OPEN

Diabetes Mellitus Type 1 has a Higher Impact on Corneal Endothelial Cell Density and Pachymetry than Diabetes Mellitus Type 2, Independent of Age: A Meta-Regression Model

Diana Patricia Amador-Muñoz, MD, MSc, Valentina Conforti, MD,† Luisa Marina Matheus, MSc, PhD,* Nicolas Molano-Gonzalez, MSc,‡ and César Payán-Gómez, MD, PhD§*

Purpose: Patients with diabetes mellitus (DM) often have keratopathy. However, the compromise of the corneal endothelium in type 1 DM (T1DM) and type 2 DM (T2DM) has so far not been well characterized.

Methods: We performed a systematic literature search to find articles on humans combining T1DM and/or T2DM and the corneal endothelium. The period was from inception to June 2020. The meta-regression evaluated the role of each type of DM on corneal endothelial cell density (CED) and pachymetry. The statistical models included age as a modulator to discriminate between the normal changes due to age and the effect of the disease and to determine the impact of the disease duration.

Results: The initial search identified 752 records, of which 17 were included in the meta-regression. Patients with T1DM had, on average, 193 cells/mm^2 lesser than control patients ($P < 0.00001$). Patients with T2DM had 151 cells/mm^2 less compared with control patients ($P < 0.00001$). The loss of corneal endothelial cells was expected because the aging was similar in patients with T1DM and T2DM and their control groups. Patients with T1DM and T2DM showed an increase in pachymetry versus control patients, and in both groups, it was associated with the duration of the disease.

Conclusions: Both types of DM reduced CED and increased pachymetry. These differences were higher in patients with T1DM versus control patients than patients with T2DM versus control patients. In T1DM, CED reduction was not correlated with the time from diagnosis. In both groups, patients had CED reduction due to aging similar to that of their matched control patients.

Key Words: diabetes mellitus type 1, diabetes mellitus type 2, endothelium, corneal, meta-analysis

(*Cornea* 2022;41:965–973)

Diabetes mellitus (DM) is a major and fast-growing global health problem. In 2019, the International Diabetes Federation estimated that 463 million people had diabetes, and this is projected to reach 578 million by 2030.[1]

Type 1 DM (T1DM) is caused by an autoimmune reaction affecting pancreatic β cells because little or no insulin is produced. ThCopyright © 2021 The Author(s). Published by Wolters Kluwer Health, Inc. This disease is more prevalent in children.[2] Type 2 DM (T2DM) affects approximately 90% of diabetic people worldwide.[1] In these patients, hyperglycemia is the result of the inability of the cells in the body to respond appropriately to insulin signaling. This prompts an increase in insulin production, and over time, it leads to pancreatic β-cell failure.[2]

Diabetic retinopathy is the most well-known ocular complication in diabetic patients. Nevertheless, it is not the only ocular complication associated with this disease. In these patients, abnormalities of the cornea have been described that predispose them to recurrent corneal erosions and reduced corneal sensitivity.[3] Studies using laboratory techniques and animal models have identified a metabolic dysfunction in corneal endothelial cells under diabetic conditions.[4] Nevertheless, corneal endothelial impairments have been less studied.

The corneal endothelial cells cover the inner side of the cornea and maintain the perfect relative dehydration of the stroma through an electroosmotic gradient and the action of cell junctions that permit a leak of fluid to the anterior chamber.[5] The CED at birth is as high as 7500 cells/mm^2 and declines

Received for publication April 13, 2021; revision received June 3, 2021; accepted June 4, 2021. Published online ahead of print September 22, 2021.

From the *Neuroscience (NEUROS) Research Group, School of Medicine and Health Sciences, Universidad del Rosario, Bogotá, Colombia; †School of Medicine and Health Sciences, Universidad del Rosario, Bogotá, Colombia; ‡Clinical Research Group, School of Medicine and Health Sciences, Universidad del Rosario, Bogotá, Colombia; and §Department of Biology, Faculty of Natural Sciences, Universidad del Rosario, Bogotá, Colombia.

The authors have no funding or conflicts of interest to disclose.

Supplemental digital content is available for this article. Direct URL citations appear in the printed text and are provided in the HTML and PDF versions of this article on the journal's Web site (www.corneajrnl.com).

Correspondence: César Payán-Gómez, MD, PhD, Department of Biology, Faculty of Natural Sciences, Universidad del Rosario, Carrera 24 No. 63 C 69, Bogotá 111221, Colombia (e-mail: cesar.payan@urosario.edu.co).

progressively during life. It has been estimated that between the ages of 20 and 80 years, the reduction in cell density is approximately 0.52% per year.[6] The normal cornea of healthy adults has approximately 3200 to 3500 cells/mm^2 organized as a mosaic in which 60% of them should have a hexagonal shape.[7] When the CED falls to 1200 cells/mm^2, the compromise of the monolayer integrity further compromises the endothelial pumping function (symptomatic threshold),[8] and finally, if the cell density drops below 500 to 700 cells/mm^2, it results in an irreversible hydration imbalance that swells the stroma and produces permanent opacity.[5] Stressors accelerate the loss expected by age, and once the cornea turns cloudy, the only therapeutic option is transplantation.[9]

It has been considered that the increase in central corneal thickness (CCT) could be an early marker of corneal endothelium dysfunction.[10] In addition, the studies suggest that diabetic patients have an increase in CCT because of Na^+/K^+ dependent adenosine triphosphatase (ATPase) impairment, increased stromal swelling pressure or disrupted barriers as a consequence of the hyperglycemia, and reduced insulin levels[11] and the compromise of the corneal endothelial cells.[12]

The results of the studies that compared the endothelium and the CCT of diabetic corneas versus matched controls are not consistent. Some of these studies did not find significant differences between CED and CCT in patients with T1DM or T2DM compared with control patients,[13,14] whereas others did.[15–18] Furthermore, studies comparing patients with T1DM or T2DM separately with control patients found differences in one group but not in the other.[18,19] In addition, it has been suggested that some of the results may be masked by the normal aging process because patients with T2DM are older, on average, than patients with T1DM.[9]

Two meta-analyses have been conducted to identify possible relations between DM and the compromise of the corneal endothelium,[20,21] but, to our knowledge, there have been no meta-regressions reporting corneal endothelial changes specifically associated with diabetic type compared with nondiabetic groups. The aim of this study was to assess the differences in CED and CCT in patients with T1DM, patients with T2DM, and nondiabetic patients. In addition, considering that DM is a chronic disease and corneal endothelial cell density and CCT change with the natural aging process, we considered important to include an age modifier in the meta-regression model. A better understanding of the effect of DM on the corneal endothelium might lead to a more comprehensive ocular evaluation of diabetic patients and might highlight areas for further research.

