolved clinical problem. Normal corneal tissue has inherent "immune privilege" [3], but not all corneal transplants have immune privilege. In clinical cases, the immune privilege of the transplanted cornea is often destroyed by certain risk factors, leading to allograft rejection. This study retrospectively analyzes corneal transplantation patient cases from January 2010 to November 2014 among Uyghur patients, with a total of 126 studied eyes.

Materials and methods

Patient selection

Patients who had undergone corneal transplantation at our hospital from January 2010 to November 2014 totaled 108 cases (126 eyes). 85 eyes were male patient eyes and 41 eyes were female patient eyes. Patient age ranged

from 10-77 years (mean age 46.14 ± 8.20 years). Surgeries included 75 cases of penetrating keratoplasty, 38 cases of lamellar keratoplasty and 13cases of combined operation. Follow-up time ranged from 0.5 to 3 years, with 106 clear eyes, 15 mildly cloudy eyes, and five significantly cloudy eyes. Of these, 25 eyes experienced allograft rejection. A total of seven suspected causes for allograft rejection were identified: walleye, corneal ulcer, bullous keratopathy, corneal degeneration. Changes to the eye included 72 cases of limbal vascularization and 15 cases of high intraocular pressure.

Pre-operative examination

In addition to a physical examination, eye exam, and other standard examinations upon admission, patients were tested for tear secretion, ocular-related conditions, as well as the location, size, and depth of lesions in the cornea, and the distribution and extent of vascularization. With ophthalmological screening, we determined whether the patients had any pathologies other than cornea-related issues that may affect their vision.

Surgical methods and administration of post-operative medication

Donor cornea (fresh cornea and stored cornea) were preserved in a wet room at 4°C. Graft size: 60 grafts with diameter of 7.0-7.25 mm; 36 grafts with diameter of 7.25-7.5 mm; 30 grafts with diameter of 7.75-8.25 mm.

Surgical methods

Penetrating keratoplasty: routine pre-operative preparation, 24-48 hours before surgery, antibiotic eye drops were administered and general anesthesia was chosen. The graft was taken from the inner layer of the donor cornea using a trephine, while the graft bed was created, using the trephine to make the initial cut. The bed itself was shaped using scissors. The graft was placed in the graft bed and 10-0 nylon suture was used for 16 interrupted stitches (graft beds with vascularization) or 20-24 continuous sutures (graft beds without vascularization). BSS was injected into the anterior chamber to help restore it, and a small amount of sterile gas was injected between the cornea and the iris to prevent adhesion. The incision was checked for leaks, and antibiotics and corticosteroid ointments were placed on the eye before bandaging.

Lamellar keratoplasty: pre-operative preparations were the same as penetrating keratoplasty. Method of anesthesia: topical anesthesia was commonly used (Alcaine eye drops). Minors or patients with poor cooperation during surgery were given general anesthesia. Graft beds were created manually and the lesion-containing cornea and sclera tissue were completely resected. Based on the depth of the graft beds, the appropriate graft section was cut. The corneal wound edge was sutured using a 10-0 nylon continuous suture, while the scleral wound edge was sutured using interrupted stitches. Post-surgical treatment was identical to that used in penetrating keratoplasty.

Post-surgery medication

After surgery, topical glucocorticoid antibiotic drops (TobraDex) were prescribed four times a day, and TobraDex ointment was prescribed once before bed. Medications to promote corneal healing and a nutritional gel (bFGF or Deproteinized Calfblood Extract Eye Gel) were prescribed four times per day. The dosage was adjusted according to the patient's condition, Three days after surgery, 1% cyclosporine A eye drops were prescribed three to four times a day. Seven to ten days after surgery, the TobraDex drops were reduced to three times a day, and were gradually replaced by 0.02% fluorometholone eye drops. Topical eye drops were prescribed for one year or longer. After surgery, stitches were removed based on their tension. Removal of stitches took place 8 months to 1 year after surgery for penetrating keratoplasty and 3-6 months for lamellar keratoplasty. Patients were followed up for a period of 3 months to 3 years.

