Alcohol Abuse Is Associated With Alterations in Corneal Endothelial Cell Morphology

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Purpose: Alcohol consumption is highly prevalent throughout the world. We sought to detect, in a large sample of cornea donors, whether alcohol abuse is associated with changes in corneal endothelial morphology after accounting for other comorbidities including tobacco use.

Methods: At a single eye bank, 10,322 eyes from a total of 5624 unique donors underwent imaging with a Konan CellChek D specular microscope. Demographic information and medical history were associated with each tissue. Images were analyzed using a standardized protocol for assessment of endothelial cell density, hexagonality, and variation. In this retrospective analysis, a multivariable regression was conducted to assess for an association between alcohol abuse and corneal endothelial metrics. Measurements were averaged across eyes for each donor. Bonferroni corrections were applied to account for multiple comparisons.

Results: Among 5624 donors, the mean (standard deviation) endothelial cell density was 2785 (383.0) cells/mm^2. Indicators of alcohol abuse were present in 1382 donors (24.5%). In a multivariable regression model that included age, sex, tobacco use, history of cataract surgery, and diabetes mellitus, alcohol abuse was associated with a decrease of 60.9 cells/mm^2 [95% confidence interval (CI), −83.0 to −38.7 cells/mm^2, $P = 7.6 \times 10^{-8}$], an increase in the coefficient of variation by 0.0048 (95% CI, 0.17–0.79, $P = 0.002$), and a decrease in percent hexagonality by 0.93% (95% CI, −1.3 to −0.6, $P = 4.5 \times 10^{-7}$).

Conclusions: Alcohol abuse is associated with significant alterations to corneal endothelial density and morphology.

Key Words: alcohol, corneal endothelium, specular microscopy

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Alcohol abuse is one of the most prevalent substance use disorders in the world, affecting approximately 283 million people in 2018.[1] Heavy alcohol consumption contributes to damage at both cellular and structural levels throughout the central nervous system.[2] Within the eye, a well-established association exists between alcohol abuse and optic nerve, retinal, and lens pathology.[3] Nevertheless, more research is needed to understand the clinical effects of alcohol on the corneal endothelium.

Comorbidities and use of additional substances among heavy users of alcohol create a challenge in conducting clinical research into the effects of alcohol use or abuse. For example, a strong association exists between use of alcohol and use of cigarettes,[4] and tobacco is associated with decreased corneal endothelial function.[5] Although a direct comparison of individuals affected and unaffected by alcohol dependence suggests a deleterious impact on corneal endothelial cell density (ECD),[6] no study to date to the best of our knowledge has examined an association between heavy alcohol use and corneal endothelial characteristics in the context of comorbidities. Analyses with a large data set and adjusting for additional variables would be helpful to address potential confounders.

Data from eye banks, which conduct specular microscopy and collect clinical data for every cornea received, offer a unique opportunity to assess the impact of environmental factors on the corneal endothelium. Large studies from eye banks have successfully identified the effects of diabetes, cataract surgery, and smoking on the corneal endothelium,[4,5] but heavy alcohol use has yet to be considered among such factors.

In this study of over 10,000 corneal endothelial images and associated medical history analyzed from an eye bank, we examined the effect of heavy alcohol use on the corneal endothelium in the setting of additional factors, such as

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Availability of data and material: The data sets generated during and/or analyzed during the current study are not publicly available but are available from the corresponding author on reasonable request.

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smoking and diabetes, to better ascertain the specific contribution of this risk factor for corneal endothelial cell loss.

METHODS

During routine evaluation, all donor corneas that were processed at the Rocky Mountain Lions Eye Bank in Colorado underwent specular microscopy for quantitative and qualitative analysis of the corneal endothelium and analyzed manually using a single Konan CellChek D. Medical history and demographic information were associated with each image. Corneal endothelial density, coefficient of variation (CV), and percent hexagonality (HEX) were calculated and documented for each image at the time of tissue processing.

All images from September 15, 2016, to August 3, 2020, were acquired for analysis. For statistical analyses, values were averaged across eyes and duplicates were removed. Three eyes were excluded from analysis because the CV value was recorded as greater than 100, which is a statistical impossibility and suggests that data entry may have been compromised for these individuals. These represent less than a tenth of 1 percent of total donors.

To determine the quality of analysis, we assessed the total number of cells counted per image, applying a standardized grading system for eye bank images.[6] In this system, we applied a grade of 4 if 100 or more cells were analyzed, 3 if 50 to 99 cells were analyzed, 2 if 15 to 49 cells were analyzed, or grade of 1 if less than 15 or no cells were analyzed.