MATERIALS AND METHODS

Formulated Questions

Following the PICO (patient, intervention/exposure, comparison groups, and outcome) reporting system, the review addressed a couple of questions: 1) What is the effect of T1DM and T2DM regarding exacerbating the reduction of CED (cells/mm^2) expected by age in nondiabetic control patients? 2) What is the effect of T1DM and T2DM regarding exacerbating the increase of CCT (μm) expected by age in nondiabetic control patients?

Search Strategy

A literature search using the Medical Subject Heading terms "corneal endothelium" OR "cornea" AND "diabetes mellitus" was conducted in PubMed, Proquest EMBASE, and Cochrane Central Register of Controlled Trials. The Health Sciences Descriptors "endotelio corneal" AND "diabetes mellitus" were used for a Spanish search in the Lilacs database. In both cases, the search was limited to studies performed in humans. No limits regarding language, publication type, or publication period were considered as of June 26, 2020, when the search was performed.

Study Selection

Study selection was performed by 2 independent reviewers (D.A.M. and V.C.). The inclusion criteria used were as follows: 1) the cases included patients clearly defined as having T1DM or T2DM and the study included matched nondiabetic control patients; and 2) corneal endothelial cell density and pachymetry are reported. Studies were excluded based on the following criteria: 1) cases included individuals with type 1 and 2 DM, and it was not possible to discriminate the results for each type of diabetes, 2) the control patients were not age matched, 3) there was no control group (nondiabetic patients) as a source of comparison, 4) case patients or control patients had diseases other than diabetes, and 5) case patients or control patients had undergone eye surgeries. Two investigators (D.A.M. and C.P.) independently evaluated the quality of eligible studies according to the Newcastle–Ottawa Scale.

Meta-Regression

The data obtained were analyzed using a meta-regression model in which the impact of each type of diabetes was tested separately. We evaluated 2 variables: one morphological, the corneal endothelial density, and one functional, the CCT using pachymetry. In addition, considering that the progressive reduction of corneal endothelial density with age is well documented, the age of the individuals was also considered in the model. Meta-regression model specifications were as follows: First, we used the raw mean as the effect size; therefore, for each study, we were able to obtain at least 2 effect measures: one raw mean of the case patients and one of the control patients.

In this setting, we specified a mixed-effect model, with a random effect associated with the study, which reports the raw means for case and control patients. Fixed effects were specified by means of a linear regression model where the status of case and control patients was included along with age. The interaction between age and case–control status was also considered in this mean structure. A complete description of the model is included in Supplemental Digital Content 1 (see Text Document, http://links.lww.com/ICO/B274).

The final set of variables and interactions were selected based on the likelihood ratio test and the Akaike information criteria. To assess the model fitness, we used the following indexes: tau, for this model, a measure of heterogeneity between studies; rho, for intrastudy correlation; the I^2

heterogeneity statistic; and the generalized determination coefficient for mixed models R^2.

This modeling strategy is based on the methodology proposed by Konstantopoulos,[22] implemented in the Metafor package[23] for the software R (version 4.0.4).[24] A second meta-regression was performed to evaluate the impact of the time from diagnosis of DM on CED and CCT values.

RESULTS

Using the search strategy, 752 records were found, of which 664 were discarded after a duplicate check. After the selection by title and abstract and after reviewing the full texts, 17 studies were used for the meta-regression (Fig. 1). Eight studies presented in 7 publications with a total of 367 patients were used for the analysis of T1DM; 14 studies reported in 13 publications with a total of 2136 patients were included in the analysis of T2DM.

Characteristics of Included Analyses

The studies examining corneal characteristics in patients with T1DM compared with control patients are summarized in Table 1. Three studies were conducted in Europe (Hungary,[19] Italy,[18] and Poland[17]), 2 in the United States,[13,25] one in Africa (Egypt[16]), and one in Asia (India[26]). All studies were used in both meta-regressions. The studies selected for T2DM are summarized in Table 2. Six studies were conducted in Europe (Hungary,[19] Italy,[18] Spain,[27,28] Lithuania,[29] and Denmark[14]), one in the United States,[13] and 6 in Asia (Turkey,[30,31] Malaysia,[32] Saudi Arabia,[33] Japan,[34] and India[15]). The study by Anbar et al[16] took independent measures of each eye. Three studies[14,15,32] were not included in the second meta-regression because they did not report the time from diagnosis of DM. Most of the studies were classified as having good quality (G). One study was classified as having fair quality (F)[19] (see Table 1, Supplemental Digital Content 2, http://links.lww.com/ICO/B265).

Meta-Regressions for Patients with T1DM and Control Patients

The meta-regression for CED involves T1DM status and mean age as moderators (see Table 2, Supplemental Digital Content 3, http://links.lww.com/ICO/B266). At 22.82 years (the