Diagnostic criteria for allograft rejection [4]

After successful corneal transplantation, patients who experienced loss of visual acuity or ocular irritation, congestion, mixed ciliary hyperemia, corneal edema, graft dissolution, appearance of epithelial rejection line, and/or bubble changes, etc. were considered to be experiencing rejection. The appearance of an endothelial rejection line, white or suet like corneal precipitation, aqueous flare phenomenon,

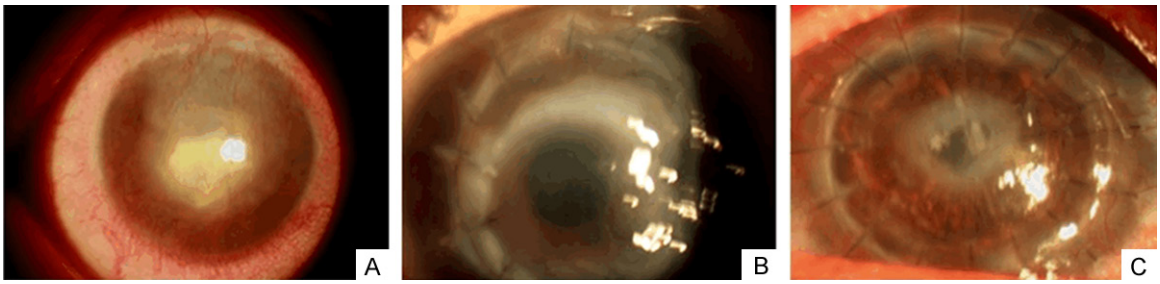

Figure 1. Allograft rejection after penetrating keratoplasty. A: Pre-operative, Condition of the cornea before surgery. B: Immune rejection appearing 53 days after penetrating keratoplasty. C: Immune rejection appearing 89 days after penetrating keratoplasty.

Table 1. Relationship between pre-operative keratopathies and rate of allograft rejection

Clinical diagnosis	Number of eyes	Number of rejections	Rate of rejection
Adhesive walleye	40	8	20%
Simple walleye	31	3	9.68%
Fungal corneal ulcer	16	3	18.75%
Viral corneal ulcer	12	4	33.33%
Bullous keratopathy	23	6	26.09%
Corneal degeneration	4	1	25%

etc. was also considered as signs of allograft rejection.

(1) Epithelial rejection: corneal epithelium rejection line, sodium fluorescein staining positive, ciliary conjunctival hyperemia, edema of various degrees at the epithelium of the rejected cornea, gray-white haze, graft dissolution.

(2) Lower epithelial rejection: visible rejection line can be seen from corneal epithelium, sodium fluorescein staining positive, ciliary conjunctival hyperemia, corneal epithelium showing varying degrees of gray-white haze, edema, limited opaque infiltration visible below the corneal epithelium, corneal pigmentation and aqueous flare in some patients.

(3) Matrix rejection: more severe eye irritation, deep vascularization into the corneal stroma, graft matrix showing edema and opacity.

(4) Endothelial rejection: endothelial folds, corneal endothelium shows pigmentation, pigmented precipitate, and aqueous flare, endothelial rejection line visible in the majority of patients.

Treatment for rejection

Once rejection was discovered, corticosteroid eye drops (e.g. TobraDex eye drops or ointment) were used 4 to 6 times/day or Flumetholon drops were used 6 times/day. Local corticosteroid injection: based on the severity of rejection, a subconjunctival injection of dexamethasone sodium phosphate was administered once a day or once every other day. Systemic corticosteroids: intravenous hydrocortisone (200 mg/day) was reduced to oral prednisone once the condition improved. Cyclosporine (1% to 2%) eye drops were administered 4-6 times/day. After treatment for one or two weeks, patients either showed improvement or had completely recovered. Evaluation standards for improvement: disappearance of epithelial or endothelial rejection line, significantly reduced graft edema, significantly reduced corneal deposits. Evaluation standards for recovery [5]: disappearance of symptoms, ciliary congestion mitigated or dissipated, disappearance of graft edema, epithelial, or epithelial rejection line, disappearance of corneal endothelial rejection line or precipitates.

Degree of pre-operative corneal vascularization

The degree of graft bed vascularization was classified by the degree of infiltration: '+' signifies corneal infiltration in less than 1/4 of the cornea, '++' signifies corneal infiltration in 1/2 of the cornea, '+++' signifies corneal infiltration in 3/4 of the cornea, while '++++' signified corneal infiltration in greater than 3/4 of the cornea.