A medical history for each patient was acquired by the Rocky Mountain Lions Eye Bank from hospital medical records and included whether alcohol dependence or its sequelae were present. To apply a conservative estimate for the association and prevent selecting for only the most well-documented, severe cases of alcohol abuse, which would artificially inflate the effect of alcohol consumption, we performed a search in the medical history of donors for the term "alcohol" or "EtOH." Once a list of all medical histories with these terms was acquired, the list was manually curated to exclude non–alcohol-related terms, such as "nonalcoholic hepatitis" that would appear in the search. Cases of secondary organ failure, such as cirrhosis, specifically mentioned as being due to alcohol ("alcoholic cirrhosis") were included.

Similarly, for tobacco use, medical histories were searched for the terms "tobacco" or "smoking." For diabetes, we included the terms "diabetes," "diabetic," "DM1," and "DM2" and then excluded cases with terms such as "pre-diabetes." For lens status, we searched for the terms "pseudophakic," "pseudophakia," and "cataract surgery."

We first conducted a descriptive analysis and assessed differences in values between donors with each factor compared with donors without any of these factors. To do so, we conducted a multivariable regression including age and sex as variables. Second, we used a multivariable regression to account for the combined effect of age, sex, cataract surgery status, diabetes, history of heavy alcohol use, and history of tobacco use on corneal endothelial characteristics. Considering these 6 factors, we applied Bonferroni corrections, and alpha was reduced from 0.05 to 0.0083.

RESULTS

The data set included 10,322 images from a total of 5624 unique donors. The average analysis included 99 cells per image. The distribution plot of the cells analyzed is included in Supplemental Digital Content 1 (see Supplemental Data 1, http://links.lww.com/ICO/B401). More than 100 cells were counted on average from 2564 donors (grade 4 of 4), between 50 and 99 cells from 3055 donors (grade 3 of 4), between 15 and 49 cells from 5 donors (grade 2 of 4), and no donors contributed tissues with a mean of less than 15 cells counted (grade 1 of 4).

Of these donors, the median age was 58, with an interquartile range of 47 (25th percentile) to 65 years old (75th percentile). A total of 2357 donors (41.9%) were female. Indicators of alcohol use were present in 1382 donors (24.5%), tobacco use in 1113 donors (19.8%), diabetes mellitus in 1271 donors (22.6%), and pseudophakia in 585 donors (10.4%). The remaining 2423 individuals (43.1%) had none of these risk factors for endothelial cell loss.

A total of 350 alcohol-related medical history phrases were identified in the data set, of which 7 appeared more than 30 times each. Of these, "alcohol abuse" (n = 958), "alcoholic cirrhosis" (n = 109), and "alcoholism" (n = 88) were by far the 3 most common descriptors. Most terms were only used once (265/350, 75.7%). Many of these terms were variations or additions to simple terms, such as "history of alcohol abuse chronically" (n = 1) or "former alcohol abuse with recent relapse" (n = 1). Table 1 includes the most common exact terms associated with heavy alcohol use in the medical history of donors. All alcohol-associated terms and their frequency are included in Supplemental Digital Content 1 (see Supplemental Data 1, http://links.lww.com/ICO/B401).

TABLE 1. Most Common Terms Associated With Heavy Alcohol Use in the Medical History of 5624 Donors to a Single Eye Bank Over a Four-Year Period

Most Common Exact Terms in the Donors' Medical History Associated With Alcohol (n > 30)	
Medical History Term	**No. of Entries**
Alcohol abuse	958
Alcoholic cirrhosis	109
Alcoholism	88
Alcoholic hepatitis	68
Alcohol withdrawal	58
Alcohol abuse with alcoholic cirrhosis	41
Alcohol abuse in the past	31

The terms in the table represent those included in the medical history of at least 30 donors. Of 350 total alcohol-related terms identified in the data set, "alcohol abuse" was the most common, whereas 265 terms were used only once. Terms not associated with alcohol abuse, such as "nonalcoholic steatohepatitis," were excluded from the data set.

TABLE 2. Corneal Endothelial Characteristics in Eyes of 5624 Cornea Donors to the Rocky Mountain Lions Eye Bank

