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Loss of endothelial cells in viral DNA-positive grafts after keratoplasty: a 2-year follow-up study

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ABSTRACT

Background To compare endothelial loss between recipients who received viral DNA-positive grafts and controls 2 years after corneal transplantation.

Methods We retrospectively analysed the clinical data and endothelial cell density of recipients of viral DNA-positive grafts and age-, sex-, aetiology- and operation-matched controls from April 2017 to July 2019 at the Peking University Third Hospital, Beijing, China.

Results A total of 23/942 (2.44%) donor corneal buttons tested virus-positive by real-time PCR. A total of 27 recipients (except for 2 recipients) of viral DNA-positive grafts and 48 recipients of viral DNA-negative grafts were included in this study. Recipients of viral DNA-positive grafts had a higher endothelial cell (EC) loss rate post-penetrating keratoplasty and post-descemet stripping automated endothelial keratoplasty ($p<0.05$), but post-deep lamellar keratoplasty, the EC loss rate was similar to that of the controls. Recipients of herpes simplex virus-1-, cytomegalovirus- and varicella-zoster virus-positive grafts all had a higher EC loss rate than the controls during the 12- and 24-month follow-up periods ($p<0.05$).

Conclusion We inferred that viruses might be hidden in corneal grafts and mainly incubate in the corneal endothelium. Viral DNA-positive grafts do not need to be replaced immediately and can be followed up for a long time.

INTRODUCTION

Donor corneas and preservation fluid are tested for bacterial and fungal infections which are believed to have a direct impact on the quality of grafts and the safety of recipients. Furthermore, cornea donors are also tested for hepatitis B virus (HBV), hepatitis C virus (HCV), syphilis and HIV before organ donation. In fact, virus detection is not a routine examination in most eye banks. Surprisingly, data on viral DNA positivity rates in donor corneas and the risk of transmission to recipients are scarce.[1] In 2009, Remeijer *et al*[2] reported that herpes simplex virus (HSV)-1 was detected in 2 of 273 corneoscleral rims, and HSV-2 and varicella-zoster virus (VZV) were not detected in donor corneoscleral rims. That study included the largest number of cases reported so far. Previous studies have mainly focused on HSV and VZV; recently, cytomegalovirus (CMV) was detected in 6 of 30 donor corneas obtained during keratoplasty.[3] There are no data regarding the Epstein–Barr virus (EBV) positivity rate in donor corneas.

HSV-1 can be transmitted from the graft to the recipient with subsequent reactivation of donor-derived HSV-1 in the transplanted cornea.[4] The donor-to-host transmission of infectious agents via corneal transplantation poses a real risk and can lead to graft failure, but there is lack of initial and follow-up data on CMV-, VZV- and EBV DNA-positive grafts after keratoplasty.

In our previous work (unpublished), we tested 942 remaining corneal rims after transplantation for HSV-1, HSV-2, CMV, VZV and EBV, and 23 donor corneal buttons tested positive for viral DNA. We continued to follow-up with recipients who received viral DNA-positive grafts and observed the influence on recipients among different virus types and operation methods. This study might allow a strategy for positive grafts after keratoplasty, for example, immediate graft replacement or long-term follow-up, to be determined.

MATERIALS AND METHODS

Donor corneas and viral DNA detection

Under sterile conditions, an 18-mm trephine was used to strip the sclera from the posterior cornea 3–4 mm within 12 hours after donor death. Then, the corneoscleral buttons obtained and stored in Optisol GS (Bausch & Lomb, Irvine, California, USA) at 4°C. The central endothelial cell density (ECD) of all donor corneas was quantified by a certified technician at our eye bank using the EB-3000 XYZ Eye bank specular microscope (HAI Laboratories, Lexington, Massachusetts, USA). Graft samples were obtained during consecutive cornea transplantation procedures. We extracted DNA from corneal tissues using a QIAamp DNA Mini Kit (catalogue no. 51 304; Qiagen, Hilden, Germany) according to the manufacturer's instructions. Briefly, corneal rim samples containing endothelium were cut into small pieces, placed in a 1.5-mL microcentrifuge tube and digested with Buffer ATL and proteinase K. The extracted DNA was diluted in water; a total of 50 ng was subjected to PCR. HSV-1, HSV-2, VZV, CMV and EBV were detected using qualitative commercial, TaqMan-based methods (HSV-1/HSV-2 Typing Real-Time PCR Kit, Z-SD-0136-02; VZV Real-Time PCR Kit, OD-0024-02; CMV Real-Time PCR Kit, Z-OD-002-02; EBV Real-Time PCR Kit, Z-OD-0023-02; Liferiver Bio-Tech Corp, China) in accordance with the manufacturer's instructions. Real-time PCR was performed using reagents from PE Biosystems (PE Applied Biosystems,

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Foster City, California). The limit of detection of viral DNA was 10 copies per sample size. Each sample was processed with the addition of an internal control for the assessment of isolation and amplification efficacy. Positive and negative controls, as well as internal controls, were provided by the kit manufacturer.

