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ORIGINAL ARTICLE

Corneal endothelial morphology changes in patients with proliferative diabetic retinopathy

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Abstract

Background Diabetes patients often suffer from diabetes keratopathy in the course of their illness. The examination of corneal endothelium in proliferative diabetic retinopathy (PDR) patients has important clinical significance. Here, we investigated the effect of PDR on corneal endothelial parameters.

Objective To analyze the associations between corneal endothelial cell parameters and proliferative diabetic retinopathy (PDR).

Methods We analyzed endothelial cell density (ECD), coefficient of variation in cell size (CV), hexagonality, and neutrophil/lymphocyte ratio (NLR) in patients with PDR and compared them with age-matched controls. The influences of duration of diabetes mellitus and level of glycosylated hemoglobin (HbA1c) were also analyzed.

Results The study group included 106 eyes of 106 PDR patients and 85 eyes of 85 control subjects. Significant differences were found in ECD ($2{,}436.11 \pm 222.08$ cells/mm^2 in PDR, 2527.16 ± 191.64 cells/mm^2 in controls; $p < 0.05$), CV (41.32 ± 7.40 in PDR, 37.71 ± 5.08 in controls; $p < 0.05$), Hex (50.07 ± 5.32 in PDR patients, 53.29 ± 5.73 in controls; $p < 0.05$), and NLR (2.94 ± 1.27 in PDR, 2.12 ± 0.56 in controls; $p < 0.05$). In the PDR group, ECD showed a decreasing trend as age increased (p trend < 0.05), and Spearman's correlation indicated a significant positive correlation between NLR and macular thickness ($p < 0.05$).

Conclusions PDR had deleterious effects on the corneal endothelium. PDR patients should undergo a rigorous corneal assessment to analyze the status of endothelial health, to identify the optimal treatment.

Keywords Proliferative diabetic retinopathy · Corneal endothelial morphology · Diabetes mellitus · Neutrophil/lymphocyte ratio

Introduction

Diabetes mellitus (DM) has become a global health problem, affecting an estimated 463 million patients, which is projected to increase to 700 million patients by 2045 [1]. Diabetic retinopathy (DR) is the most common microvascular complication of DM [2]. Epidemiological surveys showed that the global prevalence of DR among DM patients was recently estimated at 22.3%. Proliferative diabetic retinopathy (PDR) is a late stage of DR, accounting for 6.96% of DR patients [3]. The main manifestations of PDR are retinal ischemia, preretinal hemorrhage, vitreous hemorrhage, neovascularization of the optic disc or other sites, and fibrous proliferation [4–9], and it is the leading cause of acquired blindness in the working population [2].

As many as 70% of DM patients suffer from diabetes keratopathy during the course of their illness [10, 11]. Existing evidence indicates that changes in corneal structure and the biomechanics of DM patients may be potential biomarkers for the diagnosis of DM and its complications [12]. Metabolic stress induced by chronic hyperglycemia can activate a variety of pathological pathways, affecting cell morphology, cell density, ultrastructure, and barrier functions and may even change the results of internal eye

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surgery [13–15]. For patients with PDR, in addition to actively controlling the underlying disease, early intervention and timely treatment such as retinal laser photocoagulation therapy, intravitreal injection of anti-vascular endothelial growth factor (VEGF) drugs, or pars plana vitrectomy (PPV) are more important [16]. Despite the controversies, retinal laser photocoagulation therapy, intravitreal injection of anti VEGF drugs, and intraocular surgery have all been reported to damage the corneal endothelium [17–19]. In addition, the prevalence of anterior segment surgery, including cataract surgery, is high in PDR patients [20]. Therefore, preoperative detection of corneal endothelial status in PDR patients is of greater significance.

At present, there is still controversy over whether PDR affects corneal endothelial-related parameters [21–24]. It is therefore necessary to clarify possible associations between corneal endothelial cell parameters and PDR. In this study, the influence of DM and its duration and hemoglobin (HbA1c) level on corneal endothelial parameters were identified, and the relationships between the macular thickness in PDR patients, neutrophil/lymphocyte ratio (NLR), and corneal endothelial parameters of PDR patients were investigated for the first time, which provided guidance and a theoretical basis for clinical diagnosis and therapy of PDR patients.

Materials and methods

Study design

This prospective and cross-sectional study was conducted at the Second Affiliated Hospital of Nantong University from February 2022 to December 2022. The study protocol was conducted in accordance with the principles of the Declaration of Helsinki and was approved by the hospital ethics committee (2021KT004). Referring to previous relevant research [21], to achieve a power of 80%, assuming endothelial cell density (ECD) as the main observation indicator with a two-sided α of 0.05, the sample size for the research group and control group was at least 25. To ensure better analyses, more samples were included in the protocol. All participants were examined by the same ophthalmologist. Written informed consent was provided by each participant before the examination, and after explaining the significance of the study.

