Effects of Type 2 Diabetes Mellitus and Smoking on Changes in Corneal Endothelial Morphology and Cell Density

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Purpose: The purpose of this study was to compare the corneal endothelial morphology and cell density of diabetic smokers and nonsmokers with 50 to 70 age-matched healthy subjects and to determine whether smoking increases the effects of type 2 diabetes mellitus (DM) on these corneal parameters.

Methods: This prospective cohort study included 200 patients who were assigned to 4 groups, including smokers with type 2 DM (group 1), nonsmokers with type 2 DM (group 2), healthy smokers (group 3), and healthy nonsmokers (control group, group 4). Noncontact specular microscopy was used to measure central endothelial cell density (ECD), coefficient of variation of cell area, percentage of hexagonal cells, and central corneal pachymetry (CCT).

Results: According to the ECD and CCT values ($P < 0.001$ and $P = 0.013$, respectively), a significant difference was observed between the groups. The mean ECD was lowest in diabetic smokers (1917 ± 399 cells/mm^2). Healthy smokers and diabetic smokers had significantly lower ECD compared with the control group ($P = 0.03$ and $P < 0.001$, respectively). Healthy smokers and diabetic smokers had significantly lower ECD compared with diabetic nonsmokers ($P = 0.012$ and $P < 0.001$, respectively). The cornea was significantly thicker in the diabetic smokers than in the control group ($P = 0.013$).

Conclusions: The coexistence of DM and smoking causes a significant decrease in ECD and an increase in CCT. Cigarette smoking is more harmful to corneal endothelial cells than DM alone.

Diabetes mellitus (DM) is a significant public health challenge and can affect anterior and posterior eye structures.[1] It is assumed that more than 342 million people worldwide will suffer from DM by 2030, and the total health burden incurred by DM will be governed by the severity of multiorgan diabetic complications.[2] Hyperglycemia and formation of advanced glycation end products affect different layers of the cornea (epithelium, nerves, immune cells, and endothelium).[2] The ocular surface abnormalities caused by DM are manifested as dry eye, superficial punctate keratitis, recurrent corneal erosion syndrome, persistent epithelial defects, and corneal edema.[3]

Cigarette smoke contains more than 4500 chemicals, including reactive free radicals, which induce carcinogenic and proinflammatory reactions and decrease the antioxidant level in the ocular tissue, aqueous humor, and blood.[1,4] Cigarette smoke negatively affects the ocular surface and tear film characteristics. It has also been associated with various ophthalmological disorders, such as cataracts, primary open-angle glaucoma, Graves ophthalmopathy, type 2 DM, age-related macular degeneration, and ocular inflammation.[5–11]

In this study, we evaluated which entity (smoking or diabetes) is more harmful to the corneal morphological parameters and corneal endothelial cell density (ECD). Most studies have compared ECD and corneal morphology between diabetic and healthy patients or between healthy smokers and nonsmokers. This is the second study investigating corneal endothelial cell properties and central corneal pachymetry (CCT) in diabetic patients according to their smoking status. The authors in the first study showed a significant difference in ECD only between diabetic smokers and healthy subjects, but could not detect a difference in ECD among other groups.[12] This is somewhat surprising according to the published literature, and we expected more distinct results between all the investigated groups, especially when the 2 synergistic factors were involved. However, the low mean age of the study population of only 54 years might be a limitation.

A literature review of studies comparing corneal endothelial cells between diabetic and nondiabetic patients showed a decrease in ECD, usually followed by an increased

coefficient of variation of cell area (CV), whereas studies comparing only smokers and nonsmokers showed only ECD decrease with variable CV increase. However, it is expected that the decrease in ECD will be accompanied by an increase in CV, especially when 2 synergistic factors are involved.

MATERIALS AND METHODS

This prospective cohort study was performed at the Eye Department of KABEG General Hospital, Klagenfurt, Austria. The study protocol conformed to the tenets of the Declaration of Helsinki and was approved by the hospital ethics committee. Before the examination and after explaining the potential benefits of the study, all participants provided informed consent. All patients were recruited from the Ophthalmology Department of Clinic KABEG in Klagenfurt between September 2018 and December 2020.