Study subjects

A total 23/942 (2.44%) donor corneal buttons tested virus-positive by RT-PCR from April 2017 to July 2019. Twenty-five recipients (19 males and 6 females) received viral DNA-positive grafts at 0.25–86 years of age (mean age, 35.7±25.9 years), and 48 age-, sex-, aetiology- and operation-matched controls (31 males and 17 females) received viral DNA-negative grafts from 0.3 to 86 years of age (mean age, 40.3±25.6 years). Viral DNA-positive and viral DNA-negative grafts were used for keratoplasty in the same month. All recipients had no history of ocular viral infection. The study was performed according to the tenets of the Declaration of Helsinki and was approved by the local ethics committee.

Surgical technique

In this study, all procedures were performed by an experienced surgeon (Jing Hong) at the Peking University Third Hospital. Three surgical methods were included in this study.

Penetrating keratoplasty (PK): Graft suturing was performed according to standardised methods in all patients, with 16 interrupted sutures used in most patients. Sutures were removed after at least 12 months. Descemet stripping automated endothelial keratoplasty (DSAEK): The surgical procedures were the same as previously detailed.[5 6] Deep anterior lamellar keratoplasty (DALK): The anterior and middle stroma was removed using a crescent blade. Air was injected into the posterior stroma. When air injection-induced detachment of the Descemet membrane, sodium hyaluronate was injected between the posterior stroma and the Descemet membrane, and the remaining posterior stroma was completely removed using scissors. Graft suturing was performed according to standardised methods in all patients, with 16 interrupted sutures used in most patients. Sutures were removed after at least 12 months.

Postoperative treatment regimen

The standard postoperative treatment for PK, DSAEK and DALK consisted of topical levofloxacin 0.5% and artificial tears (4 times per day) for 1 month, topical dexamethasone 0.1% eye cream (once every night) for 1 week and topical prednisolone acetate 1.0% (4 times per day), tapered accordingly over 3−6 months; topical ciclosporin 1% (4 times per day) was added 1 week after the surgery and was tapered depending on the status of the graft.

Postoperative follow-up

The clinical outcome of transplantation was assessed by the best-corrected visual acuity (BCVA, LogMAR), intraocular pressure (IOP) (ICARE, TA01i, Finland), ECD, graft status and complications at 6, 12 and 24 months postoperatively. The endothelial cell (EC) loss rate was calculated according to the ECD. The average ECD of the central area was measured by in vivo confocal microscopy (HRT III, Heidelberg Engineering, Heidelberg, Germany). Graft attachment and central corneal thickness (CCT) were assessed with anterior segment optical coherence tomography (Carl Zeiss Meditec, Dublin, California, USA). The same certified ophthalmic technician performed all postoperative testing of patients using the same microscope.

STATISTICAL ANALYSIS

Statistical analysis was performed with SPSS 18.0 (SPSS, Chicago, Illinois, USA). Recipient age, BCVA, CCT, ECD and EC loss were compared between the viral DNA-positive group and the control group using an independent-samples t-test. Recipient sex was compared between the two groups using the χ^2 test. The correlation between the IOP and EC loss was examined using the Pearson test. All tests were 2-tailed, $p<0.05$ was considered statistically significant, and $p<0.01$ was considered very statistically significant.

RESULTS

Demographics

The total donor cornea viral DNA positivity rate was 2.44% (23/942). The rates of positivity for HSV-1, CMV, VZV and EBV DNA were 30.43% (7/23), 34.78% (8/23), 26.09% (6/23) and 8.7% (2/23), respectively. HSV-2 DNA was not detected in the previous study. Patient nos. 22 and 24 experienced transplant failure caused by viral infection; they underwent a second transplantation and were excluded from this study. A total of 25 recipients who received viral DNA-positive grafts and 48 recipients who received viral DNA-negative grafts were included in this study. There were no significant differences in the mean age ($t=-0.717$, $p=0.476>0.05$) or sex ($\chi^2=0.993$, $p=0.319>0.05$) between the recipients with virus-positive grafts and controls. All grafts remained transparent and showed good attachment during the 24-month follow-up period. No keratic precipitate (KP) was found in any recipient.