Participants in the study included patients with PDR (PDR group) diagnosed by FFA examinations. The healthy control subjects were recruited randomly among the patients who presented to our hospital (control group). Participants in the control group were non-diabetic subjects at the same time period, and two randomized glucose tests were conducted according to the recommendations of the American Diabetes Association to exclude diabetes that was not found [25]. Only the right eyes of participants were analyzed. The presence of type 2 DM (T2DM) was diagnosed by an endocrinologist. The inclusion criteria were as follows: (1) age ≥ 40 years and (2) diagnosed as PDR.

Potential factors affecting corneal endothelial health, including prior ophthalmic surgeries, a history of injection of intraocular anti-VEGF medications, retinal photocoagulation, severe ocular trauma, prolonged contact lens wearing, a history of chronic topical ophthalmic drugs, high myopia, glaucoma, uveitis, corneal endothelial dystrophy, and systemic diseases that impaired tear function were excluded. In addition, eyes with refractive interstitial opacity that affected the imaging quality of FFA or OCT and patients with type 1 DM were also excluded. All participants underwent routine blood tests, and PDR patients additionally underwent glycated hemoglobin tests.

Morphological characteristics of the corneal endothelium, including ECD, coefficient of variation (CV), and hexagonality (Hex), were determined using a non-contact specular microscope (SP3000P; Topcon, Tokyo, Japan) and were evaluated in the automatic mode. Three images were taken of each eye and were analyzed independently using semi-automatic technology, in which the computer program (ImageNet system, version 3.5.5) automatically outlined the endothelial cells, and we manually examined and rectified them, if necessary. The average of three measurements was used for each parameter. Corneal analysis was performed prior to any therapies for PDR or diabetic macular edema (DME).

PDR was diagnosed by a FFA examination, and the images were graded according to the international clinical diabetic retinopathy disease severity scale [6]. The image of PDR was presented as retinal neovascularization, vitreous hemorrhage, or pre-retinal hemorrhage. For all PDR patients, optical coherence tomography (OCT) performed by the same sonographer (OCT Spectralis, Heidelberg Engineering, Heidelberg, Germany) was used to acquire 19 ultrasound scans and 16 automated real-time scans in a high-resolution mode in a 20° × 15° (5.9 mm × 4.4 mm) area centered in the foveal after pupil dilatation.

Statistical analysis

The chi-squared test was used to analyze categorical variables. The Kolmogorov–Smirnov test was used to test the normality of parameters. An independent-sample *t* test was used for normally distributed data, and the rank sum test was used for non-normally distributed data, to compare the PDR and control groups. The polynomial linear correlation in one-way analysis of variance was used for the trend test (p trend). Pearson's correlation test was used to identify

the relationships between measurement parameters and the duration of DM, HbA1c level, and NLR. A value of $p \leq 0.05$ was considered statistically significant.

Results

A total of 191 participants were enrolled, including 106 PDR patients and 85 controls. The mean age of the PDR group was 55.81 ± 5.14 years with 52 females (49.06%) and 54 males (50.94%). The mean duration of diabetic disease was 10.61 ± 5.47 years, and the mean level of HbA1c level was 8.89 ± 2.16%. The mean age of the control group was 56.32 ± 8.37 years with 49 females (57.65%) and 36 males (42.35%). The demographics and age distributions of the PDR and control groups are shown in Table 1 and showed no significant difference between two groups in terms of age ($p = 0.61$) and sex ($p = 0.24$).

Comparisons of corneal parameters between the PDR and control groups are shown in Table 2. Overall, the corneal parameters in the PDR and control groups were significantly different. In the overall comparison and comparisons with most age groups, the ECD and Hex were significantly lower in the PDR group than in the control group ($p < 0.05$), and the CV and NLR were significantly greater in the PDR group ($p < 0.05$). Between the ages of 40 and 50 years in the two groups, there was no statistically significant difference in ECD and CV ($p = 0.85$ and $p = 0.43$, respectively). In the control group, as age increased, ECD and Hex showed a decreasing trend, while CV showed an increasing trend (p trend < 0.05). In the PDR group, as age increased, ECD also showed a decreasing trend (p trend < 0.05), while Hex and CV showed no change (p trend $= 0.06$ and 0.08, respectively). In both the PDR and control groups, as age increased, NLR showed no change (p trend $= 0.18$ and 0.14, respectively).

Within the PDR group (Table 3), a longer duration of T2DM (> 10 years) was associated with significantly higher macular thickness ($p < 0.05$); while the durations of T2DM and HbA1c levels were not related to corneal parameters or NLR ($p > 0.05$). Within the PDR group (Table 4), Spearman's correlation was conducted using the durations of T2DM, HbA1c levels, NLR, and ophthalmic parameters, which showed that NLR had a significant positive correlation with macular thickness ($p < 0.05$).

