Journal of Diabetes Research

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Research Article

Diabetic Retinopathy Screening in Patients with Diabetes Using a Handheld Fundus Camera: The Experience from the South-Eastern Region in Hungary

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Purpose. Diabetic retinopathy (DR) is the leading cause of vision loss among active adults in industrialized countries. We aimed to investigate the prevalence of diabetes mellitus (DM), DR and its different grades, in patients with DM in the Csongrád County, South-Eastern region, Hungary. Furthermore, we aimed to detect the risk factors for developing DR and the diabetology/ophthalmology screening patterns and frequencies, as well as the effect of socioeconomic status- (SES-) related factors on the health and behavior of DM patients. *Methods*. A cross-sectional study was conducted on adults (>18 years) involving handheld fundus camera screening (Smartscope Pro Optomed, Finland) and image assessment using the Spectra DR software (Health Intelligence, England). Self-completed questionnaires on self-perceived health status (SPHS) and health behavior, as well as visual acuity, HbA1c level, type of DM, and attendance at healthcare services were also recorded. *Results*. 787 participants with fundus camera images and full self-administered questionnaires were included in the study; 46.2% of the images were unassessable. T1D and T2D were present in 13.5% and 86.5% of the participants, respectively. Among the T1D and T2D patients, 25.0% and 33.5% had DR, respectively. The SES showed significant proportion differences in the T1D group. Lower education was associated with a lower DR rate compared to non-DR (7.7% vs. 40.5%), while bad/very bad perceived financial status was associated with significantly higher DR proportion compared to non-DR (63.6% vs. 22.2%). Neither the SPHS nor the health behavior showed a significant relationship with the disease for both DM groups. Mild nonproliferative retinopathy without maculopathy (R1M0) was detected in 6% and 23% of the T1D and T2D patients having DR, respectively; R1 with maculopathy (R1M1) was present in 82% and 66% of the T1D and T2D groups, respectively. Both moderate nonproliferative retinopathy with maculopathy (R2M1) and active proliferative retinopathy with maculopathy (R3M1) were detected in 6% and 7% of the T1D and T2D patients having DR, respectively. The level of HbA1c affected the attendance at the diabetology screening (HbA1c > 7% associated with >50% of all quarter-yearly attendance in DM patients, and with 10% of the diabetology screening nonattendance). *Conclusion*. The prevalence of DM and DR in the studied population in Hungary followed the country trend, with a slightly higher sight-threatening DR than the previously reported national average. SES appears to affect the DR rate, in particular, for T1D. Although DR screening using handheld cameras seems to be simple and dynamic, much training and experience, as well as overcoming the issue of decreased optic clarity is needed to achieve a proper level of image assessability, and in particular, for use in future telemedicine or artificial intelligence screening programs.

1. Introduction

Diabetes mellitus (DM) is a major medical and societal challenge due to its rapid increase in global prevalence and devastating late complications [1, 2]. The global occurrence of DM among adults (>18 years of age) was 8.5% in 2014, and this has nearly doubled from its 4.7% level in 1980 [3]. In 2016, 1.6 million deaths were directly attributed to DM, with more than half of them occurring in the lower- and middle-income countries. According to the WHO forecast, DM will be the seventh leading cause of death in 2030, while diabetic retinopathy (DR) will be the leading cause of vision loss among active adults in industrialized countries [4]. DR is the most common late complication of DM in people aged 20 to 64 years—the working-age population, and except for where effective screening programs have been implemented, it is the leading cause of blindness and reduced vision in this group in the developed world [5, 6]. In a study comparing data from 35 populations, the global prevalence of sight-threatening retinopathy (STR) was estimated at 10.2% for all DM patients [6].

In Hungary, a total of 865 069 patients (9.5% of the population) suffered from DM among adults (>18 years of age) in 2011 [7], and some degree of DR could be observed among 19% of the patients with type 1 DM (T1D) and 24% in those suffering from type 2 DM (T2D) for 3 or 4 years [8]. Systematic DR screening and monitoring has been proven to be cost-effective in reducing blindness and visual impairment in patients having DM. Screening enables optimized timing of laser and medical therapy that may halt disease progression [9]. The WHO guidelines [10] for DR screening state that "annual eye examinations are recommended for patients with diabetes (and every other year for persons with excellent glycemic control and no retinopathy at the previous examination...)." "Such programs need systematic evaluation for their impact on health outcomes, cost effectiveness and health equity." The WHO recommendation further states "Member States should choose the most appropriate interval between examinations" [10].

The development of optimized and effective DR screening programs is becoming eminent. The aim of this study was to investigate the prevalence of DR and its different grades in patients with DM in the Csongrád County—a South-Eastern region in Hungary, using for the first time in this country a handheld fundus camera (Smartscope Pro Optomed, Finland). Moreover, we aimed to detect the risk factors for developing DR and the diabetology/ophthalmology screening patterns and frequencies, as well as the effect of socioeconomic status- (SES-) related factors on the health and behavior of DM patients.

2. Patients and Methods

2.1. Physical Examination. A cross-sectional study was conducted between the Departments of Ophthalmology and Internal Medicine Diabetology Unit, University of Szeged, Szeged, Hungary, between November 2015 and December 2016. All examinations were voluntary and free of charge to the participants, and the patients were recruited consecutively from the Diabetology Outpatient Clinic. Written informed consent was obtained from all participants. The study was approved by the local ethical committee of the University of Szeged (No.197/2015). The detection of DR was based upon examination with a handheld fundus camera (Smartscope Pro Optomed, Finland) in a dark room by qualified professionals. The results were directly evaluated by a qualified specialist without the need to do data/file transfer. In the case of constricted pupil, another image was taken after ensuring normal intraocular pressure level and applying cyclopentolate (5 mg/mL) eye drops to achieve mydriasis. The assessment of the fundus images was performed using the Spectra DR software (Health Intelligence, UK). The recordings were safely deposited and kept inaccessible to third parties for 10 years at a designated server, so that later they can be used in further comparative studies on DR.

The images acquired with the Optomed Smartscope Pro digital handheld camera included two pictures from the participants' eyes—one with the macula—and another with the optic nerve—in the center—which is in line with the English screening requirements [11]. In case of presence of amblyopia or nontransparent media (e.g., cataract and corneal or visual axis obstructing conditions), the patients were excluded from the study. During image evaluation, the graders (A.F./G.P./G.R.) classified the signs and stages of DR and maculopathy in the standardized English-based software Spectra DR and graded the images in alignment with the English standard grading protocols [12]. Each image was evaluated in two stages: first, the referral outcome graders/ROGs (D.E./G.R.) evaluated them, and then a supervisor/ophthalmic consultant confirmed the diagnosis (A.F./G.P.). At the end, an expert opinion regarding the grade of retinopathy was provided, which included the stage of retinopathy (R0/1/2/3A) and the absence or existence of maculopathy (M0/1). Other discovered abnormalities were not diagnosed in this study, although they were recorded, as they can provide further information about other symptoms, which may have occurred in the past, and therefore may require medical attention over a specified period of time.

The classification of the DR has been described before [13]—in brief: (R0) no clinical anomaly—repeated screening was recommended one year later; (R1) mild nonproliferative—presence of microaneurysms, dot- or blot- like hemorrhages, or exudates—control examination was recommended one year later; (R2) moderate or severe nonproliferative—presence of major bleeding(s) and intraretinal microvascular abnormalities (IRMAs)—control examination was required within one month; (R3A) active proliferative—presence of neovascularization of the optic disc (NVD) or elsewhere (NVE) or preretinal bleeding(s), vitreous bleeding, preretinal fibrosis, and tractional retinal detachment—immediate medical examination was required within two weeks. All the stages were combined with sight-threatening maculopathy which was determined by the presence of exudates regardless of visual acuity (VA), or red lesions with a VA of 6/12 or worse after pinhole correction, that is within 1 disc diameter of the center of the fovea, and/or a group of exudates where the area of exudates that is greater than or equal to half the disc area, and this area is

all within the macular area (as defined by the ETDRS macular grid) when medical examination was required within a month (M1).

2.2. Self-Completed Questionnaire. Participants were asked to fill out a self-administered questionnaire which was based upon the European Health Interview Survey 2009—it included demographic characteristics such as gender, age, and place of residency. From the place of residency, the distance to the healthcare facility was calculated as <10 km or ≥10 km.

The marital status was categorized as married or lives with a partner, single, separated or divorced and widowed; due to the low sample size, categories were merged together as living alone or living in partnership. SES of the study participants was examined: education and economic status. The economic status was characterized as working—full time and working—part-time, unemployed, retired, temporarily laid off, and student; due to the lack of data between each category, the categories were allocated and merged as inactive or active. The level of education was measured as primary, secondary, or higher education (college, university, or higher).

Data were collected about self-perceived health status (SPHS) and characterized as bad satisfactory, and good. Information was also collected about "Perception of what the subject can do for his/her health status," and the information was categorized as almost nothing (nothing/little) or much more (much/very much).

Health behavior was assessed by alcohol consumption, smoking, physical activity, and diet (no/yes). Smoking was classified as yes/quit/never smoking, while alcohol consumption was classified as no/yes. Physical activity was defined according to the amount or occasions spent in the previous month in cycling, walking: daily/weekly more time, weekly, once/no activity at all (inactive).

Information was also collected about the DM-related and other health conditions, for example, if the study participant has/had hypertension: no/yes. If yes, data were collected about the duration of the hypertension (years). If the participant attended blood pressure controls, a recording was made about the last measurement of the systolic and diastolic blood pressures in millimeters of mercury (mmHg).

Information was further collected about other health conditions, for example, VA (<0.3 or ≥0.3), HbA1c level (normal <7% or elevated ≥7%), type of diabetes mellitus (T1DM or T2DM), use of medications, DM in the family or occurrence of diabetic maculopathy. In addition, data about the attendance at healthcare services like diabetology (monthly, every 3rd month, every 6th month, yearly, more than a year, or no attendance) were also collected.

2.3. Statistical Analysis. The analysis of the data was performed by descriptive statistics; percentage distribution, mean and standard deviation (SD), and in case of nonnormality of continuous variables, median and interquartile range (IQR) and range (minimum, maximum) are shown. Normality of the continuous variables was tested on a histogram, Q-Q- plot, and by Shapiro-Wilk and Kolmogorov-Smirnov test. The Independent Sample T-test was used to compare the means of the continuous, numerical variables, when the normality assumption was satisfied; otherwise, Mann–Whitney U test was used. Homogeneity of variance was analyzed with the Levene test.

Chi-square (χ^2) and Fisher test were used to test the differences of the distribution of categorical variables; for multiple comparisons, the 2-sample z-test with Bonferroni correction was applied to detect the differences in the proportions between the studied groups. If the sample within each column was 1 or less, then the z-test could not be used. The significance limit was set at $P < 0.05$. The statistical analysis of the data was performed by IBM SPSS Statistics Version 24 software.

2.4. Ethical Issues. The Regional and Institutional Human Medical Biological Research Ethics Committee of the Szent-Györgyi Albert Clinical Center, University of Szeged approved the study protocol (No. 197/2015). The research provided anonymity to the participants. Before the beginning of a test, the participants signed a voluntary written consent form in which they agreed to permit the use of data for research purposes.

3. Results

The data were collected from a total of 848 participants with known DM in the Csongrád County, South-Eastern region in Hungary (Figure 1). Out of the initial participants, 787 (92.8%) had available fundus camera images and answered the self-administered questionnaire. T1D was present in 13.5% ($N = 52$) of participants, while T2D was present in 86.5% ($N = 334$) of the participants. Among the T1D and T2D patients, 25.0% ($N = 13$) and 33.5% ($N = 112$) had DR, respectively. A large portion of the participants had unassessable fundus camera images/results 46.2% ($N = 363$) when using the handheld camera, and therefore excluded from the further analysis (Figure 1).

The data analysis was based upon the remaining 386 individuals, who had assessable fundus camera images and possessed complete data about the type of diabetes and the risk parameters studied.

Table 1 shows the characteristics of the studied participants. Gender, age, and marital status showed no significant proportion differences between the study groups, while SES showed significant proportion differences in the T1D group. The proportion of the DR differed significantly in the Education and Perceived Financial Status groups, and it was significantly higher among those with higher education (secondary/higher being 61.5%/30.8%) and perceived bad financial status (63.6%). The distance travelled to the healthcare service showed a nearly significant association with the DR—participants living more than 10 km away from the healthcare services had a higher proportion of DR (61.5%).

Tables 2 and 3 show the results of the SPHS and the health behavior of the individuals, neither of which showed a significant relationship with the disease for both, T1D and T2D groups.

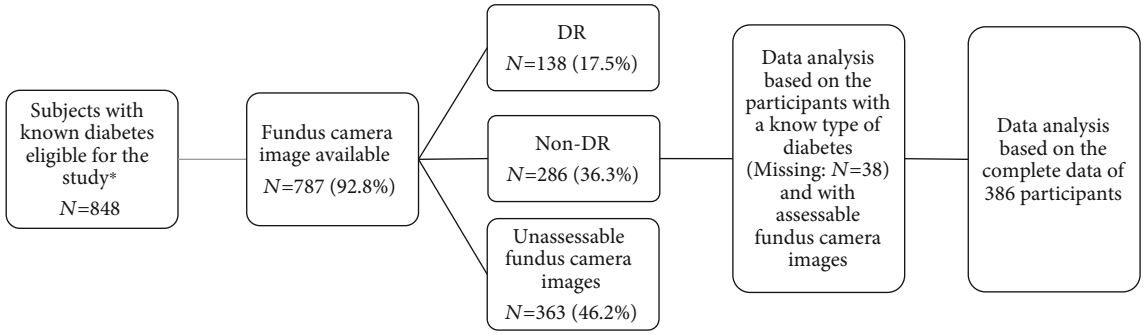

Figure 1: Flowchart of the study sample. DR: diabetic retinopathy; Non-DR: nondiabetic retinopathy; N: number. *Fulfilled the self-completed questionnaire and had a fundus camera image taken.

Table 1: Characteristics of the study sample.

	T1D $N = 52$ (%)		T2D $N = 334$ (%)	
	DR $N = 13$ (%)	Non-DR $N = 39$ (%)	DR $N = 112$ (%)	Non-DR $N = 222$ (%)
Gender				
Male	7 (53.8)	23 (59.0)	47 (42.0)	94 (42.3)
Female	6 (46.2)	16 (41.0)	65 (58.0)	128 (57.7)
Age (mean ± SD)	70.8 ± 6.0	66.4 ± 12.2	66.4 ± 12.8	65.7 ± 13.0
Distance to the healthcare services				
<10 km	*5 (38.5)*	*27 (69.2)*	75 (67.0)	140 (63.4)
≥10 km	*8 (61.5)*	*12 (30.8)*	37 (33.0)	81 (36.6)[a]
Education				
Primary	*1 (7.7)*	*15 (40.5)*	54 (48.2)	94 (43.5)
Secondary	*8 (61.5)*	*10 (11.2)*	30 (26.8)	79 (36.6)
Higher	*4 (30.8)*	*12 (32.4)*	28 (25.0)	43 (19.9)[b]
Perceived financial status				
Bad	*7 (63.6)*	*8 (22.2)*	24 (23.1)	58 (27.6)
Satisfactory	*2 (18.2)*	*23 (63.9)*	70 (67.3)	131 (62.4)
Good	*2 (18.2)*	*5 (13.9)*	10 (9.6)	21 (10.0)[c]
Marital status				
Living alone	1 (7.7)	5 (13.9)	37 (33.0)	60 (27.8)
Living in partnership	12 (92.3)	31 (86.1)	75 (67.0)	156 (72.2)[d]
Economic status				
Active	9 (69.2)	21 (55.3)	*21 (18.7)*	*63 (28.9)*

$P < 0.05$. T1D: type 1 diabetes mellitus; T2D: type 2 diabetes mellitus; DR: diabetic retinopathy; Non-DR: nondiabetic retinopathy; *N*: number; SD: standard deviation. Missing data: (a) 1; (b) 8; (c) 25; (d) 7.

Table 4 shows the characteristics of the health status of the study participants. A significant difference was only present in case of diabetes medication use and presence of diabetic maculopathy in T2D patients having DR and non-DR, with the rest of the parameters included (hypertension, VA, HbA1c, duration of DM, and familiar presence of DM) showing no significant proportion differences between the studied groups.

Mild nonproliferative retinopathy without maculopathy (R1M0) was detected in 6% of the T1D patients having DR, and 23% of the T2D patients having DR. Among the patients having DR, R1 with maculopathy (R1M1) was present in 82% of the T1D group, and 66% of the T2D group. Both moderate nonproliferative retinopathy with maculopathy (R2M1) and active proliferative retinopathy with maculopathy (R3M1) were detected in 6% of the T1D patients having DR. Among the T2D patients having DR, the prevalence of R2M1 was 4%, while the prevalence of R3M1 was 7% (Figure 2).

The level of HbA1c affected the participation in the diabetology screening, with those having HbA1c > 7% representing more than 50% of all quarter yearly attendance for both types of DM (Figure 3). About 10% of the population had no diabetology screening attendance for those having HbA1c > 7% for both types of DM and HbAc < 7% T2D. For both types of DM,

Table 2: Self-perceived health status of the study sample.

	T1D $N = 52$		T2D $N = 334$	
	DR $N = 13$ (%)	Non-DR $N = 39$ (%)	DR $N = 112$ (%)	Non-DR $N = 222$ (%)
Self -perceived health				
Bad	2 (15.4)	7 (18.4)	28 (25.2)	65 (29.3)
Satisfactory	7 (53.8)	24 (63.2)	64 (57.7)	135 (60.8)
Good	4 (30.8)	7 (18.4)	19 (17.1)	22 (9.9)[a]
What the person can do for his/her health				
Very much/much	10 (83.3)	30 (78.9)	91 (82.0)	167 (76.6)
Little/nothing	2 (16.7)	8 (21.1)	20 (18.0)	51 (23.4)[b]

$P < 0.05$. T1D: type 1 diabetes mellitus; T2D: type 2 diabetes mellitus; DR: diabetic retinopathy; Non-DR: nondiabetic retinopathy; N: number. Missing data: (a) 2; (b) 7.

Table 3: Health behavior of the study participants.

	T1D $N = 52$		T2D $N = 334$	
	DR $N = 13$ (%)	Non-DR $N = 39$ (%)	DR $N = 112$ (%)	Non-DR $N = 222$ (%)
Physical activity in the last month				
Every day/more times a week	6 (46.1)	26 (66.7)	61 (57.0)	118 (55.9)
Weekly	5 (38.5)	6 (15.4)	17 (15.9)	40 (19.0)
Only once in the last month/inactive	2 (15.4)	7 (17.9)	29 (27.1)	53 (25.1)[a]
Diet				
Yes	13 (100.0)	35 (92.1)	85 (77.3)	175 (81.8)
No	0 (0.0)	3 (7.9)	25 (22.7)	39 (18.2)[b]
Smoking				
Yes	5 (41.7)	6 (16.2)	8 (7.3)	21 (9.8)
Quit	2 (16.6)	8 (21.6)	38 (34.9)	74 (34.4)
Never	5 (41.7)	23 (62.2)	63 (57.8)	120 (55.8)[c]
Alcohol consumption				
Yes	7 (53.8)	11 (28.9)	35 (32.4)	79 (36.6)
No	6 (46.2)	27 (71.1)	73 (67.6)	137 (63.4)[d]

$P < 0.05$. T1D: type 1 diabetes mellitus; T2D: type 2 diabetes mellitus; DR: diabetic retinopathy; Non-DR: nondiabetic retinopathy; N: number. Missing data: (a) 16; (b) 11; (c) 13; (d) 11.

the yearly attendance was below 5%, while more than yearly attendance was absent for all studied groups, and low for T2D patients having HbA1c > 7% (Figure 3).

4. Discussion

DR is the most common late complication of DM in the working-age population and the leading cause of blindness in the elderly, accounting for a significant drop in the quality of life (QoL) and working ability for the patients [5, 14]. In a study comparing data from 35 populations, the global prevalence of sight-threatening retinopathy (STDR) was estimated to 10.2% for all DM patients [6]. Our study found high rates of R2M1 and R3M1, moderate and active proliferative retinopathy (6% and 7% for T1D and T2D, respectively), which is similar to the world average found so far.

A previous study in Hungary found the prevalence rate of DM in participants aged 20-69 years to be 7.47% [15]. More recently, a study from Hungary showed 24.5% of all incident DM cases to be T2D [16]. The same study also showed T1D to be the most common form of DM in children and adolescents, with its frequency having a tendency of continuous rising, while the occurrence of medically treated cases of T2D not to be increasing. The prevalence of T2D, however, is increasing due to an obesity epidemic and aging of the population, hence, one may expect a dramatic increase in DM during the next decades [1, 2, 10]. In the Csongrád County, South-Eastern region of Hungary, the studied cohort showed an approximate 1 : 7 ratio of T1D : T2D cases.

The population in the Csongrád County in Hungary is characterized by significant SES differences, and these appear to reflect upon significant proportion differences, in particular, in the T1D population. It has been previously reported that poorer populations having Medicaid insurance in the U.S. are associated with worse DR follow-up in predominantly rural patients [17]; this population appears to be similar to the rural population in the Csongrád County, Hungary. A statistically significant relationship between

Table 4: Characteristics of the health status of the study participants.

	T1D $N = 52$		T2D $N = 334$	
	DR $N = 13$ (%)	Non-DR $N = 39$ (%)	DR $N = 112$ (%)	Non-DR $N = 222$ (%)
Hypertension	4 (30.8)	21 (55.3)	97 (87.4)	190 (88.4)
Systolic blood pressure (median, IQR, range)	153 (133-162) 120-191	135 (129-150) 120-158	130 (122-140) 105-189	130 (123-140) 100-169
Diastolic blood pressure (mmHg) (median, IQR, range)	84 (80-85) 78-95	80 (70-85) 58-90	80 (75-85) 60-104	80 (70-85) 60-101
Duration of hypertension (year) (median, IQR, range)	18 (3-42) 3-52	11 (7-20) 2-53	20 (10-40) 2-56	20 (10-37) 3-56
Visual acuity				
<0.3	0 (0.0)	0 (0.0)	6 (16.7)	2 (5.5)
≥0.3	3 (100.0)	2 (100.0)	30 (83.3)	38 (95.0)[a]
HbA1c				
Elevated (≥7%)	13 (100.0)	37 (93.4)	88 (82.2)	170 (79.4)
Duration of diabetes (median, IQR, range)	20 (14-24) 10-38	20 (13-27) 1-60	13 (8-20) 0-38	15 (8-20) 0-40
Diabetes medication	5 (41.7)	13 (34.2)	*86 (77.5)*	*187 (86.6)*
Diabetes in the family	6 (46.1)	21 (53.8)	52 (46.8)	124 (56.6)
Diabetic maculopathy	*7 (53.8)*	*2 (5.1)*	*81 (73.6)*	*15 (6.8)*

$P < 0.05$. T1D: type 1 diabetes mellitus; T2D: type 2 diabetes mellitus; DR: diabetic retinopathy; Non-DR: nondiabetic retinopathy; N: number; IQR: interquartile range. Missing data: (a) 305.

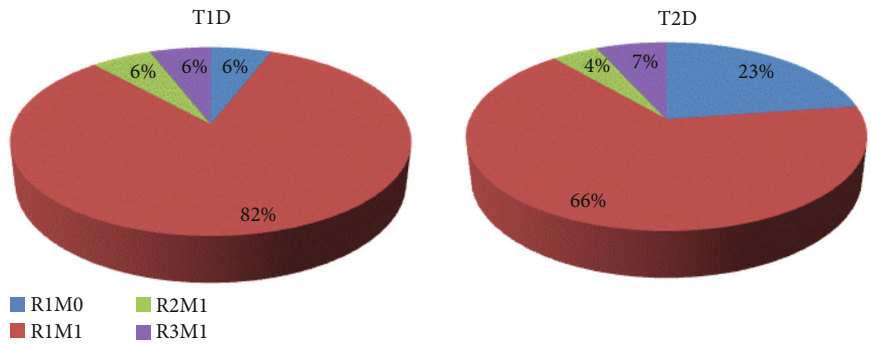

Figure 2: Distribution of the diabetic retinopathy according to the type of diabetes mellitus. DM: diabetes mellitus; T1D and T2D: type 1 and 2 DM.

diabetes complications, age group, educational level, job status, relationship with family members, number of family visits, and the reassurance provided by the family, type of leisure activities, health status, years with diabetes, smoking, type of treatment, fried food consumption and income, sense of security and communication in living environment, and daily intake of vegetables, has also been reported in a study cohort of T2D patients [18]. Furthermore, no statistical interaction could be found between SPHS and gender, while reporting the self-perceived health as poor has been associated with higher reporting of chronic diseases, including diabetes [19].

Although hypertension, VA, HbA1c, duration of DM, and familiar presence of DM showed no significant difference in our study, another study on a population having T2D found a statistically significant difference between SPHS and the levels of HbA1c; the latter study also showed age, level of education, mode of treatment, adherence to treatment, and level of exercise to be factors having statistically significant differences from, and therefore an influence on, self-reported health in a single province in Turkey [20]. Patients with T1D have been shown to have a faster decrease in the perceived health and functioning over time compared to aged persons from the general population [21].

The distribution of the DR showed similar retinopathy with maculopathy (R1M1) presence (82% in the T1D group and 66% in the T2D group) compared to an English study on both DR types (89% had a diagnosis of R1M1 in one eye in those screened positive for maculopathy (M1) in at least one eye) [22]. Our handheld camera produced unassessable fundus image results in nearly half of the participants when used by newly trained image acquisition staff (DJE and DJS); however, in an older population having T2D, this can also be due to the presence of optic axis opacities such as

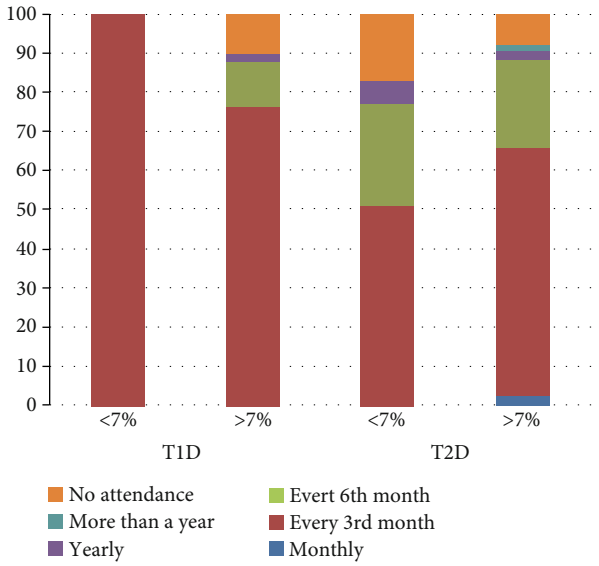

Figure 3: Attendance rate in the diabetology screening among those with normal or elevated HbA1c. T1D: type 1 diabetes mellitus; T2D: type 2 diabetes mellitus. *Data presented are based upon the result of 1 individual in case of the T1D group having HbA1c <7%.

cataract and vitreous hemorrhage. In our study, 6% and 7% of the T1D and T2D population, respectively, had R3M1 (proliferative diabetic retinopathy with maculopathy), while 6% and 4% of the T1D and T2D population, respectively, had R2M1 (preproliferative diabetic retinopathy with maculopathy); therefore, a total of 23% of the population had higher chance for DM-associated cataracts and or vitreous hemorrhages, as well as poor fixation due to macular edema. A limitation of our study is the fact that such changes were not recorded at the time the screening was conducted. Other studies have, however, shown that such handheld cameras can provide comparable results to standard fundus cameras [23]. Later versions of this camera (The Optomed Aurora) appear to have a built-in instant quality feedback software that aids the photographer to gain information when the image is assessable. In the latter study, the two cameras used reached high agreement on the diagnosis of retinopathy and maculopathy at all the levels of retinopathy. Sufficient training of paraprofessional health care staff can lead to obtaining higher quality images with a portable nonmydriatic fundus camera [24]. Known risk factors for developing DR are age, gender, duration and type of DM, elevated HbA_1c, high blood pressure, and retinopathy stage, while other risk factors are being investigated. DR is caused by damage to the retinal microvasculature. Proper screening for DR is an important milestone towards achieving early and efficient treatment for preventing visual loss [9]. For optimal effect, laser treatment must be applied as early as possible after the formation of new pathological retinal vessels, at which time most patients are asymptomatic. In addition, antivascular endothelial growth factor (VEGF) drugs or steroids injected into the vitreous of the eye may reduce diabetic macular edema [25, 26]. Other European countries like Iceland, Denmark, Sweden, and England have successfully implemented nationwide DR screening programs. In Iceland, diabetic blindness prevalence has decreased 4-5 fold after the introduction of systematic DR screening, and a similar success rate has been observed in Denmark [27].

Hungary, at present, has no coordinated national screening program for DR, despite the clear need and high number of patients with DM. Furthermore, in many parts of the country, there are no clear communication channels between GPs, diabetologists, and ophthalmologists regarding screening and sharing results from a DR assessment. Today, a newly diagnosed DM patient must be actively referred for an eye examination by his/her GP or endocrinologist, and often the patient her-/himself must book the appointment. In addition, the interval between eye examinations is at the ophthalmologist's discretion. A standardized rapid assessment of avoidable blindness (RAAB) with the DR module (DRM) has recently been used in Hungary in people aged 50 years and older: 20.0% of the 3523 participants had a known or newly diagnosed DM; 20% of the participants with known DM had a blood glucose level of ≥200 mg/dL; and 27.4% had never had an ophthalmological examination for DR. The prevalence of DR and/or maculopathy was found to be 20.7%, while the prevalence of STDR was 4.3% in one or both eyes among the participants with DM in Hungary [28]. This finding is lower than the one determined in the Csongrád County in Hungary, which can certainly underline disparities in the DR grading standards used or the distributional difference of DR throughout the different counties in the country.

A systematic DR screening in the Csongrád County, South-Eastern region in Hungary, could have significantly reduced the total load of ophthalmologist exams, and thus increase the overall capacity in ophthalmology—a field with vast capacity challenges [19]. More importantly, the lack of systematic DR screening also puts patients with a high risk of eye disease progression at an even higher risk, as they are not receiving the regular follow-up examinations needed. The WHO guidelines for DR screening [5, 14] recommend annual eye examinations for patients with diabetes and biennially for persons with excellent glycemic control and no retinopathy at the previous examination. The International Council for Ophthalmology (ICO) now recommends biennial screening for DM patients without retinopathy. In general, there is a low annual incidence of STR, and 97% of the screening visits do not lead to any active treatment [29]. However, with the increasing prevalence of DM, especially T2D, and limited eye care capacity, advocating for a personalized health care approach towards patient-tailored screening and recommendation for each individual patient has been proposed.

In Iceland for example, a path of improving cost-efficacy of screening systems has been chosen by reducing the number of unnecessary screening visits. Based on a biennial screening model, the following risk variables have been included to improve risk predictions for each individual patient: age, gender, diabetes duration, type of diabetes, HbA_1c level, blood pressure, and retinopathy stage. An European collaborative network has used this model to calculate the most appropriate interval between examinations for each

patient, the outcome of which was a reduction of 17-23% in the screening visits needed, compared to the biennial screening model [29, 30]. A personalized screening approach would have the advantage of recommending more frequent screening intervals to high-risk patients and less frequent to low-risk patients. The risk variable profile also shows significant alterations between different countries and also between different ethnic- and socioeconomic populations within the same country and region, thus, the one-size-fits-all approach may not be the best for diverse populations globally.

In conclusion, this study in the Csongrád County, South-Eastern region, Hungary, determined the prevalence of DM and DR, which appeared to follow the country trend, except for the slightly higher STDR. SES appears to affect the DR rate, in particular, for T1D. The DR screening using the Smartscope Pro Optomed handheld camera, although simple and dynamic, requires much training and experience to achieve proper levels of image assessability if future use in telemedicine or artificial intelligence screening programs or personalized medicine is planned.

Data Availability

Data from this study are available on request through the corresponding author.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

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Review Article

Optical Coherence Tomography (Angiography) Biomarkers in the Assessment and Monitoring of Diabetic Macular Edema

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Retinopathy is one of the most severe diabetes-related complications, and macular edema is the major cause of central vision loss in patients with diabetes mellitus. Significant progress has been made in recent years in optical coherence tomography and angiography technology. At the same time, various parameters have been attributed the role of biomarkers creating the frame for new monitoring and treatment strategies and offering new insights into the pathogenesis of diabetic retinopathy and diabetic macular edema. In this review, we gathered the results of studies that investigated various specific OCT (angiography) parameters in diabetic macular edema, such as central subfoveal thickness (CST), cube average thickness (CAT), cube volume (CV), choroidal thickness (CT), retinal nerve fiber layer (RNFL), retinal thickness at the fovea (RTF), subfoveal choroidal thickness (SFCT), central macular thickness (CMT), choroidal vascularity index (CVI), total macular volume (TMV), central choroid thickness (CCT), photoreceptor outer segment (PROS), perfused capillary density (PCD), foveal avascular zone (FAZ), subfoveal neuroretinal detachment (SND), hyperreflective foci (HF), disorganization of the inner retinal layers (DRIL), ellipsoid zone (EZ), inner segment/outer segment (IS/OS) junctions, vascular density (VD), deep capillary plexus (DCP), and superficial capillary plexus (SCP), in order to provide a synthesis of biomarkers that are currently used for the early diagnosis, assessment, monitoring, and outlining of prognosis.

1. Introduction

Diabetic retinopathy (DR) is the leading cause of blindness in people under 75 years of age in developed countries [1, 2]. Diabetic macular edema (DME) can occur at any stage of DR, being the major cause of central vision loss in patients with diabetes mellitus (DM) [3]. The World Health Organization estimated that by the year 2030, there will be approximately 366 million individuals suffering from DM [4]. Therefore, the study of DME with the aim to prevent vision loss is of utmost importance. The understanding and characterization of DME are essential for its prevention and for the development of new targeted treatments [5].

The transparency of ocular structures and the examining of living retina offer valuable insights into the microvascular changes subsequent to long-term exposure to hyperglycemia in patients with DM [6]. Optical coherence tomography (OCT) provides cross-sectional images of the retinal microstructures being able to measure the retinal thickness (RT) and identify DME before its clinical appearance [5]. Parallel with the development of OCT technology, various parameters have been attributed the role of biomarkers creating the frame for new monitoring and treatment strategies and offering new insights into the pathogenesis of DR. Although the pathogenesis of DME is focused mainly on the breakdown of the inner blood-retina barrier (BRB), improvements in

the visualization of the choroid by enhanced depth imaging-optical coherence tomography (EDI-OCT) and swept source-optical coherence tomography (SS-OCT) set the stage for investigating choroidal biomarkers in patients with DME.

OCT angiography (OCTA) is a noninvasive technique that allows to visualize the retinal plexuses layer by layer, to quantify microvascular parameters, and to correlate them with functional and morphological data [7].

Research is oriented towards identifying earlier preclinical biomarkers of microvascular abnormality in diabetic retina which is very important considering that early treatment is associated with better outcome [6]. Novel preclinical biomarkers could also draw attention on the pathogenesis of DR.

In this narrative review, we gathered the results of studies that investigated specific parameters in DME using OCT and OCTA in order to provide a synthesis of biomarkers that are currently used to assess, monitor, and outline the prognosis of this condition.

2. Development

Currently, OCT is an invaluable and indispensable tool for the monitoring of patients with diabetes, establishes the need for treatment, and formulates prognosis [8].

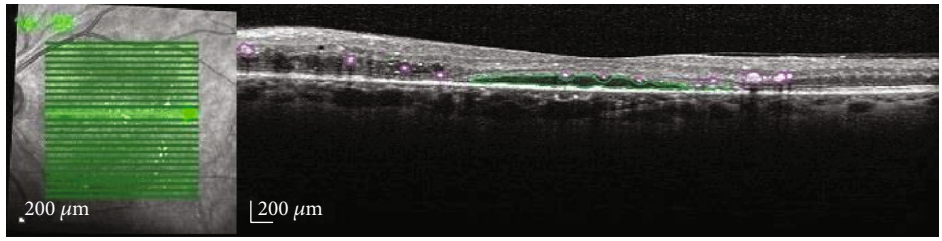
2.1. Macular Thickness and Volume. Diabetic macular edema (DME) is identified by the thickening of the retina as a result of excessive fluid accumulation [5] caused by the breakdown of (BRB) [8]. The fluid may be extracellular, intracellular, or mixed. Santos et al. aimed to characterize the type of retinal edema in the initial stages of retinopathy in patients with type 2 diabetes mellitus (T2DM) [9]. The authors used the classification proposed by Klatzo to characterize the macular edema as cytotoxic (intracellular) or vasogenic (extracellular) [10]. They showed that in the initial stages, the edema was predominantly intracellular, as a result of cytotoxic damage of the Müller cells and of other neuronal cells. As the disease progressed, the breakdown of the BRB predominated with resulting extracellular (vasculogenic) edema [5]. According to their study, macular edema occurred independently of the severity of DR. The authors found that the inner nuclear layer showed a higher and most frequent increase in retinal thickness (RT) [5]. By multimodal imaging of the initial stages of DR, the same authors found that the eyes with DME from different patients included in the same Early Treatment Diabetic Retinopathy Study (ETDRS) grading category displayed different prevalence of the main disease pathways, neurodegeneration, edema, and ischemia [9, 11]. This observation supports the theory of different phenotypes of disease progression.

In 2019, Saxena et al. suggested that three OCT biomarkers proved their validity in DME as diagnostic and predictive factors: mean central subfield thickness (CST), cube average thickness (CAT), and cube volume (CV) [12]. CST is defined as the thickness of a central circle of 1 mm diameter in the circular ETDRS grid map. CAT represents the overall average thickness of the tissue layers between internal limiting membrane and retinal pigmented epithelium (ILM-RPE) over the entire 6×6 mm square scanned area, the mean of thicknesses in nine sections. CV is defined as the overall average volume of the tissue layers between ILM-RPE over the entire 6×6 mm square scanned area. The authors revealed a statistically significant difference in CST, CAT, CV, and logMAR visual acuity between cases with DME and cases without DME, regardless the staging of DR. They concluded that CST, CAT, and CV are independent markers of severity of retinopathy and predictors of visual acuity [12].

When edema overcomes the stretching capability of the retina, bipolar axons are damaged with subsequent disruption of visual signal transmission. As a consequence of these morphological changes, the recovery of visual acuity does not parallel the resolution of edema. Therefore, according to another report, CST is not a reliable biomarker to evaluate the prognostic in patients with DME and the attention must be directed to examining the pattern of edema, its extent, and location relative to the inner and outer retina [8]. Pelosini et al. proved that the cross-sectional area between the retinal plexiform layers is a better predictor of visual acuity than macular thickness [13].

2.2. Subfoveal Neurosensory Detachment. DME can have various aspects on OCT: sponge-like swelling, cystoid macular edema, and subfoveal neuroretinal detachment (SND). The latter one has a reported prevalence of 15–30% in eyes with DME, and it appears on OCT as a hyporeflective area beneath the neuroretina [14] (Figure 1). Various hypotheses have been advanced regarding the pathogenesis of SND. The main mechanism is considered to be the leakage from the retinal or choroidal circulation into the subretinal space exceeding the reabsorption capacity [14]. In diabetic retinopathy, the RPE is altered [15] or its capacity reduced because of local hypoxia [16]. The condition of external limiting membrane (ELM) seems to be important for the pathophysiology of SND. In eyes with DME, there is a breakdown of inner BRB which causes extravasation of lipids and proteins, but as long as ELM is intact, they accumulate anterior to it causing the swelling of the outer retina. When ELM is compromised, proteins and fluid may move through it into the subretinal space, resulting in the development of SND. The study proved that the disruption of ELM correlates with the presence and height of SND in eyes with DME [14]. Vujosevic et al. found that DME with SND correlates with greater choroidal thickness (CT), more hyperreflective foci (HF), disruption of the ELM, and significant impairment of the macular function translated by the decrease of retinal sensitivity (RS) [14]. In SND+ eyes, an inverse correlation was identified between CT and RS, and in SND- eyes, a direct correlation between CT and RS was found, suggesting that DME with SND+ and SND- are two different morphologic and functional entities [14]. Functional impairment in eyes with SND+ and SND- indicates the importance of the choroid for RS [14].

