Original Article

Association between donor corneal endothelial cell counts and infectious agent reactivity: an eye bank database analysis

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Background: To evaluate the association between corneal central endothelial cell count (CECC) with reactivity for hepatitis B virus (HBV), hepatitis C virus (HCV), human immunodeficiency virus (HIV), human T-lymphotropic virus-1 (HTLV1), and syphilis from an eye bank database.

Methods: Eye bank data included 19,159 donors and 38,318 corneas screened for HBV, HCV, HIV, HTLV1, and syphilis from July 2007–May 2015. Linear and binary mixed effects models were used to determine the adjusted marginal effect a positive viral screening test had on CECC and morphology, respectively. The models were adjusted for age, race, gender, lens status, and death to preservation. Eyes with missing data were excluded from the analysis. Statistical significance was defined as P values <0.05.

Results: A total of 18,097 donors and 35,136 corneas were included in the final analysis. Average CECC for eyes with negative viral screening was 2,597±436 while the average CECC for eyes screening positive for syphilis, HBV, HCV, HIV, and HTLV1 were 2,638±392 (P=0.073), 2,569±419 (P=0.815), 2,603±363 (P=0.207), 2,615±360 (P=0.733), and 2,625±436 (P=0.362) respectively.

Conclusions: The presence of HBV, HCV, HIV, HTLV1, and syphilis display no association with a statistically significant difference in CECC when compared to normal non-diseased donors.

Keywords: Eye bank; endothelial cell count; infectious reactivity; hepatitis B virus (HBV); syphilis

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Introduction

The corneal endothelium plays a central role in maintaining the transparency of the cornea, which is crucial for the preservation of optimal visual clarity (1). It has been well established that factors which affect corneal endothelial integrity such as age (2,3), diabetes (4), and ocular conditions which impact endothelial cell counts such as pseudo-exfoliation (5), glaucoma (6), and Fuchs corneal dystrophy (7), can result in corneal edema and compromised visual acuity.

While viral and bacterial infections of the eye have been researched extensively, their impact specifically to the corneal endothelium has yet to be fully elucidated. To date, there have been only a few studies investigating the link between infectious agent reactivity and its impact on central corneal endothelial cell counts (CECC), a surrogate

Table 1 Corneal donor characteristics

Characteristic	Negative screen	+Syphilis	+HBV	+HCV	+HIV	+HTLV1
Donors						
Age, mean (SD), yr	56.1 (15.2)	58.3 (11.3)*	58.9 (11.7)*	54.7 (9.9)*	53.6 (10.7)*	53.7 (15.5)
Female sex, No. (%)	5,545 (35.4)	54 (41.1)	375 (33.8)	132 (26.6)*	27 (22.9)*	58 (45.3)*
Non-White, No. (%)	1,550 (9.9)	45 (34.4)*	240 (21.7)*	69 (13.8)*	20 (17.3)*	24 (18.4)
Eyes						
Time from death to preservation, mean (SD), hr	11.3 (5.9)	11.4 (5.5)	12.3 (6.0)*	12.4 (6.0)*	12.5 (5.9)*	13.1 (5.8)*
Phakia, No. (%)	27,666 (90.9)	233 (92.1)	1,912 (89.1)*	919 (95.1)*	213 (92.2)	222 (90.6)

*, P<0.05 compared to negative screen. HBV, hepatitis B virus; HCV, hepatitis C virus; HIV, human immunodeficiency virus; HTLV1, human T-lymphotropic virus-1.

marker for endothelial integrity. Müller *et al.* (7) showed a significant decrease in CECC in both the affected and contralateral unaffected eye of those with herpes simplex keratitis in comparison to non-affected controls (P<0.0001). Cleator *et al.* (8) reported evidence of Herpes simplex virus DNA present in "discrete foci" in the cornea, implicating Herpes simplex virus and subsequent corneal endothelial cell loss during corneal organ culture. Miyanaga *et al.* (9) showed a positive correlation between Cytomegalovirus viral load in the aqueous humor of the eye and corneal endothelial cell loss.

All of the aforementioned studies have associated viral keratitis with decreased endothelial cell density; however, to date, there have been a paucity of studies investigating the impact of systemic viral or bacterial infections on CECC. In this study of an eye bank database, we report the association of donor CECCs with infectious viral agent reactivity, specifically hepatitis B virus (HBV), hepatitis C virus (HCV), human immunodeficiency virus (HIV), and human T-lymphotropic virus-1 (HTLV1), as well as infectious bacterial agent reactivity, specifically syphilis. To our knowledge, this is the first report of its kind.

Methods

We reviewed the Lion's Eye Institute (Tampa, FL) eye bank database, which included de-identified information on all donors from July 2007 to May 2015. This encompassed a total of 38,318 corneas obtained from 19,159 donors. The database included donor information on age, sex, race, death to preservation time, and lens status.

Donors were screened for syphilis, HBV, HCV, HIV, and HTLV1. The specific assays used included Fluorescent Treponemal Antibody Absorption, Rapid Plasma Reagin, Anti-Hepatitis B Core IgM, Hepatitis B Surface Antigen, Hepatitis B Nucleic Acid Testing, Hepatitis C serology and nucleic acid testing; HIV serology and nucleic acid testing, and HTLV1 serology.