Table 1 Demographic and baseline characteristics of the PDR and control groups

Parameters	PDR group ($n = 106$)	Control group ($n = 85$)	p value
Age (years)	55.81 ± 5.14	56.32 ± 8.37	0.61
Sex (F/M)	52/54	49/36	0.24
HbA1c (%)	8.89 ± 2.16	–	–
Duration of T2DM (years)	10.61 ± 5.47	–	–
Age distribution			
40–50	25	12	< 0.05
51–60	42	58	0.69
60–70	39	15	0.17

p value by chi-square test

PDR proliferative diabetes retinopathy, *F/M* female/male, *HbA1c* hemoglobin A1c, *T2DM* type 2 diabetes mellitus

Discussion

This unique study characterized corneal endothelial cells and their influencing factors in PDR patients. Compared to the control group, ECD and HeX were significantly lower in the PDR group, while the CV and NLR were significantly greater in the PDR group.

To our knowledge, this was the first prospective study specifically studying corneal endothelial cell parameters in PDR patients, based on a relatively large sample size. Compared with DM patients with NPDR, patients with PDR are more likely to receive retinal laser photocoagulation, anti-VEGF drug therapy, and PPV [9, 26, 27]. According to previous reports, photocoagulation can cause loss of corneal endothelial cells, and there was a correlation between laser energy and a decrease in corneal endothelial cells [17, 28]. Intravitreous injection can generate sufficient concentrations of anti-VEGF drugs in the anterior chamber [29, 30], and some anti-VEGF drugs are believed to reduce corneal endothelial cell density [18, 31]. In the treatment of vitrectomy, whether it is the vitrectomy itself, possibly combined with cataract extraction surgery, or the use of postoperative vitreous cavity tamponades, all possibilities cause damage to corneal endothelial cells [32–34]. The cornea with altered morphology and functionality is susceptible to pathologies like recurrent corneal erosions, and impaired corneal sensitivity following trauma or surgical insult, leading to recurrent ulceration with impaired healing [35–37]. Considering that an adequate understanding of any potential endothelial dysfunction before treatment may be associated with more positive treatment outcomes [38], it is clinically important to analyze the corneal endothelial cell status in patients with PDR. Given that there are large numbers of patients with DM and PDR in China [24, 39], information on changes in corneal endothelial parameters in PDR patients may help ophthalmologists to choose appropriate treatment methods for patients.

Table 2 Age comparisons of the mean values of endothelial cell density, cell size, and hexagonality of proliferative diabetic retinopathy eyes and controls across all ages (mean ± SD)

	Age group (years)	PDR group ($n=106$)	Control group ($n=85$)	$p$value
ECD (cell/mm^2)	All age	2436.11 ± 222.08	2527.16 ± 191.64	< 0.05
	40–50	2625.23 ± 227.43	2609.34 ± 231.83	0.85
	51–60	2433.02 ± 198.14	2529.36 ± 185.50	< 0.05
	60–70	2328.40 ± 168.26	2440.19 ± 152.45	< 0.05
$\chi^2(F)$		3.92	3.31	
p*-value		< 0.05	< 0.05	
CV (%)	All age	41.32 ± 7.40	37.71 ± 5.08	< 0.05
	40–50	36.85 ± 6.64	35.10 ± 5.02	0.43
	51–60	41.02 ± 7.04	37.84 ± 4.96	< 0.05
	60–70	44.51 ± 6.80	39.29 ± 5.11	< 0.05
$\chi^2(F)$		1.57	11.40	
P*-value		0.06	< 0.05	
Hex (%)	All age	50.07 ± 5.32	53.29 ± 5.73	< 0.05
	40–50	53.04 ± 3.45	55.92 ± 3.48	< 0.05
	51–60	50.71 ± 5.30	53.43 ± 6.18	< 0.05
	60–70	47.46 ± 5.22	50.67 ± 4.34	< 0.05
$\chi^2(F)$		1.49	2.67	
p*-value		0.08	< 0.05	
NLR	All age	2.94 ± 1.27	2.12 ± 0.56	< 0.05
	40–50	3.21 ± 1.03	2.04 ± 0.56	< 0.05
	51–60	2.64 ± 1.10	2.17 ± 0.53	< 0.05
	60–70	3.11 ± 1.52	2.02 ± 0.66	< 0.05
$\chi^2(F)$		1.30	1.42	
P*-value		0.18	0.14	

PDR proliferative diabetes retinopathy, *ECD* endothelial cell density, *CV* coefficient of variation, *Hex* hexagonality, *NLR* neutrophil/lymphocyte ratio. p value by independent t-test

*p value by chi-square test for trend test (p trend)

Since the early 1980s, accumulating studies have been reported on corneal endothelial morphological changes in DM patients. Table 5 summarizes the main results of morphological studies on corneal endothelial cells in DM patients in recent years, which showed the presence of some differences. In most studies, compared with the control group, the ECD of DM patients decreased, and the course of the disease, HbA1c level, and severity of

