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Original Article

The Prevalence of Positive Donor Corneoscleral Rim Culture and its Association with Ocular Infection After Transplantation

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Abstract

Objectives: The aim of the study was to determine the prevalence of positive corneoscleral donor rim cultures and to report keratitis and endophthalmitis after keratoplasty.

Methods: Eye bank records and medical records of patients who underwent keratoplasty between September 1, 2015, and December 31, 2019, were retrospectively reviewed. Patients who had routine donor-rim culture taken during surgery and followed up for at least 1 year in the post-operative period were included in the study.

Results: A total of 826 keratoplasty procedures were performed. A total of 120 (14.5%) cases had a positive donor corneoscleral rim culture. Positive bacterial cultures were obtained from 108 (13.7%) of the donors. Bacterial keratitis was observed in one patient (0.83% of recipients) who had a positive bacterial culture. Positive fungal cultures were obtained from 12 (1.45%) donors, of whom one (8.33% of recipients) developed fungal keratitis. Endophthalmitis was observed in one patient whose culture result was negative. Both bacterial and fungal culture results were similar in penetrating and lamellar surgical procedures.

Conclusion: Although the donor corneoscleral rims have a high positive culture result, the rate of bacterial keratitis and endophthalmitis is low, the risk of infection is high in patients with a fungal positive donor rim. Closer follow-up of patients with fungal positive donor corneo-scleral rim result and initiation of aggressive antifungal treatment when infection occurs will be beneficial.

Keywords: Bacterial keratitis, Endophthalmitis, fungal keratitis, keratoplasty, positive donor rim culture

Introduction

Keratoplasty is a surgical procedure in which damaged or diseased cornea is replaced with donated corneal tissue. Donor-to-host transmission of infectious agents is a rare but serious complication resulting in keratitis and/or endophthalmitis, resulting in graft failure and poor visual outcomes (1,2). The incidence of microbial keratitis in grafts varies from 1.76 to 18.7% (3,4). The rates of infectious endoph-

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thalmitis after penetrating keratoplasty (PK) reported to be low, ranging from 0.11% to 2.47% in large series (5-7).

Eye banks worldwide have implemented an array of preventive strategies to avoid the contamination of donor corneas, including antiseptic measures, aseptic retrieval of donor tissue, and use of antibiotics in transport and preservation media; however, corneal button contamination remains a cause of ocular infection in the early post-operative period (7). Therefore, determination of microbial contamination and antimicrobial susceptibility of donor eyes are important for the fastest and most accurate treatment of graft infection.

The purpose of this study was to report the prevalence of positive donor corneoscleral rim cultures in a tertiary eye care center and its association with keratitis and endophthalmitis after keratoplasty.

Methods

After obtaining the approval of the Haydarpasa Numune Training and Research Hospital Clinical Research Ethics Committee (Approval number: 2020/KK/3, February 10, 2020), the study was conducted in accordance with the ethical standards of the Declaration of Helsinki. Eye bank records and medical records of patients who underwent keratoplasty between September 1, 2015, and December 31, 2019, were retrospectively reviewed. Patients who had routine donor-rim culture taken during surgery and followed up for at least 1 year in the post-operative period were included in the study.

Cases with a history of death of unknown cause, septicemia, leukemia, subacute sclerosing panencephalitis, progressive multifocal leukoencephalopathy, ocular tumor, and cases with a history of Hepatitis B, Hepatitis C, HIV, HTLV-1, Cruetzfeld-Jacob disease were excluded as donors. Donor corneas were taken in accordance with sterilization and quality standards. After the donor corneal button is removed under sterile conditions, it was transferred to a sterile storage medium (Optisol-GS, Chiron Ophthalmics, Irvine, CA) or Optisol-GS (Bausch δ Lomb, Rochester, NY) and kept at +4° until transplantation. Corneal grafts for descemet stripping automated endothelial keratoplasty (DSAEK) and descemet membrane endothelial keratoplasty (DMEK) are prepared in the operating room.

The corneal tissue storage container from the eye bank was not opened until before tissue preparation by the surgeon in the operating room. After punching the graft to the desired size, the residual corneoscleral donor rim was sent to microbiology for culture analysis.