On the other hand, many studies demonstrated the protective effect of SND in the sense that its presence was associated with better visual gains at the end of one year, including in the subgroup of patients with pars plana vitrectomy for diffuse DME [17]. It was also reported that SND at baseline



Pink lines - hyperreflective foci
Green line - subfoveal neurosensory detachment

Figure 1: Macular OCT image revealing SND and HF.

was associated with better response to intravitreal aflibercept [18] and dexamethasone implants [19].

SND is an important OCT biomarker, but its role as an anatomic and functional prognostic factor needs further investigation [8].

2.3. Intraretinal Cystoid Spaces. The formation of intraretinal cysts is the consequence of inner BRB disruption in diabetes as a consequence of elevated VEGF levels [8]. Cystic spaces within the macula (Figure 2) are the expression of coalescent extracellular fluid resulting from the malfunctioning of Müller cells that act like pumps to keep the macula dry [20]. The prognostic significance of intraretinal cystoid spaces depends on their size, location, and association of hyperreflective material. Based on their size, the cysts were classified as small (<100 μm), large (101-200 μm), and giant (>200 μm). The larger size of the cysts is associated with macular ischemia, being poor prognostic factors for visual acuity. Large and giant intraretinal cysts affect the outer nuclear layer (ONL) and damage the IS/OS junction with irreversible loss of the visual function [21]. The hyperreflective material forms septa within the cysts; it is hypothesized to be fibrin and inflammatory by-products and signifies the severe disruption of BRB, being associated with poor outcome of visual acuity following treatment with anti-VEGF agents [20, 22]. Al Faran et al. identified that bridging between the cystic cavities is associated with better functional outcomes following bevacizumab injections as opposed to its absence [23]. The bridging tissue represents residual neuronal material connecting the outer and inner retina with subsequent improvement of transmitting visual impulses to the optic nerve axons. If the bridging process does not occur, the outcome is poor with resulting retinal thinning and atrophy [23].

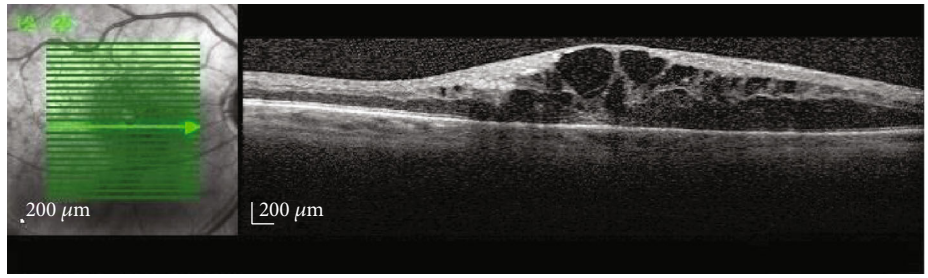
2.4. HF. Vujosevic et al. described three types of HF according to their appearance and location, with various meanings: ≤30 μm diameter, reflectivity similar to nerve fiber layer, absence of back shadowing, and location in the inner and outer retina may be associated with activated microglial cells (Figure 3); >30 μm diameter, reflectivity similar to EPR-Bruch membrane complex, presence of back shadowing, and location in the outer retina may represent hard exudates; and >30 μm diameter, reflectivity similar to EPR-Bruch membrane complex, the presence of back shadowing, and location in the inner retina may represent microaneurysms [24]. Small HF (≤30 microns) are proposed as imaging OCT biomarkers of retinal inflammation in eyes with DME [14]. They were postulated to be fine lipid or protein deposits originating in the breakdown of BRB and anticipating the appearance of hard exudates [25, 26]. According to other theories, HF result from a neurodegenerative process and they precede the development of DR [27]. A significant correlation was found between the number of small HF and the presence of SND supporting the theory of a major inflammatory condition in this pattern of DME [14].

In 2019, Liu and colleagues evaluated the role of OCT in predicting the response to anti-VEGF treatment in DME. They used conbercept (KH902; Chengdu Kanghong Biotech Co., Ltd., Sichuan, China), a new anti-VEGF drug similar to aflibercept, binding to VEGF receptors 1 and 2, which has been demonstrated to be effective in treating DME [28, 29]. When compared to ranibizumab in the treatment of DME, conbercept achieved similar clinical efficacy with longer treatment intervals and fewer intravitreal injections [29]. The authors noticed a reduction in the number of HF on the OCT scans following the administration of conbercept, asserting that HF on OCT scans are reliable biomarkers of individual response to conbercept treatment in patients with DME. A greater number of HF on the OCT scans at baseline demonstrates a more active DME and predicts worse final best-corrected visual acuity following conbercept treatment [3].

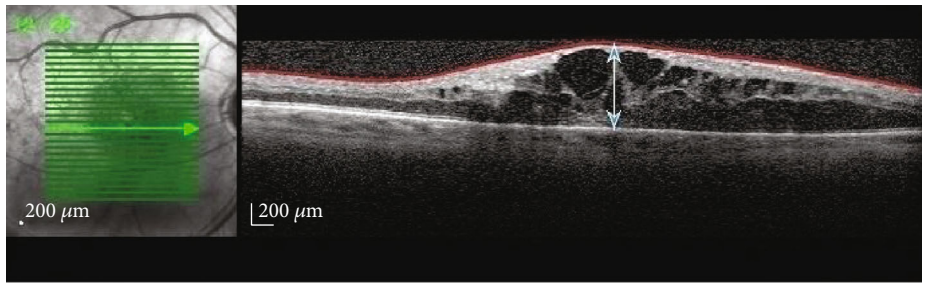
HF larger than 30 μm and with back shadowing, located in the outer retina, are suggestive for hard exudates, meaning lipoprotein deposits due to BRB breakdown. It was proved that they are associated with serum lipid levels and that elevated triglyceride levels are associated with subfoveal location of hard exudates [30]. If they are located subfoveally, intravitreal implants with steroids may be more effective than anti-VEGF agents [31]. The OCT monitoring of hard exudates could be useful in assessing the response to treatment of DME [32].

2.5. Disorganization of the Inner Retinal Layers (DRIL). DRIL is a novel and recently described biomarker which is not specific to DR but develops in multiple retinal diseases as a response to retinal stress [20, 33]. DRIL signifies the poor definition of the boundaries of the inner retinal layers [33] (Figure 4).

Nadri et al. were the first to study the correlation between DRIL, macular thickness parameters, disruption of the ellipsoid zone (EZ), and retinal nerve fiber layer (RNFL)

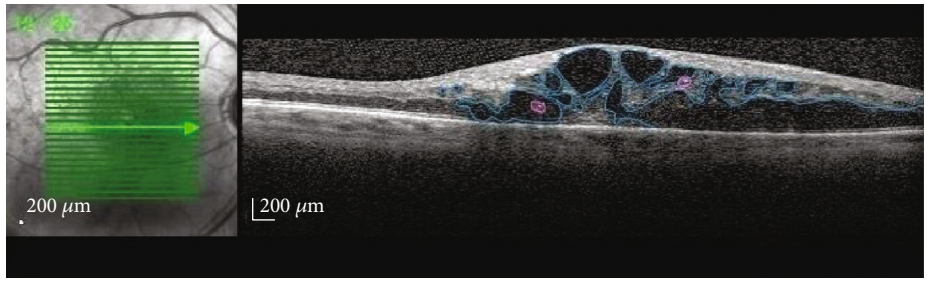


(a)



Red line - retinal surface
Arrows - increased in macular thickness with loww of the foveal pit

(b)



Blue lines - intraretinal cystoid spaces
Pink lines - hyperreflective foci

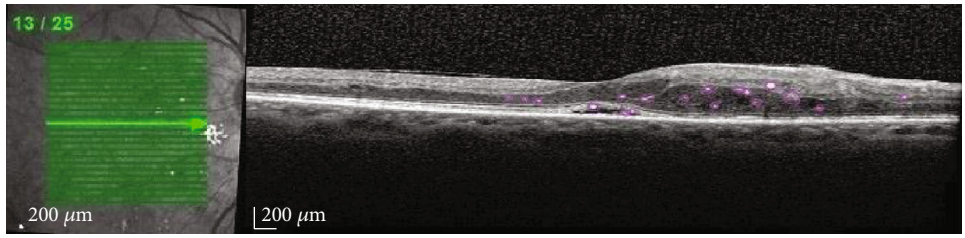
(c)

Figure 2: OCT aspects of the intraretinal cystoid spaces: (a) original OCT image; (b, c) highlighted lesions of the same image.

thickness in DR using Spectral Domain OCT (SD-OCT) [34]. DRIL was graded as 0 (absent) or 1 (present). EZ was graded as intact (grade 0), with focal disruption (grade 1) and with global disruption (grade 2). DRIL was significantly associated with the severity of DR. There was a significant positive correlation between DRIL and CST, CAT, and the grades of EZ disruption and a significant negative correlation between DRIL and RNFL thickness [34].

Das et al. formulated the question of whether retinal morphology evaluated by SD-OCT can be a potential biomarker in eyes with DME [20]. They found that DRIL was identified more frequently in eyes with increasing severity of DR and it was associated with worse outcomes of visual acuity. The authors also noted that for each 100 μm horizontal increase of DRIL, there was a negative impact on visual acuity of more than one line on the ETDRS chart [20]. One possible explanation is given by the mechanical theory according to which bipolar axons snap when their elasticity limit is exceeded by edema, leading to the disorganization of the inner retina [13]. DRIL was significantly associated with disruption of the outer retinal layers (ELM and EZ) and with the increase of the retinal thickness at the fovea (RTF) [20]. When analyzed together, these findings suggest that the same mechanisms are responsible for the disorganization of the inner retina and for the disruption of the outer retina [20]. Currently, it is not known whether the EZ line corresponds histologically to the junction of the inner and outer segments. This study underlines the clinical significance of an intact EZ and suggests that DRIL and the disruption of the outer retina have the same pathogenic mechanisms. Since DRIL correlated significantly with the severity of DR, the authors assume that the finding of worse visual acuities associated with more severe DR may be the consequence of DRIL [20].

Jolitkov et al. set the objective to elucidate the relationship between DRIL and the retinal function in patients with diabetes without DR and with nonproliferative diabetic retinopathy (NPDR) but without DME [33]. DRIL was identified



Pink lines - small hyperreflective foci (<30 microns)

Figure 3: Highlighted OCT image revealing the small HF.

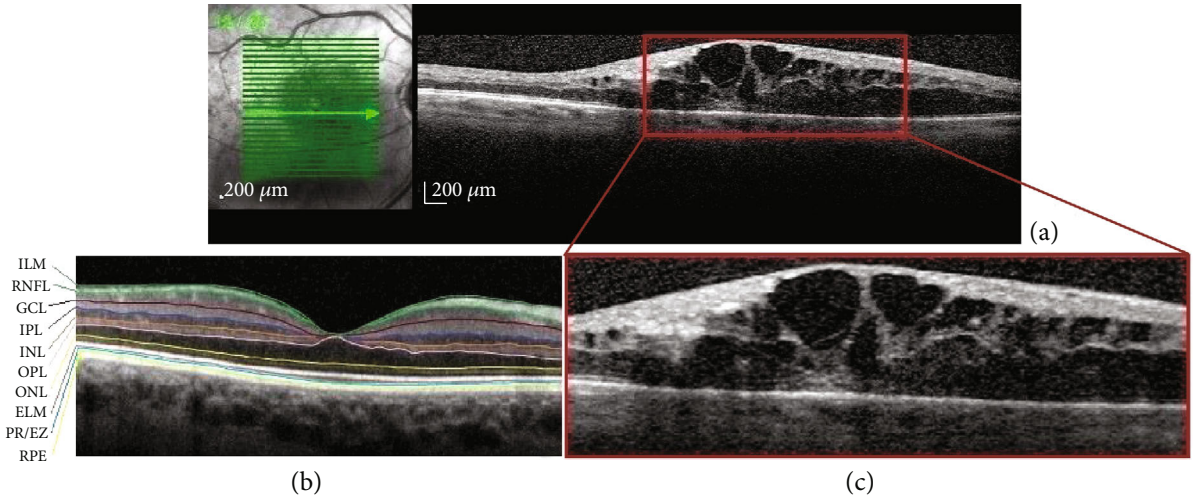


Figure 4: OCT aspect of macular DRIL. (a) Disorganization of the inner retinal layers (DRIL). (b) Normal macular segmentation. (c) Magnified segment of the first image (a); global disruption of the ellipsoid zone and RNFL. ILM: internal limiting membrane; RNFL: retinal nerve fiber; GCL: ganglion cell layer; IPL: inner plexiform layer; INL: inner nuclear layer; OPL: outer plexiform layer; ONL: outer nuclear layer; ELM: external limiting membrane; PR/EZ: photoreceptor layer/ellipsoid zone (inner and outer photoreceptor segment junction); RPE: retinal pigment epithelium.

in SD-OCT scans in 16% of patients with diabetes and in none of the controls. In addition to ETDRS visual acuity testing, the authors used an automated contrast sensitivity method and three visual field testing strategies [33]. DRIL was associated with a measurable degree of retinal dysfunction, even if the neuroretinal impairment was in an early stage [33]. When comparing DRIL with OCT thickness, the authors found that DRIL was associated with retinal thinning mostly in the inner retina but also in the outer retina [33]. The likelihood of DRIL was greater in mild to moderate NPDR as compared to patients with diabetes without retinopathy. The study also found that the patients with DRIL had higher body mass index and longer duration of DM. The findings of this study highlight the correlation between retinal structure and function, and it confers DRIL the status of a reliable and readily available biomarker to monitor the neuroretinal impairment in DM [33].

2.6. Vitreomacular Interface. In patients with diabetes mellitus, often the posterior hyaloid forms a sheet along the posterior pole with the subsequent development of traction forces and macular distortion (Figure 5). The term describing this abnormal vitreomacular relationship is taut posterior hyaloid membrane and is responsible for recalcitrant macular edema. OCT reveals taut posterior hyaloid membrane, identifying the patients with DME who could benefit from pars plana vitrectomy and removal of the posterior hyaloid [35].

2.7. Outer Retina. The OCT imaging of the outer retinal layers offers valuable information on the health of photoreceptors and RPE (Figure 6). Zur et al. described three grades of IS/OS junction aspects: continuous, partly disrupted, and completely disrupted, and concluded that eyes with intact IS/OS junctions have better outcomes following treatment with dexamethasone implant [36]. Ota et al. found that visual acuity is positively correlated with the survival rate of ELM and with the EZ which are affected by long-term DME [37].

Photoreceptor outer segment (PROS) is defined on OCT as the distance between IS/OS junction and RPE. There is evidence that shorter PROS were significantly associated with the presence of DR or DME [38] and with worse visual acuity in patients with DME [39].

2.8. Choroidal Biomarkers. The choroid provides the blood supply to the RPE and photoreceptor cells, playing a major role in the metabolic exchange to the foveal avascular zone

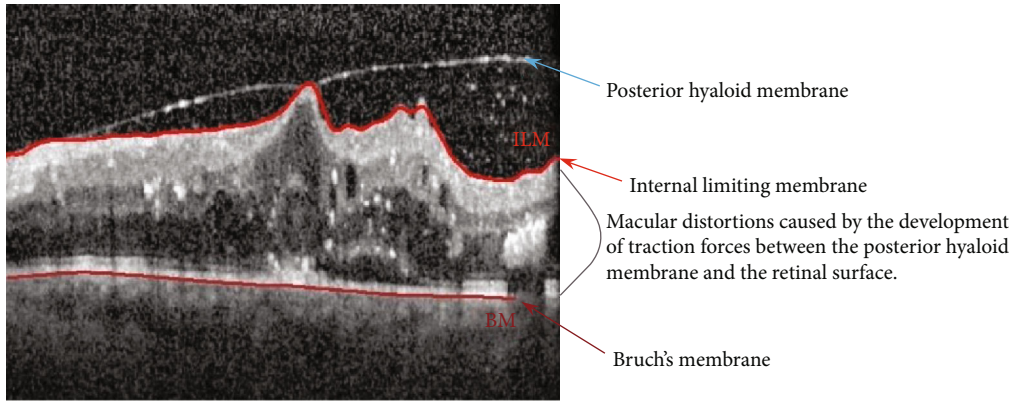
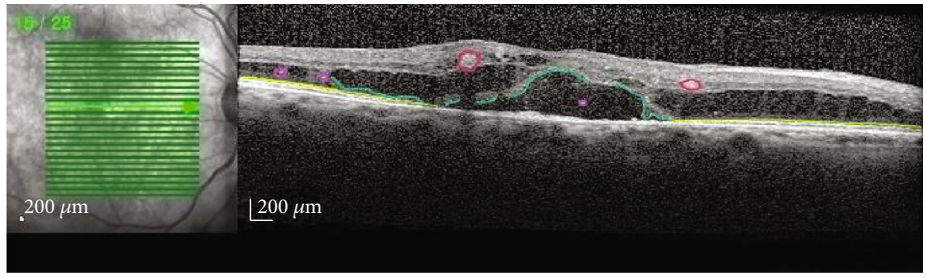


Figure 5: OCT aspects of the vitreomacular interface showing taut posterior hyaloid membrane with subsequent macular distortion.



Pink lines - small hyperreflective foci
Red - hard exudates
Green and blue - disruption of the inner and outer segment of the photoreceptor layer-ellipsoid zone

Figure 6: Highlighted OCT image showing HF, hard exudates, and the disruption of IS/OS photoreceptor segments (EZ).

(FAZ). Endo et al. determined the central choroid thickness (CCT) based on EDI-OCT in patients with treatment naïve DME in comparison to patients with diabetes without DME. CCT layer was significantly thicker in patients with treatment naïve DME as compared to patients without DME [40]. The authors selected untreated DME in order to eliminate the influence of various treatment modalities on CCT. Thus, panretinal laser photocoagulation [41], intravitreal anti-VEGF administration [42], and intravitreal triamcinolone acetonide injection [43] could affect CCT. Other studies found that the central choroid in patients with treatment naïve DME was thinned [44–47], thickened [48, 49], or unchanged [50–52]. The explanations of these conflicting results are various: different inclusion criteria regarding the staging of DR, small number of cases, patient background, and differences between races [40].

A study conducted by Sala-Puigdollers et al. evaluated the reliability of the next generation of OCT devices, the SS-OCT in DME [53]. SS-OCT operates up to 100.000 A-line scans per second and uses a laser source of a longer wavelength (1050 nm) that penetrates deeper in the retina and choroid than the conventional laser sources of the SD-OCT devices. The authors found good reliability, repeatability, and reproducibility of SS-OCT in quantifying retinal and choroidal thickness in DME cases [53]. Moreover, the authors claim that SS-OCT may become the gold standard technique for the evaluation of DME [53]. The studies that investigated the reproducibility of choroidal thickness measurements concluded that there is a low variability of this parameter acquired with SD-OCT and SS-OCT [54].

Abadia et al. compared the choroidal thickness between patients with T2DM and healthy age-matched controls using SS-OCT and found that overall, the patients with T2DM had thinner choroids than the normal controls [54]. All the measurements were performed within the same range of time during the day to avoid fluctuations due to the diurnal variations in choroidal thickness. An interesting observation was that in both groups, the choroid thickness had similar patterns: it was thickest in the subfoveal (SF) area followed by the temporal and nasal zones close to the SF area; the choroid was thinner in the temporal area far from the SF zone and thinnest nasally to the optic disc [54]. According to the same authors, within the group of patients with T2DM, the presence of DME did not influence the choroid thickness. However, the choroid was significantly thinner in patients with DME versus healthy controls, with the most important difference at the SF area. Currently, it is not known whether the thinning of the choroid is prior to the DR lesions or the DR structural changes result in the reduction of the choroidal thickness [54].

The choroidal vascularity index (CVI) is a novel OCT parameter for measuring the vasculature status of the choroid [55]. The CVI is a term introduced by Agrawal et al. and represents the ratio of choroidal luminal area to

total choroidal area [56]. The CVI was recently introduced as a novel biomarker to monitor the progression of DR. Studies proved that while choroidal thickness is unaltered in DR, the CVI correlates with progressing DR [46]. More than that, the CVI is altered before the onset of DR, supporting the theory of choroidal primary damage in DR [8].

Using an EDI SD-OCT device, Gupta et al. evaluated the structural changes of the choroid in eyes with treatment naïve DME and various grades of DR versus healthy controls [57]. Gupta et al. found that the CVI was highest in patients with mild DR and lowest in patients with proliferative diabetic retinopathy (PDR), with a statistically significant difference across the DR severities and the control group. DME did not correlate significantly with the CVI. Subfoveal choroidal thickness (SFCT) increased with the severity of DR, but not in a statistically significant manner. SFCT had a positive significant correlation with the central macular thickness (CMT) and total macular volume (TMV). A negative correlation, although insignificant, was found between SFCT and CVI. This study concludes that the CVI has the potential to be useful in monitoring the progression of DR and DME and offers an additional insight in elucidating the pathogenesis of the disease by tracking the structural changes in the choroid [57].

According to a study conducted by Rayess et al., SFCT is a predictor of response to anti-VEGF therapy [42]. The authors found that a greater SFCT at baseline is associated with better outcomes following anti-VEGF treatment. The possible explanation is that greater choroidal thickness is associated with intact choriocapillaris, less ischemic outer retina, and better preservation of photoreceptors [42].

Hyperreflective choroidal foci (HCF) were described recently, and they represent lipofuscin deposition in the choroidal layers [58]. HCF signal poor prognostic for visual acuity, their number being significantly higher in eyes with PDR versus NPDR [8].

2.9. OCTA Biomarkers. OCTA makes it possible to visualize the retinal vascular plexuses, which is impossible with fluorescein angiography [7].

AttaAllah et al. aimed to evaluate macular perfusion using OCTA automated software algorithms in patients with treatment naïve DME and moderate to severe NPDR [59]. The macular area vascular density (VD) and FAZ were assessed and compared between three groups: diabetic eyes with DME, diabetic eyes without DME, and healthy controls. The authors found that eyes with DME had significantly lower vessel densities at the level of deep capillary plexus (DCP) and FAZ was significantly larger at the level of the superficial capillary plexus (SCP) when compared with diabetic eyes without macular edema and controls. In patients with DME, eyes with larger FAZ had worse visual acuity. The authors conclude that the above-mentioned OCTA biomarkers could be used to predict the evolution of visual acuity and to monitor the response to treatment [59].

Parravano et al. combined OCT and OCTA parameters to investigate the progression of diabetic microaneurisms (MA) and to quantify their effect on the accumulation of retinal extracellular fluid at 1 year follow-up in patients with NPDR [60]. The following MA parameters were evaluated by SD-OCT: the visibility, the changes of internal reflectivity (graded as hyporeflective, moderate, or hyperreflective), and the amount of fluid surrounding each MA. The changes in the visualization of SCP and DCP and the flow in the corresponding OCTA scans were evaluated. The extracellular fluid accumulation at 1 year was strongly associated with the reflectivity pattern of the MAs at baseline, with hyperreflective MAs being significantly associated with an increased risk of fluid accumulation as compared to the hyporeflective ones. The development of extracellular fluid at 1 year was significantly associated with the presence of flow, the visibility, and the deep location of MAs [60]. The authors conclude that OCT and OCTA parameters of MAs predict the retinal extracellular fluid accumulation at one year in patients with NPDR; therefore, a better interpretation of MAs could improve the timing of treatment in DME [60].

Tang et al. focused on the investigation of OCTA parameters related to the DCP in patients with DM included in one of the following categories: without DR, mild DR, moderate DR, or severe DR [7]. Three parameters were calculated: FAZ, vascular density (VD), and fractal dimension (FD). Larger FAZ was associated with more severe DR, shorter axial length (AL), thinner SFCT, and lower body mass index (BMI) [7]. Lower VD was associated with more severe DR, shorter AL, and worse visual acuity. Lower FD was associated with more severe DR and older age [7]. The authors concluded that the effect of ocular and systemic factors should be considered in order to interpret correctly OCT and OCTA parameters [7]. Decreased VD in the DCP was associated with worse visual acuity, suggesting that VD in DCP may reflect the degree of capillary loss in patients with visual deterioration related to DME [7]. DCP supplies 10 to 15% of the oxygen for the photoreceptors. Since DCP is the first affected in DM, OCTA evaluation could predict the evolution of visual acuity at an early stage, facilitating the monitoring and management of patients with DM [7]. The severity of DR was associated with all DCP metrics, but in a multivariable analysis, only the most severe category of DR was related to an increased FAZ due to the high variability of the FAZ itself among even normal individuals. The association of lower SFCT with more advanced stages of DR suggests that choroidal vascular abnormalities occur simultaneously with or as a result of DR [7]. Older age was related to reduced FD, since aging is associated with decreased complexity of organ structure [7]. Vascular structure changes in obesity, including thickened basement membranes, increased vascular diameter, and stiffened arterioles with reduced lumen size explain the OCTA findings associated with increased BMI, namely, increased FD and FAZ in DCP [7]. The increased diameter and thickening in case of increased BMI lead to an increased occupancy of vessels in OCTA images which translate into increased FD and decreased FAZ area. However, the underlying mechanism remains unclear, so these findings should be interpreted with caution.

Rosen et al. brought evidence of preclinical DR by comparing perfused capillary density (PCD) in patients with diabetes against healthy controls using OCTA [6]. The patients with diabetes without retinopathy demonstrated consistently

higher PCD compared to the control group, reaching statistical significance. The NPDR and PDR groups showed progressively decreasing PCD. Regarding FAZ metrics, there was no statistically significant difference between the No DR group and controls [6]. Notably, PCD was more sensitive than FAZ metrics in detecting the differences between the No DR and control groups. Increased PCD values in the No DR group as compared to controls could be explained by autoregulation as a response to increased metabolic demand. The PCD decrease in the NPDR and PDR groups results from the incremental loss of capillary segments [6]. This shift of PCD from elevation to progressive loss marks the key moment of the compensatory response just preceding the appearance of clinical signs [6]. Therefore, the decline of PCD may have the value of a biomarker signaling the risk of visual loss and other systemic complications [6].

In a recent paper, Veiby et al. describe important findings in a cohort of patients with type 1 DM using OCTA [61]. The authors found that lower VD in DCP was the only OCTA factor associated with the progression of NPDR. Since the decrease of VD in DCP occurs before the presence of apparent retinopathy, it could be attributed the role of an early noninvasive biomarker for the progression of DR, being superior to OCT in detecting changes associated with NPDR progression without macular edema. According to the same study, the FAZ area measured by OCTA was not significantly associated with the NPDR level, but it was significantly higher in the severe NPDR group compared to other groups. Based on the results of their study, the authors suggest a new classification system of DR based on OCTA measurements [61].

3. Conclusions

Recent progress in OCT and OCTA imaging techniques led to the identification of new parameters having the potential of biomarkers in DME. The possibility to investigate the choroid with EDI-OCT and SS-OCT paved the way for the discovery of new biomarkers. OCTA makes it possible to investigate noninvasively and individually the retinal vascular layers, to delineate precisely the vascularized from the nonvascularized areas, and to calculate various vascular parameters. Analysis of newly discovered biomarkers and their connection with those already known offered new insights into the pathogenesis, early diagnosis, and monitoring of diabetic retinopathy and diabetic macular edema and opened new avenues of research.

Based on the data presented, in parallel with the widespread use of OCT and OCTA in the clinical practice, future screening of DR should include these examinations for the assessment of DME with subsequent earlier diagnosis and better outcomes.

Conflicts of Interest

The authors declare that there is no conflict of interest regarding the publication of this paper.

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Research Article

Longitudinal Screening for Diabetic Retinopathy in a Nationwide Screening Program: Comparing Deep Learning and Human Graders

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Objective. To evaluate diabetic retinopathy (DR) screening via deep learning (DL) and trained human graders (HG) in a longitudinal cohort, as case spectrum shifts based on treatment referral and new-onset DR. *Methods*. We randomly selected patients with diabetes screened twice, two years apart within a nationwide screening program. The reference standard was established via adjudication by retina specialists. Each patient's color fundus photographs were graded, and a patient was considered as having sight-threatening DR (STDR) if the worse eye had severe nonproliferative DR, proliferative DR, or diabetic macular edema. We compared DR screening via two modalities: DL and HG. For each modality, we simulated treatment referral by excluding patients with detected STDR from the second screening using that modality. *Results*. There were 5,738 patients (12.3% STDR) in the first screening. DL and HG captured different numbers of STDR cases, and after simulated referral and excluding ungradable cases, 4,148 and 4,263 patients remained in the second screening, respectively. The STDR prevalence at the second screening was 5.1% and 6.8% for DL- and HG-based screening, respectively. Along with the prevalence decrease, the sensitivity for both modalities decreased from the first to the second screening (DL: from 95% to 90%, $p = 0.008$; HG: from 74% to 57%, $p < 0.001$). At both the first and second screenings, the rate of false negatives for the DL was a fifth that of HG (0.5-0.6% vs. 2.9-3.2%). *Conclusion*. On 2-year longitudinal follow-up of a DR screening cohort, STDR prevalence decreased for both DL- and HG-based screening. Follow-up screenings in longitudinal DR screening can be more difficult and induce lower sensitivity for both DL and HG, though the false negative rate was substantially lower for DL. Our data may be useful for health-economics analyses of longitudinal screening settings.

1. Introduction

Blindness from diabetes is expected to rise dramatically in this new decade [1]. To reduce diabetes-associated blindness, nationwide systematic screening for diabetic retinopathy (DR) has been implemented [2]. Many countries have studied the development of systematic screening programs [3–6], resulting in several lessons learnt. First, though a large proportion of patients with well-controlled diabetes showed no retinopathy with low risk of visual loss over the years [7], nonattendance in screening programs increased risk of visual loss from sight-threatening DR (STDR) [8]. While annual DR screening is generally recommended [9, 10], studies in some resource-rich countries have found a ceiling uptake of patients [11] which was compromised by an abundance of resource investment [12]. Extending the screening interval from annual to once every 2-3 years was found to be cost-effective in several studies in Europe [13, 14].

Automated retinal disease assessment tools have been studied for DR screening since before the commercial availability of digital retinal photography [15]. Using conventional methods of machine learning, this tool reached a plateau for detecting referable DR with high sensitivity (90%) but less-stellar specificity (45%) [16] in the early 2010s. Deep learning (DL), a subfield of machine learning, has recently demonstrated robust performance with very high sensitivity (95%) and specificity (95%) [17]. Most cross-sectional studies on DL for DR screening have demonstrated this level of performance [17–21]. As a result, DR screening trends have shifted towards the use of DL in assisting or replacing trained human graders (HG) for detecting referrals in DR screening programs [18].

To assess the roles of DL in longitudinal screening for DR, a study on longitudinal performance of DL is important, particularly if the screening was to be repeated in subsequent visits. The continual screening for DR in subsequent years would encounter a shift in the case spectrum since patients correctly detected to have referable DR or STDR would be referred for treatment and exit the screening program. The cohort of patients rescreened in the following years should contain mainly cases that did not display findings of STDR in the previous screenings but might have developed new subtle changes of early STDR in the following screenings. These subtle changes may be more difficult to detect than the more obvious findings associated with well-established STDR.

In this study, we used a real-world, nationwide, longitudinal screening program for DR as a model to assess biennial screening for DR using DL and HG to grade color retinal photographs. The objective was to analyze possible changes in various screening outcomes for detecting STDR determined by DL over two years and compare them with those determined by HG.

2. Methods

This study utilized demographic information, laboratory data, and retinal fundus photographs from patients with diabetes in 13 health regions in the Thai national DR screening program. All data were deidentified. This study was conducted according to the Declaration of Helsinki with approvals from the Institutional Review Board of hospitals where the patients were recruited.

Instituted in 2013 by the Ministry of Public Health, the Thai DR screening program has been implemented in every province and conducted by the Noncommunicable Disease Unit in each Provincial Health Office. All patients with diabetes can access this program without cost thanks to the Universal Coverage insurance scheme provided by the National Health Security Office. Consistent with level 1 evidence suggesting its adequacy, this program employs nonmydriatic, single-field (45-degree, macular-centered) color fundus photography [22] as a screening tool with gradings by trained HG in each region to determine referral to ophthalmologists.

Our study included randomly selected patients in the DR screening program who underwent DR screening twice, two years apart (years 2014 and 2016 or 2015 and 2017). All patients had color retinal photographs of the both eyes taken at each screening. The color retinal photographs were captured by various fundus cameras: Topcon TRC-NW8, Nidek (AFC-210 and AFC-230), and KOWA (Nonmyd α-DIII 8300, Nonmyd 7, VX-10α, Nonmyd α-DIII, Nonmyd WX, VX-20). The diagnosis of DR was based on grading of the retinal photographs. Each photograph was graded for its DR severity level and the presence or absence of diabetic macular edema (DME) according to the International Clinical Classification of DR. The reference standard grades were provided via adjudication by three international retina specialists (from USA, India, and Thailand). As part of the study, we compared gradings from a DL system and HG to this reference standard. The HG were selected from regional DR graders within the national DR screening program. Details of gradings by the retinal specialists, DL, and HG were described previously [19].

Patients were excluded from this study if they had retinal diseases other than DR which precluded diagnosis of DR in either eye, did not have gradings from all three modalities, or if the reference standard, DL, or HG found the images ungradable. Patients were labelled as ungradable if the both eyes were ungradable, or if either eye was ungradable or the fellow eye did not have severe non-proliferative DR (NPDR), proliferative DR (PDR), or DME.

In this study, we studied a simulated setting where each patient was assigned a DR severity level based on the severity of the worse eye. Patients were labelled as STDR if either eye had either DME, severe NPDR or PDR. Those with STDR in the first screening were "referred out" for treatment and excluded from the second screening.

2.1. Statistical Analysis. We estimated the sample size for the first screening of no less than 5,530 patients, considering a margin of error of 10%, type 1 error at 0.05 and type 2 error at 0.2, and an STDR prevalence in Thailand of approximately 6.5% of all patients with diabetes screened for DR [23]. The number of patients included from each of the 13 health regions in the sample was proportional to the number of patients with diabetes in each region [19].

We then computed the prevalence, incidence rate, sensitivity, specificity, positive predictive value, negative predictive value, and accuracy, as well as the number and proportion of true positives, false positives, true negatives, and false negatives. The chi-squared test was used to evaluate statistical significance, with $\alpha = 0.05$.

3. Results

In this retrospective study, we examined 5,738 patients who were screened for DR on two separate occasions, approximately two years apart and simulated scenarios where either the DL or HG screened for STDR. To mimic a realistic scenario, all cases who were indicated for referral by either DL or HG were verified by retina specialists (our reference standard), and only patients with verified STDR were "referred" out of the screening program (Figure 1, additional details below). Patient demographics, including prevalence of DR of different severities and DME at each screening, are shown in Table 1.

3.1. Comparison between DL and HG at the First Screening. At the first screening, prevalence of STDR in both the DL and HG cohorts was 12.3% (704 out of 5,738; the cohorts have yet to diverge based on the screening outcome). The DL arm indicated a greater number of cases than HG as positive for STDR (771 vs. 590, corresponding to 13% and 10% of the cohort), resulting in a substantially higher sensitivity (95% vs. 74%). Specificities of both arms was high at 98-99%. Detailed results for positive predictive value, negative predictive value, and accuracy are presented in Table 2, and the full 2×2 contingency table (also termed "confusion matrix") is presented in Table 3.

3.2. Cohort Changes at the Second Screening. After the first screening, cases indicated as positive by the DL or HG were reviewed by retina specialists, and cases confirmed to have STDR were "referred out." This resulted in different numbers of patients and a different case spectrum presenting for the second screening in the DL and HG arms of the study: 4,148 and 4,263 (72% and 74% of the original 5,738 patients), respectively.

During the intervening period between screenings, 195 patients developed new STDR according to the reference standard, with the majority of these cases arising from patients with moderate NPDR during the first screening (Table 4). Looking across the whole cohort, the rates of STDR were substantially higher with increasing severity of DR at the first screening: 2% for no DR, 9% for mild NPDR, and 25% for moderate NPDR. This trend of increasing 2-year STDR incidence with DR severity was also preserved when stratifying patients based on the DL and HG grades at the first screening.

Despite the approximately 200 new STDR cases, because many true positive STDR cases were referred out (669 for DL and 519 for HG), the prevalence of STDR was substantially lower in the second screening than the first screening (DL arm: 5.1% vs. 12%, $p < 0.001$; HG arm: 6.8% vs. 12.3%, $p < 0.001$).

3.3. Comparison between First and Second Screening for DL and HG. Consistent with the prevalence changes, the rates of positive screens by the DL and HG were both significantly lower in the second screen than in the first (DL: 6.6% vs. 13%, $p < 0.001$; HG: 5.3% vs. 10%, $p < 0.001$). The sensitivity of the DL and HG was also both lower than at their first screening, at 90% (vs. 95%, $p = 0.008$) and 57% (vs. 74%, $p < 0.001$), respectively. For both DL and HG, the specificity remained high at 98-99% without significant changes ($p = 0.742$). The positive predictive value decreased in both arms (DL: from 87% to 69%, $p < 0.001$; HG: from 88 to 74%, $p < 0.001$). Negative predictive value remained at 99% for DL and 96-97% for HG, and accuracy remained at 97-98% for DL and 96% for HG; neither of these trends were statistically significant at the $\alpha = 0.05$ level. Confidence intervals are presented in Table 2.

When examining the full contingency table (Table 3), the fraction of true positives and true negatives differed significantly between the first and second screenings; the fraction of false positives and false negatives was not statistically significantly different. This trend was consistent in both the DL and HG arms.

3.4. Breakdown of STDR into DR and DME. Next, we examined the prevalence of severe NPDR and PDR vs. DME among the STDR cases and among the false negatives (Supplementary Table 1). Of all STDR cases, over 91% were due to DME in the first screening as well as in both arms of the second screening. When examining the false negatives specifically, rates of DME were around 90% for HG. For DL, there were only 35 and 11 false negatives in the first and second screening, respectively; the rates of DME in the two screenings were 94% and 64%, respectively.

A similar breakdown for the non-STDR cases is presented in Supplementary Table 2, showing that among all non-STDR cases, fewer than 7% were moderate NPDR without DME. For the false positive cases specifically, a much greater proportion were moderate NPDR without DME: 65% and 54% for DL and 18% and 20% for HG.

3.5. Performances of DL and HG at the Eye Level. Finally, we explored the STDR detection performance of DL and HG at the eye level (Supplementary Table 3). Similar trends were observed for both DL and HG: sensitivity and positive predictive value for STDR decreased on the second screening compared to the first screening, while specificity, negative predictive value, and accuracy remained similar. The trends for considering DME and severe NPDR/PDR separately were similar.

4. Discussion

Globally, it is estimated that Asia-Pacific accounts for the majority of patients with poor DR-induced visual outcomes, including both blindness (51%, $n = 424{,}400$) and visual impairment (56%, $n = 2.1$ million) [24]. To improve DR-related visual outcomes, several countries have established DR screening programs. In our study, we conducted a

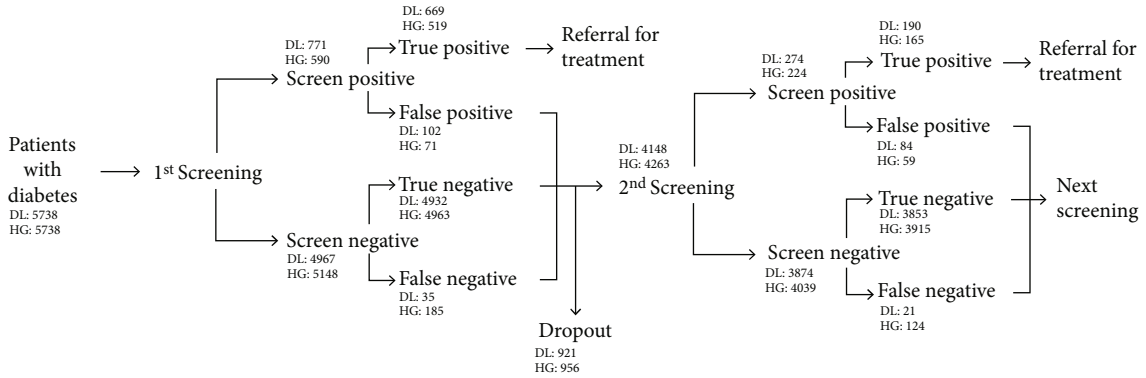

Figure 1: Flow of patients from the first to the second screening. The number of patients in the cohorts of deep learning (DL) and trained human graders (HG) is compared at each point of the screening. The reference standard for these cases was based on an overread by retina specialists (Methods). Screen positive/negative indicates patients whom the DL or HG indicated as positive/negative. In this simulated setting, only patients who were confirmed by retina specialists to have STDR (i.e., true positives) were referred for treatment. The remaining patients were entered into the second screening. Dropout before the second screening included patients with missing data in either DL or HG or determined as ungradable by the reference standard during the second screening.