Table 3 Comparison of parameters based on the duration of type 2 diabetes mellitus and HbA1c levels

Characteristic	Total ($n=106$)	ECD (cell/mm^2)	CV (%)	Hex (%)	NLR	Macular thickness (μm)
T2DM duration						
≤ 10 years	56 (47.2)	2468.03 ± 254.91	40.43 ± 6.83	50.63 ± 4.95	3.03 ± 1.30	294.27 ± 102.17
> 10 years	50 (52.8)	2400.37 ± 174.01	42.32 ± 7.93	49.44 ± 5.69	2.86 ± 1.37	333.15 ± 125.31
t/z		$t=-1.61$	$t=1.32$	$t=-1.15$	$t=-0.67$	$Z=-2.09$
p value		0.11	0.19	0.25	0.50	< 0.05
HbA1c level						
≤ 7.5%	24 (22.6)	2485.82 ± 273.51	40.45 ± 6.12	50.67 ± 5.88	3.25 ± 1.60	291.84 ± 90.82
> 7.5%	82 (77.4)	2421.56 ± 204.31	41.58 ± 7.75	49.89 ± 5.17	2.86 ± 1.23	318.69 ± 121.11
t/z		$t=1.07$	$t=-0.66$	$t=0.63$	$t=1.28$	$Z=-0.42$
p value		0.29	0.51	0.53	0.20	0.68

p value by independent t test or Mann Whitney U test

T2DM type 2 diabetes mellitus, *HbA1c* hemoglobin A1c, *ECD* endothelial cell density, *CV* coefficient of variation, *Hex* hexagonality, *NLR* neutrophil/lymphocyte ratio

Table 4 Correlation between measurement parameters and the duration of type 2 diabetes mellitus, HbA1c levels, and neutrophil/lymphocyte ratios

Factors in PDR group	ECD (cells/mm2)	CV (%)	Hex (%)	Macular thickness (μm)
T2DM duration (years)				
R-value	−0.16	0.17	−0.13	0.13
p value	0.11	0.09	0.18	0.18
HbA1c level (%)				
R-value	0.00	0.00	0.01	−0.02
p value	1.00	0.99	0.88	0.83
NLR				
R-value	−0.04	−0.12	0.03	0.21
p value	0.63	0.23	0.76	< 0.05

p value by Spearman's rho correlation test

T2DM type 2 diabetes mellitus, *HbA1c* hamoglobin A1c, *NLR* neutrophil/lymphocyte ratio, *PDR* proliferative diabetes retinopathy, ECD endothelial cell density, *CV* coefficient of variation, *Hex* hexagonality

retinopathy were significantly related to changes of the ECD [21, 23, 24, 40, 41]. However, in other studies [22, 42–44], there was no statistically significant difference in the corneal structures and endothelial characteristics between DM and non-DM participants. As shown in Table 5, in studies suggesting that there was no significant difference in corneal endothelial cells between DM patients and non-DM patients, more DM patients without DR or mild to moderate DR patients were often included in the study group. We speculated that the change of corneal endothelium in diabetes patients without DR or patients with mild to moderate DR may be slight and may be an important reason for the difference in the above results. In the present study, stricter inclusion criteria were used, and only patients with PDR were included in the study group.

In the overall comparison, and comparisons with most age groups, the ECD and Hex were significantly lower in the PDR group than in the control group ($p < 0.05$), and the CV and NLR were significantly greater in the PDR group ($p < 0.05$). These results therefore supported the view that DM does have an impact on corneal endothelial cells. DR is the main complication of DM caused by microangiopathy. Due to the common pathophysiological mechanisms of endothelial injury, such as accumulation of advanced glycation end products and increased oxidative stress [45, 46], a connection between DR and corneal endothelial cell loss should be expected.

In the present study, when we separately analyzed the corneal morphology of the diabetes group and healthy eyes, we found that with increasing age, endothelial cells and Hex cells showed a downward trend, while CV showed an upward trend (p trend < 0.05) in the control group. These results were consistent with previous studies [24]. In the PDR group, as age increased, ECD also showed a decreasing trend (p trend < 0.05), while Hex and CV showed no obvious change (p trend > 0.05). T2DM duration tended to show a negative correlation for ECD and Hex, and a positive correlation for CV, but the difference was not statistically significant ($p > 0.05$). Although some of the results from the PDR group were not completely consistent with those of other previous studies on DM patients [21, 41], it was believed to be due to the unique nature of PDR patients. PDR results from uncontrolled diabetes [47]. Use of insulin and poly pharmaceuticals has been associated with poor glycemic control among these PDR patients with T2DM [48]. There has been insufficient research on the relationships between corneal endothelial parameters and drugs and insulin [15, 49]. In addition, PDR patients have a more complex homeostasis [50, 51]. It is therefore worth determining whether this will affect corneal endothelial cells by affecting the composition of the aqueous humor.