Donor corneoscleral rim bacterial and fungal cultures were performed for all cases. The donor corneoscleral rims were cultured on blood agar, chocolate agar, MacConkey agar for gram-negative bacteria, Sabouraud dextrose agar for fungi, and thioglycolate broth for anaerobes. Brain heart infusion agar was used to detect fastidious bacteria, and the plates were incubated aerobically in an environment containing 5% CO2. The culture media were incubated at 35–37° for a week except for the Sabouraud dextrose agar, which was incubated at 30° for a month.

The post-operative treatment included topical corticosteroid eye drops every 2 h, which were tapered gradually over 2 months, and antibiotic eye drops every 2 h after surgery for 2 to 3 weeks. They were instructed to undergo an immediate ophthalmologic examination if they felt ocular pain or discomfort, decreased vision, or redness of the eye.

The primary outcome measures were the presence of a positive corneoscleral rim culture and the development of keratitis and endophthalmitis after keratoplasty.

Statistical Analysis

All statistical analyses were performed using Statistical Package for the Social Sciences (SPSS) version 13.0 (SPSS Inc). Descriptive statistical methods (mean, standard deviation, median, frequency, percentage, minimum, and maximum) were used to analyze the study data. Chi-square test was used to compare the groups.

Results

A total of 826 keratoplasty procedures were performed between September 1, 2015, and December 31, 2019. The average age of the donors with contaminated donor corneoscleral rims was 51.31±12.57 years. The time from donor to corneal removal was 4.07±2.91 h. The average interval between harvest and transplantation was 5.35±2.11 days.

A total of 120 (14.52%) cases had a positive donor corneoscleral rim culture. Positive bacterial cultures were obtained from 108 (13.7%) donors. Bacterial keratitis was observed in one patient (0.83% of the recipients) who had a positive bacterial culture. Positive fungal cultures were seen in 12 (1.45%) donors, of whom one (8.33% of the recipients with a positive fungal culture) developed fungal keratitis. Endophthalmitis was observed in one patient whose culture result was negative.

When the patients who underwent keratoplasty were divided into two groups as penetrating and lamellar surgical procedures [deep anterior lamellar keratoplasty (DALK), DSAEK, DMEK], positive donor rim culture results were not statistically different in terms of bacteria and fungi (p=0.64). When PK, DMEK, DSAEK, and DALK were compared, no difference was found in terms of bacterial and fungal positive donor corneoscleral rim culture results (p=0.52) (Table 1).

Stenotrophomonas maltophilia, Staphylococcus epidermidis, Acinetobacter baumanni complex, and *Enterococcus faecium* were the most common cornea rim contaminants (Table

Table 1. Number of bacteria and fungi grown as a result of donor corneoscleral rim culture according to keratoplasty types

	PK	DSAEK	DMEK	DALK	
Number of bacteria	87	6	7	8	P=0.52
Number of fungus	9	0	2	1	
Total number	96	6	9	9	

PK: Penetrating keratoplasty, DSAEK: Descemet stripping automated endothelial keratoplasty, DMEK: Descemet membrane endothelial keratoplasty, DALK: Deep anterior lamellar keratoplasty.

2). Candida albicans was the most common fungi (Table 3). Seventeen percent of the pathogens belonged to the ocular flora. (*S. epidermidis, Coagulase negative Staphylococcus, Staphylococcus hominis, Staphylococcus capitis, Staphylococcus haemolyticus, and Corynebacterium striatum*)

The characteristics of the three cases of ocular infection after keratoplasty in our clinic are as follows:

Case 1 — A 37-year-old female patient who underwent PC due to advanced keratoconus and apical corneal scar had severe pain, lid edema, purulent secretion, multiple foci of white infiltration, and intense anterior chamber reaction on the 1st post-operative day. Cornea-conjunctival swab and anterior chamber samples were taken for direct microscopic examination and culture. Fortified vancomycin (50 mg/mL) drops and fortified ceftazidime (50 mg/mL) drops were started hourly. Steroid drops were discontinued. Gram-negative bacilli were seen in direct microscopic examination, but there was no growth in culture. It was reported that *Klebsiella pneumonia* grew as a result of donor cornea-scleral rim culture on the 2nd postoperative day. Therefore, donor-derived multidrug-resistant *K. pneumonia* keratitis was considered in the patient. Fortified drops were discontinued and colistin drops 0.19% every half hour, Intravenous colistin 2 million IU 3X1 for 10 days was started. On the 1st day of colistin treatment, pain, anterior chamber reaction, and suppuration were reduced. On the 5th day of colistin treatment, 0.5 mg/kg oral steroid was started and tapered off, and then topical steroid treatment was added. The graft cornea healed with a small central haze and preserved its transparency.