Endothelial cells were analyzed with specular microscopy (Konan CellChek EB-10). CECCs were calculated using software after manual identification of a minimum of 100 cell centers from 3 to 5 micrographs. Additionally, the presence of polymegathism and polymorphism was determined by specular technician assessment of microscopy photographs. Specular microscopy technicians were required to partake in an annual in-house qualification process.

Linear and binary mixed effects models were used to determine the adjusted marginal effect that a positive viral screening test had on CECC and morphology, respectively. The models were adjusted for age, race, gender, lens status, and death to preservation. Eyes with missing data were excluded from the analysis. All statistical analyses were performed using Stata 14.0 (StataCorp., College Station, TX). Statistical significance was defined as P value <0.05. The study was performed in accordance with the tenets of Helsinki. Approval for the retrospective analysis of this data was obtained from the Institutional Review Board and the authors have no competing interests.

Results

Table 1 shows background demographics for 18,097 donors

Table 2 Adjusted mixed effect model results

Infectious agent	Adjusted CECC difference from negatively screened eyes	P value (SE)
Syphilis	+54	0.073 (30.1)
HBV	+3	0.815 (11.5)
HCV	−19	0.207 (15.5)
HIV	−10	0.733 (31.5)
HTLV1	−31	0.362 (34.8)

HBV, hepatitis B virus; HCV, hepatitis C virus; HIV, human immunodeficiency virus; HTLV1, human T-lymphotropic virus-1.

and 35,136 eyes. The average donor age was 56.3±15.0 years, 35.4% were female, and 9.6% tested positive for at least one viral screening test. The screening test with the highest percentage of positive results was HBV (6.1%), and the test with the lowest percentage of positive results was HIV (0.7%).

The average CECC for eyes with negative viral screening was 2,597±436. The average CECC for eyes screening positive for syphilis, HBV, HCV, HIV, and HTLV1 were 2,638±392, 2,569±419, 2,603±363, 2,615±360, and 2,625±436 respectively. *Table 2* shows the adjusted marginal difference of a positive viral screening test on CECC. None of the viral screening tests for syphilis, HBV, HCV, HIV, and HTLV1 was associated with a statistically significant difference in CECC compared to controls with P values of P=0.073, P=0.815, P=0.207, P=0.733, and P=0.362 respectively.

Discussion

Despite previous studies showing a decrease in CECC correlated with evidence of ocular viral reactivity, our study did not show any statistically significant increase or decrease in CECC correlated with disease reactivity for HBV, HCV, HIV, HTLV1 and syphilis. The correlation between a reduced CECC and disease reactivity in the eye has been explained before and is quite understandable considering that the corneal endothelium is known to be a relatively non-mitotic entity. When trauma or pathogen induced damage occurs, corneal endothelial pump function becomes significantly impaired as the corneal endothelial cells undergo apoptosis, and corneal edema ensues (1).

It has been discussed that while the exact mechanism for the reason behind the accelerated corneal endothelial cell loss following trauma such as cataract extraction is poorly understood, possible mechanisms could be due to decreased innervation, exposure to vitreous humor, and increase in subclinical inflammation (1,7). Koh *et al.* (10) showed that the protective effects of vasoactive intestinal peptide (VIP) help to prevent corneal endothelial cell apoptosis and maintain corneal integrity. Furthermore, Müller *et al.* (7) demonstrated that corneal endothelial cell loss in the setting of viral keratitis could be related to the potentially causal effect of viral reactivity on the loss of corneal sub-basal innervation and subsequent decrease in neuropeptides levels such as the aforementioned VIP.

Despite this, in our study, results appear to show no effect of viral and bacterial reactivity on CECC. Similarly, after a qualitative analysis using criteria from an Eye bank atlas (11) to determine the presence of polymegathism and polymorphism in corneal donor cells, no effect of disease reactivity was found on the presence or absence of such morphology. One explanation could be that while previous studies (7-9) looked at the effect of viral keratitis on CECC, in our study, evidence of systemic viral reactivity without confirmation of ocular involvement was used. Lee *et al.* (12) detected the presence of HCV RNA via polymerase chain reaction (PCR) in the corneas of 7 (24.1%) out of 29 corneal donors who were seropositive for HCV. Heck *et al.* (13) detected the presence of HCV RNA via Nucleic Acid Testing (NAT) in the cornea of 17 (77%) out of 22 corneal donors who were NAT seropositive for HCV, HIV RNA in the cornea of 1 (50%) out of 2 corneal donors who were NAT seropositive for HIV, and HBV RNA in the cornea of 1 (50%) out of 2 corneal donors who were NAT seropositive for HBV. While these findings represent a significant correlation between systemic viral reactivity and ocular viral presence, it also shows that not all who are seropositive display evidence of ocular involvement. Hence, it is possible that those in our study that tested seropositive for both viral and bacterial agents did not have ocular involvement and thus were equivalent to seronegative controls. Therefore, despite the large database utilized for this study, it may still be underpowered as we do not know how many, if any, of the systemically-infected donors also had intraocular virus.

In conclusion, analysis of a U.S. eye bank database shows that the presence of HBV, HCV, HIV, HTLV1, and syphilis display no association with a statistically significant correlation in CECC or morphology when compared to normal non-diseased corneas. Thus, clinically speaking, the five infectious diseases discussed here may display no significant effects on the corneal endothelium.

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Footnote

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Ethical Statement: The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. The study was performed in accordance with the tenets of Helsinki (as revised in 2013). Approval for the retrospective analysis of this data was obtained from the Institutional Review Board and the authors have no competing interests.

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