The study of NLR in PDR patients in this study was also important. NLR is a new indicator for many diseases with systemic inflammatory pathophysiology [52–54]. In the current study, the NLR of the PDR group was significantly higher than the control group, indicating the presence of chronic, systemic, and low-grade inflammations in PDR patients. Consistent with the study by Ilhan et al. that the NLR of PDR patients was 2.67 ± 1.02 [55], the NLR of PDR patients from our results was 2.94 ± 1.27. It was reported that when the NLR of diabetes patients was 2.11 or higher, there might be aggravation of DR [55], and a score of 2.26 or higher was identified as an indicator of DME pathogenesis [56]. Within the PDR group, NLR showed a significant positive correlation with macular thickness, which was also consistent with previous research [56]. In brief, our results showed the importance of NLR as a relevant indicator for PDR.

There were some limitations to this study. First, this was a cross-sectional study, so measurements were taken at one point in time, and not all clinical differences could be precisely determined, whereas prospective, controlled, and blind design studies might result in higher quality data interpretation. Second, this study lacked a comparison of corneal central thickness, as the relationship between corneal thickness and diabetes retinopathy is controversial [57–60] and some study [60] even suggest that CCT may be the earliest detectable change in eyes with diabetes. Further research will be conducted on the above issues. In addition, although more PDR patients were included, when compared with previous studies, there was still a mismatch in the 40 – 50-year age group, which may cause bias in the results.

Table 5 Recent studies on diabetes mellitus and corneal endothelial cells

Study (year) and area	Number of two groups	Staging of DR	Study design	Mean age	Main conclusion
Chowdhury et al. (2021); India [40]	T2DM: 131 Control: 131	NPDR: 33 PDR: 14	Cross-sectional observational study	53.26 ± 6.24	Poorly controlled patients with T2DM and those with macroalbuminuria have corneal endothelial abnormalities
Storr-Paulsen et al. (2014); Denmark [44]	T2DM: 107 Control: 128	Without or mild DR	Prospective clinical study	72.10 ± 11.00	T2DM has no impact on corneal cell density or morphology in subjects with good glycaemia status
Seyed-Ali-Akbar et al. (2022); Iran [22]	T2DM: 134 Control: 128	Without DR: 40 Mild to moderate NPDR: 26 Severe NPDR: 28 PDR: 40	Cross-sectional study	61.03 ± 8.08	Corneal endothelial parameters were not associated with T2DM patients without or with DR
Beato et al. (2020); Porto [42]	T2DM: 60 Control: 47	Without DR: 42 Mild to moderate NPDR: 10 Severe NPDR: 8	Cross-sectional study	72.00 ± 6.00	No statistically significant differences were found between the corneal structural and endothelial characteristics of two groups
Papadakou et al. (2020); Athens [21]	T2DM: 72 Control: 88	Without DR: 23 Mild NPDR:29 Moderate NPDR: 15 Severe NPDR: 2 PDR: 3	Case–control study	67.10 ± 10.70	ECD was decreased in the DM group while duration of disease, HbA1c levels and severity of DR were significantly associated with changes in ECD
Kim et al. (2021); Korea [41]	T2DM: 511 Control: 900	Unclassified	Retrospective cross-sectional study	65.60 ± 11.10	T2DM affects corneal endothelial cell in older age and those with long-standing T2DM and higher HbA1c
Durukan. (2019); Turkey [23]	T2DM: 120 Control: 112	Without DR: 40 NPDR: 51 PDR: 29	Cross-sectional study	59.50 ± 8.10	Increase in corneal thickness, reduction in ECD, and distortion of morphology were detected in T2DM patients
Wichai et al. (2015); Thailand [43]	T2DM: 171 Control: 156	Unclassified	Cross-sectional study	58.49 ± 9.78	The corneal endothelial structure was not different between two groups
Ashok et al. (2021); India [24]	T2DM: 592 Control: 596	Without DR: 299 Mild NPDR: 81 Moderate NPDR: 67 Severe NPDR: 74 PDR: 71	Prospective, observational study	62.17 ± 9.49	T2DM has deleterious effects on corneal endothelium and thickness

T2DM type 2 diabetes mellitus, *DR* diabetic retinopathy, *NPDR* non-proliferative diabetic retinopathy, *PDR* proliferative diabetes retinopathy, *ECD* endothelial cell density, *HbA1c* hemoglobin A1c

Conclusion

In conclusion, the present study showed that the ECD and Hex were significantly lower, while CV and NLR were significantly greater in the PDR group, when compared with the control group. It is therefore necessary to evaluate not only the retina, but also the corneal endothelial cells during follow-ups of PDR patients. Overall, PDR patients should undergo a rigorous corneal assessment to analyze endothelial health status, to determine the optimal treatment.