Table 1: Demographic characteristics of patients with diabetes in the first and second screening, including the prevalence of each diabetic retinopathy severity level and diabetic macular edema.

Characteristics	First screening, DL and HG ($n = 5{,}738$)	Second screening, DL ($n = 4{,}148$)	Second screening, HG ($n = 4{,}263$)
Age, years, mean ± SD	57.27 ± 10.44	56.51 ± 10.52	56.53 ± 10.51
Female, *n* (%)	3,945 (68.8%)	2,874 (69.3%)	2,951 (69.2%)
Hypertension, *n* (%)	3,895 (67.9%)	2,855 (68.8%)	2,921 (68.5%)
FBS, mg/dL in mean ± SD	151.26 ± 52.83	150.48 ± 50.97	150.65 ± 51.23
No NPDR, *n* (%)	4,152 (72.36%)	3,239 (78.09%)	3,256 (76.38%)
Mild NPDR no DME, *n* (%)	589 (10.26%)	448 (10.80%)	449 (10.53%)
Moderate NPDR no DME, *n* (%)	293 (5.11%)	250 (6.03%)	269 (6.31%)
Severe NPDR no DME, *n* (%)	6 (0.10%)	7 (0.17%)	6 (0.14%)
PDR no DME, *n* (%)	47 (0.82%)	11 (0.27%)	17 (0.40%)
DME, *n* (%)	651 (11.35%)	193 (4.65%)	266 (6.24%)

DL: deep learning; HG: trained human graders; FBS: fasting blood sugar; NPDR: nonproliferative diabetic retinopathy; PDR: proliferative diabetic retinopathy; DME: diabetic macular edema. The prevalence of each DR severity level and DME in each cohort was determined by the reference standard.

longitudinal analysis of data from the Thai national DR screening program.

Our DR screening program's endpoint of interest is "STDR" (severe NPDR, PDR or DME [25]). However, we note that other definitions exist (e.g., moderate NPDR or worse [7]), which can hinder comparisons across studies. In our study, the prevalence of STDR during the first screening was 12.3%, which is comparable to the prevalence of STDR estimated from a meta-analysis of 35 studies (10.2%) [26]. As part of a longitudinal analysis, we observed 2-year incident STDR rates of 1.7% and 8.6% among patients without DR and mild DR, respectively, and 3.9% across all non-STDR patients. For comparison, a meta-analysis of 17 studies found that patients without DR and mild DR at baseline had average STDR incidence rates of approximately 1% and 8% per year, respectively [27]. Trends were similar in another study in Asia, where the incidence rate was 1.5% per year in patients without retinopathy at baseline and 13.6% at 4 years [28]. Others have reported a 4-year incidence of 1.45% from no DR at baseline and a rate of 5.02% from all cases (with or without DR) [29].

Given prior work showing that DL can be used to help detect STDR, our study focused on better understanding the longitudinal implications of using DL, as compared to HG. To do so, we followed a single nationwide cohort of more than 5,000 patients across 13 regions. Our data showed that consistent with intuition, referring true positives out of the system decreases the prevalence of STDR in the cohort over time. This decrease happens because the number of true positives was detected with high sensitivity, and their removal presumably leaves behind more difficult examples (false negatives). As the cohort continued to develop STDR, new-onset STDR (i.e., more subtle cases) developed, further enriching the cohort with diagnostically challenging STDR

Table 2: The number of patients with sight-threatening diabetic retinopathy including the screening outcomes in the first and second screening determined by each modality.

Modality	Metric	First screening	Second screening	Difference (%)	p value
DL	No. of patients	5,738	4,148	*n/a*	
	No. STDR (%)	704 (12.27%)	211 (5.09%)	-7.18	<0.001*
	No. graded as STDR (%)	771 (13.44%)	274 (6.61%)	-6.83	<0.001*
	Sensitivity (95% CI)	95.03 (93.42-96.63)	90.05 (86.01-94.09)	-4.98	0.008*
	Specificity (95% CI)	97.97 (97.58-98.36)	97.87 (97.42-98.32)	-0.10	0.742
	PPV (95% CI)	86.77 (84.38-89.16)	69.34 (63.88-74.8)	-17.43	<0.001*
	NPV (95% CI)	99.3 (99.06-99.53)	99.46 (99.23-99.69)	+0.16	0.318
	Accuracy (95% CI)	97.61 (97.22-98.01)	97.47 (96.99-97.95)	-0.14	0.657
HG	No. of patients	5,738	4,263	*n/a*	
	No. STDR (%)	704 (12.27%)	289 (6.78%)	-5.49	<0.001*
	No. graded as STDR (%)	590 (10.28%)	224 (5.25%)	-5.03	<0.001*
	Sensitivity (95% CI)	73.72 (70.47-76.97)	57.09 (51.39-62.8)	-16.63	<0.001*
	Specificity (95% CI)	98.59 (98.26-98.92)	98.52 (98.14-98.89)	-0.07	0.753
	PPV (95% CI)	87.97 (85.34-90.59)	73.66 (67.89-79.43)	-14.31	<0.001*
	NPV (95% CI)	96.41 (95.9-96.91)	96.93 (96.4-97.46)	+0.52	0.169
	Accuracy (95% CI)	95.54 (95-96.07)	95.71 (95.1-96.32)	+0.17	0.681

STDR: sight-threatening diabetic retinopathy; PPV: positive predictive value; NPV: negative predictive value; DL: deep learning; HG: trained human graders; CI: confidence interval. p value was calculated from chi-squared test for the difference between the first and second screening. *p value < 0.05.

Table 3: The number of patients in the first and second screening in each cell of the contingency table (true positive, false positive, true negative, and false negative) for detecting sight threatening diabetic retinopathy by each modality.

Modality	Metric	First screening	Second screening	Difference (%)	p value
DL	No. of patients	5,738	4,148	*n/a*	
	True positives	669 (11.66%)	190 (4.58%)	-7.08%	<0.001*
	False positives	102 (1.78%)	84 (2.03%)	+0.25%	0.3671
	True negatives	4,932 (85.95%)	3,853 (92.89%)	+6.94%	0.001*
	False negatives	35 (0.61%)	21 (0.51%)	-0.1%	0.5139
HG	No. of patients	5,738	4,263	*n*/a	
	True positives	519 (9.04%)	165 (3.87%)	-5.17%	0.001*
	False positives	71 (1.24%)	59 (1.38%)	+0.14%	0.5410
	True negatives	4,963 (86.49%)	3,915 (91.84%)	+5.35%	<0.001*
	False negatives	185 (3.22%)	124 (2.91%)	-0.31%	0.3755

STDR: sight-threatening diabetic retinopathy; DL: deep learning; HG: human graders; p value was calculated from chi-squared test for the difference between the first and second screening. *p value < 0.05.

cases. This enrichment for difficult cases may help explain the decreased sensitivity and positive predictive value of both DL and HG in the second screening.

The degree to which this enrichment happens is dependent on the sensitivity of the screening modality. For example, HG had a lower sensitivity in the first screening, which led to a larger number of false negative cases (185 vs. 35) that entered the second screening, and correspondingly a relative 33% higher STDR prevalence at the second screening (HG: 6.8% vs. DL: 5.1%). Thus, we expect that more accurate DL methods or experienced HG will lead to fewer false negatives but a more rapid increase in case difficulty at follow-up visits.

False negative cases are also concerning because they represent cases missed for treatment referral and are thus at risk of vision loss. While such misses are inevitable, this proportion was relatively small when expressed as a fraction of the entire screening population: 0.5-0.6% for DL and about 3% for HG. In addition, most false negative cases were DME, with generally less than 10% being severe NPDR or PDR in both DL and HG cohorts. The increase in proportion of severe NPDR or PDR in false negatives in the second screening might reflect the limitation of both modalities in being able to detect subtle changes of new severe NPDR or PDR compared to DME. Because "screen-negative" cases (i.e., true

Table 4: The 2-year progression of patients from baseline retinopathy severity levels in the first screening into sight-threatening diabetic retinopathy detected by each modality in the second screening.

Baseline retinopathy levels at the first screening, *n*	Number of patients with STDR in the second screening, per reference standard (%)	DL, number of patients with STDR in the second screening, per reference standard (%)	HG, number of patients with STDR in the second screening, per reference standard (%)
No retinopathy, 4,136	71 (1.72%)	128 (3.09%)	73 (1.76%)
Mild NPDR, 584	50 (8.56%)	57 (9.76%)	41 (7.02%)
Moderate NPDR, 293	74 (25.26%)	97 (33.11%)	57 (19.45%)
Total of all non-STDR severity levels, 5,013	195 (3.89%)	282 (5.63%)	171 (3.41%)

STDR: sight-threatening diabetic retinopathy; NPDR: nonproliferative diabetic retinopathy; DL: deep learning; HG: trained human graders. $p < 0.001$ for the proportions of STDR of patients in the different baseline severity levels from the first screening in each modality.

negatives and false negatives) comprise more than 85% of the cohort, having retina specialists overread all such cases is likely impractical. To help improve the ability to detect more difficult or subtle STDR cases, better DL algorithms or continuing education, monitoring, and audits of HG may be useful. Nonetheless, the particularly low incidence of false negatives by DL (and even then with DME representing the majority) suggests DL-based biennial DR screening can be clinically acceptable.

In contrast to false negatives, decreasing the rate of false positives might improve costs. In our setup, overreads were performed for every "screen-positive" (i.e., true positives and false positives). Reducing the rate of this "over-triggering" can reduce the need for such overreads and help scale DR screening. We anticipate that our detailed data can aid future cost-effectiveness or cost-utility analyses into evaluating DL for DR screening and cost-benefit analysis of overreads vs. unnecessary referrals.

Our study contains some limitations. First, as a retrospective study, our inclusion criteria and desire to study longitudinal outcomes required patients to have retinal photographs in two screenings. Such a cohort may not fully reflect real-world screening settings. Similarly, cohorts do not remain static, but instead, newly diagnosed patients with diabetes enter the screening program on an on-going basis. Though we have not accounted for this, the proportion of new patients with diabetes is expected to be small (estimated at 5% by the National Health Security Office in Thailand). Second, though we expect the trends observed in increasing diagnostic-difficulty and decreasing sensitivity to hold over subsequent screenings (beyond the second), we have not conducted that analysis in this study. Third, the performance of HG may be underestimated because they did not have images from previous screenings available, whereas access to previous images is common practice in real-world settings. Finally, patients with moderate NPDR without DME were included in our biennial screening cohort. Although this group accounted for only 5% of the patients in the first screening, 25% of them progressed to STDR in the second screening. It may be advisable to stratify DR screening patients by their expected risk of developing STDR [27, 30, 31] and initiating biennial screening only for patients in the low-risk group.

The DL used in our study was developed to categorize DR severity and detect DME, and hence, the evaluation of the algorithm's capability to detect other retinal diseases was not possible. The development of DL models that are capable of detecting multiple retinal conditions is an important area of active research. Similarly, the ungradable images in our simulated cohort were "referred" based on our program's standard protocol, with the reason being that many contain cataracts. In this regard, future development of an AI that can more accurately detect DR in the eyes with cataracts may be valuable to reduce the overall referral burden.

5. Conclusion

In a longitudinal follow-up of a biennial DR screening cohort, DL performed well, with higher sensitivities and positive predictive values than HG in both the first and second screening. This was despite a case spectrum shift as STDR cases were referred for treatment, and the remaining false negative cases were joined by new STDR cases, both of which were presumably more subtle and difficult to detect. To reduce unnecessary referrals, further studies on health economics could provide guidance on whether expert overreading is required for all "screen-positive" cases.

Data Availability

The deidentified data underlying this study may be available from DR screening programs of Rajavithi Hospital, Lamphun Hospital, Somdejphrajaotaksin Maharaj Hospital, Sawanpracharak Hospital, Nakhon Nayok Hospital, Photharam Hospital, Prapokklao Hospital, Mahasarakham Hospital, Nongbualamphu Hospital, Pakchong-nana Hospital, Mukdahan Hospital, Suratthani Hospital, Sungaikolok Hospital, and Bangkok Metropolitan Administration Public Health Center 7, but restrictions apply. Researchers interested in collaborating should contact the corresponding author.

Additional Points

Code Availability. Machine learning models were developed in prior work and deployed using standard software libraries and scripts in TensorFlow. Custom deployment code was specific to our computing infrastructure and mainly used for data processing.

Conflicts of Interest

J.S., V.N., K.S., T.S., N.S., V.R. C.R., R.R., AG., and P.R. express no conflicts of interest. M.S., L.P., D.R.W., C.S., J.K., R.S., F.H., and Y.L. are Google employees and receive salary and stock as a part of the standard compensation package. R.T. provides services for Google via Optimum Solutions and expresses no conflict of interest.

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Supplementary Materials

The supplementary information includes tables showing the number of patients with STDR or false negatives classified into severe NPDR/PDR and DME; the number of patients with non-STDR or false positive classified into no/mild NPDR and moderate NPDR without DME; the number of the eyes with STDR, including DME and severe NPDR/PDR, and screening outcomes in the first and second screening determined by each modality. *(Supplementary Materials)*

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Review Article

Recent Developments in Diabetic Retinal Neurodegeneration: A Literature Review

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Neurodegeneration plays a significant role in the complex pathology of diabetic retinopathy. Evidence suggests the onset of neurodegeneration occurs early on in the disease, and so a greater understanding of the process is essential for prompt detection and targeted therapies. Neurodegeneration is a common pathway of assorted processes, including activation of inflammatory pathways, reduction of neuroprotective factors, DNA damage, and apoptosis. Oxidative stress and formation of advanced glycation end products amplify these processes and are elevated in the setting of hyperglycemia, hyperlipidemia, and glucose variability. These key pathophysiologic mechanisms are discussed, as well as diagnostic modalities and novel therapeutic avenues, with an emphasis on recent discoveries. The aim of this article is to highlight the crucial role of neurodegeneration in diabetic retinopathy and to review the molecular basis for this neuronal dysfunction, its diagnostic features, and the progress currently made in relevant therapeutic interventions.

1. Introduction

Diabetic retinopathy is a major cause of preventable vision impairment and blindness worldwide, with increasing prevalence during recent decades [1, 2]. Traditionally, vasculopathy has been considered the primary pathophysiologic mechanism responsible for diabetic retinopathy (DR). However, in recent years, the role of diabetic retinal neurodegeneration (DRN) is increasingly evident and quite possibly supersedes that of vasculopathy as the primary pathogenic event of the disease. Indeed, it has been suggested that DRN is not only a possible biomarker for early development of the vasculopathy that constitutes DR but rather that DRN is in fact a causal factor in the development of DR [3–7]. The term diabetic retinal disease (DRD) is used to integrate the retinal microvasculopathy and retinal neuropathy caused by diabetes [8]. As current focus of medical practice, in terms of early detection and treatment of DRD, lies on the vascular component of DR, new discoveries regarding DRN's significance may lead to a paradigm shift. In this review, we aim to provide a comprehensive and up-to-date overview of the rapidly expanding body of work elucidating DRN's role in DRD and its effect on diagnostics and treatment.

2. Methods

The PubMed and Medline databases were the main resources used to conduct the medical literature search. An extensive search was conducted to identify relevant articles concerning DRN published up to March 31, 2020. Emphasis was placed on recent articles, published since January 1, 2018, but earlier articles were also included if they provided significant information to the understanding of DRN. The following keywords were used in various combinations: diabetic retinal neurodegeneration, neurodegenerative, neurodegeneration, neuroprotective, diabetes, diabetic retinopathy, diabetic retinal disease, diabetic macular edema, and diabetic eye disease. We included original studies and reviews that described

incidence, pathogenesis, imaging, and therapies of retinal neurodegeneration in diabetes. Case reports were excluded. Of the studies retrieved by this method, we reviewed all publications in English and those having English abstracts. Other articles cited in the reference lists of identified publications were considered as a potential source of information. No attempts were made to discover unpublished data.

3. DRN Pathophysiology

3.1. DRN Basic Pathophysiology. Dysfunction of the retinal "neurovascular unit" (NVU) is key in the development of DRN. The term NVU refers to the intricate physical and functional relationship between neurons, glia, and vasculature in the central nervous system. In the retina, it forms the blood-retinal barrier (BRB) and maintains energy homeostasis and neurotransmitter regulation [9, 10]. The retinal NVU is damaged early in the progression of diabetes, as a result of processes of innate immunity, the complement system, and microglia activated by the disease [11]. Such damage is expressed by reduced functional reactivity, which may be detected prior to clinical appearance of DR changes [12–14]. Subsequent impairment in the NVUs leads to breakdown of the BRB and vascular leakage, with manifest retinopathy [9, 15]. The breakdown of the BRB is the culmination of processes governed by the secretion of many factors, among which are vascular endothelial growth factor (VEGF), proinflammatory cytokines (e.g., IL-1β, TNF-α, IL-6, and monocyte chemoattractant protein-1 (MCP-1)), and components of complement. These are variously secreted from RPE, glia, and immune cells [15]. In the late stages of DR, immune privilege is compromised, and the retina is infiltrated by circulating immune cells and serum proteins, further damaging blood vessels and neurons. Furthermore, even after the BRB is repaired, the blood-derived immune stimulators and responders may remain in the neuronal retina [16].

The impairment of the neurosensory retina in diabetes is governed by various mechanisms, which may be classified as inflammatory, metabolic, and genetic/epigenetic. Principal components include imbalance of neurotrophic factors, oxidative stress, and glial reactivity [17, 18]. The latter pertains to the activation and proliferation of astrocytes, Müller cells, and microglia in the diabetic retina, causing secretion of proinflammatory mediators and neurotoxic factors, with subsequent reactive gliosis, diminished retinal neuronal function, and neural-cell apoptosis [19–22]. Early-on in diabetes, changes in astrocytes are observed, such as a decrease in cell number and altered protein expression profile, coincide with inner retinal hypoxia and functional deficits in ganglion cell responses [23]. Müller cells' dysfunction due to chronic hyperglycemia causes them to release a large variety of growth factors and cytokines. This affects vascular dysfunction and angiogenesis but also serves to protect glia cells and retinal neurons [24]. Reactive Müller cells are thought to be initially neuroprotective but consequently may contribute to neuronal degeneration. This is owing to various dysfunctional Müller cell faculties, such as malfunction of glutamate uptake, and expression of nucleoside triphosphate diphosphohydrolase 1 (NTPDase1), enabling extracellular adenosine formation [25]. Microglia, the retinal macrophages, are activated in diabetes due to a complex interplay between hyperglycemia, oxidative stress, leukostasis, and vascular leakage. In turn, microglia increase proliferation and migration and demonstrate transcriptional changes, causing release of various proinflammatory mediators, including cytokines, chemokines, caspases, and glutamate. This results in apoptosis of retinal neurons, consequential thinning of the nerve fiber layer, and eventual visual loss [26–29]. Multifocal electroretinogram (mfERG) is most commonly used in studies to unveil the functional ramifications of DRN, even in patients with no DR or mild nonproliferative DR (NPDR) [30–33].

3.2. Recent Findings in DRN Pathophysiology. Galectin-3 regulates several biological processes, including ones involved in inflammation, oxidative stress, and apoptosis. It has been linked to diabetes' development and identified as a biomarker for prediabetes and diabetes [34, 35]. In streptozotocin-(STZ-) induced diabetic mice, galectin-3 knockout correlated with less macrophage infiltration/proliferation and less activation of astrocytes and microglia in the optic nerve, as well as less retinal ganglion cell (RGC) death and a higher number of myelinated nerve fibers [36]. These findings indicate galectin-3's involvement in stimulation of neuroinflammation and neurodegeneration in the diabetic retina [18].

Serine racemase (SRR) and its product, D-serine, are known to contribute to neurotoxicity, through serine's activity as an endogenous coagonist of the N-methyl-D-aspartate receptor (NMDA-R), a mediator of glutamate excitotoxicity. Previous studies show that increased retinal levels of SRR and D-serine are correlated with DRD [37, 38]. Recently, this link has been further substantiated owing to studies demonstrating an attenuation of retinal neurodegeneration in diabetic mice with SRR deletion or loss-of-function mutation [39, 40].

The stress response protein regulated in development and DNA damage-response 1 (REDD1), known to promote neuronal apoptosis, was previously demonstrated to be overexpressed in response to hyperglycemia in the retina of diabetic rodents [41]. A recent study elucidated the protein's importance in neurodegeneration. It was found that cell death occurred concomitantly with REDD1 overexpression in hyperglycemic conditions in retinal cell cultures, whereas REDD1-deficient cells were not driven to cell death by hyperglycemia. Similar results were exhibited in diabetic mice models, where retinal cell apoptosis, as well as functional deficiencies in visual acuity and contrast sensitivity, were avoided in REDD1-deficient diabetic mice [42].

The microtubule-associated protein tau is a critical mediator of neurotoxicity in neurodegenerative diseases, such as Alzheimer's disease (AD), but has not been previously studied in association with DRN. In a study of high-fat diet-(HFD-) induced diabetes mice models, hyperphosphorylated tau was found to cause vision deficits and synapse loss of RGCs and eventually retinal microvasculopathy and RGCs apoptosis [43].

Several neuroprotective factors were recently established to be associated with DRN: diabetic mice models were found to have reduced levels of αA-crystallin (molecular chaperone,

regulating neuronal cell survival in multiple neurodegenerative conditions) [44], SIRT6 (a NAD-dependent sirtuin deacylase, known to modulates aging, energy metabolism, and neurodegeneration) [45], thioredoxin (antioxidant involved in antiapoptosis and transcriptional regulation) [46, 47], and ciliary neurotrophic factor (CNTF) [48]. Of note, CNTF is known to enhance survival of photoreceptors and RGCs and has broad neuroprotective effects on damaged retinas. Another known neuroprotectant, X-box binding protein 1 (XBP1), was recently studied using a conditional retina-specific knockout mouse line. The study demonstrated that depletion of XBP1 in retinal neurons results in early onset retinal function decline, loss of RGCs and photoreceptors, disrupted photoreceptor ribbon synapses, and Müller cell activation after induction of diabetes [49]. Interestingly, both XBP1 and αA-crystallin are involved in the regulation of the unfolded protein response or endoplasmic reticulum stress response, in which they play key roles to prevent protein aggregation and subsequent cell toxicity and cell death. Recent findings regarding the dysregulation of the L-arginine pathway in plasma samples from type 2 diabetic patients with PDR [50, 51] may lead to novel therapeutic avenues using substances such as arginase 1 for treatment of DRN and other ischemic retinopathies [52].

One factor which stands out in its increasingly recognized importance to neurodegenerative processes is Sigma1 receptor (Sig1R) [53–58]. It is a pluripotent modulator with a number of biological functions, many of which are relevant to retinal disease, including involvement in calcium regulation, modulation of oxidative stress, ion channel regulation, and molecular chaperone activity. Several compelling studies have provided evidence of powerful in vivo neuroprotective effects against ganglion cell loss as well as photoreceptor cell loss [59]. The connection to diabetes was first demonstrated in a study published at 2012, revealing that the damage caused to retinal ganglion cells in mice lacking Sig1R was accelerated by STZ-induced diabetes [60].

It is known that hyperglycemia and HbA1c levels over 7% are major factors predisposing patients with diabetes to vascular complications [61]. Furthermore, recent data indicates that increased glucose variability might contribute to progression of diabetic complications even if HbA1c is in the target range. Importantly, glucose variability, as assessed by continuous glucose monitoring, has been associated with damage to the neuroretina, independently of HbA1c levels, in patients with type 1 diabetes [62, 63]. Of recent, the mechanism by which glucose variability promotes neuroretinal degeneration was shown to involve activation of Müller cells. In a study of rat retinal Müller cells, the impact of glucose variability was analyzed. Cell activation was shown to differ according to basal glucose conditions, as well as subsequent exposure (constant high glucose versus alternating low/high glucose) [64].

4. Recent Developments in DRN Imaging

Retinal neuronal apoptosis occurs early in the disease course, causing a reduction in the thickness of inner retinal layers and of the retinal nerve fiber layer (RNFL), as may be depicted on optical coherence tomography (OCT) [6, 65–67]. In the Maastricht Study, reduced thickness of the pericentral macula was found in as early as prediabetes conditions, when compared to that of people with normal glucose metabolism, and a significant linear trend was established correlating macular thinning with severity of glucose metabolism status [68]. The conclusion that retinal neurodegenerative processes commence prior to initiation of diabetes was later supported by a study demonstrating thinner inner retinal layers and photoreceptor layers in patients with metabolic syndrome [69]. This may suggest that factors such as insulin resistance and adipose tissue-derived inflammation could cause neurodegenerative effects, independently from hyperglycemia.

Diabetes duration was found to be negatively related to the RNFL thickness in type 2 diabetic patients with early stage DR, as were BMI, triglycerides, HDL, HbA1c, and albumin-creatinine ratio [70]. Longitudinal studies of type 1 and type 2 diabetes patients with no DR or mild NPDR revealed progressive thinning of inner retinal layers [71–74]. A study using Cirrus-HD OCT for grading of en face slab OCT images of the innermost retina showed progression of damage over time and with advancing stage of DR [73]. Kim et al. also found baseline macular ganglion cell–inner plexiform layer (mGCIPL) thickness and mGCIPL thinning rate to be independent risk factors of DR progression [71].

The retinal structural alterations described have clinical implications in terms of functional deficiencies such as decreased hue discrimination, contrast sensitivity, delayed dark adaptation, and abnormal visual fields [75, 76]. Such correspondence to visual impairment was recently exemplified using an Optos OCT/SLO/microperimeter, which displayed a correlation between reduced inner and total macular thickness, and reduced microperimetric sensitivity [77].

OCT angiography (OCTA) has been extensively studied in DR, but until recently, no attempts have been made to use this technology to help elucidate the crosslink between neurodegeneration and vascular changes. Hafner et al. found a significant association between the vessel density in the deep capillary plexus of the parafovea on OCTA and the inner retinal layer thickness, mainly ganglion cell layer (GCL) and RNFL [78]. These results indicate that retinal neurodegenerative features are associated with retinal microvascular perfusion. A controlled study of eyes with no DR or mild NPDR discovered a strong positive correlation between loss of mGCIPL and reduction in vessel density from baseline to 24 months. Multivariable regression analysis showed that thinner baseline mGCIPL and greater reduction in mGCIPL thickness were significantly associated with change of vessel density [74].

5. Neuroprotective Therapeutic Avenues

Currently, managing DRD involves stressing the necessity of balancing blood glucose levels and targeting the microvasculopathy that is at the core of DR. Prevention and treatment of the neurodegenerative component of DRD is tragically overlooked, though the insidious loss of neurons is irreversible. The ever-growing research in the field of DRN presents

opportunities to incorporate neuroprotective strategies as adjunct therapies with existing treatments for DR. Potential treatments tend to focus on one of the key players in DRN: neurotrophic factors, inflammation, and oxidative stress, though some putative therapies display mixed mechanisms. Many neuroprotective therapeutic avenues are continuously being investigated in the context of retinal disease, as has been the subject of several reviews [5, 79–84]. Our aim is to shine a light on the most recent studies of therapeutics at the forefront of the battle against DRN (Table 1).

5.1. Anti-inflammatory Substances. Alpha-1-antitrypsin (A1AT) commonly works as an inhibitor of serine proteases. In the context of DRD, it has been described as anti-inflammatory, involved in apoptosis avoidance and extracellular matrix remodeling and also in the protection of vessel walls and capillaries [85]. STZ-induced diabetic mice were systemically treated with A1AT (8 weekly intraperitoneal injections) and displayed a markedly reduced inflammatory status. This was evident by the downregulation of NFκB, iNOS, and TNF-α expression, all normally increased in diabetic models and related inflammation. The treatment caused a decrease in both retinal thinning and loss of ganglion cells, thus ameliorating neurodegenerative changes [86]. In an attempt to elucidate A1AT's mechanism of action on a molecular level, it was later studied in ARPE-19 cells exposed to high glucose. A1AT normalized the levels of NFκB and its targets iNOS and TNF-α, as well as regulated proteins related to glucose metabolism, awakened signals related to epithelial-mesenchymal transition, and normalized protein levels involved in essential RPE function [87].

Citicoline is an endogenous compound known to act as a neuroprotective agent and has been shown to be effective in the treatment of glaucoma [88]. Topical administration of citicoline in liposomal formulation in the db/db mouse model (a model for obesity-induced type 2 diabetes) prevented glial activation and neural apoptosis in the diabetic retina. In vivo, citicoline was able to ameliorate the functional abnormalities recorded on ERG in the diabetic mice. The main mechanism implicated was the inhibition of the downregulation of synaptophysin induced by diabetes and the prevention of upregulation of NFκB and TNF-α [89].

In a retrospective study of patients with diabetic macular edema treated with intravitreal fluocinolone acetonide, neuroretinal analysis of OCT was obtained at 3-month intervals before and after treatment. In the region located 1.5 mm to 3.0 mm from the fovea, there was a statistically significant decrease in the posttreatment rate of DRN (defined as change over time of the inner neuroretinal thickness), compared with the pretreatment rate [90]. Prospective, controlled trials are necessary to further validate this effect.

5.2. Antioxidants. A PPARα agonist used to treat dyslipidemia, fenofibrate, was found by major studies to have unprecedented therapeutic effects in DR [91–93], though the mechanism for this has not been previously elucidated (and could conceivably be caused by its lipid-normalizing effect). A later study of an experimental mouse model of type 2 diabetes indicated that neuroprotection is one of the underlying mechanisms by which fenofibrate exerts its beneficial actions in DRD [94]. Recently, in a rat model of type 1 diabetes, activation of PPARα decreased retinal cell death and had a robust protective effect on retinal function. The study revealed a neuroprotective effect of PPARα through improved mitochondrial function and subsequent alleviation of energetic deficits, oxidative stress, and mitochondrially mediated apoptosis [95]. As such, PPARα is a promising drug target, and since then, new classes of PPARα agonists were studied for proof-of-concept in vivo efficacy and preliminary pharmacokinetic assessment [96].

As previously mentioned, hyperphosphorylated tau was found to participate in DRN in a study of diabetic mice. This hyperphosphorylation, known to be induced by oxidative stress, was shown to result from an activation of glycogen synthase kinase 3β (GSK3β). Therapeutically, intravitreal administration of an short interfering RNA (siRNA) targeting tau or a specific inhibitor of GSK3β attenuated tau hyperphosphorylation and caused a reversion of RGC-synapse loss and restoration of visual function [43]. In a separate study, topical ocular application of ginsenoside Rg1 was shown to alleviate tau hyperphosphorylation and consequent synaptic neurodegeneration of RGCs in diabetic mice [97]. Notoginsenoside R1 was also found to have numerous mitigating effects in DRD, as oral treatment to diabetic mice caused dramatic alleviation of retinal vascular degeneration, of reduced retinal thickness, and of impaired retinal function [98]. Ginsenoside Rg1 and notoginsenoside R1 are two of the saponins extracted from the traditional Chinese medical herb *Panax notoginseng*. They are known to possess antioxidant and anti-inflammatory qualities, with resulting antidiabetic effects, also studied in the context of diabetic retinopathy [99, 100].

Extensive literature is available regarding Spermine oxidase (SMOX), a mediator of polyamine oxidation, and its role in neurodegenerative diseases in general and in neuroretinal damage specifically. SMOX inhibitors have been found to limit oxidative stress and reduce retinal neurodegeneration from various etiologies [101]. Recently, STZ-induced diabetic mice were systemically treated with MDL 72527-a SMOX inhibitor. Compared with placebo-treated diabetic mice, the treated mice displayed significantly improved ERG responses, inhibition of retinal thinning, and attenuation on RGC damage and of neurodegeneration [102].

Similarly, several other substances have been investigated for their antioxidative effect in DRN. Diabetic mice treated intravitreally with caffeic acid alkyl amide derivatives (CAF6 or CAF12), intraperitoneally with the amino acid taurine, or orally with the leucine analogue gabapentin, the acrolein-scavenging drug, 2-HDP, or the pigment astaxanthin, all exhibited reduction of oxidative stress and of neurodegenerative damage [103–107].

5.3. Neurotrophins and Other Neuroprotective Factors. Somatostatin (SST) is an endogenous neuroprotective peptide that is downregulated in the diabetic retina. SST downregulation is related to glial activation and neuron apoptosis, the two hallmarks of retinal neurodegeneration [108]. SST was one of the first reported topical experimental drugs

Table 1: Novel therapeutic approaches to diabetic retinal neurodegeneration in experimental studies.

Anti-inflammatory substances	Antioxidants	Neurotrophins and other neuroprotective factors	Mixed or unknown mechanisms
Alpha-1-antitrypsin	Fenofibrate and other PPARα agonists	Somatostatin	Flavonoids and other nutraceuticals
Citicoline	Inhibitors of protein tau hyperphosphorylation: ginsenoside Rg1 Notoginsenoside R1 siRNA	Ciliary neurotrophic factor	Angiotensin II type 1 receptor blockers
Fluocinolone acetonide	Spermine oxidase inhibitors	Sigma1 receptor	Angiotensin-converting enzyme 2 activators
	Caffeic acid alkyl amide derivatives	Synthetic microneurotrophin BNN27	GLP-1 receptor agonists
	Taurine	Brain-derived neurotrophic factor	Lamivudine
	Gabapentin	Eicosapentaenoic acid ethyl ester	Endothelin-1 receptor antagonists
	Acrolein-scavenging 2-HDP	Intravitreal bone marrow CD133+ stem cells transplantation	Intraocular pressure-lowering agents
	Astaxanthin		

to exert a neuroprotective effect [108]. In a randomized, placebo-controlled, phases II–III trial by the EUROCONDOR consortium, topical administration of SST and brimonidine was useful in arresting the progression of neurodegeneration in early DR with preexisting retinal neuro-dysfunction [109]. Amato et al. used the SST analog octreotide bound to magnetic nanoparticles and revealed it may be used as an octreotide intraocular delivery system, ensuring localization to the retina and enhanced bioactivity [110].

Ciliary neurotrophic factor (CNTF) is a member of the IL6 family of cytokines, and it supports the differentiation and survival of neurons. CNTF is also known to enhance survival of retinal photoreceptors and RGCs. CNTF delivered by encapsulated cell intraocular implants is approved for treatment of retinitis pigmentosa and of geographic atrophy [111]. In a recent study of STZ-induced diabetic rats, intravitreal injections of CNTF rescued RGCs and dopaminergic amacrine cells from neurodegeneration [48].

The accumulation of evidence regarding Sig1R's role in retinal neurodegeneration led to studies exploring its potential as a novel treatment target. Ligands for Sig1R, such as (+)-pentazocine [(+)-PTZ], were found confer marked retinal neuroprotection in vivo and in vitro [59]. In murine models of diabetic retinopathy, administration of intraperitoneal injections of (+)-PTZ resulted in significant neuroprotection, reduced evidence of oxidative stress, and preserved retinal architecture [112, 113].

The synthetic microneurotrophin BNN27 is a BBB- and BRB-permeable dehydroepiandrosterone (DHEA) derivative. It was injected intraperitoneally to STZ-induced diabetic rats and reversed the diabetes-induced glial activation and reduction of amacrine cells in a dose-dependent manner. Treatment was also found to target the inflammatory component of the disease, as it reduced proinflammatory and increased anti-inflammatory cytokine levels [114]. The neuroprotective effect to the diabetic retina was maintained with topical administration of BNN27 [115].

It has long been known that levels of brain-derived neurotrophic factor (BDNF) are reduced in DRN and that intraocular administration of BDNF counteracts diabetes-related neurodegenerative processes [116]. Of late, oral intake of eicosapentaenoic acid ethyl ester (EPA-E) was shown to ameliorate BDNF reduction and improve functional results on ERG in DRD. An EPA metabolite, 18-HEPE, induced BDNF upregulation in Müller glia cells and recovery of ERG results [117]. In a separate study, bone marrow CD133+ stem cells were intravitreally transplanted into STZ-induced diabetic mice and caused retinal BDNF levels to increase, with consequent retinal cell survival [118].

5.4. Mixed or Unknown Mechanisms

5.4.1. Nutritional Supplements and Nutraceuticals. A variety of nutraceuticals have been studied, both in vitro and in vivo, and found to have a significant antioxidant and anti-inflammatory effect, at times reducing both the neural and vascular damage typical of DR.

Flavonoids are bioactive compounds found largely in dietary plants, aiding in the plants' protection from ultraviolet radiation, oxidants, and pathogens [119]. A high-flavonoid diet was found to be associated with lower levels of diabetic markers and reduced the prevalence of DR by 30% [120], and green tea was found to be neuroprotective in DR [121]. Over the years, a number of experimental studies showed that dietary flavonoids, such as quercetin [122], rutin [123], naringenin [124], and others, cause a reduction in oxidative stress and ameliorate inflammation and apoptosis in DRN, as was recently extensively reviewed [125, 126].

Nonflavonoid polyphenols, such as curcumin and resveratrol, have been shown to exert antiapoptotic effects on the retina of diabetic rat models, with attenuation of retinal thinning, among other neuroprotective influences. Both these substances have been reported to inhibit apoptosis by stimulating autophagy [127–130].

A variety of studies have examined the possible protective role of Müller cell-autophagy in DR [131, 132]. Inhibition of autophagy increased retinal cell apoptosis induced by high glucose [133], whereas its activation could protect

Müller cells from high glucose-induced apoptosis [134]. Epigallocatechin-3-gallate (EGCG) is a major polyphenol in green tea that has attracted attention as a potential therapy for various pathologies, including apoptosis of retinal neurons [135, 136]. In a recent study, retinal Müller cells in high glucose conditions treated with EGCG showed activation of autophagy machinery and reestablishment of cargo degradation, which protected the cells from apoptosis. EGCG could increase the ability of cells to proliferate by increasing autophagy. In a mouse model of diabetic retinopathy, EGCG treatment reduced the reactive gliosis of Müller cells and decreased retinal damage [137].

As mentioned previously, the antioxidative effect of notoginsenoside R1 [98] and the neurotrophic effect of EPA-A [117, 118] are also the subject of recent research, as are other nutraceuticals and nutritional supplements [138–140].

5.4.2. Therapeutic Targets of the Renin-Angiotensin System (RAS) in DRN. The role of RAS in the development and progression of DRD is well established [141–144]. In recent years, research shed more light in regard to the relationship between RAS and the neurovascular unit [145]. Some researchers explored the therapeutic feasibility of RAS-related substances for DRN prevention or control. Retinal explants treated with angiotensin II demonstrated a 40% reduction in RGC survival, compared with vehicle [146]. Treatment of STZ-induced diabetic rats with telmisartan, an angiotensin II type 1 receptor blocker, caused elevated levels of neurotrophic factors in the sera and retinas compared with untreated rats, as well as an increase of endogenous antioxidant glutathione content and decreased signs of apoptosis in diabetic retina [147]. Treatment of STZ-induced diabetic rats with an angiotensin-converting enzyme 2 (ACE2) activator significantly reduced the apoptotic cell death of RGCs compared with untreated diabetic rats [148]. Verma et al. used engineered probiotic species as live vector for oral delivery of human ACE2 with enhanced tissue bioavailability, blocking RGC loss in two mouse models of diabetic retinopathy, while also reducing retinal inflammatory cytokine expression and the number of acellular capillaries [149].