Case 2 — Phacoemulsification and intraocular lens implantation and Descemet's membrane endothelial keratoplasty were performed on a 71-year-old female patient due to cataracts and Fuch's endothelial corneal dystrophy. On the 8th day, she presented with the complaint of pain. A preliminary diagnosis of fungal keratitis was made due to the presence of two small, round, dense, and white fluffy-edged corneal infiltrates in the slit lamp examination. A corneal sample could not be obtained due to the deep location of the keratitis focus but *C. albicans* was seen to grow in the donor corneoscleral rim. A combination therapy of oral fluconazole (400 mg daily loading dose and 200 mg daily maintenance dose) and hourly eye drops with voriconazole 1% and amphotericin B 0.5% were begun. Voriconazole injections were inadministered intracamerally, intrastromally, and subconjunctivally. Topical steroids were discontinued. Topical cyclosporine drop was added 4 times a day. It regressed with medical treatment without the need for graft extraction. After 4 months, membranectomy was performed. Topi-

Table 2. Bacterial species cultured from donor corneoscleral rims

Bacterial species	n=108
Stenotrophomonas maltophilia	44
Staphylococcus epidermidis	12
Acinetobacter baumanni complex	13
Enterococcus faecium	10
Pseudomonas aeruginosa	7
Klebsiella pneumonia	4
Coagulase-negative staphylococcus	3
Enterococcus hirae	2
Staphylococcus hominis	2
Staphylococcus aureus	1
Staphylococcus capitis	1
Staphylococcus haemolyticus	1
Scedosporium sp	1
Providencia rettgeri	1
Corynebacterium striatum	1
Escherichia coli	1
Enterobacter	1
Penicillium spp	1
Streptococcus pneumonia	1
Actinomyces	1

Table 3. Fungal species cultured from donor corneoscleral rims

Fungal species	n=12
Candida albicans	4
Candida glabrata	1
Candida parapsilosis	2
Candida tropicalis	1
Candida kefyr	1
Candida lusitaniae	1
Aspergillus terreus	1
Sporobolomyces (yeast)	1

cal treatment was completed in 6 months. The patient's graft remained transparent and the final best-corrected visual acuity was determined as 1.0 using the Snellen chart.

Case 3 — Endophthalmitis started on the postoperative 1st day after PK. Pars plana vitrectomy was performed. There was no reproduction in the vitreous tap. The final visual acuity was light perception.

Discussion

We found positive donor corneoscleral rim culture results in 120 (14.52%) of 826 corneal grafts. A total of 108 (13.07%) of these grafts had a positive bacterial corneoscleral rim culture and 12 (1.45%) had a positive fungal corneoscleral rim culture. In our study, bacterial keratitis developed in only 1 (0.83%) patient with a positive bacterial donor rim and fungal keratitis developed in 1 (8.33%) of the 12 positive fungal donor rims. We encountered endophthalmitis in 1 case (0.12%) whose donor rim culture result was negative. Both bacterial and fungal culture results were similar in penetrating and lamellar surgical procedures.

Rehany et al., reported that of the 469 corneal grafts, 79 (16.8%) had positive bacterial donor rim cultures and none had a positive fungal culture (1). Keyhani et al. found that of the 2,466 corneal grafts, 325 (13.2%) had positive donor rim cultures and four developed endophthalmitis (2). Ritterband et al. determined that of the 509 corneal grafts, 61 (12%) had positive donor rim cultures while none developed endophthalmitis (8). Al-Assiri et al., noted that of the 2,392 corneal grafts, 419 (17.5%) had positive donor rim cultures and one developed endophthalmitis (9). Wilhelmus and Hassan. reported that of the 17,614 corneal grafts, 2459 (14%) had positive donor rim cultures and 31 (0.2%) developed endophthalmitis (7). Similar positive donor rim culture results were found in our study, consistent with the literature but endophthalmitis was not observed in any of the cases in which positive donor rims were used. These range values may result from different culture techniques, storage media, and equipment.