Results

General patient condition

From January 2010 to November 2014, a total of 108 corneal transplant patients (126 eyes) were admitted. Among these, 85 eyes belonged

Table 2. Relationship between rate of allograft rejection and degree of vascularization

Degree of vascularization	Number of eyes	Constituent ratio	Number of eyes with allograft rejection (rate)
++++	25	19.84%	9 (36%)
+++	36	28.57%	7 (19.44%)
++	31	24.60%	5 (16.13%)
+	15	11.91%	2 (13.33%)
None	20	15.87%	2 (10%)

Table 3. Relationship between time of rejection and degree of vascularization

Degree of vascularization	Number of eyes	Number of eyes with allograft rejection	Time of allograft rejection (months post-operation)
++++	25	9	2.5
+++	36	7	8.3
++	31	5	12.6
+	15	2	16.4
None	20	2	15.5

to male patients and 41 eyes belonged to female patients. Patient age ranged from 10-77 years (mean age 46.14 ± 8.03 years). Penetrating keratoplasty was performed on 75 eyes, lamellar keratoplasty was performed on 38 eyes and combined operation was performed on 13 cases.

Corneal graft

One week after surgery, the corneal graft showed mild corneal opacity, epithelial defects, and Descemet membrane folds. One week later, the graft became transparent. Follow-up time ranged from 0.5 to 3 years. Transparent graft was observed in 106 eyes (84.13%), slightly cloudy graft was observed in 15 eyes (11.91%), and significant turbidity was observed in 5 eyes (3.97%). Allograft rejection was observed in 25 eyes that showed mixed hyperemia, graft edema, turbidity, and decreased vision. Through the use of anti-rejection treatment, the allograft rejection was gradually brought under control. However, five grafts remained completely cloudy. See **Figure 1**.

Pre-operative keratopathies and eye changes

There were seven causes of keratopathy: 15.49% of the patients had walleye (11/71), of these, 8% had adhesive walleye (17/40) and 9.68% had simple walleye (3/31); 25% had corneal ulcers (7/28), among these, 18.75% had fungal corneal ulcer (3/16), 33.33% had viral corneal ulcer (4/12), and 26.09% had bullous keratopathy (6/23); 25% had corneal degeneration (1/4). Eye changes: corneal vascularization was seen in 72 patients, while 15 patients showed high intraocular pressure. Of 160 cases of corneal transplantation (126 eyes), 25 eyes experienced allograft rejection, for a total incidence rate of 19.84%. The time of rejection was from 25 days to three years after surgery, with the peak period for rejection at one month to three months post-operation. Five eyes did not show any improvement in the final graft edema, accounting for 20% of allograft rejections.

Relationship between pre-operative keratopathies and rate of allograft rejection

The highest rates of allograft rejection were associated with the following pre-operative keratopathies: viral corneal ulcers, bullous keratopathy, adhesive walleye, fungal corneal ulcer. See **Table 1**.

Relationship between rate of allograft rejection and degree of vascularization

Our results show that the rate of rejection following standard corneal transplantation in cases with no vascularization was 10%, while the rate was 36% in cases with severe vascularization. With the increase of the degree of vascularization, there was an increase in the rate of rejection. The time at which rejection began to appear ranged from 20 days to 16.4 months. The more severe the corneal vascularization, the sooner we observed post-operative rejection. See **Tables 2** and **3**.

Relationship between the surgical method and the rate of allograft rejection

The rate of rejection after combined surgery was higher than that of penetrating keratoplasty and lamellar keratoplasty alone. The rate of rejection following penetrating keratoplasty is higher than with lamellar keratoplasty. See **Table 4**.

Table 4. Relationship between surgical method and the rate of allograft rejection

Surgical method	Number of eyes operated	Number of eyes with graft rejection	Rate of rejection
Penetrating keratoplasty	75	15	20%
Lamellar keratoplasty	38	6	15.79
Combined surgery	13	4	30.77

Table 5. Relationship between graft size and rate of graft rejection

Corneal graft (graft bed diameter)	Number of operations	Rejections (number of eyes)	Rate of rejection
7.0 mm-7.25 mm	60	9	15%
7.25 mm-7.5 mm	36	6	16.67%
7.5 mm-7.75 mm	24	7	29.17%
7.75 mm-8.0 mm	6	3	50%

Relationship between graft size and rate of allograft rejection

Our research shows that, with the increase of corneal diameter, there is an increased incidence of post-operative rejection. Graft diameters exceeding 7.75 mm result in significantly higher incidences of rejection. See **Table 5**.