Recipients with virus-positive grafts

Nine recipients (HSV-1/CMV/VZV/EBV=1/5/3/0) underwent PK, 9 recipients (HSV-1/CMV/VZV/EBV=3/2/2/2) underwent DSAEK and 7 recipients (HSV-1/CMV/VZV/EBV=4/2/1/0) underwent DALK. The diagnoses of the recipients are listed in table 1.

BCVA, CCT and ECD

No significant differences in the mean BCVA and CCT were found between the two groups ($t=0.062$, $p=0.951>0.05$; $t=0.636$, $p=0.527>0.05$). The average ECD of grafts with virus positivity was 3431±508 cells/mm^2. The average ECD of grafts in the control groups was 3316±450 cells/mm^2. No significant differences in the mean ECD ($t=0.842$, $p=0.404>0.05$) were identified between the two groups. The mean ECDs were 2290, 2052 and 1716 cells per mm^2 at 6, 12 and 24 months, corresponding to an EC loss of 25.63%, 32.68% and 43.81%, respectively, in the recipients with virus-positive grafts (n=25) (table 2). The mean ECDs were 2547, 2363 and 2156 cells per mm^2 at 6, 12 and 24 months, corresponding to EC losses of 16.85%, 12.89% and 29.13%, respectively, in the controls (n=48) (table 2).

PK

No significant differences in the mean BCVA and CCT were found between the two groups ($t=0.209$, $p=0.836>0.05$; $t=0.203$, $p=0.841>0.05$). The mean ECDs were 2232, 1942 and 1442 cells per mm^2 at 6, 12 and 24 months, corresponding to EC losses of 33.03%, 41.15% and 56.91%, respectively, in the recipients with virus-positive grafts (n=9) after PK (table 2). The mean ECDs were 2576, 2320 and 2038 cells per mm^2 at 6, 12 and 24 months, corresponding to EC losses of 19.33%, 27.22% and 35.99%, respectively, in the controls (n=16). There was a significant difference in the mean EC loss rate at all follow-up