5.4.3. Additional Novel Therapeutic Pathways. One of the more promising therapeutic agents investigated is glucagon-like peptide-1 (GLP-1) and GLP-1 receptor agonist (GLP-1RA) liraglutide. Liraglutide is currently used to treat type 2 diabetes and is known to have neuroprotective effects. It was previously reported that topical administration of GLP-1 or GLP-1RAs prevented DRN and early vascular leakage in early treatment of diabetic mice (treated at the age of 10 weeks, before retinal abnormalities are detected) [150, 151]. The same group went on to prove that the treatment with topical GLP-1 at a later stage (24 weeks) could revert the retinal neurodegeneration induced by long-term diabetes. The treatment generated anti-inflammatory effects, anti-apoptotic effects, anti-VEGF, and even neuroregenerative ones [152]. Liraglutide also incurs antiendoplasmic reticulum stress and-oxidative stress effects, in its protective action against DRN [153], as well as reversal of hyperphosphorylated tau-triggered RGCs synaptic degeneration [154].

Another drug to show encouraging results is lamivudine (3TC), a newly discovered Purinergic Receptor P2X 7 (P2rx7) inhibitor. P2rx7 is upregulated in diabetes and its inhibition via oral treatment of lamivudine reversed retinal neuronal, as well as vascular damage, incurred by diabetes. This was evident as neuroglial function on ERG was maintained, the number of GABAergic amacrine cells was improved, and the formation of acellular capillaries in the retina was prevented [155].

It has been suggested that endothelin 1 (ET-1) is involved in the development of diabetic retinal microvasculopathy [156]. Endothelin B-receptors activation mediates retinal neurodegeneration, but this was not previously proved to occur in diabetes. Recently, it was found that upregulation of ET-1 and its receptors is an early event in the diabetic retina. Topical administration of bosentan (a dual endothelin receptor antagonist) in diabetic (db/db) mice was shown to result in a significant decrease of reactive gliosis and apoptosis. Anti-inflammatory, as well as anti-VEGF mechanisms, was implicated in bosentan's auspicious effects [157].

Lastly, as allegations of an association between diabetes and glaucoma, on fluctuations in intraocular pressure (IOP), continue to arise [158], the role of serial IOP changes in DRN was investigated in STZ-induced diabetic rats. Diabetic rats exhibited higher fluctuations of IOP than normal controls or diabetic rats treated with brinzolamide and latanoprost ophthalmic solutions. IOP-lowering drugs reduced RGC-apoptosis and were suggested to decrease intermittent mechanical stress, glial activation, axoplasmic flow, and eventually neurodegeneration [159].

6. Discussion

Recent progress has provided increasing evidence of the importance of DRN in progression of DR and DRD. Therefore, improved understanding of its mechanisms, as well as novel approaches for diagnosis and treatment, is needed.

Several new molecular biomarkers of DRN have been identified recently in experimental models of diabetes. These are proteins involved in inflammation, oxidative stress, apoptosis, cell survival, endoplasmic reticulum stress response, aging, and other cell processes (Figure 1). These novel biomarkers of DRN include galectin-3 [18, 34–36] serine racemase (SRR) and its product, D-serine [37–40], REDD1 [41, 42], hyperphosphorylated tau [43], αA-crystallin [44], SIRT6 [45], thioredoxin [46, 47], CNT [48], and XBP1 [49]. They might become promising targets for timely diagnosis and treatment of DRN in the future.

Among potentially modifiable clinical factors associated with DRN, glucose variability has emerged as an important contributor to DRD, even in the presence of HbA1c levels corresponding to "well controlled diabetes" [62–64].

Diagnostic approaches of DRN include several modalities. Application of OCT has been shown to be effective in demonstrating structural changes in DRN, such as thinning of the retinal layers [6, 65–74]. For demonstration of functional deficiencies characteristic of DRN, an Optos

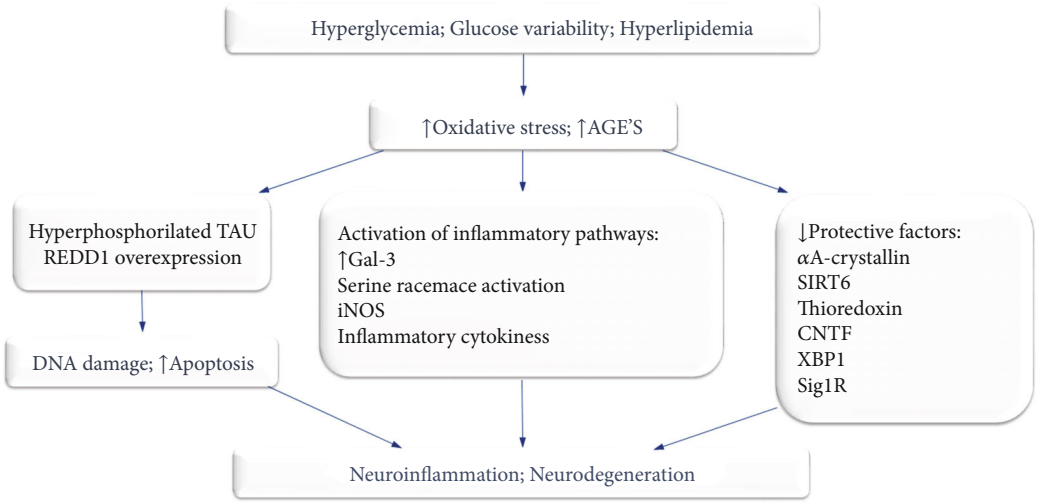

Figure 1: Key mechanisms and recent discoveries in diabetic retinal neurodegeneration.

OCT/SLO/microperimeter has been applied [77]. Finally, OCTA has been used in studies to evaluate a crosslink between neurodegeneration and vascular changes in retina [74, 78].

Several novel therapeutic approaches of DRN have been applied recently. Promising results have been obtained in experimental models of DRN with anti-inflammatory agents, such as alpha-1-antitrypsin (A1AT) [85–87], citicoline [88, 89], epigallocatechin-3-gallate (EGCG) [135–137]. Intravitreal fluocinolone acetonide, used to treat patients with diabetic macular edema, achieved a positive effect on the inhibition of DRN [90]. Of the antioxidants, fenofibrate and PPARα activation demonstrated neuroprotective effects on DRD, in both clinical and experimental studies, and results of studies with novel PPARα agonists in retinal diseases are forthcoming [91–96]. Several novel compounds targeting protein tau hyperphosphorylation are under investigation in experimental studies [43, 97, 98]. Other compounds with antioxidative actions under investigation in DRN include SMOX inhibitors [101, 102], caffeic acid alkyl amide derivatives, taurine, gabapentin, and others [103–107].

Of the neurotrophic and neuroprotective substances, CNTF [48, 111], Sig1R [54, 59, 112, 113], and synthetic microneurotrophin BNN27 [114, 115], were shown to have neuroprotective properties and ability to reduce neurodegeneration in diabetic animals. Several studies have targeted BDNF reduction via intraocular BDNF administration [116], oral intake of eicosapentaenoic acid ethyl ester [116], and intravitreal bone marrow CD133+ stem cells transplantation, leading to improved retinal cell survival [118]. Topical administration of SST was tested in a randomized, placebo-controlled, phases II–III trial and could inhibit the neurodegenerative process in early DR with preexisting retinal neuro-dysfunction [109].

Among substances with unknown or mixed mechanisms, flavonoids and other nutritional supplements show great promise [125–129, 137–140]. Substances that alter the RAS have been extensively studied, with exciting new prospects [147–149]. Other promising agents with neuroprotective effects in animal models of DRN include GLP-1RA liraglutide [150–154], lamivudine (P2rx7 inhibitor) [155], endothelin-1 receptor antagonists [156, 157], and agents lowering intraocular pressure [158, 159].

To conclude, an impressive body of evidence is accumulating regarding DRN's role in the ocular damage caused by diabetes. Disruptions of glucose and lipid status generate oxidative stress and increase formation of advanced glycation end products, setting in motion several pathologic processes, including amplification of inflammatory pathways, impediment of neuroprotective pathways, and induction of DNA damage and apoptosis. As information regarding these cellular and molecular mechanisms is revealed and as diagnostic modalities evolve, expeditious detection of DRN is made feasible. Promising results have been attained with various substances, including antioxidants, neuroprotective factors, and anti-inflammatory substances, in an attempt to attenuate DRN. Further studies are needed to facilitate clinical implementation of novel options for timely diagnosis, prevention, and treatment of this pivotal component of DRD.

The long-awaited implementation of this therapeutic approach cannot materialize without robust animal models of DR, accurately mimicking the human disease. Translating experimental success from animals to humans is often hurdled by conceptual and methodological challenges, such as the timing of the therapeutic intervention, participant follow-up, disease heterogeneity, effective drug delivery, and selecting reproducible and clinically important trial endpoints. In addition, major efforts should be devoted to standardizing methods for screening and monitoring of neurodegeneration, to ensure uniformity across studies. It is essential that a set of guidelines is established for such experimental and clinical studies. Lastly, combination therapies of DRN merit further research, as many of these approaches have potentially complimentary mechanisms, which may produce synergistic effects, thereby improving the overall neuroprotective effect.

While there is still a way to go, taking into account the volume of information accumulated thus far, the question is no longer "Will treatment of DRD include DRN-targeted

therapies?", the question now is "How and when?". A multidisciplinary collaborative effort is required in order to address this issue and offer hope that functional vision may be sustained throughout the lives of the diabetic patients.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

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Research Article

Associations between Macular OCT Angiography and Nonproliferative Diabetic Retinopathy in Young Patients with Type 1 Diabetes Mellitus

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Background/Objective. Optical coherence tomography angiography (OCTA) is increasingly used to supplement ophthalmoscopy in the diagnosis and follow-up of diabetic retinopathy. Our objective was to confirm if OCTA parameters can predict the development of nonproliferative diabetic retinopathy (NPDR) and to clarify if any single OCTA parameter is associated with NPDR independently of well-known risk factors in young type 1 diabetes (T1D) patients. *Methods*. OCTA of both eyes was performed in a cross-sectional study of 14 to 30-year-old individuals with at least 10-year duration of T1D and controls recruited from the Norwegian Atherosclerosis and Childhood Diabetes (ACD) study. Vessel density (VD) and foveal avascular zone (FAZ) area in the superficial and deep capillary plexus (SCP and DCP), total retinal volume (TRV), and central macular thickness (CMT) were calculated using automated software. Univariate and multivariate ordered logistic regression (OLR) models were used accordingly. *Results*. We included 168 control eyes and 315 T1D eyes. Lower VD in DCP (OR 0.65, 95% CI 0.51–0.83), longer diabetes duration (OR 1.51, 95% CI 1.22–1.87), and higher waist circumference (OR 1.08, 95% CI 1.02–1.14) were significantly associated with progression of NPDR. VD in SCP and DCP were significantly lower in T1D patients without diabetic retinopathy than in controls. *Conclusions*. Sparser VD in DCP is significantly associated with severity of NPDR, supporting that OCTA might detect the earliest signs of NPDR before it is visible by ophthalmoscopy.

1. Introduction

Diabetic retinopathy (DR) is the most common microvascular complication of diabetes mellitus (DM) and the leading cause of blindness in the working population of developed countries across the world. DR is asymptomatic in its early stages, and by the time visual impairment is detected, chronic and progressive pathology has already developed in the retinal microvasculature. Adolescents have a higher risk of progressing to sight-threatening retinopathy compared to adults with type 1 diabetes (T1D) and the progression may be rapid [1, 2]. Well-established risk factors for DR are poor glycemic control and longer diabetes duration. Other debated risk factors are older age, puberty, high blood pressure (BP), concomitant nephropathy, male sex, smoking, high body mass index (BMI), dyslipidemia, and celiac disease [2, 3].

Diabetic macular edema (DME) and proliferative diabetic retinopathy (PDR) are the two advanced stages of

diabetic retinopathy that are the main causes of visual loss in patients with diabetes mellitus. Diabetic macular ischemia (DMI) in the absence of DME and PDR is a less commonly recognized cause of visual loss. DMI is characterized by retinal capillary loss and enlargement of the foveal avascular zone (FAZ). The understanding of the natural pathology, risk factors, and functional outcomes of DMI is limited. This has partly been because of the need for fluorescein angiography (FA) to diagnose it. With the advent of OCTA, which enables detailed depth-resolved visualization of the 3 retinal capillary plexuses (superficial, intermediate, and deep) to be evaluated independently, without the need for dye injection, the interest in studying DMI has been reignited. With widefield OCTA, this has become even more useful and will likely replace FA in the future. OCTA can measure, among others, macular vessel density and FAZ area, but it still remains unclear whether these OCTA parameters have significant functional and prognostic implications [4]. Early microvascular changes in DR such as microaneurisms, capillary dropouts such as decreased vessel density (VD), and foveal avascular zone (FAZ) enlargement are not visible by ophthalmoscopy at the early stages but can be detected by optical coherence tomography angiography (OCTA) [5]. OCTA uses the principle of "motion contrast" for the detection of blood flow and generates high-resolution cross-sectional images of the human retina in a noninvasive and reliable manner. Considering that >90% of vision loss cases can be prevented with early accurate staging and classification of DR [6, 7], OCTA plays an ever-increasing role in the diagnosis of DR and the assessment of treating options [4, 8].

To date, most studies on OCTA and DR have included adults with type 2 diabetes (T2D) or a mix of T1D and T2D [5, 9–20]. A few have focused on only T1D in adults [21, 22]. Generally, these studies have found that eyes with DR have lower VD in the SCP and/or DCP and larger FAZ than normal eyes. DR is uncommon before puberty, and there are only a few OCTA studies on children with T1D. Some of them have found no differences in macular OCTA parameters between T1D without DR and controls [23, 24], while others have found children with T1D without DR to have significantly lower VD in the DCP and larger FAZ than controls [25–27]. Puberty significantly increases the risk of DM complications; hence, adolescence is the time when efforts should be directed to screening for early signs of DR and modifiable risk factors [2]; therefore, patients aged 15-30 years with T1D with at least 10 years of diabetes duration are a very important age group. Currently, there is scarce data regarding early macular vascular changes diagnosed by OCTA in adolescents and young adults with T1D [28].

The prospective Atherosclerosis and Childhood Diabetes (ACD) study was designed to detect early atherosclerosis in young individuals with T1D by comparing them to sex- and age-matched controls. At the 10-year follow-up, the study evaluated DR by ophthalmoscopy and OCTA. In this cross-sectional part of the study, we aim to confirm whether any detectable OCTA changes exist before DR is visible for the clinician and if OCTA parameters can predict the development of NPDR in 14 to 30-year-old individuals with at least 10-year duration of T1D. We also performed this study to evaluate which of the numerous OCTA parameters have the highest diagnostic and prognostic value for OCTA to be useful in clinical practice. We also aimed to find out if any single OCTA parameters are associated with NPDR independently of traditional risk factors.

2. Materials and Methods

2.1. Study Design, Population, Eligibility Criteria, and Ethics. The individuals included in the present cross-sectional ophthalmological study performed between 2017 and 2019 were from the Norwegian Atherosclerosis and Childhood Diabetes (ACD) study, an ongoing prospective population-based study, initiated in 2006, with follow-up every 5 years. At baseline, 314 individuals with childhood-onset T1D and 120 controls aged 8-18 years were enrolled. At the 5-year follow-up, additional 15 new T1D patients and 15 new controls in the age-group 8-18 years were enrolled. The T1D patients were all on modern intensive insulin treatment with insulin pumps or basal-bolus regimens with insulin pens (≥4 daily injections), very few being also on other medication of importance, but none of significant consequence for the data. The details of the study inclusion process and examinations have been described elsewhere [29–31]. At the 10-year follow-up, all individuals enrolled in the ACD study were invited to participate in the present ophthalmological study at the Department of Ophthalmology, Oslo University Hospital; of them, 189 T1D patients and 96 controls were willing and eligible to participate [31]. Exclusion criteria were as follows: current or recent (<3 months) pregnancy, any history of ocular disease including proliferative diabetic retinopathy (PDR) and clinically significant diabetic macular edema (CDME), ocular trauma, retinal laser treatment, intravitreal injection, ocular surgery, high ametropia (spherical equivalent (SE) > ±6 D), and poor OCTA image quality. Approval for all study-specific procedures was obtained by the appropriate Regional Committee for Medical and Health Research Ethics. The described research adhered to the tenets of the Declaration of Helsinki. Written informed consent was obtained from all individuals and their parents in the case of youngsters below the age of 18.

2.2. Clinical and Ophthalmological Examinations. All individuals were examined according to a study protocol [29] that included diastolic (DBP) and systolic blood pressure (SBP), height, weight, waist circumference, fasting blood samples, and urine samples. Mean arterial blood pressure (MAP) was calculated as DBP+1/3 (SBP–DBP). All individuals completed a questionnaire on medical history, family history of eye disease, iris color, and medication. They underwent a routine ophthalmological examination including refraction and best-corrected visual acuity (BCVA) (Early Treatment Diabetic Retinopathy Study, ETDRS, LogMAR) at 100 LUX (Hagner Model EC1), intraocular pressure (IOP) measured with Icare tonometer (ic100, Icare, Vantaa, Finland) followed by dilation of the pupils using tropicamide 1% eye drops, only supplemented with phenylephrine 10% when needed. Mean ocular perfusion pressure (MOPP) was calculated as 2/3 (MAP–IOP) [32]. Slit-lamp examination

with ophthalmoscopy, OCTA, and fundus photography of the macula and optic disc were performed after dilation. The grade of retinopathy was classified according to the International Clinical Diabetic Retinopathy (ICDR) classification system [33], and the patients with T1D were allocated into four groups: (1) T1D with no apparent NPDR (NDR), (2) mild NPDR, (3) moderate NPDR, and (4) severe NPDR. The study only comprised of individuals with nonproliferative diabetic retinopathy (NPDR) without CDME. Both eyes were included in the analyses.

2.3. OCTA Image Acquisition and Analysis. OCTA images were obtained by RS-3000 Advance AngioScan (NIDEK CO., LTD., version 1.7.0.4, Gamagori, Japan), a spectral-domain OCTA using a custom 3 × 3 mm acquisition protocol centered in the fovea. The different OCTA parameters were automatically computed by the built-in Navis-EX 1.7 software. The area of the FAZ was manually outlined in two vascular layers, SCP and DCP, and was expressed in square millimeters (mm^2) by the software (Figure 1). The VD was analyzed in two vascular layers, the SCP and DCP, between the inner limiting membrane (ILM) and the retinal pigment epithelium (RPE) from the enface OCTA (Figure 2). The SCP consists of capillaries between the ILM and the inner plexiform layer (IPL)/inner nuclear layer (INL)+8 μm. The DCP consists of capillaries in the inner nuclear layer between IPL/INL+13 μm and IPL/INL+88 μm. The Navis-EX software automatically computed VD, total retinal volume (TRV), and average central macular thickness (CMT) from the OCTA tomograms. The VD was expressed in mm^2 and converted to percentage of the surface that is occupied by capillaries per area of the entire scan (9 mm^2). We did not exclude the FAZ area when calculating the VD. TRV (mm^3) was measured within a central 6 mm diameter circle, and CMT (μm) was measured within a central 1 mm diameter circle. CDME was defined according to ETDRS as retinal thickening at or within 500 μm of the macular center, hard exudates at or within 500 μm of the macular center with adjacent retinal thickening, or one or more disc diameters of retinal thickening, part of which is within one disc diameter of the macular center (ETDRS study report number 1, no authors listed, [34]).

2.4. OCTA Quality Control. Two independent readers (NCBBV and NS) carefully evaluated each OCTA scan before the quantitative analysis. The readers were blinded to all patient characteristics. OCTA with poor image quality (SSI < 6/10) and significant image artefacts (motion lines, blurry images, and poor centration) were excluded. We also excluded those eyes that did not have all OCTA parameters measured, to avoid missing parameters.

2.5. Statistics and Data Analysis. Clinical characteristics are presented as means with standard deviations (SD), number (n) with percentages (%). Quantile-quantile (Q-Q) plots were used to check all continuous variables for normality. FAZ area in SCP and DCP was not normally distributed. We used Pearson correlation for normally distributed variables and Spearman correlation for not-normally distributed variables. We checked for multicollinearity among all the covariates with a correlation coefficient of 0.7 as a cutoff. An independent sample t-test was used to test for differences in mean OCTA parameters between NDR patients and controls. One-way ANOVA was used to test for differences in mean OCTA parameters between the four NPDR subgroups, and Tukey analysis was used as post hoc pairwise comparison after one-way ANOVA. In order to test which clinical and OCTA parameters were predictive of the NPDR level, we built an ordered logistic regression (OLR) model. The outcome retained four ordinal levels: no DR, mild DR, moderate DR, and severe DR. Robust standard errors were calculated while clustering on a patient level, to adjust for intraindividual correlation (since both eyes of each individual were included). For model building, we first conducted a univariable OLR analysis for each variable: gender, age, duration of diabetes, MAP, BMI, waist circumference, HbA1c, serum glucose, hemoglobin, total cholesterol, HDL cholesterol, LDL cholesterol, triglycerides, urine albumin-creatinine ratio, SE, BCVA, and IOP. All variables with a $p < 0.05$ were subsequently included in the multivariable model, to control for potential confounders. The final model was built from the multivariable model through a step-down approach. Odds ratios (OR) were reported with 95% confidence intervals (CI). FAZ in DCP and SCP, BCVA, and hemoglobin were scaled by their standard deviation to deal with convergence problems. To investigate OCTA parameters in NDR patients vs. controls, a generalized estimating equation (GEE) analysis was applied to adjust for intraindividual correlation (since both eyes of each individual were included) using the same modelling approach as described above. All statistics were performed on STATA (version 15, StataCorp LLC, TX, USA). A p value of <0.05 was considered statistically significant.

3. Results

3.1. Demographic and Clinical Characteristics. We examined both eyes of 285 individuals: 189 with T1D and 96 controls. After exclusion criteria were applied, 254 individuals (166 with T1D and 88 controls) and 483 eyes (315 with T1D and 168 controls) were considered suitable for analysis. Reasons for exclusion were poor OCTA image quality ($n = 28$ eyes) and poor fixation ($n = 8$ eyes), CDME ($n = 5$ eyes), PDR ($n = 4$ eyes), and spherical equivalent (SE) > 6 diopters ($n = 2$ eyes); 40 eyes could not have their OCT taken because the OCT NIDEK machine was out of order on the examination day (24 eyes with T1D without DR, 2 with moderate NPDR, and 14 control eyes).

Clinical characteristics of the patients with T1D and controls are presented in Table 1. The mean duration of T1D was 15.7 ± 3.8 years in all T1D patients. These patients had higher MAP, BMI, waist circumference, fasting blood glucose, HbA1c, and IOP and lower best-corrected visual acuity and were more myopic than the controls. Age, diabetes duration, BMI, and waist circumference increased with the increasing level of NPDR (Table 1).

3.2. Descriptive Analysis of OCT Parameters. Mean values of vascular and structural outcomes of OCTA are shown in

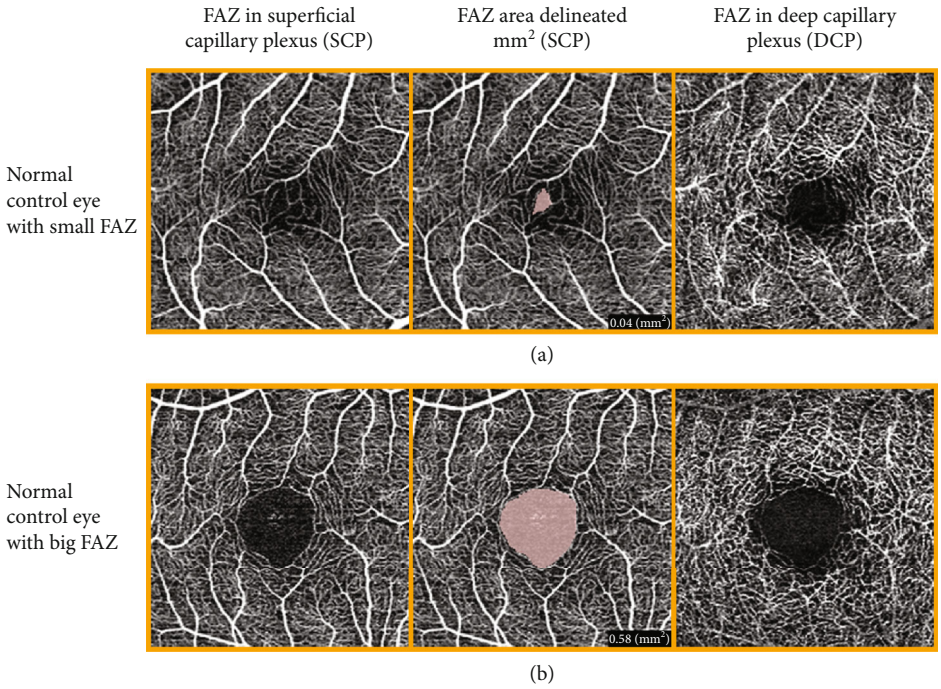

Figure 1: OCTA scans from two control eyes. This illustrates how the FAZ area is delineated and how different the size and shape can be in normal eyes. (a) The FAZ is 0.04 mm^2 in the SCP, and crossing capillaries in the FAZ area makes it difficult to decide where to measure. (b) The FAZ is 0.58 mm^2 in the SCP.

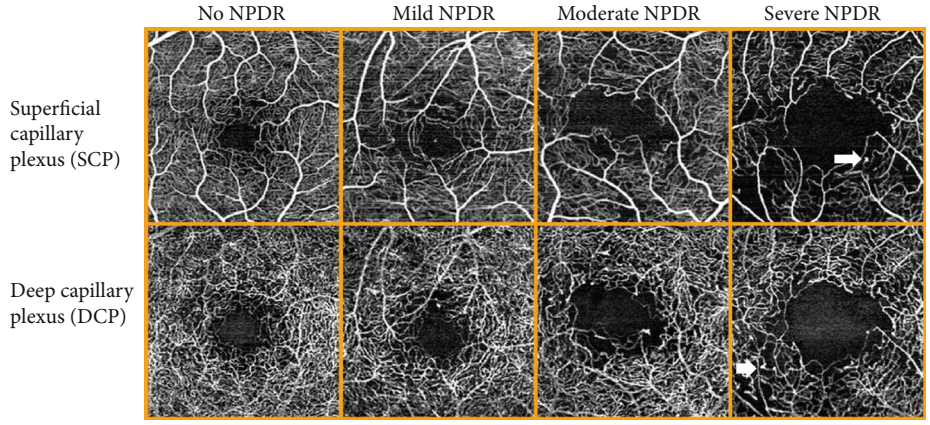

Figure 2: Representative 3 × 3 mm macular OCTA scans of the SCP and DCP for each ICDR level of NPDR in T1D patients. It is visible that the FAZ area increases and the vessel density decreases due to capillary dropout with the increasing level of NPDR. There are also some visible microaneurisms (arrows).

Table 2. After ICDR grading, there were 239 eyes with no DR (NDR) and 58 eyes with mild, 15 eyes with moderate, and 3 eyes with severe NPDR in the T1D group. None of the controls had retinopathy. There was a large interindividual variation in the FAZ area. VD and FAZ area were higher in the DCP than in the SCP in all groups. Figure 1 shows an example of a small and a large FAZ in controls, while Figure 2 shows representative OCTA scans of SCP and DCP in NDR and mild, moderate, and severe NPDR in patients from this study.

No significant difference was found in the FAZ area in neither SCP ($p = 0.140$) nor DCP ($p = 0.063$) when comparing the NDR patients ($n = 239$ eyes) with the controls ($n = 168$ eyes). The FAZ area in both capillary plexuses showed no increase from NDR to moderate NPDR but was significantly higher in the severe NPDR group compared to the other groups ($p < 0.001$, Table 2).

VD in the DCP was significantly lower in the NDR patients than in controls ($p < 0.001$), and it decreased significantly with increasing grade of NPDR ($p < 0.001$, Figure 3).

VD in the SCP, TRV, and CMT were significantly lower in NDR patients than in controls, but they did not change significantly with the increasing level of NPDR (Table 2).

3.3. Correlations between Right and Left Eyes. OCTA parameters in right and left eyes were highly correlated: CMT

Table 1: Demographic and clinical characteristics of study patients ($n = 254$ individuals).

			ICDR level in patients with type 1 diabetes					
	Controls ($n = 88$)	All T1D ($n = 166$)	NDR ($n = 113$)	Mild NPDR ($n = 40$)	Moderate NPDR ($n = 11$)	Severe NPDR ($n = 2$)	$p^{\ddagger}$	p^{*}
Gender male/female (male %)	41/47 (46.6%)	68/98 (41%)	48/65 (42.5%)	17/23 (42.5%)	3/8 (27.3%)	0/2 (0%)		
Age (years)	23.9 ± 3.4	24.3 ± 3.3	23.5 ± 3.4	25.3 ± 2.2	27.1 ± 1.9	27.6 ± 0.9	<0.001	0.471
Age onset of diabetes (years)		8.6 ± 3.4	8.8 ± 3.4	8.6 ± 3.1	7.3 ± 4.1	4.7 ± 2.5	0.222	
Duration of diabetes (years)		15.7 ± 3.8	14.8 ± 3.5	16.7 ± 3.3	19.8 ± 4.2	23.0 ± 1.7	<0.001	
Mean arterial blood pressure (mmHg)	85.9 ± 8.0	89.6 ± 8.0	89.1 ± 8.7	89.9 ± 5.5	93.0 ± 7.2	89.5 ± 0.23	0.473	0.001
Body mass index	23.6 ± 3.1	25.7 ± 4.5	25.2 ± 3.6	25.2 ± 4.8	31.2 ± 7.8	28.8 ± 0.02	<0.001	<0.001
Waist circumference (cm)	80.5 ± 10.0	85.2 ± 12.3	83.2 ± 9.7	86.3 ± 12.6	99.3 ± 22.7	98.7 ± 8.9	<0.001	0.002
Serum glucose (mmol/L)	4.8 ± 0.4	10.0 ± 4.3	9.9 ± 4.3	10.1 ± 4.1	9.9 ± 4.6	14.8 ± 5.8	0.462	<0.001
HbA1C (mmol/mol)	32.2 ± 2.9	64.9 ± 15.5	64.1 ± 15.3	65.3 ± 10.8	70.3 ± 27.1	78.1 ± 24.7	0.357	<0.001
Hemoglobin (g/dL)	14.4 ± 1.2	14.5 ± 1.2	14.4 ± 1.2	14.5 ± 1.3	15.2 ± 1.0	14.4 ± 0.0	0.590	0.535
Total cholesterol (mmol/L)	4.4 ± 0.7	4.5 ± 0.9	4.5 ± 0.9	4.3 ± 0.8	4.8 ± 1.0	4.6 ± 1.5	0.405	0.289
HDL cholesterol (mmol/L)	1.6 ± 0.4	1.6 ± 0.4	1.6 ± 0.4	1.5 ± 0.4	1.6 ± 0.6	1.8 ± 0.01	0.776	0.516
LDL cholesterol (mmol/L)	2.7 ± 0.7	2.8 ± 0.7	2.8 ± 0.7	2.6 ± 0.7	3.0 ± 0.7	2.6 ± 1.5	0.419	0.275
Triglycerides (mmol/L)	0.87 ± 0.4	1.01 ± 0.8	0.97 ± 0.9	1.04 ± 0.7	1.38 ± 0.9	0.85 ± 0.1	0.474	0.080
U-albumin-creatinine ratio (mg/mmol)	3.7 ± 17.0	1.0 ± 2.2	1.0 ± 2.4	1.0 ± 1.4	1.6 ± 1.6	1.4 ± 0.8	0.831	0.154
BCVA LogMAR right eye	−0.07 ± 0.07	−0.05 ± 0.07	−0.05 ± 0.07	−0.06 ± 0.06	−0.03 ± 0.11	0.00 ± 0.00	0.406	0.019
BCVA LogMAR left eye	−0.07 ± 0.08	−0.05 ± 0.07	−0.06 ± 0.07	−0.04 ± 0.07	−0.05 ± 0.09	−0.03 ± 0.06	0.420	0.014
IOP right eye (mmHg)	14.5 ± 2.7	16.1 ± 3.2	16.1 ± 3.4	16.5 ± 2.8	15.4 ± 3.8	17.7 ± 0.6	0.635	<0.001
IOP left eye (mmHg)	14.1 ± 3.0	15.9 ± 3.4	15.7 ± 3.5	16.2 ± 2.9	16.1 ± 4.3	17.7 ± 1.5	0.595	<0.001
Spherical equivalent, refraction right eye (diopters)	−0.37 ± 1.44	−1.03 ± 1.57	−0.98 ± 1.69	−1.12 ± 1.14	−1.39 ± 1.73	0.00 ± 0.71	0.651	0.001
Spherical equivalent, refraction left eye (diopters)	−0.33 ± 1.50	−0.98 ± 1.48	−0.92 ± 1.55	−1.07 ± 1.17	−1.41 ± 1.83	−0.13 ± 0.18	0.592	0.001
MOPP (mmHg)	42.8 ± 5.4	43.6 ± 5.8	43.4 ± 6.5	43.4 ± 3.9	46.7 ± 5.2	42.2 ± 0.5	0.341	0.316

Values are mean ± SD. $^{*}p$ value: independent sample t-test for difference between all T1D and controls. $^{\ddagger}p$ value: global one-way ANOVA analysis for the difference between the NPDR subgroups.

($r = 0.92$, $p < 0.001$), TRV ($r = 0.90$, $p < 0.001$), VD in SCP ($r = 0.66$, $p < 0.001$), VD in DCP ($r = 0.77$, $p < 0.001$), FAZ area in SCP (0.80, $p < 0.001$), and FAZ area in DCP (0.78, $p < 0.001$) in patients with T1D.

3.4. OCTA Parameters in T1D without DR vs. Controls. GEE analyses were performed to investigate clinical parameters and OCTA parameters in NDR patients vs. controls. In the final model, VD in SCP (OR 0.92, 95% CI 0.87–0.97) and DCP (OR 0.83, 95% CI 0.76–0.90), MAP (OR 1.03, 95% CI 1.01–1.06), and serum glucose (OR 1.75, 95% CI 1.53–1.99) were significantly different in NDR patients compared to controls (Table 3).

3.5. OCT Parameter Association with the NPDR Level. OLR analysis was performed to find out if any OCTA parameters were associated with DR independently of other traditional risk factors. With univariable analysis, VD in DCP was the only OCTA parameter that was associated with the level of NPDR (OR 0.55, 95% CI 0.44–0.71). In the multivariable model, we included the relevant variables from the univariable analyses to build the final model through a stepdown procedure. In the final model, lower VD in DCP (OR 0.65, 95% CI 0.51–0.83), longer diabetes duration (OR 1.51, 95% CI 1.22–1.87), and higher waist circumference (OR 1.08, 95% CI 1.02–1.14) were associated with the increasing level of NPDR. This means, for each 1% decrease in VD in the DCP, there was a 35% risk of jumping from one NPDR level to the next; for each year increase in diabetes duration, there was a 51% risk of jumping from one NPDR level to the next; for each 1 cm increase in waist circumference, there was an 8% risk of jumping from one NPDR level to the next, no matter what level the patient started with. Refraction was forced into the model to correct for possible magnification (Table 4).

3.6. Mean Ocular Perfusion Pressure (MOPP). Of all OCTA parameters, MOPP was only correlated with VD in SCP in

Table 2: Descriptive analysis of macular OCTA parameters in controls and patients with T1D with different levels of NPDR.

OCTA parameters	Controls ($n = 168$)	NPDR level in patients with type 1 diabetes: NDR ($n = 239$)	Mild NPDR ($n = 58$)	Moderate NPDR ($n = 15$)	Severe NPDR ($n = 3$)	$p^{\ddagger}$	p^{*}
Vascular outcomes							
FAZ area in SCP (mm^2)	0.26 ± 0.09 (0.05-0.59)	0.25 ± 0.10 (0.04-0.56)	0.28 ± 0.12 (0.09-0.81)	0.29 ± 0.15 (0.08-0.70)	0.77 ± 0.58 (0.29-1.42)	$p < 0.001$	$p = 0.14$
FAZ area in DCP (mm^2)	0.35 ± 0.09 (0.13-0.61)	0.33 ± 0.11 (0.07-0.72)	0.34 ± 0.12 (0.16-0.79)	0.39 ± 0.16 (0.18-0.73)	0.83 ± 0.55 (0.34-1.43)	$p < 0.001$	$p = 0.063$
Vessel density in SCP (%)	17.98 ± 3.52 (10.78-26.44)	16.57 ± 3.53 (9.78-28.78)	17.02 ± 2.86 (11.56-25.00)	16.94 ± 2.22 (13.67-22.33)	18.15 ± 0.34 (17.78-18.44)	$p = 0.679$	$p < 0.001$
Vessel density in DCP (%)	38.55 ± 1.83 (32.00-42.33)	36.60 ± 2.49 (30.00-42.44)	35.53 ± 1.92 (29.89-39.56)	33.23 ± 2.91 (29.22-38.44)	27.89 ± 2.79 (26.22-31.11)	$p < 0.001$	$p < 0.001$
Structural outcomes							
Total retinal volume, TRV (mm^3)	9.54 ± 0.34 (8.80-10.26)	9.41 ± 0.42 (8.33-10.84)	9.52 ± 0.32 (8.25-10.14)	9.4 ± 0.40 (8.64-9.97)	9.01 ± 0.41 (8.69-9.47)	$p = 0.082$	$p = 0.002$
Central macular thickness, CMT (μm)	272.74 ± 16.33 (229-309)	269.13 ± 19.80 (212-315)	269.50 ± 19.69 (232-321)	267.53 ± 28.9 (225-310)	244.67 ± 23.67 (231-272)	$p = 0.221$	$p = 0.04$

Values are mean ± SD (range). n = eyes. $^{*}p$ value: independent sample t-test for the difference between controls and T1D eyes with no NPDR (NDR). $^{\ddagger}p$ value: global one-way ANOVA analysis for the difference between all the NPDR subgroups.

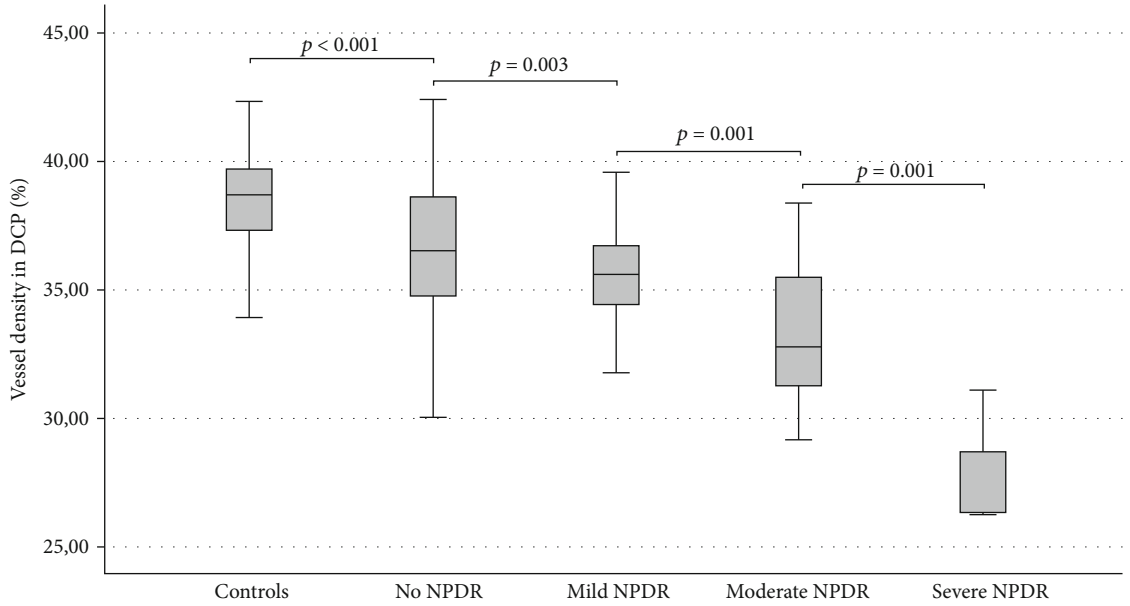

Figure 3: Vessel density in the deep capillary plexus is decreasing with the increasing level of NPDR. Post hoc pairwise comparison between all the subgroups after one-way ANOVA analysis shows a significant difference in vessel density between each level of NPDR.

controls ($r = 0.285$, $p = 0.009$, Pearson correlation) and T1D patients ($r = 0.167$, $p = 0.037$). MOPP was not correlated with VD in DCP.