The study included 200 patients, and only 1 eye from each participant was analyzed. The patients were divided into 4 groups, with 50 patients in each group. The first, second, third, and fourth groups included smokers with type 2 DM, nonsmokers with type 2 DM, healthy smokers, and healthy nonsmokers (control group), respectively.

Patients with diabetes were recruited from a retina specialist in our diabetes outpatient clinic, which takes place every Wednesday at our hospital. All other patients were healthy subjects without concomitant systemic disease, nonsmokers, and smokers, who came to the Department of Ophthalmology and Optometry for routine eye examinations and/or presbyopic glasses and were recruited from the general outpatient clinic 5 days in a week. The presence of DM was confirmed by a general practitioner, and the most recent glycosylated hemoglobin (HbA1c) values were recorded. The patients with DM who smoked had a mean HbA1c of 7.6%, and diabetic nonsmokers had a mean HbA1c of 7.0%.

The modified version of a questionnaire used in the study by Seale et al[13] was used to determine the smoking history of the participants. All the smokers had smoked for more than 20 years.

A detailed ophthalmological examination, including slitlamp biomicroscopy, best-corrected visual acuity using the Snellen chart (20 feet), intraocular pressure measurement using Goldmann applanation tonometer, and fundus examination with a 90-D lens, was conducted on all participants. Fluorescein angiography and macular optical coherence tomography (Heidelberg Engineering, Inc, Heidelberg, Germany) were performed on patients with DM, and according to the findings, the presence and stage of diabetic retinopathy (DR) were investigated. According to the Early Treatment DR Study, diabetic patients were classified into 3 groups: proliferative DR (PDR), nonproliferative DR (NPDR), and no-DR.[14]

The exclusion criteria were patients with a history of intraocular surgery; contact lens wearers; myopia more than −3.00 diopters (D); patients with glaucoma; corneal diseases such as keratoconus, Fuchs dystrophy, and corneal opacity due to inflammation; patients with dry eye or who used chronic topical therapy; and other systemic chronic conditions other than type 2 DM and hypertension.

The corneal ECD, morphology, and CCT were examined in all eyes by the same experienced technician using a noncontact specular microscope (Topcon SP-3000P; Topcon Corp, Tokyo, Japan). The patient was positioned on a chair in front of a specular microscope. After placing the head at the chin rest, the patient fixated on the light from inside the device. Three digital photographs from the central corneal endothelium were obtained, and at least 100 visible endothelial cells were marked manually with mouse clicks. Minimum, maximum, and average cell size (AVG); ECD; SD of mean cell area (SD); CV; and percentage of hexagonal cells (HEX) (%) of the endothelial cell layer were calculated using the built-in software.

The average of the 3 images was used to define the average cell density. All specular microscopy measurements were performed at the same interval of the day (from 8 to 12 AM) to lower the impact of diurnal oscillations on corneal hydration. Corneal ECD (cells/mm^2), CV, HEX, and CCT were measured using a specular microscope.[15,16] The CV in cell size (SD divided by the mean cell area) was used as an index of the extent of variation in the cell area (polymegathism). The HEX (%) in the analyzed area was used as a variation index in cell shape (pleomorphism).[17] A CV value above 0.4, an HEX (%) < 50%, and an increased corneal thickness >540 μm were considered abnormal.[18,19] The ECD table according to age was used to validate the ECD.[20]

Statistical Analysis

We used the methods of descriptive presentation of data and methods of inferential statistics. As part of the descriptive analysis, the data were presented in tabular form as absolute frequencies, percentages, and measures of the central tendency, and graphically through diagrams. Data are presented as arithmetic mean, SD, minimum and maximum values, variance, median, and interquartile range limits. To observe the correlation between the observed variables and the categories, if necessary, Pearson or Spearman correlation coefficient on a closed scale $-1 < r < 1$ was calculated to check whether the observed categories had a positive or negative degree of correlation and to determine the correlation intensity.