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Author contribution All authors contributed to the conception of the work by writing sections of the manuscript and drafting and revising it critically as well as final approval of the published version. Yue Zhou, Lili Huang, and Xiaoli Yu were involved in the design of the study. Xiaojuan Chen, Lele Li, Min Wang, and Lidan Xue were involved in data collection and data analysis.

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Data availability The raw data supporting the conclusions of this article are available from the authors, without undue reservation.

Declarations

Ethical clearance The study was approved by the Human Research Ethics Committee of the Second Affiliated Hospital of Nantong University, China (No. 2021KT004) and adhered to the Declaration of Helsinki. Informed consent in written form was obtained from all participants and the participants' guardians for those under 18 years old.

Conflict of interest The authors declare no competing interests.

References

1. Saeedi P, Petersohn I, Salpea P, Malanda B, Karuranga S, Unwin N, Colagiuri S, Guariguata L, Motala AA, Ogurtsova K, et al. Global and regional diabetes prevalence estimates for 2019 and projections for 2030 and 2045: results from the International Diabetes Federation Diabetes Atlas, 9(th) edition. Diabetes Res Clin Pract. 2019;157:107843.
2. Ferrara M, Loda A, Coco G, Grassi P, Cestaro S, Rezzola S, Romano V, Semeraro F. Diabetic retinopathy: soluble and imaging ocular biomarkers. J Clin Med. 2023;12(3).
3. Zhou J, Liu Z, Chen M, Luo ZH, Li YQ, Qi GY, Liu T. Concentrations of VEGF and PlGF decrease in eyes after intravitreal conbercept injection. Diabetes Ther. 2018;9(6):2393–8.
4. Arevalo JF, Lasave AF, Kozak I, Al Rashaed S, Al Kahtani E, Maia M, Farah ME, Cutolo C, Brito M, Osorio C, et al. Preoperative bevacizumab for tractional retinal detachment in proliferative diabetic retinopathy: a prospective randomized clinical trial. Am J Ophthalmol. 2019;207:279–287.
5. Gologorsky D, Thanos A, Vavvas D. Therapeutic interventions against inflammatory and angiogenic mediators in proliferative diabetic retinopathy. Mediators Inflamm. 2012;2012:629452.
6. Wilkinson CP, Ferris FL 3rd, Klein RE, Lee PP, Agardh CD, Davis M, Dills D, Kampik A, Pararajasegaram R, Verdaguer JT. Proposed international clinical diabetic retinopathy and diabetic macular edema disease severity scales. Ophthalmology. 2003;110(9):1677–82.
7. Fong DS, Ferris FL 3rd, Davis MD, Chew EY. Causes of severe visual loss in the early treatment diabetic retinopathy study: ETDRS report no. 24. Early Treatment Diabetic Retinopathy Study Research Group. Am J Ophthalmol. 1999;127(2):137–41.
8. Stitt AW, Curtis TM, Chen M, Medina RJ, McKay GJ, Jenkins A, Gardiner TA, Lyons TJ, Hammes HP, Simó R, et al. The progress in understanding and treatment of diabetic retinopathy. Prog Retin Eye Res. 2016;51:156–186.
9. Chaudhary S, Zaveri J, Becker N. Proliferative diabetic retinopathy (PDR). Dis Mon. 2021;67(5):101140.
10 Abdelkader H, Patel DV, McGhee CNJ, Alany RG. New therapeutic approaches in the treatment of diabetic keratopathy: a review. Clin Exp Ophthalmol. 2011;39(3):259–70.
11. Romero-Aroca P. Ocular complications of diabetes and therapeutic approaches. J Clin Med. 2022;**11**(17).
12. Wang X, Xu G, Wang W, Wang J, Chen L, He M, Chen Z. Changes in corneal biomechanics in patients with diabetes mellitus: a systematic review and meta-analysis. Acta Diabetol. 2020;57(8):973–81.
13. Coco G, Hamill KJ, Troughton LD, Kaye SB, Romano V. Risk factors for corneal epithelial wound healing: can sex play a role. Eur J Ophthalmol. 2022;32(5):2676–82.
14. Goldstein AS, Janson BJ, Skeie JM, Ling JJ, Greiner MA. The effects of diabetes mellitus on the corneal endothelium: a review. Surv Ophthalmol. 2020;65(4):438–50.
15. Pont C, Ascaso FJ, Grzybowski A, Huerva V. Corneal endothelial cell density during diabetes mellitus and ocular diabetes complications treatment. J Fr Ophtalmol. 2020;43(8):794–8.
16. Wong TY, Cheung CM, Larsen M, Sharma S, Simó R. Diabetic retinopathy. Nat Rev Dis Primers. 2016;**2**16012.
17. Murata H, Kato S, Fukushima H, Tsutsumi A, Numaga J, Amano S. Corneal endothelial cell density reduction: a complication of retinal photocoagulation with an indirect ophthalmoscopy contact lens. Acta Ophthalmol Scand. 2007;85(4):407–8.
18. Muto T, Machida S. Effect of intravitreal aflibercept on corneal endothelial cells: a 6-month follow-up study. Clin Ophthalmol. 2019;**13**373–381.
19 Kopecky A, Nemcansky J. Changes in the anterior segment of the eye following uncomplicated pars plana vitrectomy. A review. Biomed Pap Med Fac Univ Palacky Olomouc Czech Repub. 2019;163(3):207–11.
20. Grzybowski A, Kanclerz P, Huerva V, Ascaso FJ, Tuuminen R. Diabetes and phacoemulsification cataract surgery: difficulties, risks and potential complications. J Clin Med. 2019;8(5).
21. Papadakou P, Chatziralli I, Papathanassiou M, Lambadiari V, Siganos CS, Theodossiadis P, Kozobolis V. The effect of diabetes mellitus on corneal endothelial cells and central corneal thickness: a case-control study. Ophthalmic Res. 2020;63(6):550–4.
22 Mortazavi SA, Akhlaghi M, Dehghani A, Pourazizi M, Malekahmadi M, Fazel M, Tavakoli M, Noorshargh P. Diabetic retinopathy and corneal endothelial parameters: an analytical cross-sectional study. BMC Ophthalmol. 2022;22(1):427.
23. Durukan I. Corneal endothelial changes in type 2 diabetes mellitus relative to diabetic retinopathy. Clin Exp Optom. 2020;103(4):474–8.