Table 1 Clinical information of recipients of viral DNA positive-grafts

No.	Age range	Sex	Clinical diagnosis	Type of transplantation	BCVA	IOP (mm Hg)	Viral DNA	ECD of donor (cells/mm^2)	Last time of follow-up			ECD of graft at postoperative follow-up (cells/mm^2)		
									BCVA	IOP (mm Hg)	CCT (μm)	6	12	24
1	40s	M	Pseudophakic bullous keratopathy	DSAEK	2.3	8.0	HSV-1 (+)	4735	0.1	10.3	525	3045	2716	1845
6	30s	M	Ocular trauma	DSAEK	2.3	30.0	HSV-1 (+)	3077	1.0	23.4	600	1982	1859	1232
11	70s	F	Fuchs endothelial dystrophy	DSAEK	0.4	11.6	HSV-1 (+)	2722	0.1	19.0	624	2350	2005	1316
22	80s	F	Bullous keratopathy due to PHACO	DMEK	1.1	10.0	HSV-1 (+)	3189*	0.5	15.1	547	1732	1656	–
24	50s	M	Bullous keratopathy due to PHACO	DSAEK	2.3	16.0	HSV-1 (+)	3016*	0.3	17.5	912	1302	1298	–
8	30s	M	Alkaline burn of ocular surface	PK	2.3	10.0	HSV-1 (+)	3945	2.3	12.0	680	2120	1958	1458
12	20s	M	Keratoconus	DALK	2.0	6.0	HSV-1 (+)	–	0.0	9.9	500	1754	1703	1632
21	<3years	F	Congenital glaucoma	DALK	2.6	24.0	HSV-1 (+)	–	2.3	21.5	490	2698	2585	2415
23	10s	M	Keratoconus	DALK	2.0	7.0	HSV-1 (+)	–	0.1	13.0	525	3153	3089	2989
25	10s	M	Keratoconus	DALK	2.0	7.0	HSV-1 (+)	–	0.0	13.8	640	2856	2783	2603
9	30s	F	Congenital corneal leucoplakia	DSAEK	2.3	20.0	CMV (+)	3530	0.7	18.7	1050	1456	1234	994
18	20s	M	Congenital corneal leucoplakia	DSAEK	2.3	14.0	CMV (+)	3930	0.5	10.0	579	1842	1621	1556
2	<1 year	F	Congenital corneal leucoplakia	PK	2.6	26.3	CMV (+)	3130	0.5	15.6	558	2350	2023	1182
10	30s	M	Thermal burn of ocular surface	PK	2.3	9.0	CMV (+)	3764	0.3	10.2	561	2139	1898	1587
13	50s	M	Alkaline burn of ocular surface	PK	2.3	6.0	CMV (+)	2788	2.0	14.5	509	2457	1958	1297
17	30s	M	Bullous keratopathy due to PPV	PK	2.3	12.0	CMV (+)	3378	0.5	18.0	588	2165	1795	1818
20	70s	M	Alkaline burn of ocular surface	PK	2.3	6.6	CMV (+)	3332	2.0	9.8	598	2385	2047	1967
19	80s	F	Scleral melting	DALK	0.4	13.0	CMV (+)	–	0.4	11.0	519	2109	2045	1983
24	50s	F	Corneal leucoplakia	DALK	1.0	11.0	CMV (+)	–	0.3	14.5	527	1699	1602	1605
4	50s	M	Prior DSAEK failure	DSAEK	1.4	22.0	VZV (+)	2986	0.5	20.3	738	2006	1656	1433
14	<1 year	M	Congenital endothelial dystrophy	DSAEK	2.6	9.0	VZV (+)	3547	0.7	16.0	605	2848	2222	2031
3	60s	M	Fungal keratitis	PK	2.0	13.0	VZV (+)	2758	0.3	18.6	503	1301	1003	983
5	<1 year	M	Peter anomaly	PK	2.6	26.5	VZV (+)	3545	0.5	13.4	533	2421	2569	1403
7	20s	M	Fungal keratitis after trauma	PK	2.3	9.0	VZV (+)	3539	0.5	12.8	810	2753	2525	1285
15	70s	F	Corneal marginal degeneration	DALK	0.8	12.0	VZV (+)	–	0.7	12.0	590	1867	1800	1756
16	20s	M	Bullous keratopathy due to glaucoma	DSAEK	2.0	21.0	EBV (+)	3845	0.3	20.0	686	2710	2321	2292
25	<1 year	M	Congenital glaucoma	DSAEK	2.6	11.0	EBV (+)	3212	1.0	15.0	598	2789	2598	1732

BCVA, best-corrected visual acuity; CCT, central corneal thickness; CMV, cytomegalovirus; DSAEK, descemet stripping automated endothelium keratoplasty; DMEK, descemet membrane endothelial keratoplasty; DALK, deep lamellar keratoplasty; ECD, endothelial cell density; HSV, herpes simplex virus; IOP, intraocular pressure; PK, penetrating keratoplasty; PPV, pars plana vitrectomy; VZV, varicella-zoster virus.
*The ECD of the donor was measured a second time.

times (t=3.016, p=0.013<0.05; t=3.271, p=0.009<0.01; t=7.934, p=0.000<0.01) (figure 1A).

DSAEK

No significant differences in the mean BCVA and CCT were identified between the two groups (t=−0.268, p=0.791>0.05; t=0.737, p=0.468>0.05). The mean ECDs were 2336, 2026 and 1659 cells per mm^2 at 6, 12 and 24 months, corresponding to EC losses of 32.45%, 41.45% and 54.01%, respectively, in the recipients with virus-positive grafts (n=9) after DSAEK (table 2). The mean ECDs were 2656, 2471 and 2230 cells per mm^2 at 6, 12 and 24 months, corresponding to EC losses of 22.40%, 27.61% and 35.05%, respectively, in the controls (n=18). There was no significant difference in the mean EC loss rate at 6 months (t=1.866, p=0.097>0.05); there was a significant difference in the mean EC loss rate at 12 and 24 months (t=2.855, p=0.020<0.05; t=5.502, p=0.000<0.01) (figure 1B).