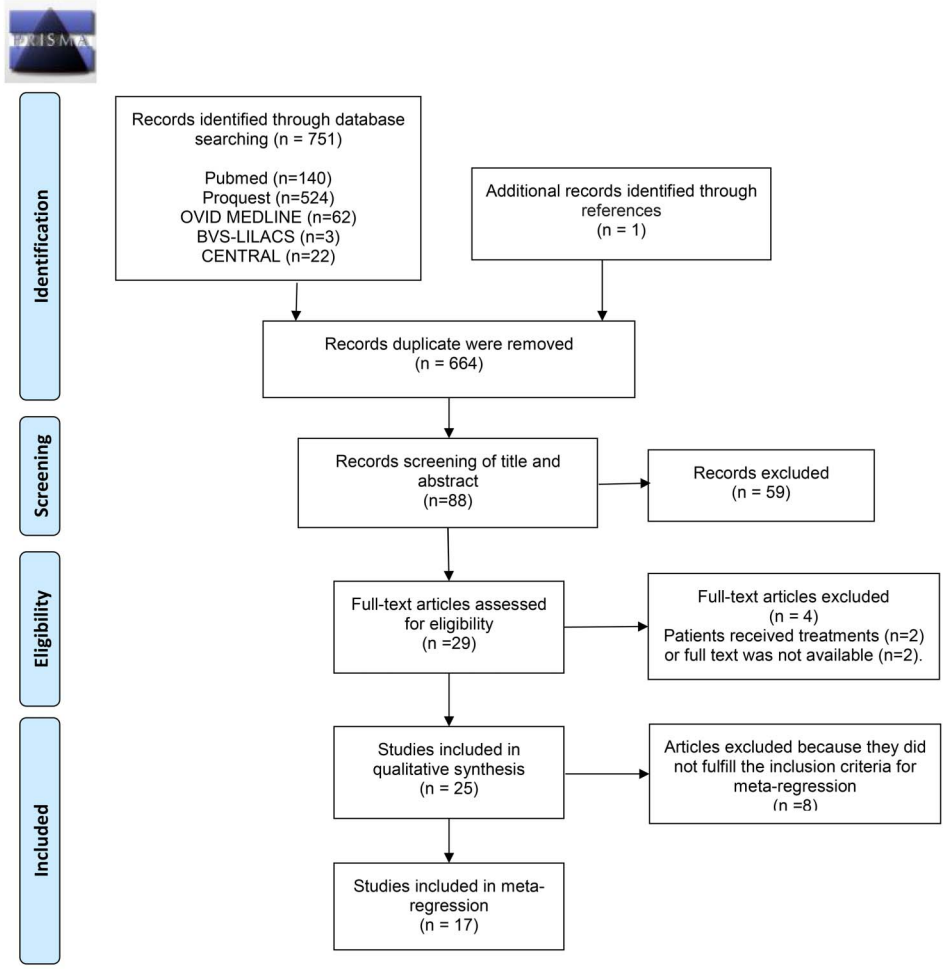

FIGURE 1. PRISMA flow diagram for study selection. (The full color version of this figure is available at www.corneajrnl.com.)

From: Moher D, Liberati A, Tetzlaff J, Altman DG, The PRISMA Group (2009). *P*referred *R*eporting *I*tems for *S*ystematic Reviews and *M*eta-*A*nalyses: The PRISMA Statement. PLoS Med 6(7): e1000097. doi:10.1371/journal.pmed1000097

TABLE 1. Studies Selected for the Analysis of Endothelial Differences Between Patients With T1DM and Nondiabetic Control Patients

		Case Patients						
Study, yr	**Country (Continent)**	**Participants (M/F)**	**Age, yr Mean ± SD**	**Disease Duration, yr Mean ± SD**	**HbA1c, % Mean ± SD**	**Cell Density, Cell/mm² Mean ± SD**	**Hexagonal Cells, % Mean ± SD**	**Pachymetry, µm Mean ± SD**
Anbar et al[16] (LE), 2016	Egypt (Africa)	80 (32/48)	8.22 ± 3.12	3.51 ± 2.24	8.33 ± 2.32	3142 ± 416	50 ± 7	539 ± 30
Anbar et al[16] (RE), 2016	Egypt (Africa)	80 (32/48)	8.22 ± 3.13	3.51 ± 2.23	8.33 ± 2.31	3150 ± 344	49 ± 5	537 ± 33
Fernandes et al,[26] 2019	India (Asia)	50 (24/26)	12.16 ± 2.63	3.91 ± 1.65	10.9 ± 2.3	3040 ± 293	NS	525 ± 33
Keoleian et al,[25] 1992	USA (America)	14 (NS/NS)	33 ± 12	22 ± 11	10 ± 1.4	2883 ± 280	68 ± 8	560 ± 20
Larsson et al,[13] 1996	USA (America)	49 (NS/NS)	36 ± 12	20 ± 11	10.4 ± 2.1	2722 ± 313	59 ± 8	580 ± 50
Módis et al,[19] 2010	Hungary (Europe)	22 (13/9)	40.97 ± 15.46	10.88 ± 8.06	8.55 ± 1.83	2428 ± 219	NS	570 ± 40
Roszkowska et al,[18] 1999	Italy (Europe)	30 (NS/NS)	29.76 ± 3.43	15.3 ± 1.2	NS	2141 ± 78	NS	580 ± 20
Urban et al,[17] 2013	Poland (Europe)	123 (60/63)	15.34 ± 3.06	8.02 ± 3.9	8.02 ± 3.9	2436 ± 443	NS	550 ± 30

	Control Patients					
Study, yr	**Participants (M/F)**	**Age, yr Mean ± SD**	**HbA1c, % Mean ± SD**	**Cell Density, Cell/mm² Mean ± SD**	**Hexagonal Cells, % Mean ± SD**	**Pachymetry, µm Mean ± SD**
Anbar et al[16] (LE), 2016	40 (14/26)	7.83 ± 2.49	4.33 ± 0.83	3316 ± 100	55 ± 10	501 ± 16
Anbar et al[16] (RE), 2016	40 (14/26)	7.83 ± 2.49	4.33 ± 0.83	3309 ± 99	56 ± 10	504.7 ± 24
Fernandes et al,[26] 2019	50 (28/22)	12.28 ± 3.00	NS	2495 ± 191	NS	513 ± 29
Keoleian et al,[25] 1992	14 (NS/NS)	33 ± 10	NS	3131 ± 483	74 ± 8	560 ± 40
Larsson et al,[13] 1996	20 (NS/NS)	36 ± 12	NS	2805 ± 368	64 ± 7	550 ± 30
Módis et al,[19] 2010	22 (12/9)	40.45 ± 15.16	NS	2495 ± 191	NS	505 ± 24
Roszkowska et al,[18] 1999	30 (NS/NS)	29.5 ± 3.11	NS	2406 ± 116	NS	540 ± 30
Urban et al,[17] 2013	124 (66/58)	14.58 ± 2.01	NS	2971 ± 207	NS	530 ± 33

LE, left eye; RE, right eye.

pooled mean age of the studies in the meta-regression), the estimated CED was 2900 cells/mm² [95% confidence interval (CI): 2712–3089] for control patients with T1DM. At this age, patients with T1DM had 193 cells/mm² lesser than its control patients (95% CI: −254 to −132; $P < 0.00001$). The model calculated that control patients for the T1DM groups had an average loss of 16 cells/mm² per year (95% CI: −32 to −0.31). Of interest, the rate of cellular loss expected by the normal aging process was similar between patients with T1DM and control patients ($P = 0.253$) (Fig. 2). In addition, the analysis showed that 44% of the variance in CED was explained by the model that included T1DM and age.

The meta-regression about time from diagnosis and CED values showed that at 10.89 years (the mean time from diagnosis of the disease) the estimated CED is 2700 cells/mm² (95% CI: 2458–2942) for patients with T1DM. This model calculated that patients with T1DM had an average loss of 16 cells/mm² per year (95% CI: −50 to 19), similar to what was found for control patients. Remarkably, the loss of cells was not associated with the duration of the disease (95% CI: −51 to 19; P =0.383) (see Table 3 and Fig. 1, Supplemental Digital Content 4, http://links.lww.com/ICO/B267 and http://links.lww.com/ICO/B263).