24. Jha A, Verma A, Alagorie AR. Association of severity of diabetic retinopathy with corneal endothelial and thickness changes in patients with diabetes mellitus. Eye (Lond). 2022;36(6):1202–8.
25. Diagnosis and classification of diabetes mellitus. Diabetes Care. 2007;30 Suppl 1:S42–47.
26. Pandit S, Ho AC, Yonekawa Y. Recent advances in the management of proliferative diabetic retinopathy. Curr Opin Ophthalmol. 2023;34(3):232–6.
27 Crabtree GS, Chang JS. Management of complications and vision loss from proliferative diabetic retinopathy. Curr Diab Rep. 2021;21(9):33.
28 Pardos GJ, Krachmer JH. Photocoagulation. Its effect on the corneal endothelial cell density of diabetics. Arch Ophthalmol. 1981;99(1):84–6.
29. Niwa Y, Kakinoki M, Sawada T, Wang X, Ohji M. Ranibizumab and aflibercept: intraocular pharmacokinetics and their effects on aqueous VEGF level in vitrectomized and nonvitrectomized macaque eyes. Invest Ophthalmol Vis Sci. 2015;56(11):6501–5.
30. Bakri SJ, Snyder MR, Reid JM, Pulido JS, Ezzat MK, Singh RJ. Pharmacokinetics of intravitreal ranibizumab (Lucentis). Ophthalmology. 2007;114(12):2179–82.
31. Ari S, Nergiz Y, Aksit I, Sahin A, Cingu K, Caca I. Evaluation of intracameral injection of ranibizumab and bevacizumab on the corneal endothelium by scanning electron microscopy. J Ocul Pharmacol Ther. 2015;31(2):100–5.
32. Koushan K, Mikhail M, Beattie A, Ahuja N, Liszauer A, Kobetz L, Farrokhyar F, Martin JA. Corneal endothelial cell loss after pars plana vitrectomy and combined phacoemulsification-vitrectomy surgeries. Can J Ophthalmol. 2017;52(1):4–8.
33. Coman Cernat CC, PatoniPopescu SI, Maliţa D, Stanca S, Muşat O, Negru Ş, Feier H, Karancsi OL, Roşca C. Endothelial corneal cell damage after pars plana vitrectomy: analogy of different intraocular tamponade agents. Rom J Ophthalmol. 2021;65(2):141–9.
34. Goezinne F, Nuijts RM, Liem AT, Lundqvist IJ, Berendschot TJ, Cals DW, Hendrikse F, La Heij EC. Corneal endothelial cell density after vitrectomy with silicone oil for complex retinal detachments. Retina. 2014;34(2):228–36.
35. Saghizadeh M, Kramerov AA, Yu FS, Castro MG, Ljubimov AV. Normalization of wound healing and diabetic markers in organ cultured human diabetic corneas by adenoviral delivery of c-Met gene. Invest Ophthalmol Vis Sci. 2010;51(4):1970–80.
36. 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):1–16.
37. Shenoy R, Khandekar R, Bialasiewicz A, Al MA. Corneal endothelium in patients with diabetes mellitus: a historical cohort study. Eur J Ophthalmol. 2009;19(3):369–75.
38. Greene JB, Mian SI. Cataract surgery in patients with corneal disease. Curr Opin Ophthalmol. 2013;24(1):9–14.
39 Tan H, Wang X, Ye K, Lin J, Song E, Gong L. Prevalence and risk factors of diabetic retinopathy among Chinese adults with type 2 diabetes in a suburb of Shanghai. China PLoS One. 2022;17(10):e0275617.
40. Chowdhury B, Bhadra S, Mittal P, Shyam K. Corneal endothelial morphology and central corneal thickness in type 2 diabetes mellitus patients. Indian J Ophthalmol. 2021;69(7):1718–24.
41 Kim YJ, Kim TG. The effects of type 2 diabetes mellitus on the corneal endothelium and central corneal thickness. Sci Rep. 2021;11(1):8324.
42. Beato JN, Esteves-Leandro J, Reis D, Falcão M, Rosas V, Carneiro Â, Falcão Reis F. Evaluation of corneal structure and endothelial morphological characteristics in type 2 diabetic and non-diabetic patients. Clin Ophthalmol. 2020;14:1993–9.