DALK

No significant differences in the mean BCVA and CCT were observed between the two groups (t=−0.041, p=0.968>0.05; t=0.000, p=1.000>0.05). The mean ECDs were 2305, 2230 and 2140 cells per mm^2 at 6, 12 and 24 months, corresponding to EC losses of 7.35%, 10.52% and 13.84%, respectively, in the recipients with virus-positive grafts (n=7) after DALK (table 2). The mean ECDs were 2375, 2274 and 2198 cells per mm^2 at 6, 12 and 24 months, corresponding to EC losses of 6.85%, 10.91% and 13.67%, respectively, in the controls (n=14). No significant difference in the mean EC loss rate was found at any follow-up times (t=0.566, p=0.58>0.05; t=−0.373, p=0.713>0.05; t=0.197, p=0.846>0.05) (figure 1C).

DALK was not related to changes in the corneal endothelium and showed no differences between the two groups; thus, from this point on, the ECD does not include recipients who underwent DALK.

Table 2 Average BCVA, CCT and ECD during the follow-up period in the graft viral DNA+ group and the control group

	Last time of follow-up		Average ECD during follow-up period (cells/mm^2)		
	BCVA	CCT (μm)	6 months	12 months	24 months
Total					
Graft viral DNA+ (n=25)	0.71±0.70	605±121	2290±481	2052±491	1716±476
Control (n=48)	0.70±0.62	590±79	2547±430	2363±372	2156±369
PK					
Graft viral DNA+ (n=9)	1.01±0.83	593±97	2232±378	1942±439	1442±291
Control (n=16)	0.95±0.62	587±44	2576±296	2320±205	2038±166
DASEK					
Graft viral DNA+ (n=9)	0.54±0.34	667±156	2336±512	2026±458	1659±366
Control (n=18)	0.59±0.50	631±99	2656±400	2471±331	2230±385
DALK					
Graft viral DNA+ (n=7)	0.54±0.81	541±81	2305±545	2230±543	2140±497
Control (n=14)	0.55±0.72	541±51	2375±530	2274±511	2198±469
HSV-1					
Graft viral DNA+ (n=4)	0.88±1.04	607±64	2374±409	2059±399	1588±211
Control (n=7)	0.77±0.78	598±54	2571±391	2320±135	2066±115
CMV					
Graft viral DNA+ (n=7)	0.96±0.73	606±111	2113±329	1797±267	1486±322
Control (n=15)	0.93±0.66	611±86	2550±295	2343±264	2079±282
VZV					
Graft viral DNA+ (n=5)	0.50±0.14	637±132	2266±565	1995±593	1427±341
Control (n=8)	0.54±0.16	616±110	2651±304	2468±326	2203±362
EBV					
Graft viral DNA+ (n=2)	0.65±0.49	642±62	2750±40	2460±139	2012±280
Control (n=4)	0.57±0.33	616±33	2893±456	2616±349	2366±434

The average ECD of grafts for HSV-1, CMV, VZV and EBV DNA does not include the data of recipients who underwent DALK.
BCVA, best-corrected visual acuity; CCT, central corneal thickness; CMV, cytomegalovirus; DALK, deep lamellar keratoplasty; ECD,endothelial cell density; EBV,Epstein–Barr virus; HSV, herpes simplexvirus; PK, penetrating keratoplasty; VZV, varicella-zostervirus