There was a significant positive association between T1DM and CCT. At 22.82 years, the control patients included in the meta-regression had an average corneal thickness of 531 µm (95% CI: 524–537; $P < 0.00001$) as measured by pachymetry, and because of the disease, we calculated that patients with T1DM had a pachymetry 24 µm greater than that of the control patients (95% CI: 15–33; $P < 0.00001$) (see Table 4, Supplemental Digital Content 5, http://links.lww.com/ICO/B268). However, the change in pachymetry expected because of the normal aging process was similar between patients with T1DM and control patients for the different groups analyzed ($P = 0.563$) (Fig. 3). The model including T1DM disease and age explained 88.4% of the difference in the pachymetry between patients with T1DM and control patients (see Table 4, Supplemental Digital Content 6, http://links.lww.com/ICO/B268).

The meta-regression about time from diagnosis and CCT values showed that at 10.89 years (the mean time from diagnostic of the disease), the estimated CCT was 555 µm (95% CI: 546–564) for patients with T1DM. In contrast to the findings for CED, the CCT increase was significant (2 µm per year 95% CI: 0.8–3.5; $P = 0.0015$) (see Table 5 and Fig. 2,

TABLE 2. Studies Selected for the Analysis of Endothelial Differences Between Patients With T2DM and Nondiabetic Control Patients

		Case Patients						
Study, yr	**Country (Continent)**	**Participants (M/F)**	**Age, yr Mean ± SD**	**Disease, Duration, yr Mean ± SD**	**HbA1c, % Mean ± SD**	**Cell Density, Cell/mm² Mean ± SD**	**Hexagonal Cells, % Mean ± SD**	**Pachymetry, µm Mean ± SD**
Calvo-Maroto[27] (ST), 2014	Spain (Europe)	37 (16/21)	45.5 ± 2.5	0.38 ± 0.12	7.66 ± 0.78	2581 ± 175	NS	546 ± 13
Calvo-Maroto[27] (LT), 2014	Spain (Europe)	40 (17/23)	52.2 ± 1.8	10.2 ± 0.8	7.78 ± 0.66	2205 ± 194	NS	569 ± 11
Cankurtara et al,[30] 2019	Turkey (Asia)	153 (76/77)	54.9 ± 6.6	11.5 ± 6.3	8.7 ± 1.8	2483 ± 326	54 ± 10	534 ± 34
(+)Choo et al,[32] 2010	Malaysia (Asia)	100 (NS/NS)	NS	NS	NS	2542 ± 516	45 ± 21	517 ± 53
Durukan et al,[31] 2019	Turkey (Asia)	120 (67/53)	59.5 ± 8.1	11.5 ± 6.2	9.2 ± 1.5	2295 ± 311	49 ± 10	544 ± 38
El-Agamy et al [33]	Saudi Arabia (Asia)	57 (27/30)	57.08 ± 8.37	12.87 ± 8.03	8.57 ± 2.09	2492 ± 261	33 ± 10	546 ± 30
Galgauskas et al,[29] 2016	Lithuania (Europe)	62 (31/31)	45.5 ± 13.5	9.3 ± 6.6	9.8 ± 2.3	2722 ± 264	60 ± 11	566 ± 36
Inoue et al, [34] 2002	Japan (Asia)	99 (53/46)	65.5 ± 7.5	9.1 ± 8.2	6.9 ± 1.3	2493 ± 330	56 ± 8	538 ± 36
Larsson et al,[13] 1996	USA (America)	60 (NS/NS)	60 ± 10	13 ± 8	9.9 ± 2.1	2457 ± 367	57 ± 8	570 ± 50
Modis et al, [19] 2010	Hungary (Europe)	30 (10/20)	64.36 ± 10.47	13.61 ± 6.5	8.79 ± 2.01	2330 ± 251	NS	560 ± 30
Roszkowska et al, [18] 1999	Italy (Europe)	45 (NS/NS)	49.6 ± 6.16	17.2 ± 2.2	NS	1915 ± 70	NS	570 ± 20
Sanchis-Gimeno,[28] 2016	Spain (Europe)	35 (17/18)	33.8 ± 3.2	5.9 ± 1.2	6.7 ± 0.3	2101 ± 184	NS	567 ± 11
(+)Storr-Paulsen et al, [14] 2014	Denmark (Europe)	107 (51/56)	72.1 ± 11	NS	7.3 ± 0.2	2578 ± 77	58 ± 1	546 ± 7
(+)Sudhir et al, [15] 2012	India (Asia)	1191 (637/554)	54.8 ± 9.5	NS	NS	2550 ± 96	57 ± 7	525 ± 35

	Control Patients					
Study, yr	**Participants (M/F)**	**Age, yr Mean ± SD**	**HbA1c, % Mean ± SD**	**Cell Density, Cell/mm² Mean ± SD**	**Hexagonal Cells, % Mean ± SD**	**Pachymetry, µm Mean ± SD**
Calvo-Maroto[27] (ST), 2014	38 (20/18)	45.3 ± 2.1	NS	2603 ± 155	NS	545 ± 11
Calvo-Maroto[27] (LT), 2014	43 (22/21)	52.3 ± 2.6	NS	2554 ± 254	NS	547 ± 11
Cankurtara et al,[30] 2019	146 (71/75)	53.9 ± 7.3	5.2 ± 0.2	2530 ± 276	53 ± 10	523 ± 35
(+)Choo et al,[32] 2010	101 (NS/NS)	NS	NS	2660 ± 516	41 ± 20	511 ± 72
Durukan et al,[31] 2019	112 (60/52)	57.3 ± 7.2	5 ± 0.3	2501 ± 302	55 ± 10	522 ± 32
El-Agamy et al [33]	45 (22/23)	50.8 ± 8.39	NS	2630 ± 293	34 ± 9	539 ± 29
Galgauskas et al,[29] 2016	65 (32/33)	45.4 ± 19.5	NS	2967 ± 264	59 ± 11	550 ± 56
Inoue et al, [34] 2002	97 (52/45)	67.6 ± 7.3	NS	2599 ± 278	56 ± 6	537 ± 38
Larsson et al,[13] 1996	20 (NS/NS)	59 ± 12	NS	2604 ± 385	61 ± 6	560 ± 30
Modis et al, [19] 2010	30 (15/15)	62.69 ± 15.38	NS	2354 ± 186	NS	560 ± 40
Roszkowska et al, [18] 1999	32 (NS/NS)	49.75 ± 7.01	NS	2032 ± 82	NS	550 ± 30
Sanchis-Gimeno,[28] 2016	48 (23/25)	33.4 ± 3	NS	2505 ± 182	NS	542 ± 12
(+)Storr-Paulsen et al, [14] 2014	128 (49/79)	75.6 ± 8.9	NS	2605 ± 66	58 ± 1	538 ± 5
(+)Sudhir et al, [15] 2012	121 (58/62)	51.9 ± 8.7	NS	2634 ± 256	57 ± 7	526 ± 33

(+) Studies not included in meta-regression about time from diagnosis.
LT, long-term diabetes; ST, Short-term diabetes.