Depending on the distribution of the obtained data, we used parametric or nonparametric statistical methods. The decision to apply parametric and nonparametric tests was considered by testing the distribution of variables' normality in the study, and the distribution normality was tested using the Kolmogorov–Smirnov test and the Shapiro–Wilk test. If the deviation of the observed variables from the normal (Gaussian) distribution was determined, the testing would be performed using nonparametric versions of the tests, that is, the χ^2 test and, if necessary, Fisher exact test, Mann–Whitney U test, and Kruskal–Wallis test. If the normality of the distribution was confirmed, analysis was performed using a parametric t test and analysis of variance.

The significance of all tests during testing was set at 5%, which represents a reliability level of 95%. Based on the obtained significance, a decision was made to accept or reject the set hypotheses, and all the measured *P* values were 2-

sided. Statistical analyses were performed using the SPSS statistical software (version 21.0; SPSS Inc, Chicago, IL).

RESULTS

The study included 200 patients (89 women and 111 men) aged between 50 and 70 years. There were no statistically significant differences in age or sex between the 4 groups ($P > 0.05$, for all). Table 1 presents the demographic characteristics of each group.

Table 2 presents the corneal ECD, morphology, and CCT of the patients in all 4 groups. Significant differences were observed between the groups in ECD and CCT values ($P < 0.001$ and $P = 0.013$, respectively; Table 2).

To determine in which group a statistically significant difference occurred in ECD and CCT, we performed post hoc testing. Bonferroni corrections were used for multiple comparisons between 6 subgroups. Smokers without DM and smokers with DM had significantly lower ECD compared with the control group ($P = 0.03$ and $P < 0.001$, respectively; Table 3). Smokers without DM and smokers with DM had significantly lower ECD compared with nonsmokers with DM ($P = 0.012$ and $P < 0.001$, respectively; Table 3).

The cornea was significantly thicker in the diabetic smokers than in the control group ($P = 0.013$; Table 4). The CCT was thicker in the diabetic patients than in the nondiabetic group, but the difference was not significant. No statistically significant differences were observed between CV and HEX among the 4 groups.

Of the 100 patients in the study with DM, 31 patients had PDR (16 smokers and 15 nonsmokers), 54 patients had NPDR (25 smokers and 29 nonsmokers), and the remaining patients in the DM group did not have any signs of DR (no-DR) ($P = 0.916$). The ECD, CV, HEX, and CCT did not differ significantly among diabetic patients and their subgroups of no-DR, NPDR, and PDR ($P > 0.05$, for all).

DISCUSSION

A literature search revealed that diabetes-related corneal endothelial cell changes have been studied since 1984 and the effects of smoking on corneal endothelial cells since 1994. To date, only 1 study has investigated the coexistence of DM and smoking on corneal endothelial cell characteristics.[12] In this study, Cankurtaran and Tekin[12] reported a significant decrease in ECD in diabetic smokers compared with healthy

nonsmokers and a significant increase in CCT in diabetic nonsmokers compared with healthy smokers. Our CCT was significantly thicker in diabetic smokers compared with the control group. In addition, Cankurtaran and Tekin[12] showed no statistically significant difference in AVG. This was somewhat surprising because one would expect an increase in CV and AVG with a loss of ECD. However, the results could be explained by the average age of the study population, and we therefore studied an older group of patients.

In our study, healthy smokers had a statistically significant lower ECD compared with the control group ($P = 0.030$), indicating that smoking alone decreased ECD by approximately 9%. The ECD of diabetic smokers was significantly lower than that of the control group ($P = 0.001$). The 15% reduction in ECD in diabetic smokers confirms that smoking exacerbates the deleterious effects of DM on the corneal endothelium. Our work brings a new discovery: ECD was significantly lower in healthy smokers than in diabetic nonsmokers ($P = 0.012$), implying that smoking has a more deleterious effect on ECD than diabetes alone.