	All (n = 5624)		Alcohol Abuse (n=1382)				Pseudophakia (n = 585)			
	Mean (SD)	Median (IQR)	Mean (SD)	Median (IQR)	Effect Estimate (95% CI)	P	Mean (SD)	Median (IQR)	Effect Estimate (95% CI)	P
Age (yr)	54.2 (14.0)	58 (47 to 65)	52.4 (12.5)	55 (43 to 62)	**−2.4 (−3.3 to −1.6)**	**3.6×10^{-8}**	63.4 (7.6)	65.0 (60 to 68)	**10.1 (9.0 to 11.3)**	**3.9×10^{-63}**
ECD (cells/mm²)	2785 (383.0)	2780 (2548 to 3027)	2756 (343.5)	2756 (2538 to 2977)	**−60.9 (−83.0 to −38.7)**	**7.6×10^{-8}**	2631.7 (408.7)	2630.5 (2392 to 2884)	**−88.1258 (−120.0 to −56.3)**	**6.0×10^{-8}**
HEX (%)	56 (6.2)	56 (52 to 60)	56 (6.1)	56 (52 to 60)	**−0.93 (−1.3 to −0.57)**	**6.6×10^{-7}**	55.7 (5.6)	55.5 (52.0 to 59.0)	0.44 (−0.085 to 0.97)	0.10
CV (×100)	36.5 (5.2)	36 (33 to 39)	37 (4.9)	36 (34 to 39)	**0.48 (0.17 to 0.79)**	**0.002**	37.0 (5.4)	36.0 (34.0 to 40.0)	−0.23 (−0.68 to 0.21)	0.31

	Unaffected by Selected Risk Factors (n = 2423)		Diabetes Mellitus (n = 1271)				Tobacco Abuse (n = 1113)			
	Mean (SD)	Median (IQR)	Mean (SD)	Median (IQR)	Effect Estimate (95% CI)	P	Mean (SD)	Median (IQR)	Effect Estimate (95% CI)	P
Age (yr)	51.9 (15.9)	57 (43 to 64)	59.1 (9.4)	61.0 (54.0 to 66.0)	**6.2 (5.4 to 7.1)**	**4.4×10^{-45}**	55.7 (12.7)	60.0 (48.0 to 65.0)	**1.9 (1.0 to 2.8)**	**4.2×10^{-5}**
ECD (cells/mm²)	2838 (386)	2825 (3595.5 to 3075.3)	2731.8 (396.4)	2735.0 (2492.0 to 2976.0)	−15.8 (−38.9 to 7.3)	0.18	2737.7 (367.5)	2732.0 (2500.0 to 2985.0)	**−42.9 (−66.7 to −19.0)**	**4.3×10^{-4}**
HEX (%)	56.8 (6.5)	56.5 (52.5 to 56.5)	55.7 (5.6)	56.0 (52.0 to 60.0)	−0.092 (−0.48 to 0.29)	0.64	56.0 (5.8)	56.0 (52.0 to 60.0)	−0.16 (−0.56 to 0.24)	0.42
CV (×100)	36.1 (5.3)	35.5 (33 to 38.5)	37.1 (5.2)	36.5 (34.0 to 40.0)	0.31 (−0.011 to 0.64)	0.058	36.8 (5.1)	36.0 (34.0 to 40.0)	0.18 (−0.150 to 0.52)	0.28

For each risk factor, a multivariable regression was conducted including age and sex as variables. Assessments for age included sex and the risk factor in question as variables. Values in bold have surpassed the threshold for statistical significance.

The median endothelial cell density in the data set was 2780 (IQR 2548–3027). Table 2 summarizes demographic and endothelial (corneal endothelial density, CV, and HEX) values. Including age and sex as additional variables, alcohol abuse, tobacco abuse, diabetes mellitus, and pseudophakia were all associated with significantly different values relative to controls without any such factors.

In the multivariable regression model of multiple factors (Table 3), alcohol abuse was significantly associated with changes to each of the endothelial characteristics studied. The data demonstrated a decrease of approximately 60.9 cells/mm² relative to donors without a history of alcohol abuse [95% confidence interval (CI), −83.0 to −38.7 cells/mm², $P = 7.6 \times 10^{-8}$]. A small increase in the CV (+0.0048, 95% CI, 0.17–0.79, $P = 0.002$) and a decrease in HEX of approximately one percentage point (−0.93%, 95% CI, −1.3% to −0.6%, $P = 4.5 \times 10^{-7}$) were appreciated in donors with a history of alcohol abuse. In this model, tobacco use and cataract surgery also each contributed to a decreased endothelial cell count, with an estimated decrease of 36 ($P = 0.003$) and 88 cells/mm² ($P = 8.7 \times 10^{-8}$), respectively, among those harboring these risk factors.

DISCUSSION

This study contributes a large data set to explore the effect of heavy alcohol use on the corneal endothelium. Quantitative and qualitative changes in endothelial morphology appeared even after adjusting for additional factors.