Supplemental Digital Content 7, http://links.lww.com/ICO/B269 and http://links.lww.com/ICO/B264).

Meta-Regressions for Patients With T2DM and Control Patients

The meta-regression for CED involving T2DM status and mean age as moderators determined that mean age was not significant in this case ($P = 0.357$). CED is estimated to be 151 cells/mm² lower for patients with T2DM compared with control patients (95% CI: −230 to −72; $P = 0.0002$) (see Table 6, Supplemental Digital Content 8, http://links.lww.com/ICO/B270). Only 14% of the variance in CED was explained by the model including T2DM disease and age.

Various articles reported different conclusions either in favor or against an association between T2DM and CED

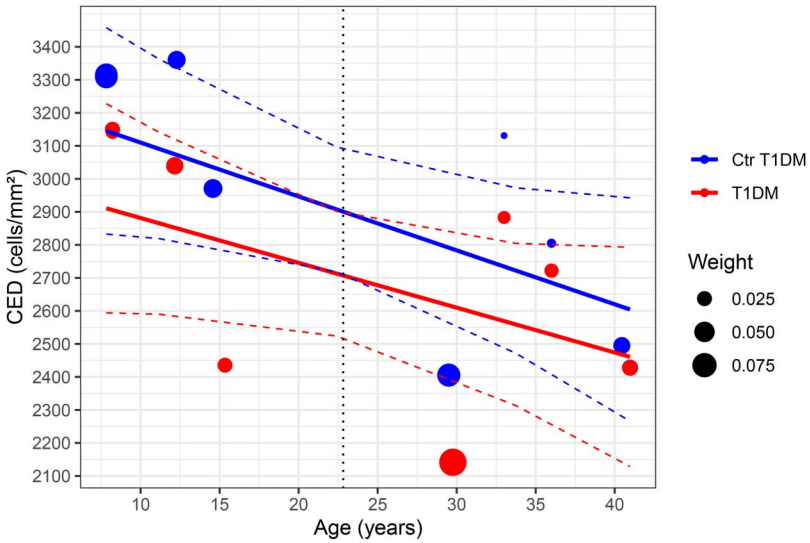

FIGURE 2. Meta-regression scatterplot showing the correlation between endothelial cell density (cells/mm²) and age (years) in patients with T1DM (red) and control patients (blue). Each circle represents the CED of each group with the average age. The size of the circle is proportional to study weighting. The solid lines represent the regressions fitted by the model. The dashed lines show the 95% CI around the regression lines. (The full color version of this figure is available at www.corneajrnl.com.)

changes. In addition, the meta-regression showed that the CED changes associated with age did not have a significant effect on the differences between patients with T2DM and control patients. Then, we used a forest plot to display the different populations on a single set of axes to demonstrate where there is a difference between patients with T2DM and nondiabetic control patients (Fig. 4).

Unlike the results obtained for T1DM, the meta-regression about time from diagnosis and CED values showed that duration of T2DM disease had a significant effect on the differences between patients and control patients (−37 cells/mm² per year 95% CI: −45 to −29; $P < 0.00001$). At 10.41 years (the mean time from diagnosis of the disease for the T2DM group), the estimated CED is 2386 cells/mm² (95% CI: 2251–2520) (see Table 7 and Fig. 1, Supplemental Digital Content 9, http://links.lww.com/ICO/B271 and http://links.lww.com/ICO/B263).

The meta-regression for pachymetry involved T2DM status and mean age as moderators, and it explained 15% of the variance between patients with T2DM and control patients. This analysis determined that the mean age was not significant in this case ($P = 0.093$) (see Table 8, Supplemental Digital Content 10, http://links.lww.com/ICO/B272). The CCT of patients with T2DM was estimated to be 13 μm greater than that of its control patients (95% CI: 7–19; $P < 0.0001$). These results can be seen in Figure 5.

The meta-regression about time from diagnosis and CCT showed that at 10.41 years (the mean time from diagnosis of the disease), the estimated CCT is 554 μm (95% CI: 545–563) for patients with T2DM. The average CCT increase from the diagnosis of T2DM was 2 μm per year (95% CI: 1.7–2.8; $P < 0.00001$) (see Table 9 and Fig. 1, Supplemental Digital Content 11, http://links.lww.com/ICO/B273 and http://links.lww.com/ICO/B263).

DISCUSSION

DM is a chronic disease whose incidence and prevalence are growing worldwide. Two meta-analyses had been conducted to identify possible relations between DM and the compromise of the corneal endothelium.[11,12] This study adds to the available knowledge by making an independent estimation of CED and pachymetry for each type of diabetes (type 1 and type 2) and by including the mean age in the model of evaluation. This helps us to understand the impact of the disease considering the reduction in CED expected over time and the increase in pachymetry at different ages. Our analysis demonstrated that DM types 1 and 2 are associated with reduced CED and increased pachymetry. These differences were bigger between patients with T1DM and control patients than between patients with T2DM and control patients, independently of the reduction in the endothelial cell density and the increase of pachymetry expected by the normal aging process.

The meta-regressions for T1DM included 448 case patients and 340 control patients from Europe, the United States, Africa, and Asia. Our model showed a significant reduction in CED of 193 cells/mm² in diabetic patients compared with control patients. The meta-regression for T2DM included 1064 case patients and 1026 control patients from Europe, Asia, and the United States. Our model estimated a significant reduction of 151 endothelial cells/mm² in patients with T2DM compared with control patients, which suggested a lower impact than the one identified in the T1DM group versus nondiabetic control patients. The results of the T1DM and T2DM meta-regressions showed that the reduction in CED due to the aging process was similar between diabetic and nondiabetic groups, but older people seem to have less density loss than the younger ones. However, the time from diagnosis was related with the impact of the disease only for patients with T2DM.