Discussion

Pre-surgical keratopathy

Corneal transplantation is the type of organ and tissue transplantation that carries the highest rate of success. However, post-operative allograft rejection is still the main reason for surgical failure. With increased corneal transplant surgery at our hospital, post-operative complications, the most important of which is blindness caused by allograft rejection, have become an urgent problem. In Western countries, the most common pathologies for which keratoplasty is indicated include Fuchs endothelial dystrophy, bullous keratopathy, walleye, keratoconus, etc. [6-8]. In Asian countries, keratoconus and infectious corneal disease are the most common causes for keratoplasty [9, 10]. This clinical study showed that the most common pre-operative keratopathies in Xinjiang Uygur patients are viral corneal ulcer, bullous keratopathy, corneal degeneration, and adhesive walleye.

Rate of rejection and the number of corneal blood vessels

According to statistics, the rejection rate after standard corneal transplantation when there is no vascularization is only 10%, while the rejection rate in cases with severe vascularization is 20-60% [11-13]. Studies have shown that allograft rejection and corneal vascularization are closely related. The microenvironment near the transplanted cornea is destroyed by vascularization, removing the immune privilege of the cornea and creating a major risk factor for corneal graft rejection [14, 15]. In the vascularized graft bed, graft antigen receptors can rapidly enter the circulatory and lymphatic system, sensitizing the host and leading to severe rejection. Inoue et al [16] reported that with increasing corneal vascularization, the rate of corneal rejection increases. With more advanced vascularization on the graft bed, rejection occurs sooner, with more serious consequences. Our results suggest that the rate of rejection in Uygur patients who have undergone keratoplasty with severe corneal vascularization was 61.5%, while the rejection rate with avascular graft beds was 10%. In cases with pre-operative corneal vascularization, the rate of rejection was high, and the rate increased with increasing degrees of vascularization.

Surgical method and corneal allograft rejection rate

This clinical study shows that the rejection rate is higher after penetrating keratoplasty when compared to lamellar keratoplasty. A combination of the two surgical approaches resulted in higher rates of rejection than either method on its own. Banerjee et al. [17] found that during the first corneal transplant, the antigens from the donor cornea may induce a local immune response, resulting in much faster and significantly more severe immune rejection with the second transplant. During multiple corneal transplants, cellular and humoral immunity together play important roles and disorders in

the interactions between various immune factors results in much more severe rejection after transplantation. Penetrating keratoplasty is the main surgical approach taken for corneal transplantation and accounts for over 90% of corneal transplants performed abroad. However, this surgical procedure can easily damage eye tissue and lead to allograft rejection, intraocular infection, high astigmatism, persistent post-operative endothelial cell loss, and other complications after surgery [18]. Due to large quantities of donor antigens that penetrate the corneal graft, donor antigens are not only delivered through the vasculature, but antibodies can also cause allograft rejection by stimulating the body to produce aqueous humor. Lamellar keratoplasty can expand indications for surgery, reduce complications caused by intraocular operations, and help post-operative recovery of the expanded central and peripheral cornea. Based on our experience, the success rate depends on the selection of the proper surgical method and the technical proficiency of the surgeon.

Graft size and post-operative allograft rejection rate

Since the recipient is afflicted with a variety of keratopathies, the location and size of lesions differ and individual differences exist in the preparation of the graft bed. Therefore, it is necessary to match the graft bed with the graft both in size and shape and there are differences in the preparation of the graft bed between patients. The size of the graft and graft rejection show a very close relationship. Patients with increased corneal diameter show increased incidences of post-operative rejection, among which graft diameters exceeding 7.75 mm show significantly increased rates of rejection. Avascular corneal tissue and corneal Langerhans cells are two important factors for success in corneal transplants. Langerhans cells found in increased numbers in the limbus. The larger the graft, the closer the limbus, and large corneal grafts are rejection-prone. There are no systemic or local immune parameters that can be used for the early diagnosis of allograft rejection after corneal transplantation. Clinical symptoms are mainly used as exclusion criteria for diagnosis. It is necessary for ophthalmologists to discover and correct post-operative allograft rejection through years of experience, careful observation, and rigorous post-operative follow-up.

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Disclosure of conflict of interest

None.

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