3.7. HbA1c and Waist Circumference. HbA1c was significantly correlated with waist circumference ($r = 0.173$, $p = 0.006$).

4. Discussion

In a population of young patients with T1D (mean age 24.3 years) imaged with macular OCTA, the VD in the DCP was found to be the only OCTA parameter associated with the increasing level of NPDR, and it could predict the development of NPDR. Lower VD in DCP, longer diabetes duration, and wider waist circumference were the three risk factors that were significantly associated with the progression of NPDR. In addition, VD in the SCP and DCP were significantly lower in T1D patients without NPDR than in controls, when adjusting for clinical confounders. VD in DCP was not associated with visual acuity. Our findings indicate that a decrease in VD in both SCP and DCP is an early process in DR and that changes in OCTA parameters are detectable before the patients have any apparent retinopathy. TRV

Table 3: Association between clinical risk factors and OCTA parameters in T1D patients without retinopathy vs. controls calculated by GEE analysis.

	Univariable model OR (95% CI)	p	Multivariable model OR (95% CI)	p	Final model OR (95% CI)	p
Clinical features						
Gender	1.06 (0.83-1.35)	0.643				
Age (years)	0.99 (0.95-1.03)	0.542				
Mean arterial blood pressure (mmHg)	1.03 (1.01-1.05)	<0.001	1.03 (1.01-1.06)	0.015	1.03 (1.01-1.06)	0.007
Waist circumference (cm)	1.00 (1.01-1.03)	0.002	1.01 (0.99-1.03)	0.384		
Hemoglobin pr SD (g/dL)	1.26 (0.51-3.13)	0.617				
Serum glucose (mmol/L)	1.78 (1.57-2.03)	<0.001	1.74 (1.52-1.98)	<0.001	1.75 (1.53-1.99)	<0.001
Total cholesterol (mmol/L)	1.13 (0.97-1.13)	0.112				
HDL cholesterol (mmol/L)	0.90 (0.67-1.21)	0.498				
LDL cholesterol (mmol/L)	1.16 (0.97-1.38)	0.085				
Triglycerides (mmol/L)	1.15 (0.95-1.40)	0.157				
U-albumin-creatinine ratio (mg/mmol)	0.98 (0.95-1.01)	0.116				
Spherical equivalent (diopters)	0.84 (0.77-0.92)	<0.001	0.90 (0.78-1.04)	0.144		
Best-corrected visual acuity pr SD	1.18 (1.03-1.36)	0.020	1.09 (0.92-1.28)	0.315		
Vascular OCTA outcomes						
FAZ area in SCP pr SD (mm^2)	0.88 (0.75-1.04)	0.133				
FAZ area in DCP pr SD (mm^2)	0.86 (0.73-1.01)	0.070				
Vessel density in SCP (%)	0.93 (0.90-0.97)	<0.001	0.93 (0.88-0.99)	0.014	0.92 (0.87-0.97)	0.002
Vessel density in DCP (%)	0.78 (0.73-0.83)	<0.001	0.84 (0.76-0.92)	<0.001	0.83 (0.7-0.90)	<0.001
Structural OCT outcomes						
Total retinal volume (mm^3)	0.59 (0.43-0.82)	0.002	0.73 (0.40-1.32)	0.294		
Central macular thickness (μm)	0.99 (0.98-1.00)	0.044	1.00 (0.99-1.01)	0.931		

and CMT that can also be measured with conventional OCT were not associated with the increasing level of NPDR, indicating that OCTA is superior to conventional OCT to detect changes associated with NPDR progression without macular edema. It also shows that VD in macular plexuses has a higher index to discriminate patients with T1D from individuals without T1D than FAZ area, TRV, and CMT, indicating that vascular pathology precedes thinning of the central macular area. Since the nerve fiber layer thickness was not measured in this study, it cannot be concluded whether retinal neuropathy precedes the vascular changes described. Progression of NPDR was not associated with gender, age, HbA1c, serum glucose, MAP, lipid profile, hemoglobin, and U-albumin-creatinine ratio, which may be due to the study population being young. Despite earlier studies which have shown HbA1c, after the duration of TD1, to be the most important factor in disease progression, our population showed no association between HbA1c and the level of NPDR [35, 36]. Waist circumference was strongly associated with disease progression, likely higher waist circumference reflecting better, a high level of HbA1c cumulatively over many years compared to a single blood test on the day of the eye examination. This is supported by the fact that HbA1c was significantly correlated with waist circumference in this population.

High systemic blood pressure is a well-known risk factor for retinopathy [37], which in our young population showed no association with NPDR probably because the individuals were normotensive and too young to have any significant damaging effect of it. In addition, mean ocular perfusion pressure (MOPP) was not significantly different between the NPDR groups and the controls and it was only correlated with VD in SCP; accordingly, MOPP was not an important risk factor in this population.

Our data confirm and add knowledge to previously published data by demonstrating VD in the DCP to be the most robust OCTA parameter for the differentiation of clinical stages of NPDR in young T1D patients [10, 18, 38, 39]. Other studies found lower VD in both SCP and DCP in eyes with retinopathy compared to normal eyes [9, 40]. All these earlier studies were smaller and conducted on individuals older than the ones in our study, most of them including T2D patients with comorbidities.

We used the same OLR analysis and included both eyes, almost the same clinical characteristics and OCTA parameters as a recent study [18], but their population was older (mean age 62.6 years), had a high prevalence of hypertension, included both T1D and T2D with a longer duration of diabetes (mean 14-23 years), and did not include BMI and waist circumference. They found that a higher level of HbA1c and lower VD in the DCP were associated with the increasing level of NPDR in the final model. In our younger study population, waist circumference and diabetes duration were stronger predictors for retinopathy than HbA1c. Even

Table 4: The associations of clinical risk factors and OCTA parameters with the increasing level of NPDR calculated as odds ratios with ordered logistic regression analysis.

	Univariable model OR (95% CI)	p	Multivariable model OR (95% CI)	p	Final model OR (95% CI)	p
Clinical features						
Gender	2.51 (0.62-10.2)	0.198				
Age (years)	1.90 (1.39-2.60)	<0.001	1.31 (0.99-1.74)	0.061		
Duration of diabetes (years)	1.74 (1.36-2.22)	<0.001	1.38 (1.11-1.73)	0.004	1.51 (1.22-1.87)	<0.001
Mean arterial blood pressure (mmHg)	1.06 (0.97-1.15)	0.182				
Body mass index	1.24 (1.05-1.48)	0.014				
Waist circumference (cm)	1.12 (1.05-1.20)	0.001	1.07 (1.01-1.13)	0.014	1.08 (1.02-1.14)	0.005
HbA1C (mmol/mol)	1.57 (0.96-2.56)	0.070				
Serum glucose (mmol/L)	1.06 (0.90-1.25)	0.456				
Hemoglobin pr SD (g/dL)	1.26 (0.51-3.13)	0.617				
Total cholesterol (mmol/L)	0.98 (0.46-2.09)	0.967				
HDL cholesterol (mmol/L)	0.92 (0.18-4.82)	0.920				
LDL cholesterol (mmol/L)	0.81 (0.32-2.06)	0.659				
Triglycerides (mmol/L)	1.74 (0.78-3.89)	0.180				
U-albumin-Creatinine ratio (mg/mmol)	1.13 (0.84-1.51)	0.295				
Spherical equivalent (diopters)	0.90 (2.29-1.37)	0.619	0.98 (0.65-1.48)	0.913	0.86 (0.58-1.29)	0.477
Best-corrected visual acuity pr SD	2.29 (1.11-4.71)	0.025	1.59 (0.83-3.02)	0.159		
IOP (mmHg)	1.08 (0.89-1.30)	0.443				
Vascular OCTA outcomes						
FAZ area in SCP pr SD (mm^2)	1.83 (0.96-3.50)	0.068				
FAZ area in DCP pr SD (mm^2)	1.67 (0.89-3.12)	0.108				
Vessel density in SCP (%)	1.10 (0.93-1.30)	0.258				
Vessel density in DCP (%)	0.55 (0.44-0.71)	<0.001	0.67 (0.52-0.85)	0.001	0.65 (0.51-0.83)	<0.001
Structural OCT outcomes						
Total retinal volume, TRV (mm^3)	0.92 (0.18-4.77)	0.925				
Central macular thickness, CMT (μm)	0.98 (0.95-1.02)	0.367				

though the two study populations were different, both studies found that VD in DCP was the most robust OCTA parameter for detecting the level of NPDR.

Similar findings were also reported in recent publications, where the FAZ area was not different between NDR patients and normal controls, while NDR patients had lower VD limited to the DCP when compared to normal eyes. However, these studies were smaller and did not perform an OLR analysis accordingly [20, 21].

Axial length (AL) can affect the magnification of OCTA scans and may affect the quantitative results of VD. In our population, the T1D patients were more myopic than the controls, but the VD was lower in the T1D group even though myopia could have influenced the vessel density result in the other direction. In addition, refraction did not change significantly with the increasing level of NPDR. Our study found the refractive error not to be a confounder accordingly. Other studies came to the same conclusion [18, 41, 42]. Even if an algorithm was used to correct for the AL, eyes with minimal NPDR had a decreased capillary complexity and decreased vessel density compared to normal eyes, especially in the deep vascular layer in a previous study [10].

The VD was greater in the DCP than SCP in both controls and T1D patients, which is in line with other previous studies [9, 10, 14, 18]. The question remains why the DCP is more susceptible to damage than the SCP. Indeed, the same feeding retinal artery supplies the SCP in the ganglion cell layer and the DCP in the inner nuclear layer. However, anatomically, the SCP consists mainly of arterioles and venules, while the DCP of capillaries [43] makes the latter to be more susceptible to capillary closure. This theory is supported by previous histologic findings showing abnormalities to be more severe in the DCP than in the SCP [19, 44]. Also, studies have shown more microaneurisms in the DCP than in the SCP, and that the microaneurisms in the DCP contributed to the pathogenesis of macular edema [45–47]. One study has confirmed the hypothesis that diabetic macular ischemia at the level of the DCP, seen as either focally absent or low-intensity flow within the DCP on OCTA, contributes to outer retinal disruption on OCT [44]. It argues that DCP ischemia contributes to disruption of the outer retina including thinning of the outer nuclear layer and photoreceptors in eyes with DR [44]. Disturbances in vasomotion in the retinal capillary microcirculation are key factors in the development of diabetic maculopathy [48]. It has been suggested that the

DCP may contribute more to the metabolic demands of photoreceptor metabolism in eyes with diabetic macular ischemia than previously thought [4, 44, 49]. Recent studies have found that ischemia or nonperfused areas in the DCP leading to lower VD as measured by OCTA is associated with abnormalities in the cone photoreceptor layer in DR as revealed by adaptive optics imaging; this suggests that the outer retinal hypoxia contributes to cone loss [49] and that complementary use of density, spacing, and packing arrangement of cones is valuable to detect early abnormalities of the parafoveal cone mosaic in adult patients with T1D. The results from this pilot study support the neurodegenerative theory, for which the retinal neuronal cells, including photoreceptors, are involved early in the course of DR [50].

Enlargement of the FAZ area is caused by the loss of capillaries in the inner vascular ring around the FAZ. We found that the FAZ area was not significantly associated with the NPDR level, but it was significantly higher in the severe NPDR group compared to other groups. A recent review paper concluded that most studies on DR found increased FAZ area in patients with diabetes compared to controls and that this was more evident in patients with advanced levels of DR [17].

According to national standards, our study was considered a big study population, and it followed a well-planned protocol in which all data were collected within a few hours in each individual. A young T1D cohort is well suited to examine retinal vascular changes due to metabolic dysregulation, since these individuals have no other vascular comorbidities such as hypertension and atherosclerosis or ocular disease, which can affect the retinal blood vessels in other ways (e.g., reduced confounders that can influence the results were avoided, so a clear influence of diabetes was obtained). Careful statistical planning was performed, and valid models were implemented to test for multiple risk factors and adjusted for potential confounders and intrapatient correlation on both eyes. The International Clinical Diabetic Retinopathy (ICDR) Disease Severity Scale was used here, since it is a more practical and valid method for use in the clinical practice than the ETDRS, thus making this study more similar to the actual clinical practice.

There are some limitations of the study as well. First, only three eyes with severe NPDR were detected, so selection bias may be possible and weak statistics due to that. Second, the current macular OCTA protocol has a small field of view; thus, we could not evaluate peripheral vascular pathology. Third, the examination time is long (between 30-45 seconds), resulting in motion artefacts, since it is hard for the patients to fixate for so long. Motion and projection artefacts may alter the interpretation of the deeper vessels, but the software has an artefact removal option that was set on default and used equally for all groups in the study. Fourth, the FAZ area was measured subjectively by the grader and could not be reliably delineated with the current NIDEK OCTA system. FAZ is irregular, difficult to measure objectively, and has considerable intergrader variability; in addition, overlap in size between the normal individuals and those with T1D was found in our study, thus not discussed further in the Results. Finally, the study was cross-sectional; therefore, it can only analyze associations between VD and DR at a given time, and not describe how VD changes over time. We excluded 36 images because of image artefacts, which may have introduced selection bias; nevertheless, we believe that it does not affect our results as the sample size is large ($n = 483$).

The traditional subjective DR grading of fundus photographs will remain clinically relevant when screening large populations, but it may fail to discover early capillary pathology which is important and only reliably detected by OCTA. We hereby suggest to make a new classification system for DR based on OCTA measurements. Automated quantification of vascular changes in the retina, primarily in the macula and on the optic disc, could translate the theoretical research usefulness of OCTA into a tool which can be easily used in "clinical practice." OCTA may indeed be included in screening programs of patients with T1D and T2D in the future. There is evidence that vascular changes detected by the noninvasive OCTA precede the progression to more advanced levels of DR, and it may also reflect the status of the microvasculature in other organs that are only accessible by invasive biopsies. OCTA has an advantage over fluorescein angiography (FA), which only shows the superficial plexus, cannot be automatically quantified, is invasive and time consuming, and has many side effects [51–54]. Widefield OCTA will likely soon replace fluorescein angiography [55] in the near future.

In conclusion, we found that longer duration of T1D, higher waist circumference, and a sparser VD in the DCP in the macula are significantly associated with a higher odds ratio of having a worse level of NPDR. VD in DCP is associated with NPDR independently of traditional risk factors. VD in DCP measured by OCTA has a high ability to detect the earliest signs of DR, before they are actually visible by ophthalmoscopy, and it has a high ability to discriminate between different levels of NPDR. The FAZ area measured by OCTA was not a good early biomarker for DR. OCTA is a much more sensitive tool to diagnose early NPDR than conventional OCT and funduscopic ICDR grading. The objective quantification of vessel density in OCTA scans is a useful early noninvasive biomarker for the progression of DR.

Data Availability

All data will be made available and deposited according to journal policy.

Disclosure

The funding organizations had no role in the design or conduct of this research.

Conflicts of Interest

The authors declare that there is no conflict of interest regarding the publication of this paper.

Authors' Contributions

Nina C. B.B. Veiby and Aida Simeunovic shared first authorship. Hanna D. Margeirsdottir and Goran Petrovski shared last authorship.

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Review Article

Identifying Genetic Risk Factors for Diabetic Macular Edema and the Response to Treatment

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Diabetic retinopathy (DR) is the most common microvascular complication of diabetes mellitus (DM). DR is complex and the term encompasses several clinical subtypes of diabetic eye disease, including diabetic macular edema (DME), the most frequent cause of central vision loss in DR patients. Both genetic and environmental factors contribute to the pathophysiology of DR and its subtypes. While numerous studies have identified several susceptibility genes for DR, few have investigated the impact of genetics on DME susceptibility. This review will focus on the current literature surrounding genetic risk factors associated with DME. We will also highlight the small number of studies investigating the genetics of response to antivascular endothelial growth factor (anti-VEGF) injection, which is used to treat DME.

1. Introduction

Diabetic retinopathy (DR), the most common microvascular complication of diabetes mellitus (DM), is a leading cause of vision loss in the working-age population [1]. It is a heterogeneous condition with multiple subtypes. DR can present as mild nonproliferative retinal changes anywhere in the retina having little or no effect on vision, to severe nonproliferative retinopathy characterized by severe retinal hemorrhages and vascular changes. A portion of patients will progress to proliferative retinopathy, characterized by aberrant neovascularization. This has a profound effect on vision, leading to permanent vision loss or blindness [2]. Diabetic macular edema (DME) is another retinal complication of diabetes and is often included under the umbrella of DR. It can occur at any stage of the progression from nonproliferative to proliferative disease, with or without other features of DR [3] and in conjunction with type 1 (T1) or type 2 (T2) DM. It is the most frequent cause of central vision impairment in patients with diabetes [4] with a reported global prevalence of 4.6% amongst diabetics between 2015 and 2019 [5]. DME presents as a collection of fluid in the central part of the retina, mainly in the inner and outer plexiform layers. It can be associated with hard exudates, which present clinically as yellow-white plaque deposits in the macular region. The gold standard and most widely used classification of DME is clinically significant macular edema (CSME), defined by the Early Treatment for Diabetic Retinopathy Study (ETDRS) as (1) retinal thickening at or within 500 microns of the macular center; (2) hard exudates at or within 500 microns of the macular center, associated with adjacent retinal thickening; or (3) one or more disc diameter of retinal thickening, any part of which lies within one disc diameter of the macular center [6]. DME is often included in the broader classification of diabetic maculopathy, which also includes diabetic macular ischemia [7]. It should also be noted that many studies do not necessarily consider DME separately from the larger collective of DR phenotypes.

From studies to date, primarily under the umbrella phenotype of DR, it appears that conventional risk factors like diabetes duration, poor glycemic control, hyperlipidemia, microalbuminuria, and high diastolic blood pressure explain only a small portion of the risk for development and progression of diabetic microvascular complications, including DME

[8, 9]. Moreover, a significant proportion of participants remain free of diabetic complications or progression even after a long disease duration [10]. Thus, other factors, including genetics, likely contribute to DR and DME risk.

The genetics of DR has been studied extensively in the last decade; however, most of these studies failed to distinguish DME as a separate phenotype of DR. The majority of studies that have made this distinction consisted of small sample sizes, limiting statistical power. Here, we review the literature related to the genetics of DME. The limitations of these studies are discussed and our current understanding of the genetic architecture of DME is summarized. In addition, we discuss the studies that have evaluated genetic factors involved in a patient's response to antivascular endothelial growth factor (anti-VEGF) injection. This group of drugs is now a frontline treatment for DME, but patient outcomes remain mixed, and understanding this variability is critical if we wish to improve outcomes. Articles published in English before January 2020 were identified through searches of PubMed, Embase, and Web of Science. Also, we manually searched the reference lists of included papers to identify other potentially eligible studies. Case reports, editorials, abstracts, reviews, and unpublished reports were excluded. A total of 61 genetic studies had DME/diabetic maculopathy mentioned in their study, of which 48 specifically state that their cohort included DME patients. Of these 48, only 24 studies conducted a separate analysis for DME.

2. Candidate Genes

The candidate gene approach focuses on establishing a genetic association between predefined genes and disease status or phenotypes [11]. Genes are selected based on prior knowledge of the molecular pathways underlying the pathophysiology of a disease and the known or presumed function of the gene in those pathways. To date, less than a dozen candidate genes have been found to be associated with DME, and findings for most have been variable (Table 1).

2.1. Apolipoprotein E (APOE). In DME, macular exudates contribute to significant visual loss when present in the foveal center and are frequently associated with a high level of serum lipids [12, 13]. Apolipoprotein E (APOE) is mainly known for lipid transportation and metabolism. It is highly expressed in the retina and has been explored as a possible DME susceptibility gene. The gene is polymorphic with three major alleles; epsilon 2 ($\varepsilon 2$), epsilon 3 ($\varepsilon 3$), and epsilon 4 ($\varepsilon 4$) [14]. Santos et al. [15] conducted a study on 36 T2 DME patients (compared to 22 healthy individuals) to determine the relationship between *APOE* polymorphisms and the severity of macular edema. DME severity was graded based on the number and extent of macular hard exudates using standardized retinal photographs based on the Early Treatment Diabetic Retinopathy Study (ETDRS) guidelines [16]. In their study, the frequency of macular hard exudates was higher in $\varepsilon 4$ carriers ($p < 0.05$). However, there was poor correlation between degree of visual impairment and presence of the $\varepsilon 4$ allele ($p = 0.057$). Estimation of lipid levels found significantly higher total lipids in the $\varepsilon 4$ carrier group ($p < 0.05$). Given the small sample size with borderline significant results, extreme caution should be taken when interpreting this study and much larger studies are required to draw conclusions about the role of *APOE* variants in DME.

2.2. Nitric Oxide Synthase (NOS). Damage to vascular endothelial cells can lead to exudation of fluid into the retinal space, a hallmark feature of DME. Damage can be caused through a range of mechanisms, including oxidative damage from free radicals. One such molecule is nitric oxide, produced by the conversion of L-arginine to L-citrulline by nitric oxide synthase (NOS) [17]. There are three recognized isoforms of NOS: the constitutively expressed, neuronal NOS (nNOS/NOS-1), endothelial NOS (eNOS/NOS-3), and the inducible NOS (iNOS/NOS-2), upregulated in response to stimuli [17]. The isoform *eNOS* has been posited as a candidate gene for DME due to its role in endothelial cells of the vasculature. The most commonly studied polymorphisms in eNOS are: -786T>C (rs2070744) in the promoter region; 894G>T (rs1799983) substitution in exon 7; and a 27-bp variable number tandem repeat in intron 4 with "a" and "b" alleles that differ in their number of repeats (27-bp *VNTR* (a/b)). Only two studies to date have analyzed *eNOS* gene polymorphisms specifically for association with DME. Awata et al. [18] studied *eNOS* gene polymorphisms in a Japanese cohort of T2DM patients compared with healthy controls. Subgroup analysis of DME patients (DME = 48, DR without DME = 69) revealed that the -786T>C polymorphism and 27-bp *VNTR* were significantly associated with the risk of developing DME. Specifically, the -786C allele ($p = 0.029$) and the 27-bp *VNTR* "a" allele ($p = 0.006$) appeared to increase the risk of DME, with significantly different genotype frequencies between the cohort with and without DME. The results were consistent when clinical covariates were also included in the analysis model ($p = 0.001$, OR = 3.57, 95%CI = 1.65 – 7.69). The 894G>T polymorphism was not associated with DME risk in either the allelic or genotypic model. In a similar study, Uthra et al. [19] tested the association between the 27-bp *VNTR* and DR in a South Indian T2DM cohort but did not identify any significant association. In a subgroup analysis, the frequency of genotypes and alleles of the 27-bp *VNTR* was compared between DR with, ($n = 100$) and without DME ($n = 87$), but no significant association with DME was observed ($p > 0.05$) [19]. Thus, there are conflicting reports for this gene and further larger studies are required for a better understanding of the role of the e*NOS* gene in the pathogenesis of DME.

2.3. Manganese Superoxide Dismutase (SOD2). Another gene involved in oxidative stress is *SOD2*, encoding manganese superoxide dismutase (MnSOD) [20]. This enzyme protects against the damaging effects of superoxide radicals, which are postulated to trigger several biochemical pathways underlying the pathophysiology of DR and DME [21]. The Ala16-Val (rs4880) polymorphism in *SOD2* results in a 30-40% lower enzymatic activity of MnSOD and hence a greater cellular susceptibility to oxidative stress [22]. This polymorphism has been studied in association with DR risk in different cohorts across many ethnicities and countries [23,

Table 1: Candidate genes evaluated for association with diabetic macular edema.

Gene	Chr	Cohort size	DM type	Country	Variant	p value	Reference
APOE	19	DME = 36 Healthy controls = 22*	T2	Mexico	$\varepsilon 2$, $\varepsilon 3$, $\varepsilon 4$	$p < 0.05$	Santos et al. [15]
eNOS	7	DME = 48 DR without DME = 69*	T2	Japan	-786T>C (rs2070744) 27-bpVNTR Glu298Asp (rs1799983)	$p = 0.029$ $p = 0.006$ $p > 0.99$	Awata et al. [18]
eNOS	7	DME = 100 DR without DME = 87*	T2	South India	27-bpVNTR	$p > 0.05$	Uthra et al. [19]
MnSOD	6	DME = 37 DR without DME = 93*	T2	South Korea	Ala16Val (rs4880)	$p < 0.05$	Lee et al. [25]
EPO	7	Combined DME = 90 (DM without DR = 233*) T1DME = 24 (DM without DR = 67*) T2DME = 66 (DM without DR = 166*)	T1+T2	Australia	rs1617640 rs507392 rs551238	For all three SNPs $p = 0.018$ (T2) $p = 0.040$ (T1 + T2)	Abhary et al. [30]
VEGFA	6	DME = 63 DR without DME = 112*	T2	Japan	-2578C>A (rs699947) -1154G>A (rs1570360) -634C>G (rs2010963)	$p = 0.148$ $p > 0.999$ $p = 0.047$	Awata et al. [37]
VEGFA	6	DME = 64 DR without DME = 148*	T2	Egypt	-634C>G (rs2010963)	$p = 0.019$ (genotype) $p = 0.022$ (allele)	Shazly et al. [40]
VEGFA	6	Combined DME = 93 DM without DR = 281*	T1 + T2	Australia	rs699946 rs833068 rs10434	$p = 0.039$ (T1) $p = 0.017$ (T1) $p = 0.027$ (T2) and $p = 0.003$ (T1 + T2)	Abhary et al. [36]
VEGFC	4	Combined DME = 425 (DR without DME = 952*) T1DME = 64 (DR without DME = 241*) T2DME = 361 (DR without DME = 711*)	T1 + T2	Australia	rs17697515 rs17697419 rs2333526	$p = 0.004$ (T2) and $p = 0.009$ (T1+T2) - -	Kaidonis et al. [46]
PEDF	17	DME = 66 DM without DR = 229*	T2	Japan	rs12150053 rs12948385	$p = 0.004$ $p = 0.008$	Iizuka et al. [50]
ALR2 *	7	Proliferative DR with DME = 20 Non-proliferative DR with DME = 35 Proliferative DR without DME = 35 Non-proliferative DR without DME = 15	T2	South India	(CA)n (Z-2) allele	$p < 0.05$	Kumaramanickavel et al. [59]
MiRNA-146a	5	Combined DME = 1026 T1DME = 170 (no/minimal DR = 258*) T2DME = 856 (no/minimal DR = 895*)	T1 + T2	Australia	rs2910164	$p = 0.025$ (T2)	Kaidonis et al. [64]
MiRNA	5	DME = 89 (DM without DR = 228*)	T1	Australia	rs10061133 rs1049835	- -	Liu et al. [65]
CA	8	DME = 93 DM without DR = 235*	T1+T2	Australia	10 tag SNPs across *CA* gene	$p > 0.05$	Abhary et al. [68]
MCP1*	17	DME = 446 (mild DME = 207, moderate DME = 173, severe DME = 66)	T2	North China	-2518A>G (rs1024611)	$p > 0.05$	Dong et al. [69]
	4		T2				Dong et al. [70]

Table 1: Continued.

Gene	Chr	Cohort size	DM type	Country	Variant	p value	Reference
CXC chemokine family∗		DME = 446 (mild DME = 207, moderate DME = 173, severe DME = 66)		North China	-251T>A in CXCL8 -1596C>T in CXCL10	$p > 0.05$ $p > 0.05$	
SLMAP	3	DME = 49 DM without DR = 160*	T2	Qatar	rs17058639	$p_{trend} = 0.0425$	Upadhyay et al. [71]

Chr: chromosome; DM: diabetes mellitus; DR: diabetic retinopathy; DME: diabetic macular edema; T1: type 1 diabetes mellitus; T2: type 2 diabetes mellitus; APOE: apolipoprotein E; EPO: erythropoietin; eNOS: endothelial nitric oxide synthase; VEGFA: vascular endothelial growth factor A; VEGFC: vascular endothelial growth factor C; MnSOD: manganese super-oxide dismutase; MiRNA: micro-ribonucleic acid; ALR2: aldose reductase 2; PEDF: pigment epithelium growth factor; CA: carbonic anhydrase; MCP1: monocyte chemoattractant protein 1; CXCL8: C-X-C motif chemokine ligand 8 (also known as interleukin 8, IL8); CXCL10: C-X-C motif chemokine ligand 8 (also known as interferon-inducible cytokine IP10); SLMAP: sarcolemma associated protein; *controls; ∗subtypes of DME were compared with each other.

24]. A study by Lee et al. [25] in a Korean T2DM cohort is the only one to explore an association between the Ala16Val polymorphism and DME. The DME subgroup ($n = 37$) was found to have a significantly lower Ala allele frequency ($p < 0.05$) when compared to the non-DME group (DR without DME = 93). In multivariate logistic regression, the Ala allele of *SOD2* was associated with DME ($p = 0.03$, OR = 1.59, 95%CI = 1.02 – 2.02). Further, disparate Ala allele frequencies were observed in the three DME subtypes; focal = 0.188 ($n = 8$), diffuse = 0.109 ($n = 23$), and ischaemic = 0.0 ($n = 6$); however, this could not be statistically evaluated due to the small sample size. Overall, due to the limited numbers of DME patients in this study, the results should be interpreted with caution and additional better-powered studies need to be undertaken to determine whether *SOD2* has a role in DME risk.

2.4. Erythropoietin (EPO). Human erythropoietin (EPO) is a potent angiogenic factor secreted in response to hypoxia by a mechanism dependent upon the hypoxia-inducible factor (HIF). EPO regulates the production of red blood cells via its receptor, erythropoietin receptor (EPOR), which is expressed in retinal tissue [26]. There are several studies demonstrating the protective role of EPO in maintaining the integrity of the blood-retinal barrier, the structure primarily responsible for the pathogenesis of DME [27–29], thus making *EPO* an important candidate gene. Abhary et al. [30] genotyped *EPO* gene polymorphisms in both T1 and T2 diabetic patients exhibiting different severity levels of DR. In this study, the GG (rs1617640), CC (rs507392), and CC (rs551238) genotypes were found to be associated with increased risk of DR in the combined DM group ($p = 0.008$), and the T2DM group alone ($p = 0.006$). This study also analyzed *EPO* polymorphisms in the DME cohort separately. All three *EPO* SNPs were associated with DME in the combined DM group (DME ($n = 90$) vs. DM without DR ($n = 233$), $p = 0.04$) as well as in the T2DM only group (DME ($n = 66$) vs. DM without DR ($n = 166$), $p = 0.018$). Additionally, the GCC haplotype of all three SNPs was significantly associated with DME both in the combined DM group ($p = 0.04$) and T2DM alone ($p = 0.031$). Analogous reports of positive *EPO* genotype associations with DR have been presented by several other studies [31, 32]; however, only Abhary et al. [30] study analyzed DME separately. Taken together, there is evidence that *EPO* has a role in DR, but a larger cohort of DME patients should be assessed to replicate the DME-specific findings.

2.5. Vascular Endothelial Growth Factor-A (VEGFA). The vascular endothelial growth factor (VEGF) family is a group of five structurally related glycoproteins [33]: VEGF-A, VEGF-B, VEGF-C, VEGF-D, and placental growth factor (PGF), each encoded by a separate gene corresponding to their respective names. VEGF-A, commonly referred to as VEGF, is mainly responsible for vasculogenesis (formation of new blood vessels during embryogenesis) and angiogenesis (formation of new blood vessels from preexisting blood vessels) [34]. Both serum and vitreous VEGF protein levels are significantly elevated in diabetic compared to nondiabetic individuals, and anti-VEGF agents are the latest standard of care for the management of DME [35]. The *VEGFA* gene is highly polymorphic and extensively studied in relation to DR; however, its role in DME risk is relatively unexplored. Of the seven studies on *VEGFA* polymorphism related to DR [36–40], only three have analyzed DME patients separately.

Awata et al. [37] studied three polymorphisms in the promoter and upstream region of the *VEGFA* gene (-2,578C>A [rs699947], -1,154G>A [rs1570360], and -634C>G [rs2010963]) in a cohort of T2 diabetic patients. In the subgroup analysis (DME = 63, DR without DME = 112), the frequencies of both the -634C>G CC genotype ($p = 0.023$) and C allele ($p = 0.023$) were significantly increased in DME patients. The CC genotype remained significantly associated with DME risk after adjusting for clinical covariates ($p = 0.047$, OR = 1.81, 95%CI = 1.01 – 3.26). Furthermore, macular thickness measured by optical coherence tomography was also found to be correlated with the same allele ($p = 0.006$), independent of the duration of diabetes. There were no significant differences in the genotype and allele frequencies of -2,578C>A or -1,154G>A in the overall cohort analysis or any subgroup analyses.

More recently, Shazly et al. [40] undertook a similar analysis of the *VEGFA* -634C>G polymorphism in a cohort

of T2 diabetic patients. In the subgroup analysis for DME (DME = 64, DR without DME = 148), they observed a significant association between DME risk and the CC genotype ($p = 0.019$) and C allele ($p = 0.022$), corresponding to the genotypic and allelic model, respectively. Multivariate logistic regression, taking into account both genetic and clinical covariates, also identified the CC genotype of the -634C>G polymorphism as a significant risk variant for DME ($p = 0.003$). However, a subgroup analysis dividing DME patients according to the proliferative state of DR (nonproliferative DR = 20, proliferative DR = 44) failed to show any significant association in either of the models assessed in this small cohort. Interestingly, the CC genotype was associated with a significantly higher serum concentration of VEGF in DME patients ($p = 0.016$).

Using a slightly different approach, Abhary et al. [36] investigated the association between 15 *VEGFA* tag SNPs with DME in a cohort of diabetic patients (T1+T2). The minor allele of rs699946 (A; $p = 0.039$, OR = 5.7, 95%CI = 1.1 – 29.3) and rs833068 (G; $p = 0.017$, OR = 5.1, 95%CI = 1.3 – 19.5) were significantly associated with DME risk in T1DM. In T2DM, the G allele of rs10434 was associated with DME ($p = 0.027$, OR = 2.9, 95%CI = 1.1 – 7.6). Combined analysis for both types of DM (DME = 93, DM without DR = 281) found a significant association of DME with the G allele of rs10434 ($p = 0.003$). This result remained significant after correcting for multiple testing ($p = 0.03$). Thus far, studies on *VEGF* polymorphisms in association with DME show encouraging results. Although the cohorts of DME patients are relatively small, associations of *VEGFA* SNPs with DME are consistently observed across studies. Given the known role of VEGFA in DME pathogenesis and the success of treatments that target this protein, these results are not unexpected. The relative contribution of this gene to the overall risk profile of DME remains to be determined and larger studies to better investigate the true effect size would be warranted.

2.6. Vascular Endothelial Growth Factor-C (VEGFC). VEGF-C is a dimeric glycoprotein of the VEGF family encoded in humans by the *VEGFC* gene. Along with VEGF-D, it acts as a major lymphangiogenic factor, leading to the formation of new lymph vessels [41] by binding to VEGF receptor 3 (VEGFR-3). Though the eye was historically considered to lack a lymphatic system, recent studies suggest lymphangiogenesis may have a role in macular edema [42]. Furthermore, VEGF-C is believed to promote retinal neovascularization independent of its widely reported counterpart VEGF-A [43, 44]. Interestingly, VEGF-C and its receptor VEGFR-3 are markedly increased in the retinal vessels of DR patients [45]. To date, only one study has investigated genetic polymorphism in the *VEGFC* gene and its association with DME risk [46]. Kaidonis et al. [46] investigated the association of 13 *VEGFC* tag SNPs with DR risk in Caucasian diabetics (T1+T2). In the overall analysis including "any DR" across both types of DM, three *VEGFC* SNPs (rs17697515, rs17697419, and rs2333526) were significantly associated with DR risk even after adjustment for clinical covariates and multiple testing. Further analysis stratified by diabetes type resulted in similar trends of association with the above-mentioned SNPs. In subset analyses of DME, the T allele of rs17697515 was negatively associated with DME risk in T2DM patients (DME = 361, DR without DME = 711, $p = 0.004$, OR = 0.53, 95%CI = 0.35 – 0.82); however, no associations were detected in T1DM patients (DME = 64, DR without DME = 241), and only a nominal association between rs17697515 and DME risk (DME = 425, DR without DME = 952, $p = 0.009$) was observed in combined DM patients after correcting for multiple testing. Thus, evidence to date suggests that *VEGF-C* might play a role in DME pathogenesis along with *VEGFA*, but the exact mechanism(s) of action is not yet fully elucidated. Further, despite a large cohort of DME, there was only a nominal association; thus, the results of this study need to be replicated.

2.7. Pigment Epithelium-Derived Factor (PEDF). Another candidate gene involved in angiogenesis pathways is pigment epithelium-derived factor (*PEDF*). PEDF, also known as serpin F1 (SERPINF1), is widely expressed in many organs and tissues, including the retinal pigment epithelial layer of the retina [47]. It is a member of the serine proteinase inhibitor (SERPIN) family, widely known as the most potent natural antiangiogenic factor [48]. In the retina, *PEDF* is known to inhibit and downregulate proangiogenic factors [49], and an imbalance between VEGF and PEDF in the vitreous has been implicated as one mechanism responsible for the development and progression of DME. To date, only one study, by Iizuka et al. [50], has investigated the association of *PEDF* gene polymorphisms with DME risk. In this study, DR cases were compared with diabetics without DR. The C allele of rs12150053 and A allele of rs12948385 were associated with DR risk in dominant and codominant models but were also observed to be associated with DME risk in a subgroup analysis (DME = 66, DM without DR = 229, $p < 0.05$). It should be noted that there is strong linkage disequilibrium between these two polymorphisms. Subsequently, two studies by Uthra et al. [51] and Yamagishi et al. [52] evaluated *PEDF* gene polymorphisms with DR risk in Asian cohorts. Uthra et al. [51] observed a moderately protective association between a polymorphism in exon 4 (Thr130Thr) and DR risk, whilst Yamagishi et al. [52] failed to observe any association between a polymorphism in exon 3 (Met72Thr) and DR risk. Neither study performed subset analyses for associations with DME risk. As only one study to date has observed a significant association between DME and *PEDF* polymorphisms, the findings require replication in additional, larger datasets.

2.8. Aldose Reductase (ALR2). Aldose reductase (ALR2), also known as aldo-keto reductase family 1 (AKR1B1/ALDR1), catalyzes the first rate-limiting step during glucose metabolism in the polyol pathway. Hyperglycaemia in DM leads to altered activity of *ALR2* and accumulation of sorbitol, which is responsible for various complications related to the disease [53]. Various polymorphic variants of the *ALR2* gene have been linked to the development of microvascular complications related to DM. Of note, the (CA)n dinucleotide repeat has been studied extensively for association with DR

susceptibility across many ethnicities [54, 55]. The CA dinucleotide repeat has three common alleles consisting of 24 repeats (labeled the Z allele), 23 repeats (the Z-2 allele), and 25 repeats (the Z+2 allele). These allelic polymorphisms have been hypothesized to alter *ALR2* mRNA levels and hence enzyme activity, thus contributing to diabetic microvascular complications [56]. However, to date, conflicting evidence has been presented; some studies have reported an association between the Z-2 allele and DR risk [57, 58], whereas others have reported no association [54, 55]. In a meta-analysis by Mi et al. [57], comprising 17 studies, the Z-2 allele was reported as a risk polymorphism for DR in both Asian and Caucasian T1 and T2DM cohorts. Notably, only one study by Kumaramanickavel et al. [59] has explored the association between the *ALR2* dinucleotide repeat and DME risk. They evaluated a South Indian T2DM population and reported that the Z-2 allele showed a significant association with overall DR risk ($p = 0.029$). DR patients were then subclassified into "proliferative DR+maculopathy" ($n = 20$), "nonproliferative DR+maculopathy" ($n = 35$), "proliferative DR" ($n = 35$), and "nonproliferative DR" ($n = 15$). There was significant difference in the Z-2 allele frequency in the "proliferative DR+maculopathy" when compared with the proliferative DR ($p = 0.004$) and nonproliferative DR ($p = 0.002$) groups, but not when compared with the nonproliferative DR+maculopathy group. This study has attempted a detailed stratified analysis by various subtypes of DR involving DME but was unable to identify a robust association. Although meta-analysis suggests this variant is associated with DR risk, its role in DME specifically is yet to be elucidated.