Zhang et al[12] and Tang et al[11] suggested that DM has a negative impact on CED. However, these studies were performed including both types of diabetes at the same time; therefore, it was not possible to see the impact of each diabetes type separately. We believe that making this distinction is useful considering the variations in pathophysiology, the difference in the age of onset of disease, and the

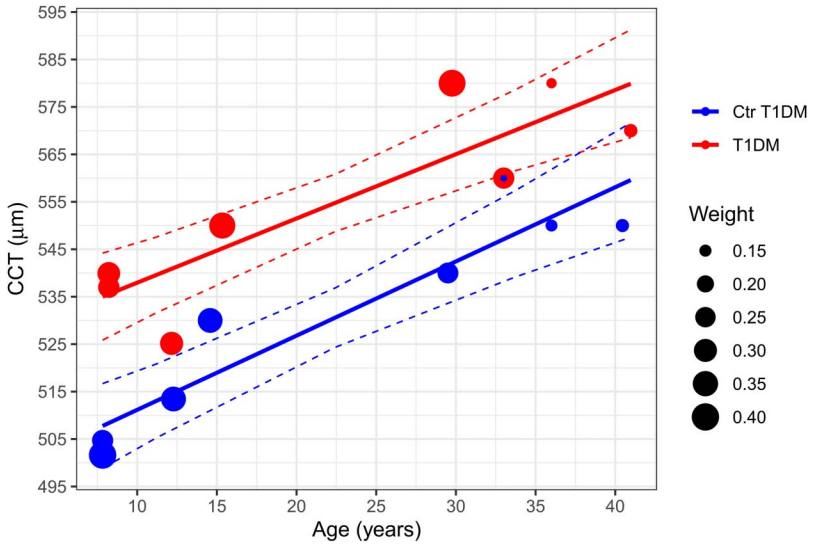

FIGURE 3. Meta-regression scatterplot showing the correlation between CCT (μm) and age (years) in patients with T1DM (red) and control patients (Ctr T1DM) (blue). Each circle represents a group of patients (control or T1DM group) of one study included in the meta-analysis, and the size of the circle is proportional to study weighting. The solid black line represents the regression line based on variance-weighted least squares. The dashed line shows the 95% CI around the regression line. (The full color version of this figure is available at www.corneajrnl.com.)

high and rapid metabolic compromise in T1DM compared with the progressive course of the T2DM. These comparisons were useful to identify that DM affects the corneal endothelium extensively; the analysis of each type separately gives us additional information to understand whether it is necessary to have a different clinical approach to the endothelial damage according to the type of DM. The reduction of CED found in both diabetic groups is not a physiological issue by itself; however, it puts diabetic patients in an unfavorable situation, especially the patients with T1DM who have the most reduced densities.

Our model with pachymetry and mean age showed a significantly increased CCT in patients with T1DM and T2DM compared with control patients, and in both, there was also a correlation between the duration of the disease and the change in CCT. As was expected, pachymetry showed an opposite trend compared with the results obtained in the meta-regression for CED. When Tang et al[20] evaluated the CCT, they did not find a significant difference between diabetic patients and nondiabetic control patients. It could be explained by the low number of studies available when they did the study; however, they reported a slightly greater CCT in patients with DM. Our results showed that the difference in CCT between patients and control patients was higher with the population with T1DM, the same group in which we found the major impact on the CED. This could imply a greater risk because, in addition to cell reduction, excessive hydration promotes folding in Descemet membrane, which increases the stromal thickness even more. In addition, the persistence of this condition also predisposes to corneal vascularization, infection, and scarring.[35]

Two main factors associated with the pathophysiology of the corneal endothelium in diabetes are the high glycemic environment and the reduced or null amount of insulin. Faced with glucose in excess, cells, such as the corneal endothelium, in which the entrance of glucose does not depend on insulin, are exposed to the task of modulating the entry of glucose, so they became particularly prone to complications.[36]

Because all diabetic patients are exposed to glucose in excess and other inductors of damage such as polyols and advanced glycation end products, we hypothesize that the differences between T1DM and T2DM are related with the impact of the disease at different points of the life span in diabetic populations. Damage mediated by diabetes has a higher impact in T1DM because it starts in the moment of life where the loss of cells is higher (16 cells/mm^2 per year in nondiabetic control patients), and DM could enhance it. In addition, T1DM has an immunological background that could also affect the level of cellular loss. Genetic analyses of T1DM have linked the HLA complex with susceptibility to T1DM in at least 50% of patients.[36] HLA class I (HLA-A, HLA-B, and HLA-C) are constitutively expressed on the corneal endothelium,[37] and HLA class II (-D) can be expressed on the corneal endothelium when it is exposed to an inflammatory trigger.[38] In addition, T1DM is associated with autoantibodies that could also affect the cornea. The antibodies against the enzyme glutamic acid decarboxylase have been recognized in 65% of patients with T1DM and might have a long-term impact on the corneal endothelium. This enzyme is highly expressed in corneal endothelial cells,[39,40] and the mutations in the coding gene are related with the development and progress of Fuchs corneal dystrophy.[41]

The proportion of the variance of CED and CCT that is explained by the meta-regression, including the DM type and mean age variables, was higher for T1DM (CED, R^2 = 44%; CCT, R^2 = 88%) than for T2DM (CED, R^2 = 14%; CCT, R^2 = 15%). This suggests that T1DM and its young age of onset are the causes of the greater difference found between its case patients and nondiabetic control patients in comparison with that between T2DM and its control group. It would be interesting to evaluate these changes in the extremes of life to identify the moment when the risk for cellular loss is higher and the cumulative impact of T1DM over time.