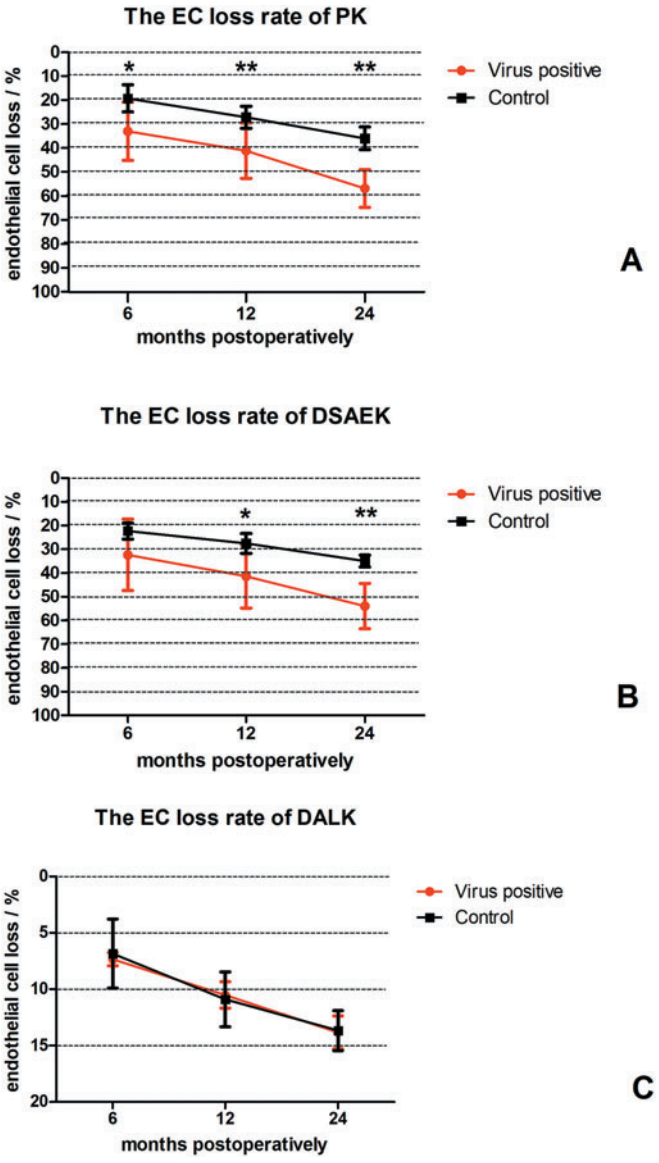

Figure 1 The EC loss rate after different operations in viral DNA-positive grafts and controls. (A) EC loss rate after PK. There was a significant difference in the mean EC loss rate at all follow-up times. (B) EC loss rate after DSAEK. There was no significant difference in the mean EC loss rate at 6 months and a significant difference in the mean EC loss rate at 12 and 24 months. (C) EC loss rate after DALK. There was no significant difference in the mean EC loss rate at any follow-up time (*p<0.05, **p<0.01). EC, endothelial cell; PK, penetrating keratoplasty; DSAEK, descemet stripping automated endothelial keratoplasty; DALK, deep lamellar keratoplasty.

HSV-1+

No significant differences in the mean BCVA and CCT were found between the two groups (t=0.189, p=0.854>0.05; t=0.254, p=0.809>0.05). The mean ECDs were 2374, 2059 and 1588 cells per mm^2 at 6, 12 and 24 months, corresponding to EC losses of 32.80%, 39.73% and 58.92%, respectively, in the recipients with HSV-1-positive grafts (n=4) (table 2). The mean ECDs were 2571, 2320 and 2066 cells per mm^2 at 6, 12 and 24 months, corresponding to EC losses of 20.08%, 27.52% and 35.47%, respectively, in the controls (n=7). No significant difference in the mean EC loss rate was noted at 6 months (t=1.897, p=0.090>0.05); a significant difference in the mean EC loss rate was observed at 12 and 24 months (t=2.986, p=0.015<0.05; t=11.418, p=0.000<0.01) (figure 2A).

CMV+

No significant differences in the mean BCVA and CCT were identified between the two groups (t=0.098, p=0.932>0.05; t=−0.132, p=0.896>0.05). The mean ECDs were 2113, 1797 and 1486 cells per mm^2 at 6, 12 and 24 months, corresponding to EC losses of 36.60%, 46.28% and 56.14%, respectively, in the recipients with CMV-positive grafts (n=7) (table 2). The mean ECDs were 2550, 2343 and 2079 cells per mm^2 at 6, 12 and 24 months, corresponding to EC losses of 20.98%, 27.35% and 35.6%, respectively, in the controls (n=15). There was significant difference in the mean EC loss rate at all follow-up times (t=2.486, p=0.046<0.05; t=3.842, p=0.007<0.01; t=5.027, p=0.001<0.01) (figure 2B).

VZV+

No significant differences in the mean BCVA and CCT were observed between the two groups (t=−0.429, p=0.676>0.05;