Fungal cultures have substantial predictive value because a positive fungal donor rim culture increases the risk of fungal endophthalmitis by 247 times (3). The overall rate of positive donor rim fungal cultures in the cornea preservation time study was 1.9%, with post-operative recipient fungal infections developing in 6.7% of corneas with positive cultures (10). Similar to our study, other studies have also reported an incidence of 1.1% to 2.1% for positive donor rim fungal cultures, and a post-operative recipient fungal infection rate of up to 7.5% when fungal contaminated tissue is received (2,3,7,11).

In our study, positive culture results in terms of bacteria and fungi were found to be similar between the cases who underwent PK, DALK, DSAEK, and DMEK. Garg et al. reported that the prevalence of positive donor rims was similar in patients who underwent PK, DSAEK, femtosecond laser-enabled keratoplasty, and DALK (12). In another study, although the increase did not reach statistical significance, it was reported that the rate of fungal infections was more common after endothelial keratoplasty than PK (13) Gao et al. reported that interface keratitis developed in 30% of positive donor rims (14). Fungal infections are primarily from *Candida* species and were reported to be difficult to eradicate in 15–24 cases requiring surgical intervention. In our case, we encountered fungal keratitis in the early post-operative period after DMEK, and the patient responded well to medical treatment. She recovered full visual acuity following membranectomy performed after the eye was calm. The deep localization of infiltration in lamellar keratoplasties makes it difficult to take samples for culture and causes a delay in the treatment process. Having a donor rim culture result and antibiogram available accelerates the treatment process and can prevent devastating results.

S. maltophilia, S. epidermidis, A. baumannii complex, and *E. faecium* were the most common corneal rim contaminants. *C. albicans* was the most common fungus, as observed in the previous studies. Matsumoto et al. reported that *Staphylococcus* was the most frequent isolate in conjunctival swabs (15). Rehany et al. determined Staphylococci and Streptococci to be the most commonly isolated bacteria (1). In our study, *Streptococcus* was isolated from one rim only. *S. maltophilia* has emerged as an opportunistic pathogen. It is an uncommon keratitis pathogen with the total number of cases reported in the literature being <40 (16,17). In our series, we did not encounter postoperative keratitis although *S. maltophilia* constituted 40.74% of the positive bacterial donor rims.

We encountered keratitis in the early period after PK in a patient with *Klebsiella* growth as a result of donor rim culture. Multidrug-resistant *Klebsiella* is an emerging group of bacteria and considered as the leading cause of nosocomial infection. In a study evaluating infectious interface keratitis after lamellar surgery, Gao et al. reported that one of nine bacterial keratitis cases was associated with *K. pneumonia*-positive donor rim results (14). Zarei-Ghanavati et al. also reported keratitis caused by *K. pneumonia* in the 2nd postoperative day of deep anterior lameller keratoplasty, which was consistent with the isolate obtained from the transplanted graft (18).

The limitations of our study concern its retrospective nature. However, to the best of our knowledge, our study is the largest series reporting donor corneoscleral rim culture results in our country and is also the only publication in the past 15 years showing donor corneoscleral rim microbial culture results.

Conclusion

Although the donor corneal rims have a high positive culture result, the rate of bacterial keratitis and endophthalmitis is low, the risk of infection is high in patients with a fungal positive donor rim. Closer follow-up of patients with fungal growth as a result of donor corneal rim culture and initiation of aggressive antifungal treatment when infection occurs will be beneficial.

Disclosures

Ethics Committee Approval: After obtaining the approval of the Haydarpasa Numune Training and Research Hospital Clinical Research Ethics Committee (Approval number: 2020/KK/3, February 10, 2020), the study was conducted in accordance with the ethical standards of the Declaration of Helsinki.

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