The heterogeneity (I^2) was high for CED and pachymetry in the T1DM and T2DM groups. This could be partially explained because the studies used for analysis were cross-sectional and case–control studies. These studies

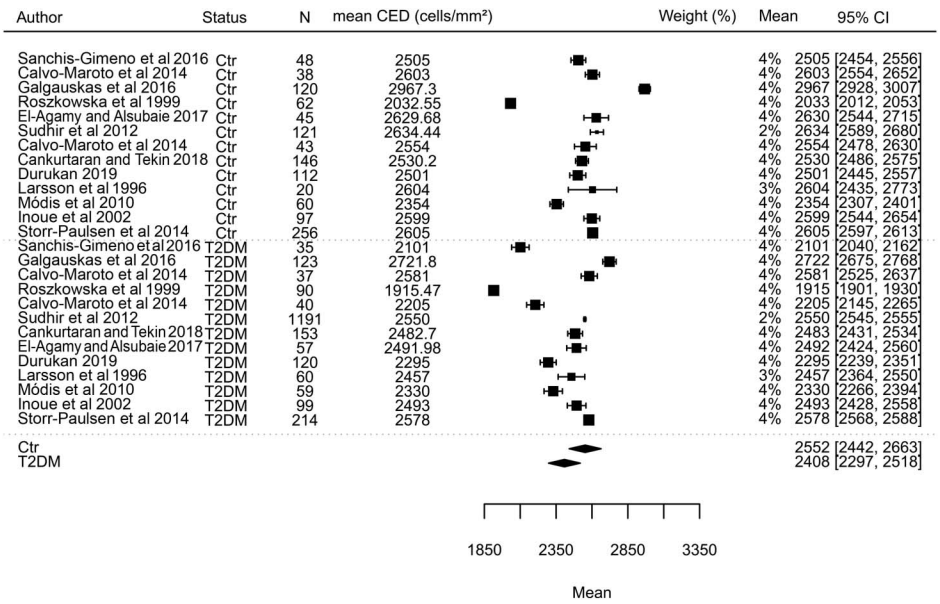

FIGURE 4. Forest plot of CED in patients with T2DM and control patients (Ctr). The mean corneal endothelial cell density is shown for patients with T2DM and control patients.

provide information on different populations at a specific period in patients' lives and the course of the disease, which is not consistent between the different studies, and increase the variability once they are pooled for analysis. In addition, the different places where the studies were performed and the age of the participants could increase the variability because it has been described that the average CED for a specific age is not the same in all geographic areas and the rate of change is different over time, even in the same population.[32,42] Because this meta-regression was performed using data obtained in different studies, it relied on the quality of the information provided by them.

We evaluated the impact of T1DM and T2DM separately, but it was not possible with the available studies to directly compare T1DM with T2DM to establish quantitative relations between these conditions. Despite this, it is interesting that a significant relation between the duration of the DM and the progressive reduction in CED was only found in the T2DM group, whereas for both types of DM, the CCT correlated with the duration of the disease. Moreover, the calculated rate of CCT change was very similar in both types of DM. Overall, these findings point out that there must be a pathophysiological mechanism associated with T1DM that affects the corneal endothelial cells and is not correlated with the duration of the disease.

Our meta-regression model allowed a more accurate estimation of the association between each type of DM and the reduction in CED or the increase of CCT compared with nondiabetic control patients. This confirms that each type of DM and the duration of disease contribute differently to

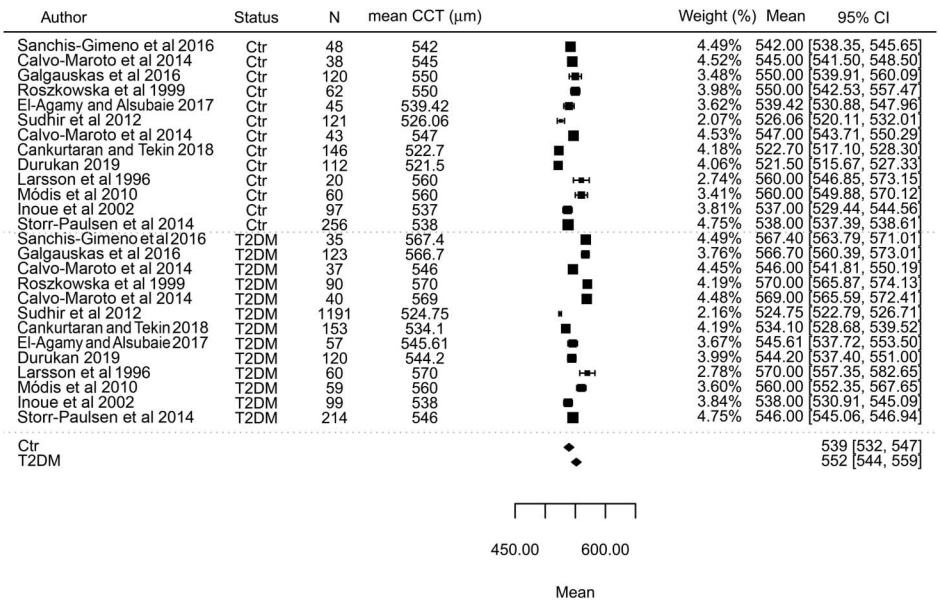

FIGURE 5. Forest plot of pachymetry (CCT) in patients with T2DM and control patients (Ctr). The mean of the pachymetry value is shown for patients with T2DM and for control patients.

corneal endothelial compromise, and this clinically requires further attention.