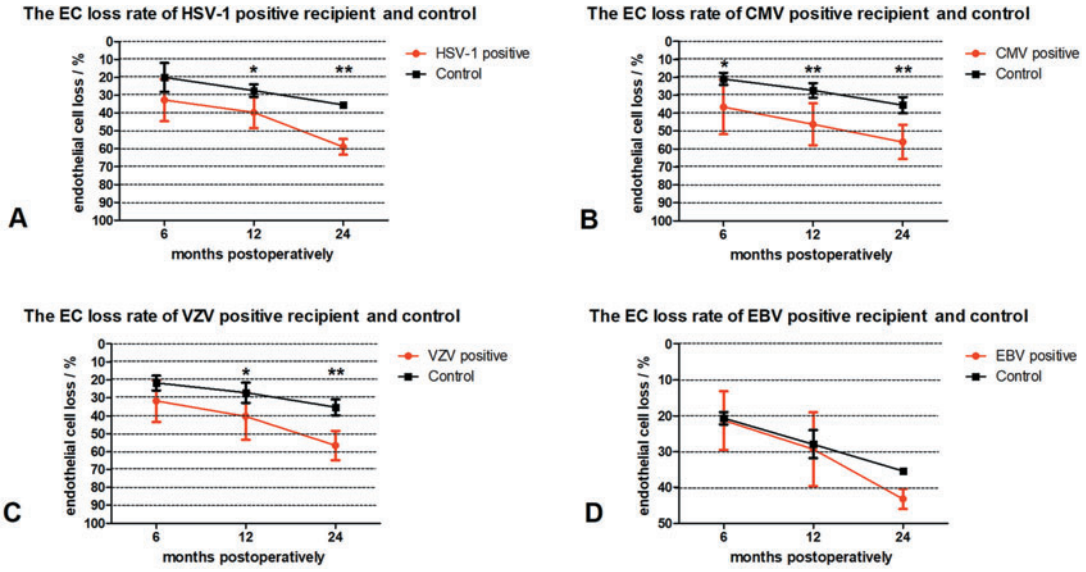

Figure 2 The EC loss in different virus-positive recipients and controls after PK and DSAEK. (A) EC loss rate in HSV-1-positive recipients and controls. There was no significant difference in the mean EC loss rate at 6 months and a significant difference in the mean EC loss rate at 12 and 24 months. (B) EC loss rate in CMV-positive recipients and controls. There was significant difference in the mean EC loss rate at all follow-up times. (C) EC loss rate in VZV-positive recipients and controls. There was no significant difference in the mean EC loss rate at 6 months and a significant difference in the mean EC loss rate at 12 and 24 months. (D) EC loss rate in EBV-positive recipients and controls. There was no significant difference in the mean EC loss rate at any follow-up time (*p<0.05, **p<0.01). CMV, cytomegalovirus; DALK, deep lamellar keratoplasty; DSAEK, descemet stripping automatedendothelial keratoplasty; EBV, Epstein–Barr virus; EC, endothelial cell; HSV,herpes simplex virus; PK, penetrating keratoplasty; VZV, varicella-zoster virus.

t=−0.311, p=0.762>0.05). The mean ECDs were 2266, 1995 and 1427 cells per mm^2 at 6, 12 and 24 months, corresponding to EC losses of 31.85%, 40.34% and 56.64%, respectively, in the recipients with VZV-positive grafts (n=5) (table 2). The mean ECDs were 2651, 1468 and 2203 cells per mm^2 at 6, 12 and 24 months, corresponding to an EC loss of 21.81%, 27.23% and 35.32%, respectively, in the controls (n=8). No significant difference in the mean EC loss rate was noted at 6 months (t=2.044, p=0.066>0.05); a significant difference in the mean EC loss rate was found at 12 and 24 months (t=2.274, p=0.044<0.05; t=5.56, p=0.000<0.01) (figure 2C).

EBV+

No significant differences in the mean BCVA and CCT were found between the two groups (t=0.229, p=0.830>0.05; t=0.687, p=0.53>0.05). The mean ECDs were 2750, 2460 and 2012 cells per mm^2 at 6, 12 and 24 months, corresponding to EC losses of 21.34%, 29.38% and 43.23%, respectively, in the recipients with EBV-positive grafts (n=2) (table 2). The mean ECDs were 2893, 2616 and 2366 cells per mm^2 at 6, 12 and 24 months, corresponding to EC losses of 20.73%, 27.96% and 35.47%, respectively, in the controls (n=4). No significant difference in the mean EC loss rate was noted at any follow-up times (t=0.074, p=0.953>0.05; t=0.135, p=0.931>0.05; t=2.694, p=0.216>0.05) (figure 2D).

IOP

There was no correlation between the IOP and EC loss in the graft-positive or control group (p=0.918, p=0.891).