2.9. MicroRNA Genes (miRNA). MicroRNAs (miRNAs or miRs) are a class of short, noncoding, single-stranded RNA molecules responsible for regulating a plethora of biological processes [60]. Several miRNAs have been reported to be expressed in the retina, and their dysregulation has been linked to various retinal disorders [61]. Moreover, miRNAs have been shown to play a significant role in angiogenesis and oxidative stress [62] and have thus been proposed as biomarkers of DR and disease progression [63]. To date, very few studies have explored whether genetic variation in microRNAs is associated with DME risk. Kaidonis et al. [64] were the first group to report an association between microRNA-146a (miR-146a) and DME. The miR-146a SNP, rs2910164, was tested for association with microvascular complications in both T1 and T2DM patients. A subgroup analysis found an association between the C allele of rs2910164 and DME risk (DME = 856, no/minimal DR = 895) in the T2DM cohort ($p = 0.025$, OR = 1.25, 95%CI = 1.03 – 1.53). However, there was no association with T1 DME (DME = 170, no/minimal DR = 258) or proliferative DR.

A more recent study investigating the relationship between microRNA genes and DR risk was conducted by Liu et al. [65]. Imputed SNP array data was extracted from a previous T1DM GWAS [66] and tested for association with different DR phenotypes, including DME. No SNPs reached genome-wide significance for any of the subtypes of DR; nevertheless, the top SNPs from the proliferative DR and sight-threatening DR analyses were genotyped in a second cohort and the data from both samples combined. SNP rs10061133 in *MIR449b* was found to be protective against sight-threatening DR ($p = 3.68 \times 10^{-4}$, OR = 0.32, 95%CI = 0.17 – 0.60) and proliferative DR ($p = 8.12 \times 10^{-4}$, OR = 0.30, 95% CI = 0.15 – 0.61). The sight-threatening DR phenotype included DME patients as well as proliferative DR, with significant overlap of phenotype between patients, but the number of patients with DME was small compared to the number with proliferative DR (total sight − threatening = 223, DME = 89, proliferative DR = 181, DM without DR = 228). Given the much larger number of patients with proliferative DR, the association signal was assumed to be driven by these patients.

In another study, McAuley et al. [67] found a significant association between a polymorphism in miRNA-126 and sight-threatening DR. The A allele of rs4636297, known to be the nonfunctional allele for posttranslational regulation of miR-126, was associated with severe sight-threatening DR ($p = 0.006$, OR = 2.02, 95%CI = 1.22 – 3.35). However, DME was not included in their definition of sight-threatening DR. Larger studies specifically evaluating the role of these miRNA variants in DME risk are required to confirm and replicate.

2.10. Other Candidate Genes. Several other genes have been studied in relation to DME risk but failed to show an association. Abhary et al. [68] investigated *carbonic anhydrase* (*CA*) sequence variation as a risk factor for DME (DME = 93 /DM without DR = 235) but found no associations in their cohort. Dong et al. [69] investigated a *monocyte chemoattractant protein-1* (*MCP1*) polymorphism in an Asian DM cohort, and while they observed a significant association with overall DR risk and proliferative DR, they found no association with severity of DME (mild DME = 207, moderate DME = 173, severe DME = 66). Another study by Dong et al. [70] using the same cohort of patients as above [69], analyzed an association between DR susceptibility and polymorphisms in the CXC chemokine family genes, *interleukin 8* (*IL8* or *CXCL8*; -251T>A) and *interferon gamma-induced protein 10* (*IP10* or *CXCL10*; -1596C>T) but failed to observe a significant association in a subgroup analysis of DME patients. Finally, Upadhyay et al. [71] studied the association between the *sarcolemma-associated protein* (*SLMAP*) gene polymorphism, rs17058639, and DR risk. Interestingly, in the subgroup analysis of DR, they did report a significant association of rs17058639 with DME ($p = 0.0425$), but the sample size was very small (DME = 49, DM without DR = 160) and this result may be a false positive.

3. Genome-Wide Association Studies (GWAS)

Genome-wide association studies (GWAS) are aimed at identifying differences in the frequency of common genetic variants across the entire genome between groups of individuals. This technique has revolutionized the field of complex disease genetics [72]. Unlike the candidate gene approach, which depends on an *a priori* hypothesis, GWAS is considered a powerful hypothesis-free tool to identify genotype-phenotype associations and discover associations with

variants in genes that have not been previously considered [72]. To account for the heavy burden of multiple hypothesis testing in a GWAS, the threshold for statistical significance is usually set at 5×10^{-8} when common variants with a population frequency > 5% are analyzed [73]. Small studies will only have the power to reach this stringent threshold when the effect size is very large. However, for many complex diseases, including DME, the expected effect size for most variants is quite low, requiring very large sample sizes to detect. To date, a total of 13 GWAS related to DR risk have been published, of which only two specifically focussed on the DME phenotype [74, 75]. The largest GWAS where DME risk was considered was reported by Meng et al. [75]. This study was done in a well-defined Scottish cohort of T2DM involving 469 DME cases (defined as diabetic maculopathy with decreased visual acuity) and 1,374 controls (DM without DR or maculopathy). A SNP in the *TTC39C* gene, rs9966620, reached genome-wide significance with a p value of 4.13×10^{-8} (OR = 1.95, 95%CI = 1.53 – 2.47). Two nearby SNPs, in linkage disequilibrium with rs9966620, also approached significance (rs7243626, $p = 5.64 \times 10^{-8}$, and rs7240470, $p = 8.05 \times 10^{-7}$). However, a GWAS considering a broader DME phenotype (maculopathy irrespective of vision loss, n = 1,240) failed to identify any SNPs reaching genome-wide significance. Whilst the *TTC39C* gene product is expressed in the eyes, the function of the protein is yet to be elucidated.

Another GWAS related to DR by Graham et al. [74] performed analyses for DME (DME = 270, DM without DR = 435) and proliferative DR. The authors found no genome-wide significant associations with DME risk. Their two highest hits were rs1990145 ($p = 4.10 \times 10^{-6}$, OR = 2.02, 95%CI = 1.50 – 2.72) and rs4771506 ($p = 6.94 \times 10^{-6}$, OR = 1.97, 95%CI = 1.46 – 2.64). The SNP rs1990145 is located in an intron of the *MRPL19* gene on chromosome 2 and rs4771506 is on chromosome 13 near the *LINC00343* gene. Further, this study also evaluated the top SNPs reported in a previous DR GWAS study (T1DM) by Grassi et al. [76]. Two SNPs reported in that study to be associated with DR, rs12267418 near *MALRD1* and rs16999051 within *PCSK2* on chromosome 20, were found to be nominally associated with DME ($p = 0.008$ and $p = 0.007$, respectively). Whilst the above studies provide some evidence for possible novel candidate DME risk genes, given the size of most of the cohorts, these findings need to be replicated in larger studies.

4. Genetic Predictors of Treatment Response

Based on the Early Treatment Diabetic Retinopathy Study (ETDRS), macular laser photocoagulation was the gold standard treatment for managing DME for many years [6]. However, intravitreal injections of VEGF inhibitors have now revolutionized the management of DME. Today, anti-VEGF intravitreal injection with or without adjunct focal laser is the standard of care for treating center-involving DME in most countries [77]. Three commonly used intraocular anti-VEGF agents are aflibercept (Eylea, Regeneron Pharmaceuticals), bevacizumab (Avastin, Genentech), and ranibizumab (Lucentis, Genetech) [77]. Despite the widespread use of anti-VEGF agents, there is wide variability in patient outcomes. This variability was initially apparent in clinical trials, where a significant proportion of patients failed to achieve a functional or anatomical response [78] but is even more striking in the real-world clinical setting [79]. While some of these variations in treatment response can be explained by clinical and environmental factors, it has been postulated that inherited genetic variation may also play a role. Many post hoc analyses from clinical trials [80, 81] and real-world clinical studies [79, 82] have attempted to identify ocular and systemic predictors of response to anti-VEGF treatment. The relationship between genetic variation and response to anti-VEGF has been studied quite extensively in other disorders that use these drugs, including age-related macular degeneration (AMD) [83] and cancer [84], but only four studies (Table 2) have specifically investigated genetic differences between responders and nonresponders to anti-VEGF injections in DME patients [40, 85–87].

Shazly et al. [40] is the only group to report a significant correlation between a patient's genetic profile and response to anti-VEGF (bevacizumab) therapy in DME. The response arm of the study (mentioned above in the Candidate Genes section) involved 64 DME patients. The distribution and allele frequency of the *VEGFA* -634C>G polymorphism (rs2010963) was compared between poor ($n = 24$) and good ($n = 40$) responders. Response was defined based on the change in best-corrected visual acuity and central macular thickness (Table 2) and patients were followed up every month for 9-12 months. The -634C>G polymorphism was selected due to its strong association with DME and DR from previous studies [37, 88]. The study identified a significantly higher CC genotype frequency amongst good responders compared to the poor responder group ($p < 0.001$), even after adjusting for clinical and demographic covariates. Likewise, the frequency of the -634C allele was significantly higher in the good responders compared to the poor responders ($p < 0.001$).

A study by Tetikoglu et al. [85] also investigated *VEGFA* gene polymorphisms (rs2010963, rs2146323, rs10434, rs833069, and rs6921438) and their association with response to intravitreal ranibizumab treatment. The response criteria in this study were less stringent (two lines improvement in visual acuity compared to three lines in Shazly et al.) and the 95 DME patients (good responders = 53, poor responders = 42) were followed up for only 5 months. Despite a significant difference in visual outcome clinically ($p = 0.001$), there was no association between the *VEGFA* polymorphisms and treatment response ($p > 0.05$).

In a pilot study by Dabir *et al.* [86], the authors conducted a gene expression analysis to identify biomarkers that distinguish bevacizumab responders from nonresponders. RNA from whole blood was assessed to identify systemic gene expression signatures relevant to treatment response. The Agilent Human Gene Expression microarray kit was used to generate gene expression data. Analysis of bevacizumab responders ($n = 5$) versus nonresponders ($n = 5$) identified 61 differentially expressed genes (2.5-fold change), 35 of which were upregulated and 26 downregulated. The majority of differentially expressed genes, both up and downregulated,

Table 2: Summary of studies evaluating the association of genetic variants and changes in gene expression with response to anti-VEGF therapies in diabetic macular edema.

Gene and variants	Anti-VEGF drug	DM type	Response criteria	Cohort size	Follow-up (months)	p value	Country	Reference
VEGFA -634C>G (rs2010963)	Bevacizumab	T2	Non-responder: increase in VA < 3 lines AND <50% decrease in CMT Responder: increase in VA ≥ 3 lines AND ≥50% decrease in CMT	64 DME Responder = 36 eyes (24 patients) Non – responder = 68 eyes (40 patients)	9-12	$p < 0.001$	Egypt	Shazly et al. [40]
VEGFA rs2010963 rs2146323 rs10434 rs833069 rs6921438	Ranibizumab	NA	Non-responder: increase in VA < 2 lines AND CMT > 300 microns Responder: increase in VA ≥ 2 lines AND CMT ≤ 300 microns	95 DME Responder = 53 Non – responder = 42	5	$p > 0.05$ for all variants	Turkey	Tetikoğlu et al. [85]
Transcriptome-wide gene expression analysis1	Bevacizumab	T2	Non-responder: stable/worsening/<10% reduction in CMT Responder: >10% reduction in CMT	Responder = 5 Non – responder = 5	NA	35 genes upregulated 26 genes downregulated	India	Dabir et al. [86]
Expression of multiple lncRNA genes2	Aflibercept	T2	Not defined	75 DME Responder = 51 Non – responder = 9 (missing data = 15)	1	$p > 0.05$ for all lncRNAs	Egypt	Toraih et al. [87]

Study evaluated mRNA expression in peripheral whole blood following bevacizumab treatment. Study evaluated serum levels of hyperglycaemia sensitive long non-coding RNA following aflibercept treatment. DM: diabetes mellitus; NA: not available; DME: diabetic macular edema; VA: visual acuity; CMT: central macular thickness; VEGFA: vascular endothelial growth factor A; lncRNA: long non-coding ribo-nucleic acid.

were present in transcription regulation ($n = 25$) or receptor activation ($n = 21$) pathways. However, due to the very limited number of samples in these analyses and no comparison of the same individuals before receiving bevacizumab treatment, the results need to be interpreted with caution.

Recently, Toraiha et al. [87] conducted a study on a Middle-East population exploring the relationship between levels of serum hyperglycemia-related long noncoding ribonucleic acids (lncRNAs) and response to anti-VEGF injection (aflibercept). LncRNAs encode RNA transcripts longer than 200 nucleotides, and despite not being translated into protein, they are capable of regulating several critical biological processes. There is a growing body of evidence implicating lncRNAs in various pathological conditions, including DR. However, Toraih *et al.* [87] found no association with aflibercept response (DME = 75, responder = 51, nonresponder = 9, missing data = 15) and circulating levels of hyperglycemia-related lncRNAs, including retinal noncoding RNA 2, nuclear-enriched abundant transcript 2, cyclin-dependent kinase inhibitor 2B antisense RNA 1, and plasmacytoma variant translocation 1. In contrast to other studies, this study evaluated the treatment response only after a single dose of intravitreal aflibercept, which may not be sufficient to induce robust gene expression changes in the circulation. Evidence shows that not all patients benefit after a single dose of anti-VEGF and it is advisable to wait until at least 3-4 monthly injections have occurred before defining treatment response [89, 90]. Furthermore, the study failed to define the response criteria clearly.

5. Conclusions

A major concern with most studies of DME genetics to date relates to the size of the study cohort. Some studies were conducted with a sample size of less than 50 [15, 86], and caution is warranted when interpreting the results. Even in larger DME cohorts, only nominal associations with genetic polymorphisms were detected [46, 74], not reaching robust statistical significance. It should be noted that there is significant overlap in phenotype between DME and other subtypes of DR. Many patients display multiple phenotypes and attempts to separate and analyze only DME might not always be feasible and practical, contributing to small sample sizes. The spectrum of overlapping DR and DME phenotypes makes it extremely challenging to distinguish genetic effects of relevance to specific subtypes, and also potentially creates heterogeneity when attempting to analyze phenotypes as a group.

Comparing results for specific genes between studies is complicated considering that for most genes, there was no single polymorphism or genetic model consistently investigated. With the exception of the -643G > C variant in *VEGFA*, none of the studies performed replication analyses in an independent cohort, nor directly replicated findings from other studies. In the handful of published treatment response studies, there was striking variation in the follow-up period, the definition of response, and a different anti-VEGF agent was used in each study. These factors also make meta-analysis of multiple studies extremely challenging. Consideration should be given to including commonly used definitions as well as treatment and follow-up regimes when designing new studies to evaluate the genetics of treatment response in DME.

In summary, the role of inherited genetic polymorphisms in DME development and treatment response is still poorly understood, with a paucity of dedicated, well-powered studies in this field. Given the social and economic burden of DME and its impact on an individual's visual morbidity, genetic studies of larger, homogeneous patient cohorts are warranted, including meta-analysis of multiple studies where appropriate. Such studies will not only lead to a greater understanding of DME but may also impact clinical practices including better screening of at-risk populations and distinguishing patients who are more or less likely to respond to anti-VEGF agents. Robust genetic findings may even identify new therapeutic targets to complement and extend the success seen with anti-VEGF agents.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

Authors' Contributions

RLG wrote the main manuscript and prepared tables. LMF, BJM, NV, and KPB reviewed and edited the manuscript.

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Research Article

Optical Coherence Tomography Biomarkers of the Outer Blood—Retina Barrier in Patients with Diabetic Macular Oedema

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Background. Numerous studies confirmed the main role of the inner blood-retinal barrier in the development of Diabetic Macular Oedema (DMO). Lately, the focus of research shifted towards the external retinal barrier with potential involvement in the pathogenesis of DMO. *Objective*. We aim to identify the OCT changes of the external blood-retinal barrier in patients with DMO and to define them as biomarkers with predictive value. *Materials and method*. We set up retrospectively 3 groups of patients diagnosed with nonproliferative diabetic retinopathy (NPDR) and DMO, proliferative diabetic retinopathy (PDR) and DMO, and controls. We compared the RPE thickness in every quadrant between groups and performed correlations between best-corrected visual acuity (BCVA) and the thickness of the retinal layers. The Social Science Statistics platform was used for statistical tests. *Results*. The NPDR-DMO group consisted of 18 eyes, the PDR-DMO group consisted of 19 eyes, and the control group included 36 eyes. In the PDR-DMO group, RPE thickness was decreased in almost all quadrants ($p < 0.001$); in the NPDR-DMO group, only the central minimum and central maximum values of the RPE thickness were significantly different from the control group. We did not find any strong correlation between BCVA and the thickness of the retinal layers. *Conclusion*. The thickness of the RPE layer is an OCT biomarker able to predict the functioning of the outer BRB. Eyes with PDR-DMO exhibited decreased thickness of the RPE layer in almost all quadrants, highlighting the degenerative changes occurring in a hypoxic environment. The thickness of a specific layer could not be identified as a biomarker to correlate significantly with BCVA, most likely because we did not analyze specific morphologic features, such as continuity and reflectivity. The analysis of the RPE thickness could clarify the unexplained decrease of BCVA and predict early the evolution of DR.

1. Introduction

Diabetic macular oedema (DMO) is the main cause of visual impairment within the group of working-age population in developed countries [1]. DMO affects 1 in 15 patients diagnosed with diabetes mellitus (DM), and its prevalence is constantly increasing worldwide. Fluid accumulation in the macular area translates clinically by the decrease of visual acuity (VA), but also by difficulty with facial recognition, reading, or driving [1].

The retina is one of the most metabolically active tissues in the organism, requiring important amounts of glucose and lactose [2]. The need for two distinct blood-retinal barriers (BRB), inner and outer, confirms the complexity of the retina and enhances the need to maintain a homeostatic retinal microenvironment [2]. The primary role of the internal BRB's disruption in the pathogenesis of DMO was confirmed by numerous studies, but it is becoming more and more obvious that also outer BRB is involved in its evolution. Outer BRB separates the neural retina from the choroidal vascularisation which is responsible for approximately 80% of the ocular blood supply [2]. Retinal pigment epithelium (RPE) plays important roles in retinal metabolism: it provides nutrition for the photoreceptors, it removes the metabolic waste

resulted from the phagocytosis of the photoreceptors' outer segments [3], and it is responsible for pumping the extravasated fluid from the internal retinal vessels towards the choriocapillaris, driven by the transport of Cl^- and K^+ [4], thus filling the lack of lymphatics [5]. Furthermore, BRB is involved in the transport and recycle of docosahexaenoic acid, a major component of the photoreceptors [2]. The diabetic retina, characterised by a highly hypoxic environment, stimulates the overexpression of hypoxia-inducible factor (HIF-) 1α and of vascular endothelial growth factor (VEGF). VEGF is also responsible for the depletion of the occludin in RPE, with subsequent disruption of the tight junction's integrity in the outer BRB [6].

Electronic microscopy demonstrated the degeneration of RPE in DMO induced in animal models: shrank nuclei, reduced endoplasmic reticulum, in-folding of the cell membrane, altered melanosome, and even loss of RPE cells [5]. When electroretinogram was performed on a diabetic mice model, a decreased c wave was identified before the occurrence of photoreceptors' dysfunction [7]. Other studies that used fluorescein angiography-based technology distinguished endothelial barrier leakage from RPE barrier-specific leakage [2, 8]. In cell cultures, like RPE-51 and ARPE-19, VEGF upregulated ZO-1α^- and ZO-1α^+ mRNA and proteins, causing an increased TER (transepithelial resistance) which is an indicator of RPE's barrier function. In addition, when soluble VEGF was neutralized with an antibody, it led to partial recovery of the RPE barrier's function [9]. Exposure of ARPE-19 cell line and primary human retinal pigment epithelial to hypoxia increased the secretion of IL-6 and IL-8 and also of VEGF, as shown by Arjama et al. [10], which describes the same environment as in a retina with proliferative diabetic retinopathy (PDR). When RPE proteome was analyzed in diabetic eyes without retinopathy, sixty-two percent of RPE's proteins involved in retinoid metabolism, regulating energy and chaperone proteins, were found to be altered. Moreover, they were also changed in nonretinal tissue, suggesting that RPE is compromised as part of the systemic impact of diabetes [11].

The aim of the present study was to investigate whether the thickness of RPE is modified in patients with DMO associated with nonproliferative diabetic retinopathy (NPDR) or proliferative diabetic retinopathy (PDR), using Spectral Domain-Optical Coherence Tomography (SD-OCT). The novelty of our approach comes from the observation that even if there is evidence in the literature that RPE thickness decreases in patients with DMO [12], there is no distinction so far between the cases with NPDR and PDR associated to the DMO.

The primary outcome of this research is to find out whether there was a difference between groups in RPE thickness. The secondary outcomes are to identify the differences between DMO with NPDR and DMO with PDR versus control. Furthermore, we aimed to investigate if there is a correlation between RPE and inner retinal thickness, photoreceptors, and central macular thickness (CMT). Finally, we intended to identify the OCT biomarkers that correlate best with BCVA: central macular thickness (CMT), inner retinal thickness, photoreceptor layer thickness, or RPE thickness.

2. Materials and Methods

2.1. Study Design. A retrospective, single-centre, observational, and comparative study was carried out. The study was approved by the Ethics Committee belonging to "Iuliu Hatieganu" University of Medicine and Pharmacy (IHUMP), Cluj-Napoca, Romania, and the study protocol adhered to the rules of the Declaration of Helsinki.

2.2. Study Sample. We included in the study the patients diagnosed with type 1 or type 2 DM and with NPDR or PDR associated with DMO. The patients were examined in the Department of Ophthalmology belonging to IHUMP, between January 2017 and September 2019. Patients with an ophthalmological examination in the same setting between July and September 2019, with no history of DM, were selected for the control group. Thus, we set up 3 groups of patients: NPDR with DMO, PDR with DMO, and control. The algorithm according to which the 3 groups were created is presented in Figure 1.

2.2.1. Diabetic Retinopathy Group. An eye was eligible for diabetic retinopathy (DR) group if the following criteria were met: NPDR (level 20-53E of the Early Treatment Diabetic Retinopathy Study (ETDRS) classification) or PDR (61-65 of the ETDRS classification). All the recruitments were performed by an ophthalmologist with experience in medical retina, and they were validated by OCT (Spectralis HRA +OCT, Heidelberg Engineering, Heidelberg, Germany) examination. Patients with a history of vitreoretinal surgery, laser or anti-VEGF injections, AMD or other macular diseases, ocular trauma, lens or corneal opacification, vitreous hemorrhage, tractional retinal detachment, segmentation errors on OCT examination, OCT segmentation quality less than 20 db, or subretinal fluid were excluded. If a patient was confirmed with bilateral DR meeting the selection criteria, but different ETDRS stages, both eyes were included in the study.

2.2.2. Control Group. Every eye included in the study had BCVA equal or above 20/40, refraction with spherical equivalent less than ±5 dpt and had undergone macular OCT imaging. Patients who were confirmed with DM, macular diseases, ocular trauma, glaucoma, significant opacification of the lens or cornea, segmentation errors on OCT examination, OCT segmentation quality less than 20 db were excluded. One eye was randomly selected for the final analysis for each patient within this group.

All participants underwent VA testing measured with the Snellen acuity chart, slit lamp biomicroscopy, dilated eye fundus examination.

The following baseline clinical characteristics were recorded: age, gender, BCVA, DR, and DMO classification.

Demographic data and ophthalmic examination were collected from the hospital's informatic system. OCT data were collected from the OCT database.

2.3. Assessment of OCT Data. OCT was performed using Spectralis OCT (Heidelberg Engineering, Inc., Heidelberg, Germany). The fast macular protocol was used: 25 raster

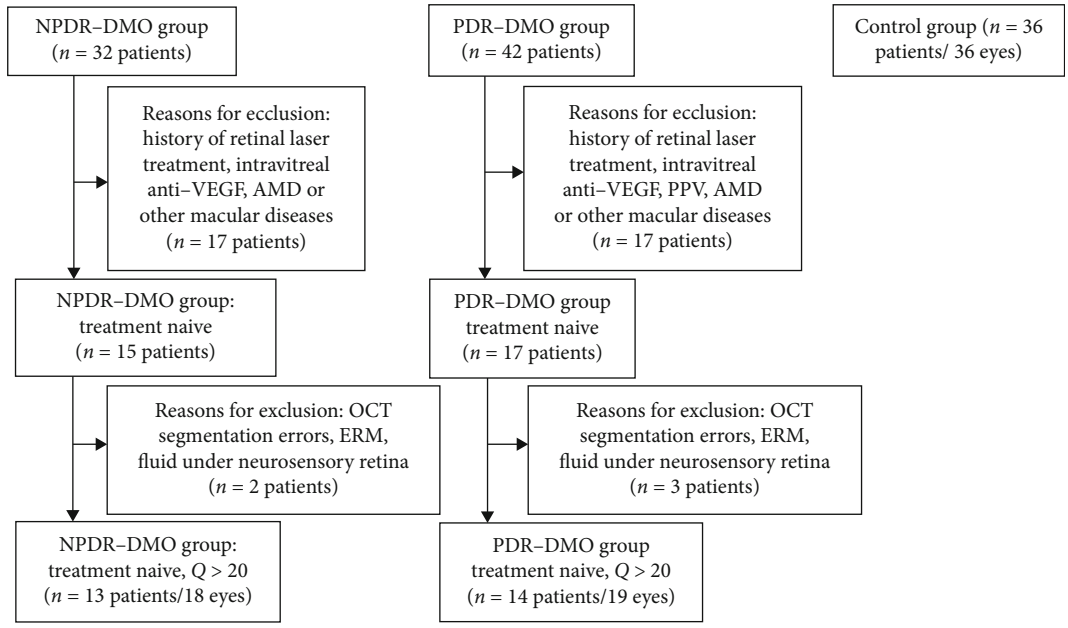

Figure 1: Flow diagram illustrating the study selection process. AMD: age-related macular degeneration; anti-VEGF: anti-vascular endothelial growth factor; PPV: pars plana vitrectomy; ERM: epiretinal membrane, Q: OCT segmentation quality; NPDR-DMO: nonproliferative diabetic retinopathy-diabetic macular oedema; PDR-DMO: proliferative diabetic retinopathy-diabetic macular oedema.

lines per eye separated by 240 μm, with a $20 \times 20^{\circ}$ scan and an automatic real mean value (ART value) set at 9. All scans were performed by the same experienced technician. Segmentation was automatically performed using the Spectralis software version 6.0. Only images with more than 20 db signal strength and with individual retinal layers that could be identified were used for the analysis. ETDRS macular maps were used to report macular thickness: 1, 3, and 6 mm concentric rings. The central 1 mm ring was defined as central thickness. The 3 mm ring, known as the intermediate, was divided into four quadrants: inner superior, inner inferior, inner nasal, and inner temporal, and the 6 mm ring, known as the outer ring, was divided into outer superior, outer inferior, outer nasal, and outer temporal. The numerical values such as thickness and volume recorded for each quadrant were used in the analysis.

The boundaries between the retinal layers are illustrated in Figure 2. We define the following parameters: central macular thickness (CMT)—between ILM and Bruch's membrane; RPE layer—between the outer limit of photoreceptor layer (PR1/2) and Bruch's membrane; outer retina—between ELM and Bruch's membrane, and ONL (outer nuclear layer)—between the outer plexiform layer (OPL) and ELM. The inner retinal thickness was considered from ILM to ELM (Figure 2).

In order to check out the relationship between RPE and the photoreceptors, we approximated the thickness of the photoreceptor layer as follows: from the outer retina, we subtracted the RPE thickness to get the thickness of photoreceptors' inner and outer segments (PR 1/2); then, we added to PR 1/2 the thickness of ONL (rod and cone cell bodies). As a result, the boundaries of the photoreceptor layer are the inner limit of the RPE band and the outer limit of the OPL.

We further detailed the segmentation of the outer retina. Thus, we defined the inner segments of the photoreceptors (IS), the outer segments of the photoreceptors (OS), and the interdigitation zone (IZ). IS are divided into two parts: myoid zone (MZ) and ellipsoid zone (EZ). MZ is a hyporeflective region located between ELM and EZ. It corresponds to the myoid portion of the inner photoreceptors' segments. EZ is a hyperreflective band between MZ and OS, previously known as the junction between photoreceptors' inner and outer segments; it represents the ellipsoid layer of the outer portion of the inner photoreceptors' segments. The OS layer is a hyporeflective band between EZ and IZ. IZ is a hyperreflective band representing the contact between the apices of the RPE cells and the outer segments of the photoreceptors; it was previously called the cone outer segment tips (COST) and rod outer segment tips (ROST).

We defined the thickness of the inner quadrant as the average thickness from all the four inner sectors and the thickness of the external quadrant as the average thickness from all the four outer sectors.

The images were reviewed by the investigator before data analysis, and manual adjustments to retinal layer segmentation were made if necessary.

2.4. Statistical Analysis. In order to perform statistical analysis, the Snellen Visual Acuity fraction was converted into an approximate ETDRS letter score. Numerical variables are summarized with means and standard deviations, whereas the nominal variables are expressed in frequencies and percentages. The nonparametric Kruskal-Wallis test was applied to assess the differences between the thickness of retinal layers among groups followed by post hoc analysis with the Mann-Whitney test if an overall significance was found.

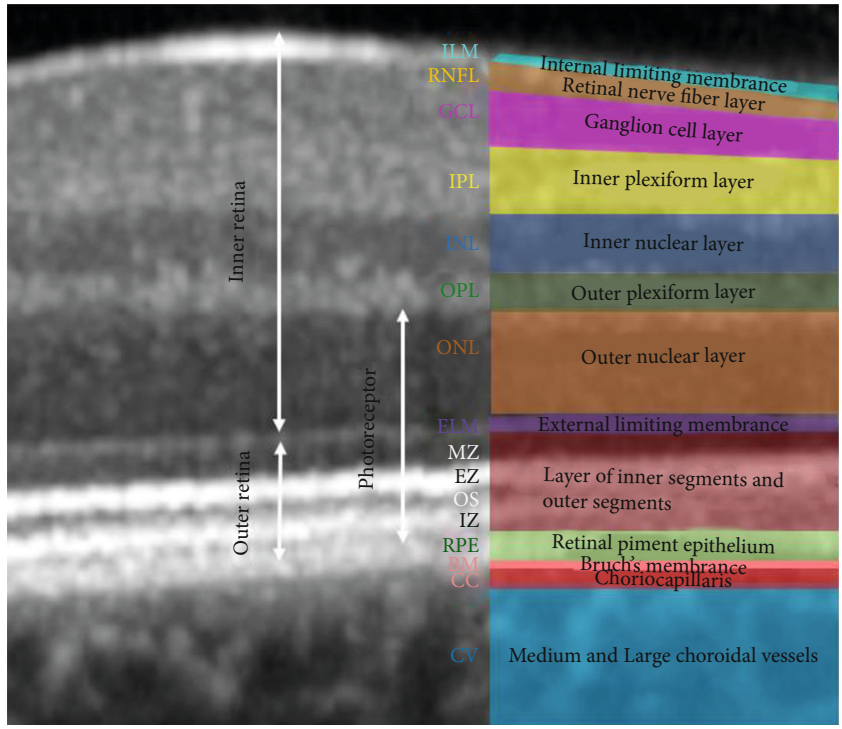

Figure 2: Retinal layer segmentation.

We corrected for the effect of multiple comparisons by conducting a posteriori Bonferroni adjustment. The gender difference between the groups was compared using the chi-squared test.

The Spearman correlation coefficient was used for the detection of correlations between quantitative variables such as the different thickness of layers and VA or age.

p values ≤ 0.05 were considered statistically significant. The platform Social Science Statistics (https://www.socscistatistics.com/) was used to perform the tests.

3. Results

3.1. Demographics and Clinical Characteristics of the Study Samples. A total of 73 eyes were included in the analysis, as follows: 18 eyes within the NPDR-DMO group, 19 eyes within the PDR-DMO group, and 36 eyes within the control group. We used the Kruskal-Wallis test for age and BCVA and the chi-squared test for gender. No statistically significant difference emerged regarding the age and gender distribution between the groups. BCVA was significantly different between NPDR-DMO and control ($p < 0.00001$), PDR-DMO and control ($p < 0.00001$), but not significantly different between NPDR-DMO and PDR-DMO ($p = 0.3125$). The baseline characteristics of these patients are presented in Table 1.

3.2. RPE Thickness and Volume. The RPE thickness and volume in every quadrant (see Table 2) were compared between the groups, and significant results such as internal quadrant ($p < 0.00001$), central subfield ($p = 0.026$), central minimum ($p < 0.00001$), central maximum ($p < 0.00001$), inner nasal ($p = 0.0005$), inner superior ($p = 0.0002$), inner inferior ($p = 0.0017$), outer nasal ($p = 0.02$), and outer superior ($p = 0.009$) were further analysed with the Mann-Whitney test.

The mean RPE thickness in the eyes with PDR-DMO compared to controls was decreased in most quadrants: central minimum (-33.8%), temporal inner (-2.09%), nasal inner (-11.1%), superior inner (-12.5%), nasal outer (-6.76%), superior outer (-8.69%), inferior outer (-3.1%), average RPE (-6.33%), inner quadrant (-7.04%), and outer quadrant (-4.58%). In contrast, in the eyes with NPDR-DMO, the RPE thickness was decreased as compared to controls, for central minimum (-33%), superior inner (-3.97%), and superior outer (-3.62%), but increased for the remaining quadrants (see Figure 3).

After post hoc analysis with the Mann-Whitney test and Bonferroni adjustment, the differences between NPDR-DMO and controls were statistically significant for the central minimum ($p < 0.00001$) and central maximum ($p < 0.00001$) thickness values. Regarding PDR-DMO and controls, differences between central thickness ($p = 0.00008$), central minimum ($p < 0.00001$), central maximum ($p < 0.00001$), inner nasal quadrant ($p = 0.00044$), inner superior ($p < 0.00001$), inner inferior ($p = 0.00058$), and internal quadrant ($p = 0.0014$) were statistically significant. Between NPDR-DMO and PDR-DMO, only the thickness of the inner nasal quadrant ($p = 0.009$) was statistically different (see Table 3).

3.3. Correlations. In the NPDR-DMO group, we identified a high positive correlation between CMT and central RPE ($r = 0.719$) (see Figure 4(a)), inner retina and central RPE ($r = 0.735$) (see Figure 4(e)), a low positive correlation

Table 1: Baseline characteristics of the patients included in the study.

	Control ($n = 36$)	NPDR-DMO ($n = 18$)	PDR-DMO ($n = 19$)	p value
Age, years	53.3 ± 14.19 CI 95% (11.51 to 18.51)	61 ± 8.57 CI 95% (6.43 to 12.85)	57.8 ± 9.56 CI 95% (7.23 to 14.14)	0.151
Gender (F/M)	20 (55.6%)/16 (44.4%)	9 (50%)/9 (50%)	13 (68.4%)/6 (31.6%)	0.497
BCVA, letters	83.75 ± 2.50 CI 95% (2.03 to 3.26)	55.52 ± 24.46 CI 95% (18.22 to 37.23)	45.94 ± 27.57 CI 95% (20.83 to 40.77)	<0.00001

*The results are expressed as mean ± *SD* or frequency with percentages in parentheses. *N*: number; F: female; M: male; BCVA: best-corrected visual acuity; NPDR-DMO: nonproliferative diabetic retinopathy-diabetic macular oedema; PDR-DMO: proliferative diabetic retinopathy-diabetic macular oedema.

Table 2: RPE thickness and volume in each ETDRS macular map quadrant.

RPE	Control	NPDR-DMO	PDR-DMO	Kruskal-Wallis p
Central subfield (μm)	16.2 ± 1.7	18.6 ± 6.9	15.8 ± 2.8	*0.026*
Central minimum (μm)	12.4 ± 1.3	8.3 ± 4.3	8.2 ± 2.8	*<0.00001*
Central maximum (μm)	21.4 ± 2.6	41.1 ± 27.3	32.8 ± 11.6	*<0.00001*
Central volume (mm^3)	0.0106 ± 0.0023	0.0117 ± 0.0038	0.0111 ± 0.0031	0.972
Temporal inner quadrant (μm)	14.3 ± 1.3	14.5 ± 1.7	14 ± 2.58	0.173
Temporal inner volume (mm^3)	0.0218 ± 0.0038	0.0217 ± 0.0038	0.0216 ± 0.0050	0.993
Nasal inner quadrant (μm)	15.3 ± 1.6	15.4 ± 1.5	13.6 ± 1.2	*0.0005*
Nasal inner volume (mm^3)	0.0231 ± 0.0047	0.0233 ± 0.0048	0.0210 ± 0.0032	0.890
Superior inner quadrant (μm)	15.1 ± 1.6	14.5 ± 2	13.2 ± 1.2	*0.0002*
Superior inner volume (mm^3)	0.0222 ± 0.0042	0.0222 ± 0.0043	0.0205 ± 0.0023	0.928
Inferior inner quadrant (μm)	14.2 ± 1.4	14.8 ± 3.8	12.9 ± 1	*0.0017*
Inferior inner volume (mm^3)	0.0214 ± 0.0035	0.0217 ± 0.0051	0.02	0.956
Temporal outer quadrant (μm)	12.7 ± 0.9	12.9 ± 0.8	12.7 ± 2.2	0.277
Temporal outer volume (mm^3)	0.0669 ± 0.0052	0.0683 ± 0.0062	0.0658 ± 0.0126	0.798
Nasal outer quadrant (μm)	13.3 ± 1.2	14 ± 4.1	12.4 ± 1.1	*0.020*
Nasal outer volume (mm^3)	0.0075 ± 0.0711	0.075 ± 0.0218	0.0642 ± 0.0067	0.504
Superior outer quadrant (μm)	13.8 ± 1.4	13.3 ± 1.2	12.6 ± 1.1	*0.009*
Superior outer volume (mm^3)	0.0728 ± 0.0085	0.0706 ± 0.0072	0.0668 ± 0.0075	0.708
Inferior outer quadrant (μm)	12.9 ± 1.1	13 ± 1.6	12.5 ± 1.1	0.120
Inferior outer volume (mm^3)	0.0686 ± 0.006	0.0689 ± 0.0096	0.0674 ± 0.0148	0.832
Average thickness (μm)	14.2 ± 1.1	14.6 ± 1.4	13.3 ± 0.9	0.316
Total volume (mm^3)	0.3845 ± 0.0296	0.3917 ± 0.0371	0.3637 ± 0.0295	0.758
Internal quadrant (μm)	14.4 ± 1.3	14.8 ± 1.4	13.4 ± 1.1	*<0.00001*
External quadrant (μm)	13.1 ± 0.9	13.3 ± 1.4	12.5 ± 1.2	0.165

The results are expressed as mean ± *SD*. The italicized values indicate a statistically significant difference between the groups: $p < 0.05$. RPE: retinal pigment epithelium; NPDR-DMO: nonproliferative diabetic retinopathy-diabetic macular oedema; PDR-DMO: proliferative diabetic retinopathy-diabetic macular oedema.

between photoreceptors and central RPE ($r = 0.383$) (see Figure 4(g)), and a low negative correlation between the central RPE and BCVA (-0.362) (see Figure 4(c)), CMT and BCVA (-3.68), and the inner retina and BCVA ($r = -0.3686$) (see Table 4). In the PDR-DMO group, we found a low positive correlation between the outer retina and BCVA ($r = 0.451$). The remaining correlations were negligible (see Table 4). We compared photoreceptor thickness between the groups: NPDR-DMO vs. control: $p < 0.00001$; PDR-DMO vs. control: $p < 0.00001$; and NPDR-DMO vs. PDR-DMO: $p = 0.4009$.