REFERENCES

1. International Diabetes Federation. *IDF Diabetes Atlas. International Diabetes Federation Web Site*. 2019. Available at: https://www.diabetesatlas.org/upload/resources/material/20200302_133351_IDFATLAS9e-final-web.pdf. Accessed November 3, 2020.
2. Powers AC, Niswender KD, Evans-Molina C. Diabetes mellitus: diagnosis, classification, and pathophysiology. In: Jameson JL, Fauci AS, Kasper DL, et al. eds. *Harrison's Principles of Internal Medicine*. New York, NY: McGraw-Hill Education; 2018:2850–2858.
3. Jeganathan VS, Wang JJ, Wong TY. Ocular associations of diabetes other than diabetic retinopathy. *Diabetes Care.* 2008;31:1905–1912.
4. Aldrich BT, Schlötzer-Schrehardt U, Skeie JM, et al. Mitochondrial and morphologic alterations in native human corneal endothelial cells associated with diabetes mellitus. *Invest Opthalmology Vis Sci.* 2017;58:2130–2138.
5. Dawson DG, John LU, Edelhauser HF. Cornea and sclera. In: Levin L, ed. *Adler's Physiology of the Eye*. St Louis, MI: W B Saunders Company; 2011:71–130.
6. Tuft SJ, Coster DJ. The corneal endothelium. *Eye.* 1990;4:389–424.
7. McCarey BE, Edelhauser HF, Lynn MJ. Review of corneal endothelial specular microscopy for FDA clinical trials of refractive procedures, surgical devices and new intraocular drugs and solutions. *Cornea.* 2008;27:1–16.
8. Malhotra D, Casey JR. Molecular mechanisms of Fuch's and congenital hereditary endothelial corneal dystrophies. In: Pedersen SH, ed. *Reviews of Physiology, Biochemistry and Pharmacology*. Cham, Switzerland: Springer International Publishing; 2020:41–81.
9. Goldstein AS, Janson BJ, Skeie JM, et al. The effects of diabetes mellitus on the corneal endothelium: a review. *Surv Ophthalmol.* 2020;65:438–450.
10. Busted N, Olsen T, Schmitz O. Clinical observations on the corneal thickness and the corneal endothelium in diabetes mellitus. *Br J Ophthalmol.* 1981;65:687–690.
11. Hatou S, Yamada M, Akune Y, et al. Role of insulin in regulation of Na+-/K+-dependent ATPase activity and pump function in corneal endothelial cells. *Invest Ophthalmol Vis Sci.* 2010;51:3935–3942.
12. Bikbova G, Oshitari T, Tawada A, et al. Corneal changes in diabetes mellitus. *Curr Diabetes Rev.* 2012;8:294–302.
13. Larsson LI, Bourne WM, Pach JM, et al. Structure and function of the corneal endothelium in diabetes mellitus type I and type II. *Arch Ophthalmol.* 1996;114:9–14.
14. Storr-Paulsen A, Singh A, Jeppesen H, et al. Corneal endothelial morphology and central thickness in patients with type II diabetes mellitus. *Acta Ophthalmologica.* 2014;92:158–160.
15. Sudhir RR, Raman R, Sharma T. Changes in the corneal endothelial cell density and morphology in patients with type 2 diabetes mellitus: a population-based study, Sankara Nethralaya Diabetic Retinopathy and Molecular Genetics Study (SN-DREAMS, Report 23). *Cornea.* 2012;31:1119–1122.
16. Anbar M, Ammar H, Mahmoud RA. Corneal endothelial morphology in children with type 1 diabetes. *J Diabetes Res.* 2016;2016:7319047.
17. Urban B, Raczyńska D, Bakunowicz-Łazarczyk A, et al. Evaluation of corneal endothelium in children and adolescents with type 1 diabetes mellitus. *Mediators Inflamm.* 2013;2013:1–6.
18. Roszkowska AM, Tringali CG, Colosi P, et al. Corneal endothelium evaluation in type I and type II diabetes mellitus. *Ophthalmologica.* 1999;213:258–261.
19. Módis L, Szalai E, Kertész K, et al. Evaluation of the corneal endothelium in patients with diabetes mellitus type I and II. *Histol Histopathol.* 2010;25:1531–1537.
20. Tang Y, Chen X, Zhang X, et al. Clinical evaluation of corneal changes after phacoemulsification in diabetic and non-diabetic cataract patients, a systematic review and meta-analysis. *Sci Rep.* 2017;7:14128.
21. Zhang K, Zhao L, Zhu C, et al. The effect of diabetes on corneal endothelium: a meta-analysis. *BMC Ophthalmol.* 2021;21:78.
22. Konstantopoulos S. Fixed effects and variance components estimation in three-level meta-analysis. *Res Synth Methods.* 2011;2:61–76.
23. Viechtbauer W. Conducting meta-analyses in R with the metafor package. *J Stat Softw.* 2010;36:1–48.
24. *R: The R Project for Statistical Computing [computer Program]. Version 4.0.4*. Viena, Austria: R Foundation for Statistical Computing; 2020.
25. Keoleian GM, Pach JM, Hodge DO, et al. Structural and functional studies of the corneal endothelium in diabetes mellitus. *Am J Ophthalmol.* 1992;113:64–70.
26. Fernandes SI, Nagpal S. Corneal thickness and endothelial cell density in children with type 1 diabetes mellitus. *Oman J Ophthalmol.* 2019;12:186–190.
27. Calvo-Maroto AM, Cerviño A, Perez-Cambrodí RJ, et al. Quantitative corneal anatomy: evaluation of the effect of diabetes duration on the endothelial cell density and corneal thickness. *Ophthalmic Physiol Opt.* 2015;35:293–298.
28. Sanchis-Gimeno JA, Alonso L, Rahhal M, et al. Corneal thickness differences between type 2 diabetes and non-diabetes subjects during preoperative laser surgery examination. *J Diabetes Complications.* 2017;31:209–212.
29. Galgauskas S, Laurinavičiūtė G, Norvydaitė D, et al. Changes in choroidal thickness and corneal parameters in diabetic eyes. *Eur J Ophthalmol.* 2016;26:163–167.
30. Cankurtaran V, Tekin K. Cumulative effects of smoking and diabetes mellitus on corneal endothelial cell parameters. *Cornea.* 2019;38:78–83.
31. Durukan I. Corneal endothelial changes in type 2 diabetes mellitus relative to diabetic retinopathy. *Clin Exp Optom.* 2020;103:474–478.
32. Choo M, Prakash K, Samsudin A, et al. Corneal changes in type II diabetes mellitus in Malaysia. *Int J Ophthalmol.* 2010;3:234–236.
33. El-Agamy A, Alsubaie S. Corneal endothelium and central corneal thickness changes in type 2 diabetes mellitus. *Clin Ophthalmol.* 2017;11:481–486.
34. Inoue K, Kato S, Inoue Y, et al. The corneal endothelium and thickness in type II diabetes mellitus. *Jpn J Ophthalmol.* 2002;46:65–69.
35. Feizi S. Corneal endothelial cell dysfunction: etiologies and management. *Ther Adv Ophthalmol.* 2018;10:2515841418815802.
36. Forbes JM, Cooper ME. Mechanisms of diabetic complications. *Physiol Rev.* 2013;93:137–188.
37. Treseler PA, Foulks GN, Sanfilippo F. The expression of HLA antigens by cells in the human cornea. *Am J Ophthalmol.* 1984;98:763–772.
38. Donnelly JJ, Li WY, Rockey JH, et al. Induction of class II (Ia) alloantigen expression on corneal endothelium in vivo and in vitro. *Invest Ophthalmol Vis Sci.* 1985;26:575–580.
39. Crotti C, Selmi C. Chapter 46—glutamic acid decarboxylase antibody. In: Shoenfeld Y, Meroni PL, Gershwin ME, eds. *Autoantibodies*. San Diego, CA: Elsevier; 2013:385–390.
40. Zhang W, Li H, Ogando DG, et al. Glutaminolysis is essential for energy production and ion transport in human corneal endothelium. *EBioMedicine.* 2017;16:292–301.
41. Zhang J, McGhee CN, Patel DV. The molecular basis of Fuchs' endothelial corneal dystrophy. *Mol Diagn Ther.* 2019;23:97–112.
42. Islam QU, Saeed MK, Mehboob MA. Age related changes in corneal morphological characteristics of healthy Pakistani eyes. *Saudi J Ophthalmol.* 2017;31:86–90.