Drawing on eye bank data offers unique insights into disease associations in a region and selects from a patient population broader than a specific hospital. Previous work drawing on such data has assessed the effects of hypertension, glaucoma, depression, dementia and neurodegenerative diseases, thyroid dysfunction, and tobacco use.[7] In this article, we confirm heavy alcohol use among known deleterious factors to the corneal endothelium and identify a strong association with ECD loss. Replication of this finding by eye banks in additional regions of the world would be helpful to confirm that this finding is generalizable across populations. Given the strength of this association, it may be helpful to consider alcohol abuse as a potential comorbidity when studying the effects of other clinical variables on corneal endothelial health.

Further research is needed to understand the specific pathological changes that take place in the setting of heavy alcohol use. It has been postulated that conversion of alcohol to acetaldehyde may result in direct cytotoxicity to the corneal endothelium,[8] which offers one possible pathway, but the physical, social, and behavioral aspects of alcohol abuse, which can include malnutrition and metabolic alterations, offer alternative mechanisms worthy of study.

By including all medical histories specifically mentioning alcohol use, we sought to identify a conservative estimate;

TABLE 3. Multivariable Linear Regression of Risk Factors for Corneal Endothelial Damage in a Set of Specular Microscopy Images From 5624 Unique Donors

	Endothelial Cell Density		Coefficient of Variation		Hexagonality	
Intercept	3263.7		32.4		62.2	
Factor	Coefficient (cells/mm²)	*P*	Coefficient (×100)	*P*	Coefficient (%)	*P*
Age	−8.2	6.8×10^{-111}	0.07	2.2×10^{-43}	−0.10	2.5×10^{-63}
Female sex	−0.67	0.95	0.28	0.044	−0.39	0.016
Alcohol abuse	−60.7	9.8×10^{-8}	0.49	0.002	−0.94	4.8×10^{-7}
Tobacco use	−35.8	0.003	0.15	0.39	−0.07	0.729
Diabetes	−12.8	0.28	0.42	0.011	−0.21	0.285
Pseudophakia	−87.7	8.7×10^{-8}	−0.30	0.19	0.45	0.099

Even after adjusting for additional risk factors, alcohol abuse is strongly associated with changes in endothelial cell density, coefficient of variation, and hexagonality. Numbers in bold are those that reached the threshold for statistical significance after adjusting for multiple comparisons.

the true association of alcohol abuse with endothelial cell loss may be even greater than what was described in this study. Further research into a dose–response curve between light and heavy alcohol use would offer additional insights into the alcohol-associated risks to the endothelium. In this study, we did not distinguish between former and recent alcohol abuse because endothelial cell loss is sustained over time and corneal endothelial cells do not replicate. Therefore, any endothelial cell loss would be expected to sustain over the life span. However, whether there is a difference in endothelial cell morphology between patients with varying histories of alcohol dependence could be an area of additional future research.

This study suggests that, in addition to decreased endothelial cell density, changes occur in HEX and the CV values with heavy alcohol use. Although a previous clinical study that assessed alcohol dependence did not identify a difference in these characteristics,[5] these patterns may be more discernible in this larger data set and particularly when adjusting for confounding variables. The variability in the shape and size of cells is of particular importance because it is associated with cell stress and has been associated with a higher likelihood of corneal decompensation after intraocular surgery.[9,10]

The estimated effect of alcohol abuse on ECD was modest at 60.9 cells/mm², a small percentage of the median ECD in this study. This suggests that the findings, although statistically significant, may not reach the threshold for clinical significance for most donors. Nevertheless, the strength of the association was particularly notable, and this knowledge may be of particular interest for people with compromised corneal endothelium, for whom the cell density is under 1000 cells/mm², and who are seeking specific strategies to maintain as much clarity of the cornea as possible. Further research will be needed to determine whether certain individuals are more susceptible to alcohol-associated ECD loss.

Given the multiple comparisons made, we applied a strict threshold for statistical significance. Although this more conservative estimate allows the observer to have confidence in the associations identified, it reduces statistical power and increases the likelihood of type II error. Therefore, it is possible that some true associations may not be recognized. Several associations demonstrated a *P* value between 0.0083 and 0.05; we interpret these as trends worthy of investigation with additional data sets. Moreover, we were unable to quantify tobacco use, and future work could investigate this phenomenon.

In this study, the proportion of specular microscopy images analyzed by counting at least 100 cells was less than half, with most images analyzed by counting 50 to 99 cells. To rule out the possibility that the findings in the study were affected by the number of cells counted, we conducted 2 post hoc analyses of ECD, one analysis adjusting for the cells counted and a second analysis stratified for those donors whose images averaged 100 cells counted per image across both eyes. The results are given in Supplemental Digital Content 1 (see Supplemental Data 1, http://links.lww.com/ICO/B401) and demonstrate the persistent association with alcohol in both analyses.

In summary, data from this large set of corneal endothelial images from an eye bank point to significant changes in endothelial cell density and morphology associated with heavy alcohol use.

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