4. Discussion

Since 1995, when the first study regarding the status of OCT in the diagnosis of macular diseases was published, this new technology has provided important insights into the pathophysiology and treatment of retinal diseases

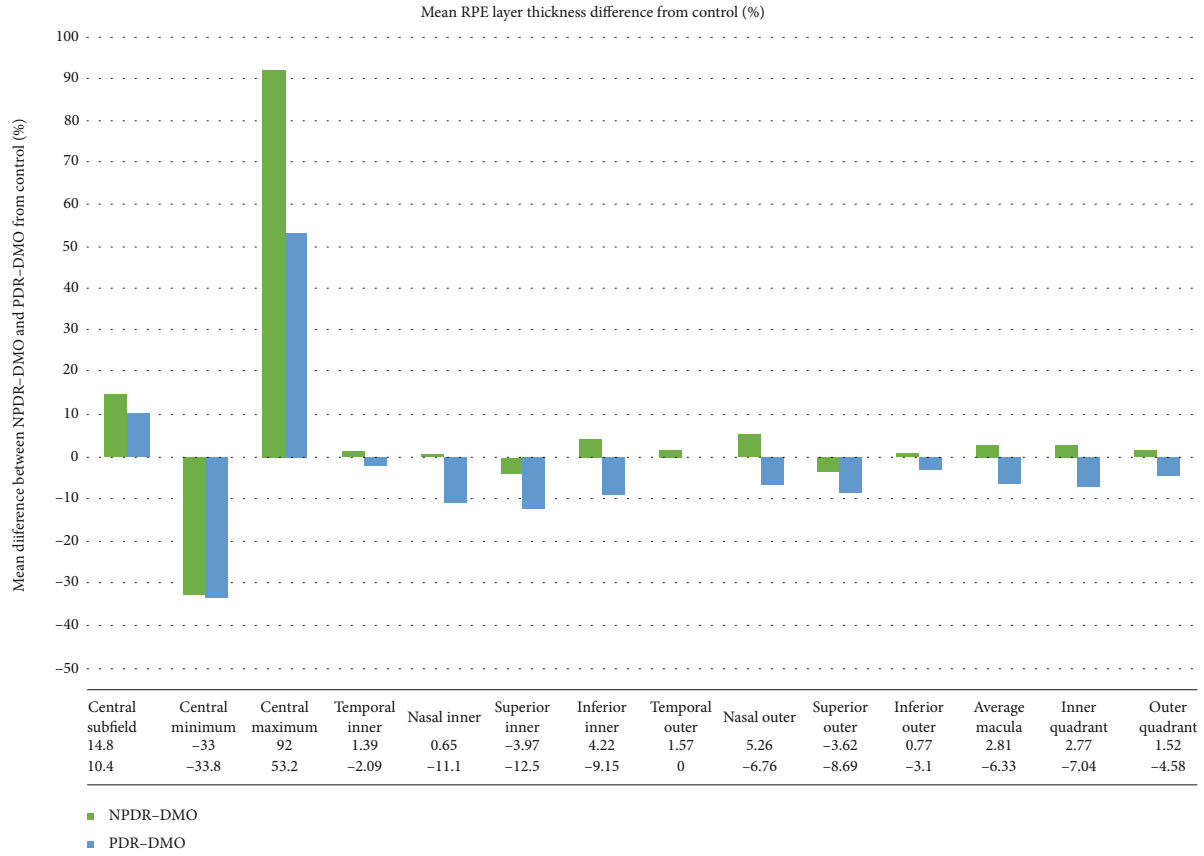

Figure 3: Mean RPE layer thickness difference (%) between the eyes from the control group and NPDR-DMO or PDR-DMO.

Table 3: Post hoc analysis for ETDRS quadrants with a statistically significant difference after the Kruskal-Wallis test.

RPE thickness	Control vs. NPDR-DMO	Control vs. PDR-DMO	NPDR-DMO vs. PDR-DMO
Central subfield	0.039	*0.00008*	0.017
Central minimum	*<0.00001*	*<0.00001*	0.447
Central maximum	*<0.00001*	*<0.00001*	0.741
Nasal inner quadrant	0.936	*0.00044*	*0.0009*
Superior inner quadrant	0.322	*<0.00001*	0.022
Inferior inner quadrant	0.660	*0.00058*	0.009
Nasal outer quadrant	0.841	0.0110	0.019
Superior outer quadrant	0.208	0.0028	0.101
Internal quadrant	0.976	*0.0014*	0.003

The italicized values indicate a statistically significant difference between groups. $p < 0.001$ adjusted Bonferroni. RPE: retinal pigment epithelium; NPDR-DMO: nonproliferative diabetic retinopathy-diabetic macular oedema; PDR-DMO: proliferative diabetic retinopathy-diabetic macular oedema.

[13]. OCT has enhanced the ophthalmologist's understanding of retinal microstructure, to the extent that currently we are able to analyse the anatomy of the photoreceptors and RPE and to anticipate their functioning [14].

For a long time, CMT has been the only biomarker according to which macular oedema was analyzed. However, progress in OCT technology revealed other structural changes, like intraretinal cysts, the disintegration of the retinal structure, flattening of the central fovea, haemorrhages, hard exudates, and subretinal fluid [13].

Since age was similar within our groups, the differences in the thickness between layers cannot be assigned to an age-related diffuse loss of neural tissue, nor to an accumulation of excessive metabolic strain causing an increased thickness [15] or an optical "pseudothickening" due to hyperreflectivity [15].

In the context of increased retinal thickness, especially on the account of INL and OPL [16], the external layers such as RPE and photoreceptors seem to decrease, proving the complex pathogenetic mechanism of DMO.

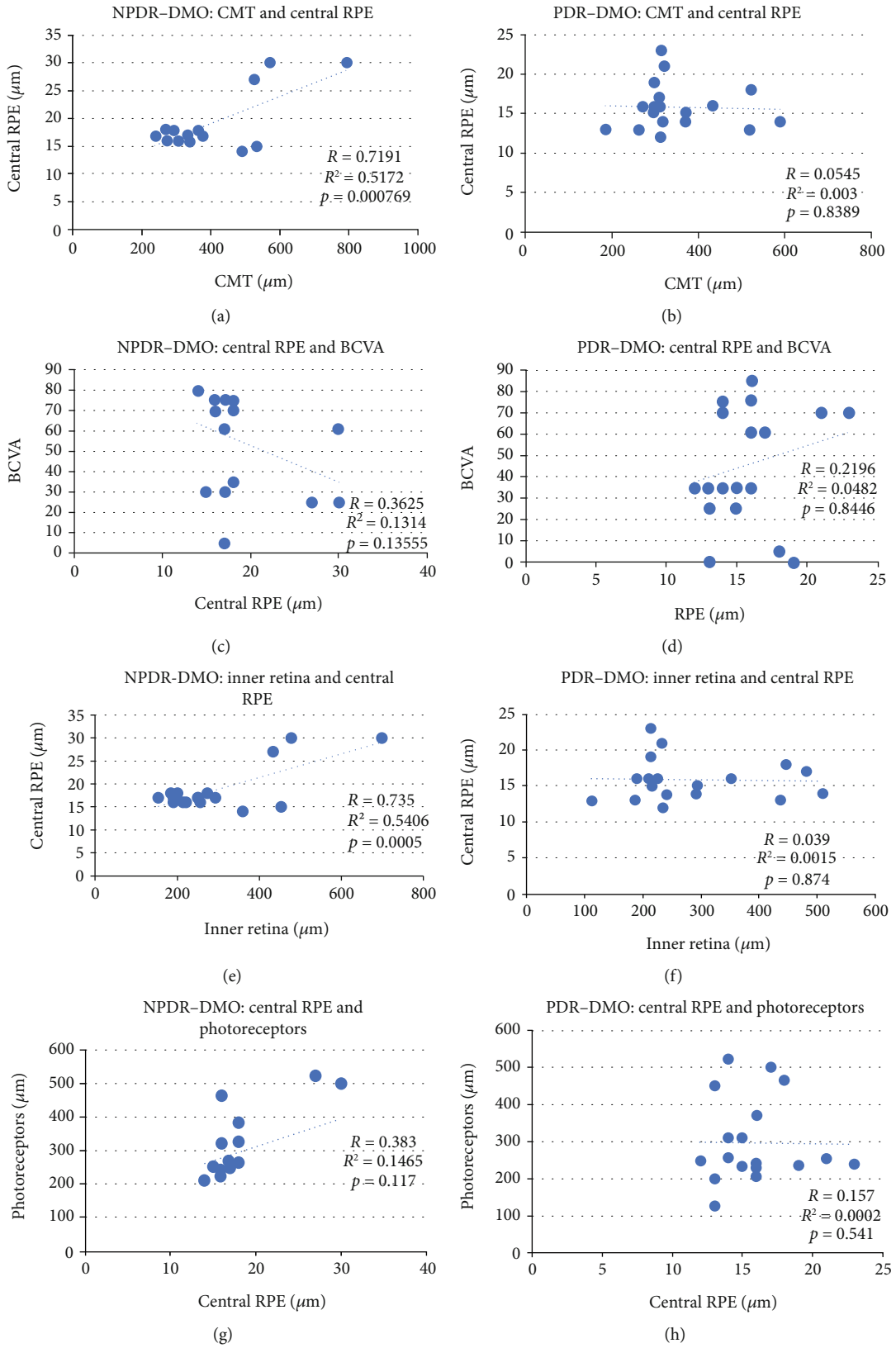

Figure 4: Scatterplots between different variables: (a) CMT and central RPE in NPDR-DMO; (b) CMT and central RPE in PDR-DMO; (c) central RPE and BCVA in NPDR-DMO; (d) central RPE and BCVA in PDR-DMO; (e) inner retina and central RPE in NPDR-DMO; (f) inner retina and central RPE in PDR-DMO; (g) central RPE and photoreceptors' thickness in NPDR-DMO; (h) central RPE and photoreceptors' thickness in PDR-DMO.

Table 4: Correlations between BCVA and retinal layers thickness.

Correlation	NPDR-DMO	R^2	p	PDR-DMO	R^2	p
Central RPE and BCVA	-0.362	0.131	0.153	0.220	0.048	0.845
Outer retina and BCVA	0.086	0.007	0.743	*0.451*	0.203	0.053
CMT and BCVA	-0.368	0.136	0.146	-0.119	0.014	0.654
Photoreceptors and BCVA	-0.0066	0	0.981	-0.102	0.010	0.687
Inner retina and BCVA	*-0.386*	0.149	0.127	-0.069	0.047	0.782
CMT and central RPE	*0.719*	0.517	0.0007	-0.054	0.003	0.839
Inner retina and central RPE	*0.735*	0.541	0.0002	-0.039	0.001	0.874
Photoreceptors and RPE	0.383	0.146	0.117	0.061	0.005	0.785

RPE: retinal pigment epithelium; NPDR-DMO: nonproliferative diabetic retinopathy-diabetic macular oedema; PDR-DMO: proliferative diabetic retinopathy-diabetic macular oedema; CMT: central macular thickness; BCVA: best-corrected visual acuity.

In the PDR-DMO group, apart from CMT, the RPE thickness was decreased in all quadrants. The reason for this finding seems to be a disruption of the RPE-photoreceptors complex [12], possibly due to ischemia, as demonstrated by Reznicek et al. [17] and by Boynton et al. [12]: the thickness of the outer retinal layers, meaning RPE and photoreceptors, was slightly reduced by $\pm 9\,\mu$m and $\pm 8\,\mu$m, respectively. Constant oxidative stress which is a feature of DR impairs autophagy (the removal of damaged organelles and protein aggregates from the same cell) and heterophagy (phagocytosis of exogenous photoreceptor outer segments in RPE cells), as proved by Kaarniranta et al. [18]. In contrast, in the NPDR-DMO group, the number of quadrants with decreased RPE thickness was lower as compared to the PDR-DMO group. This is a reasonable finding when considering that the level of inflammation and ischemia varies according to the stage of DR.

However, higher than the normal values were found occasionally when measuring RPE thickness, as proved within the groups of CMT in PDR-DMO and NPDR-DMO. One possible explanation is that over the RPE cells, new cells grow in order to compensate and to minimise the fluid leakage within the retina [5]. Another hypothesis is that the disturbance of the RPE cells' phagocytosis induces the accumulation of shed outer segments that are not timely engulfed in the RPE-photoreceptors' complex [19].

When we examined the RPE volume, in the PDR-DMO group in all quadrants, the values were decreased as compared to controls, but the differences were not statistically significant. In the NPDR-DMO group, in some quadrants, the volume was increased, whereas in other quadrants, it was decreased. This is probably due to the oedema within the layers and the lower ischemic status.

Besides its leading role in the diagnosis and monitoring of the response to treatment, OCT delivers biomarkers able to predict BCVA. Over time, multiple hypotheses were tested. The most frequently used OCT biomarker was CMT, but scenarios in which the normalization of CMT was not paralleled by the improvement of BCVA or with a modest correlation between the two variables were described [20]. Further on, the correlation between BCVA and the inner retina was evaluated; Sun et al. described the disorganization of the inner retinal layers and he named it DRIL. He proved that although associated with worse BCVA, it predicts better the BCVA outcome [21]. Later on, the integrity of ELM and IS/OS was found to be positively correlated with BCVA [22–26].

Taking into account the multiple roles played by the RPE for the normal functioning of the photoreceptors, the search for a correlation with BCVA is mandatory. In the PDR-DMO group, we found only a low positive correlation between the outer retina and BCVA. In the NPDR-DMO group, a low negative correlation was identified between CMT, central RPE thickness, inner retina thickness, and BCVA. Our results are limited by the analysis of cell thickness, not morphology. Therefore, thickness within the normal range is compatible with altered cellular anatomy. IS/OS and ELM are useful hallmarks to evaluate the integrity of the foveal photoreceptor layer, being closely associated with the final BCVA [27]. BCVA before treatment and photoreceptor status can predict the potential restoration of photoreceptor integrity and subsequent visual recovery in DMO [28].

Further on, we intended to find out if there is any correlation between the CMT and the central thickness of the RPE, namely, whether the RPE thickness will influence the CMT. In the NPDR-DMO group, the correlation was highly positive, whereas in the PDR-DMO group, it was negative, but negligible. This finding could be explained by a higher level of oedema within the retinal layers in the NPDR-DMO group, as compared to the PDR-DMO one.

We also set out to identify if there was any correlation between the internal and external retinal barriers, by approximating an overlap with the OCT layers: inner retina = internal BRB and RPE = external BRB. As Das et al. [21] have found, DRIL was strongly associated with the disruption of ELM and EZ, and the retinal thickness at the fovea (RTF) was increased in the presence of DRIL, suggesting that the inner retinal disorganization could be responsible for the disruption of the outer retinal architecture. They concluded that the breakdown of BRB in DMO could set the stage for the damage of ELM and EZ. We found a highly positive correlation between the thickness of the inner retina and the thickness of the central RPE in the NPDR-DMO group, but a low negative one in the PDR-DMO group. Therefore, it appeared obvious that in patients with DMO, the level of retinopathy is of utmost importance. Thereby, in NPDR, oedema involves the entire retina, whereas in PDR, macular oedema is driven mainly by ischemia and to a lesser extent by a vasogenic

mechanism. Moreover, as Zhang et al. [29] underlined, high glucose promotes the production of reactive oxygen species (ROS) and cell apoptosis, and it inhibits mitophagy, whereas low glucose, although it induces ROS production and cell mitophagy, has a lesser impact on cell apoptosis and proliferation.

It is well known that RPE is a monolayer of pigmented cells that are vital for the photoreceptors' functioning, survival, and maintenance. After having proved the role of RPE damage in the pathogenesis of DMO, we aimed to quantify its effect on the photoreceptor layer. RPE and photoreceptor layers are regarded as a functional unit due to their interdependence. Structural and functional changes of this complex were found also in patients with DR without DMO [19]. When analysing the total thickness of the photoreceptors (inner and outer segment plus ONL), the decreased values we found in the PDR-DMO group and in the NPDR-DMO group could be attributed to a thinner PROS (photoreceptor outer segment) length in the context of a relative outer retinal hypoperfusion induced by hypoxia, as shown by Verma et al. [30]. As Nesper et al. [31] and Muir et al. [32] pointed out, the decrease of choroidal blood flow creates a hypoxic environment for the RPE and photoreceptor cells with subsequent disruption of phagocytosis and increased fragility of the RPE cells. In a feedback loop, more superoxide and soluble inflammatory factors are produced that aggravate the condition [19].

Ferreira et al. [33] have reported a thicker RPE layer and a thinner photoreceptor layer in patients with DM without DR, as opposed to the nondiabetic controls.

When comparing the results between studies, we must pay attention to the type of OCT machine and to the segmentation algorithm of the outer retina because different results could emerge [19]. Xia et al. reported that the increase of the RPE-photoreceptor thickness precedes the alterations of the retinal nerve fibre layer (RNFL) or of the ganglion cell layer (GCL) [19].

Our study has several limitations: the small sample size, the quantitative assessment of the RPE layer, and the selection bias. The strength of our study comes from the different approach of making the distinction between the NPDR and PDR within the group of patients with DMO. Our results add to previous research serving as evidence for the key part played by the changes in the RPE layer during the evolution of DR.

5. Conclusions

In the PDR-DMO group, apart from CMT, RPE thickness was significantly decreased in almost all quadrants in our series. In the NPDR-DMO group, the number of quadrants with significantly decreased RPE thickness was lower as compared to the PDR-DMO group, proving the key impact of DR staging on DMO.

In the PDR-DMO group, we found only a low positive correlation between the outer retina and BCVA. In the NPDR-DMO group, a low negative correlation was identified between the central RPE thickness and BCVA.

In the NPDR-DMO group, the correlation between the CMT and the central RPE thickness was highly positive, whereas in the PDR-DMO group, it was negative, but negligible.

The photoreceptors' thickness was significantly lower in both groups, PDR-DMO and NPDR-DMO.

Further and more refined studies are needed to provide definite OCT biomarkers by analyzing the outer BRB in patients with DMO.

Data Availability

The data used to support the findings of this study are available from the corresponding author upon request.

Conflicts of Interest

The authors declare that there is no conflict of interest regarding the publication of this paper.

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Research Article

Renal Biomarkers for Treatment Effect of Ranibizumab for Diabetic Macular Edema

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Aims. To investigate the correlations between renal biomarkers and the treatment outcomes of ranibizumab for diabetic macular edema (DME). *Methods*. This hospital-based study retrospectively enrolled 88 eyes from 67 patients who had received one-year intravitreal ranibizumab treatment for DME. Best-corrected visual acuity (BCVA) and optical coherence tomography (OCT) at baseline and during the follow-up period were recorded. BCVA and OCT characteristics at baseline and their changes after ranibizumab treatment were compared between different proteinuria and estimated glomerular filtration rate (eGFR) groups. *Results*. Of the 88 eyes studied, those with moderately increased proteinuria had a thicker central subfield foveal thickness (CFT) and a higher proportion of intraretinal cysts than those with no proteinuria ($P = 0.012$ and 0.045, respectively) at baseline. After one year of ranibizumab treatment, the reduction in CFT was greater in patients with severely increased proteinuria than those with normal to mildly increased proteinuria ($P = 0.030$). On the other hand, patients with an eGFR <30 tended to have poorer visual improvements than those with normal eGFR ($P = 0.044$). *Conclusions*. After ranibizumab treatment for DME, patients with severe proteinuria tended to gain better anatomical improvement, while those with poor eGFR tended to have poorer visual improvement.

1. Introduction

Diabetic macular edema (DME) is the main reason for the visual deterioration in patients with diabetes [1]. With the advent of optical coherence tomography (OCT), investigators have classified the morphological patterns into diffuse retinal thickening, intraretinal cyst, and subretinal fluid [2]. The disorganization of the blood-retinal barrier was regarded as a key event in the development of DME. This process encompasses a wide variety of cytokines under chronic hyperglycemia, among which hypoxia-induced release of vascular endothelial growth factor (VEGF) played an essential role [3]. Ranibizumab (Lucentis; Genentech, South San Francisco, CA) is a humanized monoclonal antibody Fab fragment against all isoforms of VEGF-A. Intravitreal ranibizumab (IVR) have shown anatomical and visual improvements in several large randomized clinical trials (RCTs) [4, 5]. In light of these discoveries, anti-VEGF drugs have been the treatment of choice for DME in recent years [3].

Recently, researchers have shown an increasing interest in the association of DME and chronic kidney disease (CKD). Some cross-sectional studies revealed that macroalbuminuria, but not eGFR, was related to DME [6–8]. In a prospective cohort study conducted by our group [9], patients with DME at baseline also had higher serum creatinine and lower eGFR at baseline; but for those without DME, abnormal baseline urinary albumin/creatinine ratio (UACR), not eGFR, was significantly associated with the development of new-onset DME during the follow-up period. This implies that proteinuria and eGFR may play different roles in the pathophysiology of DME.

Given the growing studies concerning the association of CKD and DME, far too little attention has been paid to the impact of renal profiles on the treatment effect for DME. In

the post hoc analysis of 2 randomized trials, RISE and RIDE, serum creatinine or eGFR was irrelevant to visual change after 2 years of ranibizumab treatment [10]. However, a retrospective study reported that patients with higher serum creatinine level were prone to poorer visual improvement following bevacizumab treatment for DME [11]. Despite the presumably distinct effects of proteinuria and eGFR as proposed above, no previous studies have dealt with the role of UACR in the treatment for DME. In this study, we aim to evaluate the association between renal biomarkers and the treatment outcomes of intravitreal ranibizumab for DME.

2. Materials and Methods

2.1. Study Population. This study retrospectively collected data from patients who started receiving IVR for DME at the National Taiwan University Hospital between January 2013 and December 2017. Inclusion criteria included the following: (1) diabetic retinopathy documented by fundus photography or fluorescein angiography (FA); (2) macular edema with the presence of retinal thickening, intraretinal cysts, or subretinal fluid and a central subfield foveal thickness (CFT) greater than 300 μm as documented by optical coherence tomography (OCT); (3) best-corrected visual acuity (BCVA) between 20/400 and 20/40 at baseline; (4) available record of estimated glomerular filtration rate (eGFR) at baseline or during the first year of treatment; and (5) available record of UACR, urinary protein-creatinine ratio (UPCR), hemodialysis, or peritoneal dialysis at baseline or during the first year of treatment. Exclusion criteria included the following: (1) eyes with vitreomacular traction or tractional retinal detachment with or without macular involvement demonstrated by OCT or fundoscopy; (2) eyes with choroidal neovascularization or any other retinal vascular diseases such as retinal vein occlusion documented by FA; and (3) eyes that did not receive regular treatment and follow-up during the first year of treatment. After recruitment, a total of 88 eyes from 67 patients were enrolled in this study. All cases received three consecutive, monthly intravitreal injections of ranibizumab as the loading treatment, and then received treatment as needed after month 3 based on the combination of clinical presentation, doctors' suggestions, and patients' decision. Generally, if the macular edema had been subsided, or the BCVA and CFT had been stationary for two consecutive visits, no injection would be given. If recurrent macular edema was noted during the follow-up visits, ranibizumab injection would be given again. The subsequent follow-up interval was on a monthly basis and could be extended to up to 3 months given stable treatment outcome. This research adhered to the tenets of the Declaration of Helsinki, and approval was obtained from the Institutional Review Board of the National Taiwan University Hospital.

2.2. Data Collection. The following data was collected for all cases: best-corrected visual acuity (BCVA) and OCT measured at baseline, 3 months, 6 months, and 12 months. BCVA was measured with Snellen charts and was converted to the logarithm of the minimum angle of resolution (logMAR). CFT and the presence of intraretinal cysts or subretinal fluid at central fovea were obtained from OCT. Age, sex, status of hypertension, serum HbA1c level, and staging of diabetic retinopathy at baseline were recorded. Serum creatinine, UACR, and UPCR at baseline and during the follow-up periods were also recorded.

2.3. Measurements for Proteinuria and eGFR. The extent of proteinuria was classified into four groups: normal to mildly increased proteinuria, moderately increased proteinuria, severely increased proteinuria and dialysis. Those who had received hemodialysis or peritoneal dialysis before or during the study period belonged to the group of dialysis. Severely increased proteinuria was defined as having a UACR more than 0.3 or a UPCR more than 0.5 at baseline or any point in time during the first year of treatment. Moderately increased proteinuria was defined as having a UACR more than 0.03 or a UPCR more than 0.15, but no severely increased proteinuria at baseline or any time point during the first year of treatment. Those who always had a UACR less than 0.03 or a UPCR less than 0.15 were thought to have normal to mildly increased proteinuria. The eGFR was calculated using the equation recommended by the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI). The eGFR levels were divided into four groups for regression analysis: >90 mL/min, 61–90 mL/min, 30–60 mL/min, and <30 mL/min.

2.4. Statistical Analysis. BCVA was converted to the logarithm of the minimum angle of resolution (logMAR) for calculation. Wilcoxon rank-sum tests were used to compare the continuous variables, and Fisher's exact tests were used to examine the categorical variables between different groups of proteinuria and eGFR. Paired t-tests were used to compare the logMAR and CFT before and after treatment. Multiple linear or logistic regression models were used to evaluate the correlations between changes of BCVA or OCT characteristics and proteinuria or eGFR. When evaluating the effect of proteinuria, eGFR was adjusted in the regression models, and vice versa. Other covariates including age, hypertension, serum HbA1c level, DR staging, panretinal photocoagulation, baseline BCVA or OCT characteristics, and total injection numbers were also adjusted in the regression models. Stepwise covariate selection was used for all models to avoid over parametrization. A P value less than 0.05 was considered statistically significant. SAS 9.4 (SAS Institute Inc., Cary, NC, USA) was used for all statistical analyses.

3. Results

3.1. Baseline Characteristics. The mean age of the 65 patients was 62.0 ± 10.1 years (32 to 83 years); 33 were female and 32 were male. Seventy- four percent of them had hypertension, and the mean serum HbA1c level was 7.2 ± 1.1%. Of the 86 eyes studied, 2 had mild nonproliferative diabetic retinopathy (NPDR), 12 had moderate NPDR, 24 had severe NPDR, 15 had treatment-naive proliferative diabetic retinopathy (PDR), and 33 had PDR with previous panretinal photocoagulation. The mean logMAR of BCVA was 0.78 ± 0.38, and

Table 1: Demographic data, visual acuity, optical coherence tomography characteristics, and injection number in four different proteinuria groups at baseline.

	A: normal to mildly increased proteinuria ($n = 16$)	B: moderately increased proteinuria ($n = 16$)	C: severely increased proteinuria ($n = 43$)	D: dialysis ($n = 11$)	P value A vs. B	P value A vs. C	P value A vs. D
Age (year)	63.2 ± 9.3	60.8 ± 5.4	64.4 ± 11.3	57.5 ± 9.8	0.41	0.74	0.13
Sex (female)	44%	69%	63%	18%	0.29	0.24	0.23
Hypertension	56%	56%	88%	73%	1	0.011	0.44
HbA1c (%)	7.7 ± 1.4	7.2 ± 0.8	7.2 ± 0.9	6.5 ± 0.8	0.50	0.45	0.027
DR staging					0.13	0.23	0.75
Mild NPDR	6%	0%	0%	9%			
Moderate NPDR	25%	6%	14%	9%			
Severe NPDR	31%	19%	30%	27%			
Treatment-naive PDR	19%	19%	16%	18%			
PDR with PRP	19%	56%	40%	36%			
LogMAR of BCVA	0.73 ± 0.36	0.65 ± 0.29	0.81 ± 0.42	0.90 ± 0.37	0.84	0.56	0.26
CFT (μm)	383 ± 73	469 ± 96	430 ± 127	440 ± 103	0.025	0.49	0.19
Intraretinal cysts	75%	100%	93%	100%	0.10	0.078	0.12
Subretinal fluid	19%	25%	21%	9%	1	1	0.63
Injection number	3.8 ± 2.1	6.0 ± 2.1	5.4 ± 2.2	4.0 ± 1.5	0.008	0.015	0.41

CFT: central foveal thickness; DR: diabetic retinopathy; eGFR: estimated glomerular filtration rate; LogMAR of BCVA: logarithm of the minimum angle of resolution of best-corrected visual acuity; NPDR: nonproliferative diabetic retinopathy; PDR: proliferative diabetic retinopathy; PRP: panretinal photocoagulation.

the mean CFT was 430 ± 112 μm at baseline. Table 1 showed the baseline characteristics in different proteinuria group. Eyes with moderately increased proteinuria had a thicker mean CFT (469 ± 96 μm) than those with normal to mildly increased proteinuria (383 ± 73 μm, $P = 0.025$). Table 2 showed the baseline characteristics in different eGFR group. There were no differences in baseline BCVA or CFT among different eGFR groups ($P > 0.05$ for all).

3.2. Visual and Anatomical Improvements after Ranibizumab Treatment. After the ranibizumab treatment, the mean logMAR of BCVA improved from 0.78 ± 0.38 at baseline to 0.64 ± 0.36, 0.62 ± 0.34, and 0.63 ± 0.38 at months 3, 6, and 12, respectively ($P < 0.001$ for all). The mean CFT decreased from 430 ± 112 μm at baseline to 308 ± 90 μm, 323 ± 99 μm, and 302 ± 93 μm at months 3, 6, and 12, respectively ($P < 0.001$ for all). The mean injection number during the first year of ranibizumab treatment was 5.0 ± 2.2.

3.3. Correlations between Proteinuria and the Treatment Effects of Ranibizumab. Compared to eyes with normal to mildly increased proteinuria (3.8 ± 2.1), those with moderately or severely increased proteinuria received more ranibizumab injections within 12 months (6.0 ± 2.1, $P = 0.008$; and 5.4 ± 2.2, $P = 0.015$, respectively). Figure 1 showed the changes in BCVA and OCT characteristics after ranibizumab use in four different proteinuria groups. No obvious differences in visual improvement were noted among the four groups. Those with proteinuria or under dialysis had thicker CFTs at baseline; however, they responded well to ranibizumab, and the CFTs became thinner than those without proteinuria at month 12 after the ranibizumab treatment. Similarly, those with proteinuria or under dialysis had higher proportions of intraretinal cysts at baseline, but they responded well to ranibizumab treatment with obvious resolution of intraretinal cysts. On the contrary, the proportion of intraretinal cyst did not decrease after ranibizumab treatment in those with normal to mildly increased proteinuria. As for subretinal fluid, the proportions were similar among the four groups at baseline; however, only those with normal to mildly increased proteinuria responded poorly to ranibizumab treatment in subretinal fluid resolution. After adjustment for baseline characteristics and injection numbers in multiple regression models, the reduction in CFT was still 69 μm greater in eyes with severely increased proteinuria than those with normal to mildly increased proteinuria ($P = 0.016$) (Table 3).

3.4. Correlations between eGFR and Treatment Effects of Ranibizumab. Compared to eyes with an eGFR >90 (5.7 ± 2.2), those with an eGFR between 61 and 90 received

Table 2: Demographic data, visual acuity, and optical coherence tomography characteristics in four different eGFR groups at baseline.

	A: eGFR >90 ($n = 23$)	B: eGFR 61-90 ($n = 21$)	C: eGFR 30-60 ($n = 23$)	D: eGFR <30 ($n = 19$)	*P* value A vs. B	*P* value A vs. C	*P* value A vs. D
Age (year)	59.0 ± 10.2	61.2 ± 8.0	69.2 ± 8.7	60.5 ± 10.2	0.84	0.002	0.50
Sex (female)	61%	67%	48%	42%	0.33	0.37	0.20
Hypertension	65%	57%	100%	74%	0.76	0.004	0.74
HbA1c (%)	7.5 ± 1.2	7.2 ± 0.9	7.5 ± 1.1	6.6 ± 0.8	0.57	0.77	0.030
DR staging					0.56	0.51	0.92
Mild NPDR	0%	0%	4%	5%			
Moderate NPDR	17%	10%	17%	11%			
Severe NPDR	26%	19%	39%	26%			
Treatment-naive PDR	17%	10%	22%	21%			
PDR with PRP	39%	62%	17%	37%			
LogMAR of BCVA	0.76 ± 0.35	0.68 ± 0.42	0.79 ± 0.42	0.88 ± 0.37	0.17	0.97	0.35
CFT (μm)	439 ± 116	450 ± 128	423 ± 110	407 ± 93	0.99	0.50	0.54
Intraretinal cysts	83%	95%	91%	100%	0.35	0.67	0.11
Subretinal fluid	22%	24%	26%	5%	1	1	0.20
Injection number	5.7 ± 2.2	4.0 ± 2.3	5.7 ± 2.1	4.7 ± 1.8	0.018	0.95	0.18

CFT: central foveal thickness; DR: diabetic retinopathy; eGFR: estimated glomerular filtration rate; LogMAR of BCVA: logarithm of the minimum angle of resolution of best-corrected visual acuity; NPDR: nonproliferative diabetic retinopathy; PDR: proliferative diabetic retinopathy; PRP: panretinal photocoagulation.

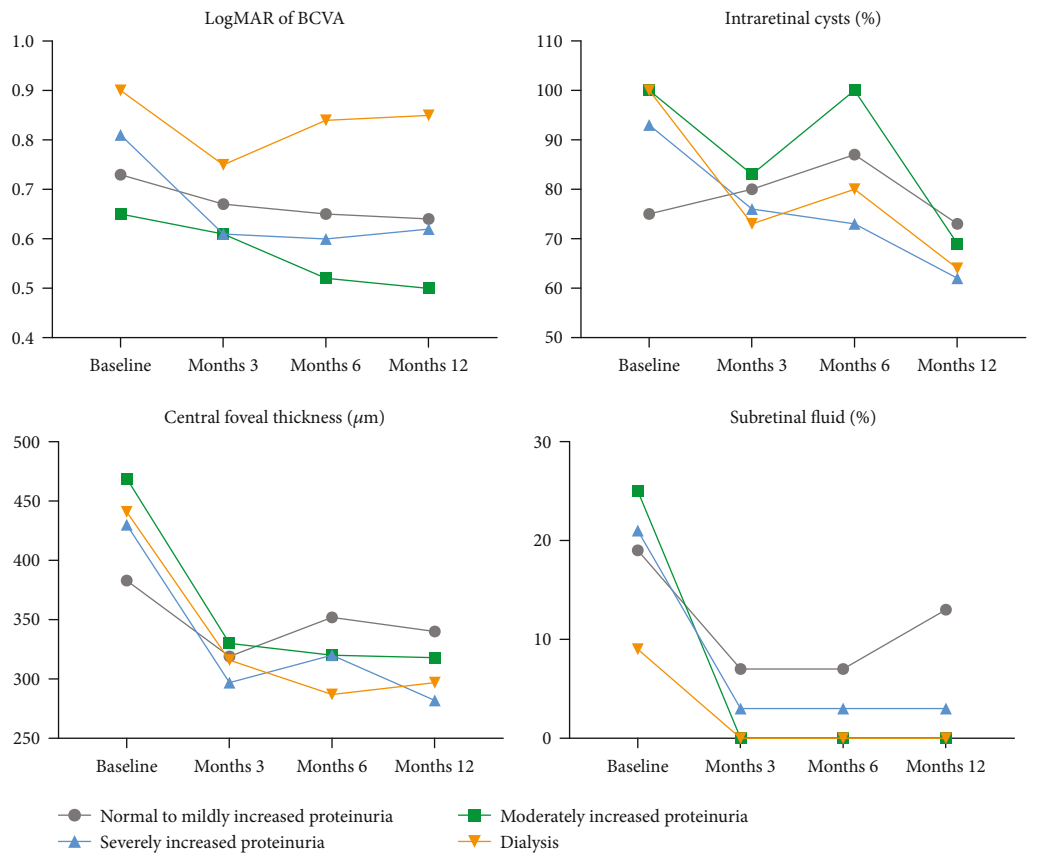


Figure 1: Changes in visual acuity (presented in logMAR of BCVA), central foveal thickness, presence of intraretinal cyst, and presence of subretinal fluid after intravitreal ranibizumab injection for diabetic macular edema in different groups of proteinuria.

Table 3: Correlation between proteinuria status and changes in visual acuity and optical coherence tomography characteristics after month 12 after ranibizumab treatment.

Changes at month 12	Correlation coefficient or odds ratio				*P* value		
	A: normal to mildly increased proteinuria ($n = 16$)	B: moderately increased proteinuria ($n = 16$)	C: severely increased proteinuria ($n = 43$)	D: dialysis ($n = 11$)	A vs. B	A vs. C	A vs. D
LogMAR of BCVA	Reference	-0.26	-0.21	-0.21	0.053	0.063	0.28
CFT (μm)	Reference	-39	-69	-56	0.24	0.016	0.15
Intraretinal cysts	Reference	3.27	4.47	4.62	0.28	0.079	0.17
Subretinal fluid	Reference	*	*	*	*	*	*

CFT: central foveal thickness; LogMAR of BCVA: logarithm of the minimum angle of resolution of best-corrected visual acuity.

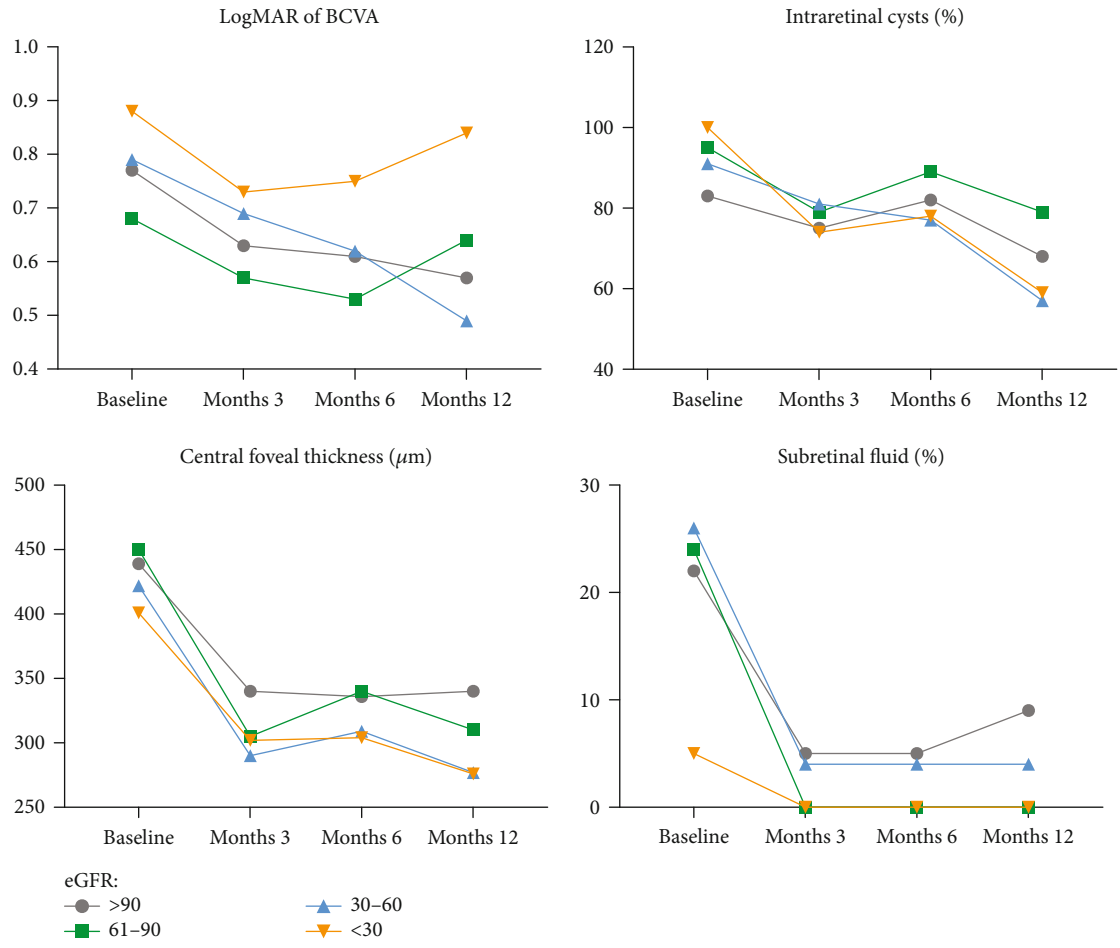


Figure 2: Changes in visual acuity (presented in logMAR of BCVA), central foveal thickness, presence of intraretinal cyst, and presence of subretinal fluid after intravitreal ranibizumab injection for diabetic macular edema in different groups of eGFR level.

less ranibizumab injections with 12 months (4.0 ± 2.3, $P = 0.018$). Figure 2 showed the changes in BCVA and OCT characteristics after ranibizumab use in four different eGFR groups. Those with an eGFR <30 tended to have poorer baseline and final BCVA. No obvious trends in changes of CFT were noted among the four groups. The proportion of intraretinal cysts seemed to be higher in those with poor eGFR at baseline (although of no statistical significance), but the final results after treatment varied. As for subretinal fluid, those with normal eGFR seemed to respond worse to ranibizumab treatment in subretinal fluid resolution. After adjustment for baseline characteristics and injection numbers in multiple

Table 4: Correlation between estimated glomerular filtration rate and changes in visual acuity and optical coherence tomography characteristics after Month 12 after ranibizumab treatment.