DISCUSSION

During our observation, except for two recipients who received HSV-1-positive grafts, acute viral infection occurred within 1 week after endothelial keratoplasty (aqueous humour tested HSV-1 DNA-positive, and endothelial grafts showed viruses by electron microscopy on replacement). Interestingly, two recipients who underwent DALK showed no evidence of viral infection. This study was recently published by our research group.[7] Other grafts remained completely transparent over the 24-month follow-up period. However, the EC loss rate in the viral DNA-positive graft group was higher at 1 and 2 years after PK or DSAEK than that in the control group, but there was no significant difference between the viral DNA-positive graft group and the control group after DALK. Therefore, we inferred that viruses might be hidden in the grafts and mainly incubate in the corneal endothelium. In PK and DSAEK, viruses can be transmitted from donors to recipients. The presence of the virus in the endothelium continued to affect the morphology and function of the corneal endothelium, resulting in a higher EC loss rate than that observed in the controls.

The EC loss rate also varied by type of viral DNA. The EC loss rates in HSV-1 and VZV DNA-positive recipients were higher than those in the control groups from 1 year after the operation. HSV-1 has been proven to be transmitted from donors to recipients; we also observed this phenomenon. Similar results pertaining to latent viral infections in donor corneas and infections in recipients have been confirmed in animal experiments.[1 8] The cornea might serve as a reservoir of latent HSV-1 and a source of virus reactivation.[9] Polcicova *et al*[10] found that in mice infected with a strain of HSV that could not move from the sensory ganglion back to the cornea, herpes simplex keratitis developed because the virus was still in the cornea. Our study showed that HSV-1 might exist in the corneal endothelium. VZV has been found hidden in multiple ganglia throughout the body.[11] The two most frequently involved are the thoracic ganglion (87%) and the trigeminal ganglion (53%).[12] However, the means by which VZV enters the sensory ganglia remains uncertain. Our findings might provide a possible explanation for why VZV was not found in some ganglia but still established a latent infection. VZV could be hidden in the corneal endothelium.

However, CMV DNA-positive recipients had a higher EC loss rate at 6 months after surgery. A previous study inferred that graft-to-host transmission scarcely occurred in cases of CMV DNA-positive grafts.[3] However, our study found that CMV might exist in corneal ECs and continue to affect corneal ECs. According to anterior chamber-associated immune deviation (ACAID), we can infer that if the viral load is large or immune system is abnormal, the virus can enter the anterior chamber of recipients from the infected corneal endothelium and replicate. If the viral load is low and immune function is normal, the virus might exist in corneal ECs and not replicate or continue to affect corneal ECs. The EC loss rate in EBV DNA-positive recipients showed no significant differences from that in the controls during the 24-month follow-up. However, the number of cases was small, and further study is needed.

In our study, the EC loss rates in the control group were 19.33%, 27.22% and 35.99% at 6 months, 1 year and 2 years after PK, respectively. The EC loss rates in the control group were 22.90%, 27.61% and 35.05% at 6 months, 1 year and 2 years after DSAEK, respectively. In the literature, the reported EC loss rates after PK were 11–33%, 16–42% and 29–49% at 6 months, 1 year and 2 years,[13–17] respectively, while the EC loss rates after DSAEK were 16–40%, 16–44%, and 23–44% at 6 months and 1 and 2 years, respectively.[18–20] Our EC loss rates are similar to those reported by others.

Ideally, corneal tissue and other materials intended for transplantation should be free of pathogens. Donor corneas and preservation fluid are tested for bacterial and fungal infections, but viral DNA detection in grafts is difficult to complete before keratoplasty. Recipients who of viral DNA-positive grafts in keratoplasty are at risk of a hidden danger. This aspect has not been emphasised in the past. Over a 2-year follow-up, we observed rapid loss of the corneal endothelium. We suggest that viral DNA-positive grafts do not need to be replaced immediately and can be followed up for a long time.

CONCLUSIONS

Recipients who received viral DNA-positive grafts had a higher EC loss rate post-PK and post-DSAEK, but post-DALK, the EC loss rate was similar to that in the controls. Therefore, we inferred that viruses might be hidden in the grafts and mainly incubate in the corneal endothelium. Viral DNA-positive grafts do not need to be replaced immediately and can be followed up for a long time.

LIMITATIONS

The follow-up period in this study was 24 months, and further research is needed. Therefore, we plan to continue observing patients with positive DNA results to further confirm our hypothesis.

Contributors JH-Q, GG-X J-H: design of the study(); JH-Q, T-Y: conduct of the study; S-Z, HQ-Q: collection and management of the data; RM-P: analysis and interpretation of the data; JH-Q: writing of the manuscript; J-H: review or approval of the manuscript.

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