43. Leelawongtawun W, Suphachearaphan W, Kampitak K, Leelawongtawun R. A comparative study of corneal endothelial structure between diabetes and non-diabetes. J Med Assoc Thai. 2015;98(5):484–8.
44. Storr-Paulsen A, Singh A, Jeppesen H, Norregaard JC, Thulesen J. Corneal endothelial morphology and central thickness in patients with type II diabetes mellitus. Acta Ophthalmol. 2014;92(2):158–60.
45. Gui F, You Z, Fu S, Wu H, Zhang Y. Endothelial dysfunction in diabetic retinopathy. Front Endocrinol (Lausanne). 2020;**11**591.
46. Goodman D, Ness S. The role of oxidative stress in the aging eye. Life (Basel). 2023;**13**(3).
47. Shukla UV, Tripathy K:StatPearls. Treasure Island (FL), 2023.
48. Wang J, Li J, Wen C, Liu Y, Ma H. Predictors of poor glycemic control among type 2 diabetes mellitus patients treated with antidiabetic medications: a cross-sectional study in China. Medicine (Baltimore). 2021;100(43):e27677.
49. Liaboe CA, Aldrich BT, Carter PC, Skeie JM, Burckart KA, Schmidt GA, Reed CR, Zimmerman MB, Greiner MA. Assessing the impact of diabetes mellitus on donor corneal endothelial cell density. Cornea. 2017;36(5):561–6.
50. Loukovaara S, Sandholm J, Aalto K, Liukkonen J, Jalkanen S, Yegutkin GG. Deregulation of ocular nucleotide homeostasis in patients with diabetic retinopathy. J Mol Med (Berl). 2017;95(2):193–204.
51. Mao D, Peng H, Li Q, Wang J, Li P, Hu K, Zhang X, Lei B. Aqueous humor and plasma adiponectin levels in proliferative diabetic retinopathy patients. Curr Eye Res. 2012;37(9):803–8.
52. Valero C, Pardo L, López M, García J, Camacho M, Quer M, León X. Pretreatment count of peripheral neutrophils, monocytes, and lymphocytes as independent prognostic factor in patients with head and neck cancer. Head Neck. 2017;39(2):219–26.
53. Zhao W, Wu Z, Li Y, Jia H, Chen M, Gu X, Liu M, Zhang Z, Wang P, Cheng W. Pretreatment neutrophil-to-lymphocyte ratio and its dynamic changes are associated with the overall survival in advanced cancer patients undergoing palliative care. Sci Rep. 2016;**6**31394.
54. Kaya MG. Inflammation and coronary artery disease: as a new biomarker neutrophil/lymphocyte ratio. Turk Kardiyol Dern Ars. 2013;41(3):191–2.
55. Ilhan C, Citirik M, Uzel MM, Tekin K. The optimal cutoff value of neutrophil/lymphocyte ratio for severe grades of diabetic retinopathy. Beyoglu Eye J. 2019;4(2):76–81.
56. Ilhan C, Citirik M, Uzel MM, Kiziltoprak H, Tekin K. The usefulness of systemic inflammatory markers as diagnostic indicators of the pathogenesis of diabetic macular edema. Arq Bras Oftalmol. 2020;83(4):299–304.
57 Canan H, Sahinoglu-Keskek N, Altan-Yaycioglu R. The relationship of central corneal thickness with the status of diabetic retinopathy. BMC Ophthalmol. 2020;20(1):220.
58 Wang L, Liu S, Wang W, He M, Mo Z, Gong X, Xiong K, Li Y, Huang W. Association between ocular biometrical parameters and diabetic retinopathy in Chinese adults with type 2 diabetes mellitus. Acta Ophthalmol. 2021;99(5):e661–8.
59. Galgauskas S, Laurinavičiūtė G, Norvydaitė D, Stech S, Ašoklis R. Changes in choroidal thickness and corneal parameters in diabetic eyes. Eur J Ophthalmol. 2016;26(2):163–7.
60. Busted N, Olsen T, Schmitz O. Clinical observations on the corneal thickness and the corneal endothelium in diabetes mellitus. Br J Ophthalmol. 1981;65(10):687–90.