The results of several studies comparing ECD, corneal endothelium morphological characteristics, and corneal thickness between patients with DM and healthy subjects showed a decrease in ECD with varying significance in CV, HEX, and CCT.[18,21] El-Agamy et al[18] and Tasli et al[21] documented a significant decrease in ECD and an increase in CV in diabetics. Cankurtaran and Tekin reported no statistically significant differences in ECD, endothelial morphology (CV and HEX), and CCT between patients with diabetes and their subgroups: no-DR, NPDR, and PDR ($P > 0.05$, for all), which was confirmed by El-Agamy et al.[12,18] However, these results do not agree with those of Tasli et al, who found that with an increase in the stage of DR, ECD and HEX statistically decreased and CCT increased.[21] Islam et al[22] and Sudhir et al[23] reported a decrease in ECD in diabetic subjects compared with healthy controls, but they did not find statistically significant changes in CV and HEX. When comparing the significance of HEX and CV, no statistically significant difference was found between the diabetic and control groups in our study, but some studies reported an increase in CV and a decrease in HEX.[18,21,24–27]

Karakurt et al[28] examined the effects of smoking on corneal endothelial cells based on the calculation of pack-years and found that ECD and HEX decreased and CV

TABLE 1. Demographic Data of Study Participants Among all Subgroups

	Group–Median (IQR)				
	DSC (n = 50)	DNC (n = 50)	NSC (n = 50)	NNC (n = 50)	*P*
AGE (median—range)	59.34 ± 7.358	61.48 ± 6.322	60.72 ± 5.838	60.62 ± 6.824	0.430*
Sex, female/male, (n/n)	20/30	16/34	27/23	26/24	0.088†
Eye, OD/OS	30/19	30/19	31/18	24/26	0.388†

*Kruskal–Wallis H test.
†χ² test.
DNC, diabetic and nonsmoker, DSC, diabetic and smoker; IQR, interquartile range; NNC, nondiabetic and nonsmoker; NSC, nondiabetic and smoker.

TABLE 2. Comparison of Corneal ECD, Morphology, and CCT Between Observed Groups

	Group–Median (IQR)				
	DSC (n = 50)	DNC (n = 50)	NSC (n = 50)	NNC (n = 50)	*P**
ECD	1917 ± 399	2263 ± 352	2065 ± 285	2267 ± 292	**<0.001**
CV	36 ± 11	32 ± 9	34 ± 7	33 ± 9	0.389
HEX	51 ± 28	57 ± 21	54 ± 29	55 ± 22	0.762
CCT	535 ± 37	532 ± 39	518 ± 30	515 ± 35	**0.013**

Bold values indicate $P < 0.05$.
Data are presented as arithmetic mean and SD.
*Kruskal–Wallis H test.
DNC, diabetic and nonsmoker, DSC, diabetic and smoker; IQR, interquartile range; NNC, nondiabetic and nonsmoker; NSC, nondiabetic and smoker.

increased as the number of pack-years increased. Moreover, Zoega et al[29] found that smoking more than 20 pack-years (1 pack per day for 20 yrs) or half a pack per day for 40 years increased the odds ratio for corneal guttata by more than 2-fold. Recently, Ali et al[30] conducted a study on pregnant, adult, and newborn mice that were exposed to a smoking chamber, where they found that cigarette smoking influenced corneal endothelial cell.

Smoking is known to generate free radicals in the body, causing peripheral vasoconstriction and leading to decreased tissue oxygenation.[30–32] Chronic hyperglycemia leads to the accumulation of advanced glycation end products that promote inflammation and oxidative stress. Advanced glycation end products are believed to be responsible for the chronic macrovascular and microvascular complications of Descemet membrane.[2]

Whether it is a long-standing smoking habit or the number of packs themselves that cause more deleterious changes in corneal endothelial cell morphology and density than diabetes alone is a matter of speculation. The patients in our study were exposed to the toxic effects of cigarette smoking for at least 25 years, and DM type 2 occurs more often in older than 45 years. Further studies might reveal whether the long exposure or the toxic effects of the cigarettes

themselves, or both, are stronger than the 5- to 10-year average duration of advanced glycation end product toxic effects (corresponding to our age group). Because this is the second study to investigate the effect of synergistic factors on corneal endothelial cells and morphology, further studies are needed.

A limitation of this study is that the exact time of onset of DM in a large proportion of patients could not be determined (as DM is insidious) because it has been shown that the onset of DM type 2 at a younger age is associated with a higher risk of cardiovascular disease and death.[33] The second limitation of this study is that healthy subjects and nondiabetic smokers were not tested for hyperglycemia or A1c testing. Anamnestic to none of patients in this group DM diagnosis was known, but despite this, (according to our data) we cannot exclude, that some of those patients had diabetes in this group. According to the results of our study, any patient with DM, who is also a smoker, should be screened for ECD before cataract surgery because this may explain the unsatisfactory outcome after primary uncomplicated cataract surgery. In addition, special care should be taken in these patient groups to protect the corneal endothelial cells during phacoemulsification, for example, by using a dispersive viscoelastic.

TABLE 3. Pairwise Comparisons of Group for ECD

Sample 1–Sample 2[1]	Test Statistic[2]	Std. Error[3]	Std. Test Statistic[4]	Sig.[5]	Adj. Sig.[6],*
DSC-NSC	−15.240	11.576	−1.317	0.188	1.000
DSC-NNC	−47.730	11.576	−4.123	0.000	**0.000**
DSC-DNC	−51.070	11.576	−4.412	0.000	**0.000**
NSC-NNC	−32.490	11.576	−2.807	0.005	**0.030**
NSC-DNC	35.830	11.576	3.095	0.002	**0.012**
NNC-DNC	3.340	11.576	0.289	0.773	1.000

Each row tests the null hypothesis that the sample 1 and sample 2 distributions are the same. Asymptotic significances (2-sided tests) are displayed. The significance level is 0.05.
[1]Multiple comparison in the observed groups; [2]The value of test statistics; [3]Standard error; [4]Standardized value of test statistics; [5]Significance (p); [6]Corrected significance (p) by Bonferroni correction.
*Significance values have been adjusted by the Bonferroni correction for multiple tests.
DNC, diabetic and nonsmoker, DSC, diabetic and smoker; NNC, nondiabetic and nonsmoker; NSC, nondiabetic and smoker.

TABLE 4. Pairwise Comparisons of Group in CCT

Sample 1–Sample 2[1]	Test Statistic[2]	Std. Error[3]	Std. Test Statistic[4]	Sig.[5]	Adj. Sig.[6],*
NN-NS	2.060	11.575	0.178	0.859	1.000
NN-DN	24.360	11.575	2.105	0.035	0.212
NN-DS	30.740	11.575	2.656	0.008	**0.047**
NS-DN	22.300	11.575	1.927	0.054	0.324
NS-DS	28.680	11.575	2.478	0.013	0.079
DN-DS	6.380	11.575	0.551	0.582	1.000

Each row tests the null hypothesis that the sample 1 and sample 2 distributions are the same. Asymptotic significances (2-sided tests) are displayed. The significance level is 0.05.
[1]Multiple comparison in the observed groups; [2]The value of test statistics; [3]Standard error; [4]Standardized value of test statistics; [5]Significance (p); [6]Corrected significance (p) by Bonferroni correction.
*Significance values have been adjusted by the Bonferroni correction for multiple tests.
DNC, diabetic and nonsmoker, DSC, diabetic and smoker; NNC, nondiabetic and nonsmoker; NSC, nondiabetic and smoker.

For DM diagnosis, there is already a screening algorithm that focuses more on the retina. However, according to this study, it is necessary to include the cornea as well. In diabetics, awareness of multiorgan toxicity follows the diagnosis of DM, whereas this awareness occurs much later in smokers. A smoker can stop the toxic effects immediately (if he is able to do so, as it is a chemical dependency), but a diabetic cannot simply eliminate diabetes by his own decision. Our study confirms that the coexistence of DM and smoking leads to a significant decrease in ECD and is responsible for thicker corneas. Cigarette smoking is more harmful to corneal endothelial cells than DM alone.

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