Changes at month 12	Correlation coefficient or odds ratio				*P* value		
	A: eGFR >90 ($n = 23$)	B: eGFR 61-90 ($n = 21$)	C: eGFR 30-60 ($n = 23$)	D: eGFR <30 ($n = 19$)	A vs. B	A vs. C	A vs. D
LogMAR of BCVA	Reference	0.115	-0.04	0.23	0.19	0.67	0.040
CFT (μm)	Reference	-29	-50	-58	0.31	0.080	0.056
Intraretinal cysts	Reference	1.10	2..81	3.88	0.91	0.20	0.11
Subretinal fluid	Reference	*	*	*	*	*	*

CFT: central foveal thickness; eGFR: estimated glomerular filtration rate; LogMAR of BCVA: logarithm of the minimum angle of resolution of best-corrected visual acuity.

regression models, although those with an eGFR <30 had a borderline tendency of more reduction in CFT ($P = 0.056$), they still had poorer visual improvement when compared with those with an eGFR >90 ($P = 0.040$) (Table 4).

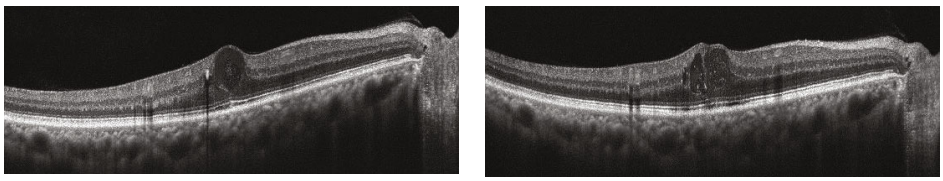
4. Discussion

In this study, we found that the severity of proteinuria was correlated with baseline CFT and the presence of intraretinal cysts at baseline. The association between albuminuria and DME had been reported in the previous studies [7–9, 12]. Both microalbuminuria and macroalbuminuria were recognized as risk factors for the development of DME, with the latter exerting a greater impact [6, 8]. Similar to our study, investigators have found no significant effects of eGFR on the baseline characteristics of DME [7–9, 13]. While albuminuria and eGFR were the two most important markers of diabetic CKD progression [14], distinct associations of DME with albuminuria and with eGFR might suggest different pathophysiological processes.

The current opinion on the development of DME has been largely focused on the breakdown of retinal barrier mediated by VEGF and other inflammatory cytokines [3, 15, 16]. Patients with CKD were found to have elevated serum VEGF, to which the eGFR level was inversely related [17]. Meanwhile, increased VEGF could also lead to higher vessel permeability and protein filtration in glomeruli [18] and in retina [3]. Nonsignificant associations between eGFR and DME, however, defied the pathogenesis of DME with the detrimental effects of VEGF alone. Admittedly, VEGF was a key to barrier dysfunction, but altered capillary dynamics controlled by the Starling forces could even compound the extent of fluid leakage upon barrier disruption [19]. Albuminuria with marked protein loss may lower the oncotic pressure and thus drove intravascular fluid into the interstitial tissue [20]. On the other hand, it was reported that overhydration in CKD was associated with DME [21]. The resolution of DME with systemic furosemide treatment, which was intended for volume expansion, was also observed in a few cases [22, 23]. Furthermore, proteinuria was an independent predictor for overhydration in CKD [24]. Intraglomerular hydrostatic pressure was proved to be correlated with urinary albumin excretion [25]. Considering these factors together, we hypothesized that proteinuria and DME may both result from increased hydrostatic pressure and were thus associated with each other.

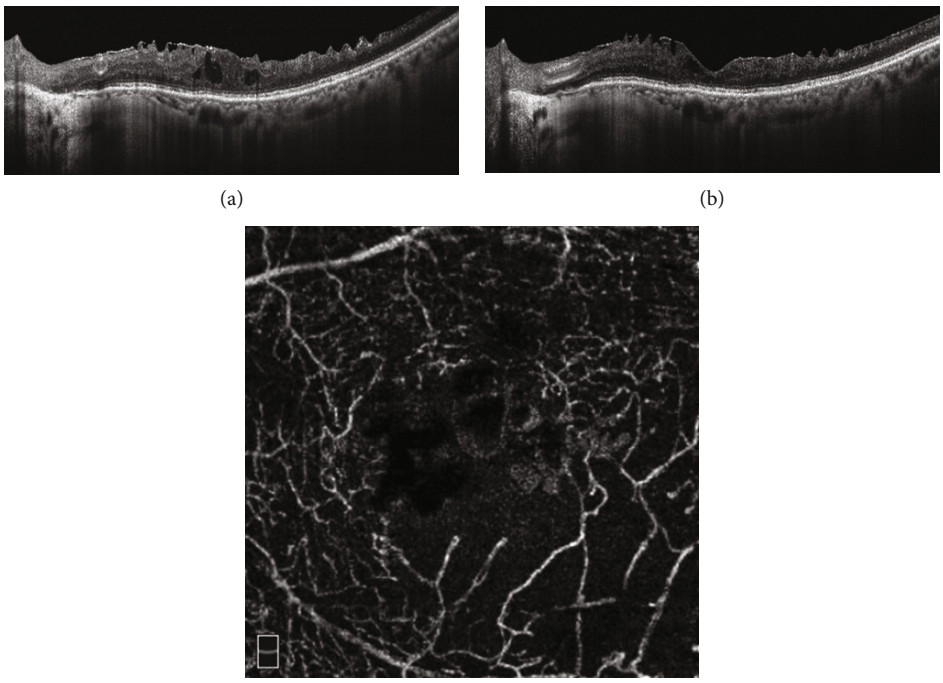
Interestingly, though bearing a worse baseline condition, patients with more severe proteinuria showed better anatomical improvement after ranibizumab treatment for DME. Those with moderately to severely increased proteinuria even had thinner CFTs than those with normal to mildly increased proteinuria after ranibizumab treatment. As far as we know, this is a novel finding that has never been reported before. The mechanism underlying the formation of DME may furnish some clue to our observation. Macular edema was a consequence of fluid imbalance, which could be accredited to increased fluid entry, decreased drainage function, or the combination of both. VEGF held a global effect on retinal vascular endothelial and pericytes, including alteration of barrier junctional integrity, promotion of leukostasis, and increase in transcellular permeability [19]. Anti-VEGF therapy aimed at blocking the abovementioned processes, however, seemed unable to drain the fluid per se. Absorption of the edema relied on passive diffusion and the pumping function of retinal pigment epithelium and retinal Müller cells, which would be disturbed in streptozotocin-induced diabetic rat model [26–28]. On the other hand, patients with more severe proteinuria, as discussed in the previous section, may possess lower intravascular oncotic pressure or higher hydrostatic pressure, thus resulting in thicker CFTs. However, once the VEGF pathway was blocked by ranibizumab, the synergistic effect from the Starling forces would also diminish, ending up with unremarkable CFTs compared to those with normal to mildly increased proteinuria. We inferred that those with normal to mildly increased proteinuria were involved more in pumping dysfunction or inflammatory reaction than those with proteinuria did, thus leaving thicker CFTs after treatment. Figure 3 showed an example of a case with normal to mildly increased proteinuria. The eye received 8 ranibizumab injections within 12 months, while the macular edema did not improve.

It was also worth noting that patients with an eGFR <30 tended to gain poorer visual improvements despite comparable CFT reduction to the other groups. In a population-based study [29], it is found that retinal perfusion density in both superficial and deep layers decreased in subjects with lower eGFR. In diabetic patients, lower eGFR was also demonstrated to be independently associated with decreased retinal



(a) (b)

Figure 3: A 74-year-old male had an UACR of 0.029. (a) At baseline, there was cystic edema involving fovea in the right eye. (b) At month 12, the cystic edema still persisted after 8 ranibizumab injections were given.



(a) (b)

(c)

Figure 4: A 55-year-old male had an eGFR of 3.8. (a) At baseline, there was macular edema with intraretinal cysts involving fovea in the left eye. (b) At month 12, the macular edema improved a lot after 6 ranibizumab injections were given, while the vision did not improve. (c) OCTA showed severe ischemia at parafoveal areas.

blood flow [30]. Nephropathy with either albuminuria or increased serum creatinine was associated with the presence of macular ischemia in eyes with diabetic retinopathy [31]. According to these results, we proposed that retinal perfusion may decline with the deterioration of eGFR, contributing to visual impairment caused by macular ischemia. Previous studies on OCT angiography (OCTA) can provide some hint about this. Although the vessel density in both the superficial and deep capillary plexus, which was lower in patients with DME, could improve after anti-VEGF therapy [32, 33], our study group reported that lower parafoveal vessel density in the superficial layers was associated with poorer visual improvement after adjustment for baseline BCVA and CFT [32]. Larger foveal avascular zone was also shown to be correlated with poorer VA in patients with resolved DME [34]. These suggest that besides macular edema, macular ischemia itself may contribute to visual impairment in cases with low eGFR, which results in the discrepancy between the anatomical improvement and visual improvement after ranibizumab treatment. However, only few cases in this study had received OCTA examinations, so that we could not evaluate the correlation between macular ischemia and eGFR. Figure 4 showed an example of a case with an eGFR <30. After receiving 6 ranibizumab injections within 12 months, the macular edema improved a lot, while the vision did not improve due to severe macular ischemia as shown in OCTA.

Injection number could also affect the treatment outcomes. In this study, patients with moderately and severely increased proteinuria tended to receive more intravitreal injections. Treated with a PRN strategy after the loading phase, patients with frequent recurrence were supposed to receive more additional doses. Under the consideration of Starling forces as discussed previously, patients with proteinuria, even under a similar barrier condition to those without proteinuria, may suffer from edema recurrence in a shorter period due to hydrostatic or oncotic pressure. On the other hand, some patients might receive less-than-needed injections due to personal reasons or

the reimbursement restriction. Therefore, the injection number was adjusted in all regression models to adjust for its effect on treatment outcomes.

The major limitation of this study was its retrospective design. We had no information about the duration and medication of diabetes, the body fluid status, or the duration and previous management for DME. In addition, our study population contained only 86 eyes from a single center, and thus, there was some inevitably inherent bias. Notwithstanding these limitations, this work offers valuable insights into the effects of renal profile on the treatment for DME. To the best of our knowledge, this is the first study to underscore the impact of renal function on the treatment effects of anti-VEGF agents for DME. Further prospective studies and randomized clinical trials are necessary for the determination of their causal relationships.

In conclusion, proteinuria rather than eGFR was associated with central retinal thickness and the presence of intraretinal cysts in DME patients at baseline. After ranibizumab treatment, those with severe proteinuria tended to gain better anatomical improvement, while those with poorer eGFR tended to have poorer visual improvement. These findings suggest a role of renal biomarkers for the evaluation of anti-VEGF treatment response for DME.

Data Availability

Dataset will be available under request.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

Acknowledgments

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Research Article

Total Bilirubin Predicts Severe Progression of Diabetic Retinopathy and the Possible Causal Mechanism

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Early detection and treatment are key to delaying the progression of diabetic retinopathy (DR), avoiding loss of vision, and reducing the burden of advanced disease. Our study is aimed at determining if total bilirubin has a predictive value for DR progression and exploring the potential mechanism involved in this pathogenesis. A total of 540 patients with nonproliferative diabetic retinopathy (NPDR) were enrolled between July 2014 and September 2016 and assigned into a progression group ($N = 67$) or a stable group ($N = 473$) based on the occurrence of diabetic macular edema (DME), vitreous hemorrhage, retinal detachment, or other conditions that may cause severe loss of vision following a telephonic interview in August 2019. After further communication, 108 patients consented to an outpatient consultation between September and November 2019. Our findings suggest the following: (1) TBIL were significant independent predictors of DR progression (HR: 0.70, 95% CI: 0.54–0.89, $p = 0.006$). (2) Examination of outpatients indicated that compared to stable group patients, progression group patients had more components of urobilinogen and LPS but a lower concentration of TBIL. The relationship between bilirubin and severe DR was statistically significant after adjusting for sex, age, diabetes duration, type of diabetes, FPG, and HbA1c (OR: 0.70, 95% CI: 0.912–0.986, $p = 0.016$). The addition of serum LPS and/or urobilinogen attenuated this association. This study concludes that total bilirubin predicts an increased risk of severe DR progression. Decreasing bilirubin might be attributed to the increased levels of LPS and urobilinogen, which may indicate that the change of bilirubin levels is secondary to intestinal flora disorder and/or intestinal barrier destruction. Further prospective investigations are necessary to explore the causal associations for flora disorder, intestinal barrier destruction, and DR.

1. Introduction

Due to the aging of population, urbanization, and lifestyle changes, diabetes mellitus (DM) has increased rapidly in both developed and developing countries. Diabetic retinopathy (DR) is the most common microvascular complication of diabetes and is the main cause of blindness among working-age people in industrialized countries [1]. In the early stage of nonproliferative diabetic retinopathy (NPDR), chronic hyperglycemia can damage the microvessels supplying the retina, leading to ischemia, vascular leakage, and central vision loss caused by diabetic macular edema (DME) [2]. As the disease progresses to proliferative DR (PDR), vision loss associated with secondary neovascularization of the retina and subsequent hemorrhage and/or retinal detachment occurs [3]. Previous studies have shown that DR progression and vision loss can impair the patient's quality of life; however, without appropriate intervention, about half of high-risk PDR patients will develop visual impairment due to DME, vitreous hemorrhage, and/or retinal detachment within five years of diagnosis [4]. Therefore, early detection and treatment are vital for delaying the progression of DR, avoiding vision loss, and reducing the burden of advanced disease.

Bilirubin has effective antioxidant properties and is a protective agent against diabetes and cardiovascular diseases [5]. A meta-analysis assessing 132,240 subjects recruited from 27 studies found a negative nonlinear relationship between bilirubin concentration and the risk of diabetic complications

[6]. Prospective studies showed that the level of serum total bilirubin was independently correlated with DR in both Type-1 Diabetes Mellitus (T1DM) and Type-2 Diabetes Mellitus (T2DM) [7]. However, there is no current consensus on lower serum total bilirubin having predictive value for the development of DR. Further, the biological mechanisms for the relationship between serum bilirubin levels and DR remain unclear.

Therefore, the present study examined whether total bilirubin has a predictive value for DR progression. The potential mechanism involved in this pathogenesis was also examined.

2. Materials and Methods

2.1. Participants. In this study, a total of 540 patients with an initial diagnosis of NPDR were enrolled in the Department of Endocrinology, the Third Affiliated Hospital of Anhui Medical University (Hefei Binhu Hospital) between July 2014 and September 2016. Retinopathy was diagnosed by binocular indirect slit-lamp fundoscopy and fundus photography after mydriasis with eye drops containing 0.5% tropicamide and 5% phenylephrine by a single grader. The final diagnosis of DR was made by fundus photographs. Color fundus photographs of two fields (macular field, disc/nasal field) of both eyes were taken with a 45 fundus camera (VISUCAM, Zeiss), according to the EURODIAB retinal photography methodology [3]. In this study, NPDR was defined as the presence of one or more microaneurysms, hemorrhages, and/or hard exudates. These evaluations were performed independently by two different ophthalmologists after training. After a telephone interview in August 2019, based on having DME, vitreous hemorrhage, retinal detachment, or other conditions induced by diabetes that caused severe vision loss, patients were assigned into a progression group ($N = 67$; among them, 47 patients were diagnosed in our hospital, and the rest were diagnosed in other hospitals at the same level) or a stable group ($N = 473$). The exclusion criteria were as follows: (1) no history of T1DM or T2DM; (2) vision loss due to nondiabetic causes during pregnancy or lactation or both; (3) presence of cancer, hepatic disease, or other coexisting illnesses, including a history of coronary stent implantation, cerebral infarction, and severe CKD (defined as eGFR ≤ 30 ml/min/1.73 m^2); and (4) inability to communicate using standard methods. After further communication, 108 patients were scheduled to visit as outpatients between September and November in 2019. Written informed consent was obtained from subjects or parents/legal guardians during the outpatient visit.

2.2. Methods. Data regarding the duration and type of diabetes, along with age and gender, were obtained from participants' medical records. All patients were tested for biochemical data (ALT, AST, Cr, TBILI, DBIL, IBIL, γ-GT, and ALP) and glucose metabolism (FPG, HbA1c) to obtain baseline levels. Outpatient visits were performed between September and November 2019. Overnight fasting blood samples (patients fasted for at least 8 hr.) were taken, and plasma from these samples was examined to obtain biochemical data such as HbA1c and lipopolysaccharide (LPS). The eGFR was calculated as follows: $194 \times Cr^{-1.094} \times age^{-0.287}$ ($\times 0.739$ for female patients). CKD stage was divided into six categories based on eGFR levels as follows: G1, ≥90; G2, 60–89; G3a, 45–59; G3b, 30–44; G4, 15–29; and G5, <15 ml/min/1.73 m^2. Urine protein was divided into three categories as follows: normoalbuminuria (urinary albumin-to-creatinine ratio (UACR), <30 mg/gCr); microalbuminuria (UACR, 30–299 mg/gCr), and macroalbuminuria (UACR, ≥300 mg/gCr). First-morning urine samples were collected to test for urobilinogen. Urobilinogen in urine was measured by direct spectrophotometry using a modified Ehrlich's method. A LPS/LOS ELISA Kit (USCN Life Science Inc., Houston, Texas, USA) was used to estimate the concentration of plasma LPS. A flow chart of the process is shown in Figure 1.

2.3. Statistical Analysis. IBM SPSS Statistics ver. 22.0 (IBM Co., Armonk, NY, USA) was used. Continuous measurements, such as the mean (SD), were utilized if data were normally distributed; however, if the data were not normally distributed, the median (IQR) was utilized. Categorical variables were described utilizing frequency and percentages (%). Independent tests, including the t-test, chi-square test, or Mann-Whitney U test, were used to compare the two patient groups. A Cox proportional hazards regression model to estimate the hazard ratio (HR) with 95% CI was used to analyze the risk factors for DR progression, or stability. Kaplan-Meier survival curves of NPDR progression by serum TBIL stratification were determined. Logistic regression analysis was adopted to calculate the odds ratio (OR) and a 95% confidence interval (CI) for the risk of DR progression, and this was determined after adjusting for potential confounding variables. Statistical significance was set at $p < 0.05$.

3. Results

3.1. Demographic and Metabolism Characterization of Study Subjects. Data from a total of 540 patients with DR (47.41% male and 20.74% T1DM) were evaluated. The average follow-up time was 48 (40–54) months, and the mean age of the population was 61.36 ± 15.49 years. Subject age varied from 24 to 87 years. The patients had been diabetic for 0–37 years. Patients in the progression group had a significantly higher duration of diabetes and lower TBIL, DBIL, IBIL, and FPG than those in the stable group (both $p < 0.05$). There were no significant differences in sex, age, type of diabetes, HbA1c, ALT, AST, γ-GT, ALP, and Cr between the two groups (all $p > 0.05$) (Table 1).

3.2. Independent Risk Factors Associated with the Progression of DR. The Cox proportional hazards regression model was used to analyze the risk factors for DR progression. After adjusting for all factors with significant associations emerging from the univariate analysis, duration of diabetes and TBIL were significant independent predictors of DR progression (HR: 1.73, 95% CI: 1.24–2.64, $p < 0.001$; HR: 0.70, 95% CI: 0.54–0.89, $p = 0.006$, respectively), as presented in Figure 2.

3.3. Kaplan-Meier Survival Curves of NPDR Progression by Serum TBIL Stratification. As mentioned above, serum TBIL was correlated with NPDR progression outcome. A further

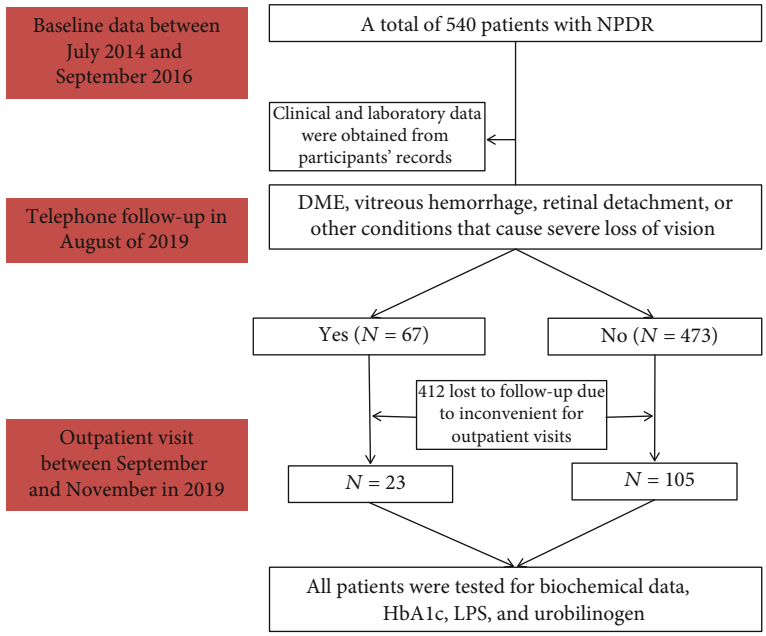

Figure 1: Flow chart of inclusion participants.

analysis with TBIL stratification was performed to estimate the ratio in different serum TBIL levels. TBIL was categorized into four groups according to the interquartile range as follows: Q1 ($N = 121$), <9.45; Q2 ($N = 140$), 9.45–10.30; Q3 ($N = 151$), 10.30–11.20; and Q4 ($N = 128$), ≥11.20 μmol/l. The Q2, Q3, and Q4 groups were significantly different when compared with the Q1 group (log-rank $\chi^2 = 4.85$, $p = 0.0277$; $\chi^2 = 16.03$, $p < 0.001$; and $\chi^2 = 15.07$, $p < 0.001$, respectively) (Figure 3).

3.4. Characterization of DR Individuals on Outpatient Visit. After further communication, 108 patients (23 from the progression group and 105 from the stable group) visited as outpatients between September and November 2019. The study groups were similar as regards age, gender distribution, diabetes type, and duration. The progression group had more components of urobilinogen and LPS but lower concentrations of TBIL. The general data and data on biochemical indexes of the two groups are summarized in Table 2.

3.5. Odds Ratios for Severe DR. To explore the effects of bilirubin on DR progression, we performed multivariable analyses using logistic regression models. The relationship between bilirubin and severe DR was statistically significant after adjusting for sex, age, diabetes duration, type of diabetes, FPG, and HbA1c (odds ratio (OR): 0.967, 95% CI: 0.912–0.986, $p = 0.016$; Model 1). The addition of serum LPS (Model 2), urobilinogen (Model 3), LPS, and urobilinogen (Model 4) in Model 1 attenuated this association. The details of the models are summarized in Table 3.

3.6. Relationship between TBIL, Urobilinogen, and LPS. Table 4 shows a strong negative correlation between TBIL/urobilinogen ($r = -0.796$, $p < 0.001$) and TBIL/LPS ($r = -0.708$, $p < 0.001$) in DR individuals.

4. Discussion

Previous studies have shown serum bilirubin plays a protective role against the development of diabetic microvascular complications, such as neuropathy, nephropathy, and DR [8]. To our knowledge, this is the first study that investigates the predictive value of serum total bilirubin level at risk of DR progression. In Cox proportional hazards regression analysis, the duration of diabetes and serum total bilirubin level were independently related to DR progression. Subsequent Kaplan-Meier survival analysis with stratification by TBIL interquartile range indicates that the Q2, Q3, and Q4 groups were significantly different when compared with the Q1 group in the incidence of DR progression. The outpatient visit shows that the relationship between bilirubin and severe DR was statistically significant after adjusting for sex, age, diabetes duration, type of diabetes, FPG, and HbA1c (OR = 0.967); however, the addition of serum LPS and/or urobilinogen to a model utilized in this work attenuated the association. A further analysis indicates a strong negative correlation between TBIL/urobilinogen ($r = -0.796$) and TBIL/LPS ($r = -0.708$) in those DR individuals.

In recent years, diabetes has become a rapidly growing threat around the world. Many of its complications not only cause a significant burden but also has an important impact on physical health and quality of life [9]. Therefore, it is important to identify diabetic individuals with a higher risk of complications. This may improve prevention and reduce the burden of the disease. DR is a common and special microvascular complication that develops with the passage of time [10]. Severe stages of DR, including DME and PDR, lead to visual impairment and blindness [11]. Epidemiological studies have shown that about one in three diabetic patients suffers from DR, and one in ten has PDR or DME. Demographic surveys indicate that half of the population

Table 1: Demographic and metabolism characterization of study subjects.

Group	Progression group ($N = 67$)	Stable group ($N = 473$)	$T/F/\chi^2$ value	p value
General data				
Gender (male/female)	32/35	224/249	0.004	0.951
Age (year)	62.34 ± 19.78	61.12 ± 13.27	0.657	0.512
Average follow-up time (month)	49 (40–56)	47 (38–54)	0.301	0.743
Duration of diabetes (year)	12.79 ± 6.31	10.12 ± 4.29	4.46	<0.001
Type of diabetes (T1DM/T2DM)	19/48	93/380	2.79	0.248
Glucose metabolism				
FPG (mmol/l)	8.92 ± 1.97	8.43 ± 1.88	1.985	0.048
HbA1c (%)	8.69 ± 3.06	8.64 ± 3.77	0.104	0.917
Biochemical data				
ALT (IU/l)	18 (13–24)	16 (10–22)	0.258	0.784
AST (IU/l)	17 (9-26)	17 (8-30)	0.135	0.876
TBIL (μmol/l)	8.36 ± 2.89	11.36 ± 3.65	6.450	<0.001
DBIL (μmol/l)	1.38 ± 0.74	2.3 ± 1.04	6.992	<0.001
IBIL (μmol/l)	6.98 ± 1.89	8.06 ± 3.57	2.427	0.016
Γ-GT (IU/l)	18 (14–27)	19 (15–30)	0.368	0.697
ALP (IU/l)	102 (72–140)	92 (83–147)	1.368	0.205
Cr (μmol/l)	86 (32–102)	81 (37–98)	1.231	0.274
eGFR (ml/min/1.73 m^2)	61.43 ± 13.12	62.56 ± 14.37	0.609	0.543
CKD stage (number)			0.333	0.954
G1	12	97		
G2	23	162		
G3a	18	125		
G3b	14	89		
Urine protein categories (number)			0.395	0.821
Normoalbuminuria	39	287		
Microalbuminuria	21	147		
Macroalbuminuria	7	39		

suffering from diabetes have not been diagnosed. In addition, individuals with an early stage of DR have not been given sufficient attention [3]. In the current study, at approximately four years subsequent to the first diagnosis of NPDR, 12.41% of the 540 patients developed severe DR, such as DME, vitreous hemorrhage, retinal detachment, or other conditions that led to severe vision loss. Consequently, it is vital to find predictive factors for desire progression during the early course of DR.

A majority of previous studies suggested a negative relationship between TBIL and DR [8, 12]. A study of T2DM patients indicated that a higher TBIL was independently associated with a reduced risk of DR (OR: 0.242, 95% CI: 0.096-0.615) [13]. A population-based cross-sectional study indicated that patients with a serum bilirubin level in the fourth quartile were less likely to develop DR than those in the first quartile for serum bilirubin level (OR: 0.55; 95% CI: 0.33~0.91) [14]. A meta-analysis indicated a significant, nonlinear, and negative correlation between TBIL and DR risk (OR: 0.19, 95% CI: 0.14-0.25) [15]. However, there are few studies regarding the predictive value of bilirubin in the development of NPDR [16]. In a prospective cohort study, a higher baseline bilirubin level was associated with a significantly reduced risk of progression from microalbuminuria to macroalbuminuria [17]. Our results are consistent with this study, indicating that serum bilirubin concentration is negatively correlated with the development of NPDR and may be a useful predictor of serious progress of DR over time.

The pathogenesis of DR has not been adequately studied. Oxidative stress caused by high glucose is an area of focus in current studies [18]. TBIL is not only a metabolite of hemoglobin but also an important endogenous antioxidant [19]. Previous studies have demonstrated that TBIL has a significant protective effect against cardiovascular disease, diabetes, and diabetic macrovascular complication. This is a result of antioxidant and anti-inflammatory effects [20]. As a natural antioxidant, uric acid has similar antioxidant effects, such as resisting oxidative stress, scavenging oxygen-free radicals, preventing apoptosis, and protecting vascular endothelial cells from DNA [21]. A large number of studies have shown that higher levels of serum uric acid are associated with a higher risk of DR [22–24]. Those findings seem to contradict previous studies regarding the antioxidant effects of bilirubin on the pathogenesis of DR. This suggests that other potential mechanisms are probably involved in this process.

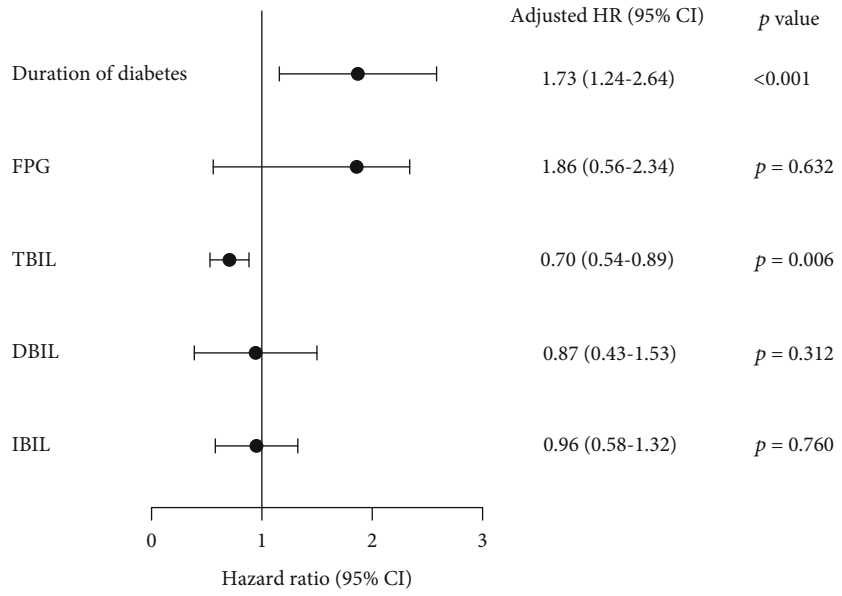

Figure 2: Cox proportional hazards regression model: the risk factors for DR progression.

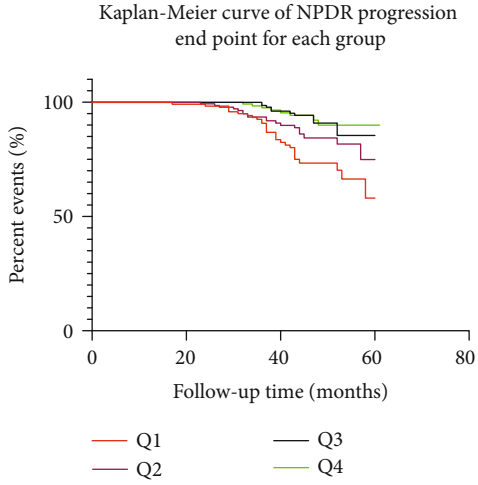

Figure 3: Kaplan-Meier survival curves by TBIL stratification ($n = 540$). Patients were categorized into four groups: Q1 ($N = 121$), Q2 ($N = 140$), Q3 ($N = 151$), and Q4 ($N = 128$).

Anatomically, the portal system transports intestinal blood to the liver, which contains not only nutrients but also molecular patterns related to pathogens, including LPS, and peptidoglycan among other substances [25]. Studies in rodents and humans have shown that a long-term, high-fat diet (HFD) can lead to intestinal barrier defects, which may promote the transport of intestinal contents (food antigens, bacterial by-products, and bacteria themselves), especially bacterial LPS, into systemic circulation, resulting in low-grade inflammation [26]. Previous studies have also demonstrated that obese individuals and animals fed HFD exhibit changes in the composition of intestinal microflora and a two-three factor increase in serum LPS concentration [27]. In our study, the progressive group had more LPS components, suggesting that these patients may be experiencing more severe intestinal flora disorders and intestinal barrier disruptions. Urobilinogen refers to a group of colorless tetrapyrrole formed when intestinal anaerobes reduce intestinal unconjugated bilirubin (conjugated bilirubin secreted to the upper small intestine is hydrolyzed to unconjugated bilirubin). Up to 20% of the urine bilirubin produced daily is reabsorbed from the intestine and undergoes enterohepatic recirculation [28]. Most of the reabsorbed urobilinogen is taken up by the liver and subsequently reexcreted into bile, while a small amount is excreted into the urine. In this study, we found that the progression group had a higher level of urobilinogen but a lower concentration of TBIL when compared to the stable group. The relationship between bilirubin and DR progression was statistically significant after adjusting for known risk factors; however, the addition of serum LPS or urobilinogen in the corresponding model attenuated this association. These results may support the assertion that for intestinal flora disorder and intestinal barrier destruction, the reabsorption of urobilinogen will be increased through enterohepatic circulation or local damage of the vascular barrier which would lead to the positive feedback of bilirubin excretion. In other words, the decreasing of bilirubin is secondary to intestinal flora disorder and/or intestinal barrier destruction.

Several limitations of this study should be mentioned. First, as this was a single-centre study in China, the results might not be directly applicable to other ethnicities and regions. Second, there were 20 patients diagnosed with DR progression in other hospitals, which might have led to potential heterogeneity. Finally, unmeasured confounding factors might not have been fully addressed.

5. Conclusions

In conclusion, our study indicates a negative relationship between total bilirubin concentration and the progression of DR, which might be attributed to the increased levels of

Table 2: Characterization of DR individuals in an outpatient visit.

Group	Progression group ($N = 23$)	Stable group ($N = 105$)	$T/F/\chi^2$ value	p value
Gender (male/female)	9/14	53/52	0.972	0.324
Age (year)	65.14 ± 19.43	67.09 ± 23.75	0.367	0.714
Average follow-up time (month)	50 (39–55)	49 (37–54)	0.269	0.835
Duration of diabetes (year)	13.76 ± 6.47	12.46 ± 5.93	0.937	0.351
Type of diabetes (T1DM/T2DM)	6/17	28/77	0.003	0.955
ALT (IU/l)	21 (13–39)	19 (14–33)	0.943	0.316
TBIL (μmol/l)	8.04 ± 3.14	12.46 ± 3.42	6.147	<0.001
Γ-GT (IU/l)	21.5 (10–36)	19 (12–35)	1.845	0.124
Cr (μmol/l)	91 (45–106)	87 (42–104)	1.654	0.189
eGFR	57.46 ± 16.35	59.04 ± 14.27	0.468	0.640
FPG (mmol/l)	8.14 ± 3.17	7.06 ± 2.38	1.850	0.067
HbA1c (%)	8.19 ± 2.76	7.93 ± 1.87	0.55	0.583
Urobilinogen (mg/dl)	0.75 (0.23–1.04)	0.48 (0.05–0.67)	5.568	<0.001
LPS (Eu/ml)	0.71 (0.34–1.79)	0.58 (0.20–1.45)	2.263	0.037

Table 3: Odds ratios for severe DR.

	Bilirubin (μmol/l)	p value	LPS (Eu/ml)	p value	Urobilinogen (mg/dl)	p value
Unadjusted	0.894 (0.765–0.943)	<0.001	—	—	—	—
Model 1	0.967 (0.912–0.986)	0.016	—	—	—	—
Model 2	0.969 (0.934–1.023)	0.084	2.476 (1.632–3.091)	<0.001	—	—
Model 3	0.992 (0.960–1.104)	0.136	—	—	1.734 (1.234–2.430)	0.009
Model 4	1.013 (0.893–1.347)	0.422	1.985 (1.346–2.808)	0.016	1.702 (1.141–2.336)	0.027

Model 1: adjusted for sex, age, diabetes duration, type of diabetes, FPG, and Hba1c; Model 2: Model 1+LPS; Model 3: Model 1+urobilinogen; Model 4: Model 1 +LPS+urobilinogen.

Table 4: Relationship between TBIL, urobilinogen, and LPS.

Variable	TBIL	
	β	p value
Urobilinogen	-0.796	0.000
LPS	-0.708	0.000

LPS and urobilinogen. This may indicate that a decrease of bilirubin is secondary to intestinal flora disorder and/or intestinal barrier destruction. Further prospective investigations are necessary to explore the causal associations for flora disorder, intestinal barrier destruction, and DR.

Data Availability

The datasets analyzed during the current study are available from the corresponding author.

Ethical Approval

The study protocol was approved by the ethics committee of the Third Affiliated Hospital of Anhui Medical University.

Consent

It was agreed that the requirement for informed consent be waived because this study was designed to collect available data from participants' medical records retrospectively. Written informed consent was obtained from subjects or parents/legal guardians during the outpatient visit before data collection.

Conflicts of Interest

The authors declare that they have no competing interests.

Authors' Contributions

Yu Ding designed the study. Yu Ding and Junmin Zhao managed the study. Gangsheng Liu, Yinglong Li, Jiang Jiang, Yun Meng, Tingting Xu, and Kaifeng Wu conducted the study. Yu Ding analyzed the study. All authors read and approved the final manuscript.

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Research Article

Qualitative and Quantitative Analysis of B-Cell-Produced Antibodies in Vitreous Humor of Type 2 Diabetic Patients with Diabetic Retinopathy

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Aim. To analyze the levels of B-cell-produced antibodies in the vitreous humor of patients with or without diabetic retinopathy (DR) both qualitatively and quantitatively. *Methods*. A total of 52 type 2 diabetes mellitus (T2DM) with DR patients and 52 control subjects without diabetes mellitus or inflammatory diseases were included in this prospective study. The levels of immunoglobulin (Ig)A, IgM, and IgG subtypes were measured using a magnetic color-bead-based multiplex assay. *Results*. The concentrations of IgA, IgM, and total antibodies in the DR group were significantly higher than those in the control group (all $p < 0.001$), but there was no significant difference in the 4 IgG subtypes between the two groups after Bonferroni correction. Pearson's correlation analysis revealed low negative correlations between levels of antibodies (IgA, IgM) and estimated glomerular filtration rate (eGFR, $r = -0.443$, $r = -0.377$, respectively, both $p < 0.05$). Furthermore, multiple linear regression analysis yielded three equations to predict the concentrations of IgA, IgM, and total antibodies in the vitreous humor according to eGFR and other clinical variables ($r = 0.542$, $r = 0.461$, and $r = 0.312$, respectively, all $p < 0.05$). *Conclusion*. Increased levels of IgA, IgM, and total antibodies produced by B cells were observed in the vitreous humor of T2DM patients with DR. There were low negative correlations between levels of antibodies (IgA, IgM) and eGFR.

1. Introduction

Diabetes mellitus (DM) is a chronic metabolic disorder that is characterized by hyperglycemia, resulting in insulin resistance. According to the latest statistics, there are 463 million people currently with DM in the world, and this continues to rise [1]. Type 2 diabetes mellitus (T2DM) is the most common form of DM, accounting for 91% of DM. Hyperglycemia control reduces the mortality and microvascular complications associated with the disease [2, 3]. Diabetic retinopathy (DR) is one of the important microvascular complications of DM and is the leading cause of blindness in DM population. Inflammation is regarded as a critical component in the pathogenesis of DR [4, 5]. The clinical findings in patients with DR include (1) increased levels of inflammatory biomarkers such as vascular endothelial growth factor (VEGF) and C-reactive protein in the serum [6]; (2) increased levels of inflammatory cytokines and chemokines such as tumor necrosis factor-alpha, interleukin- (IL-) 1, IL-6, and C-C motif ligand (CCL) 3 in the aqueous and vitreous humor [7, 8]; and (3) detection of inflammatory cells such as neutrophils, macrophages, and lymphocytes in the proliferative

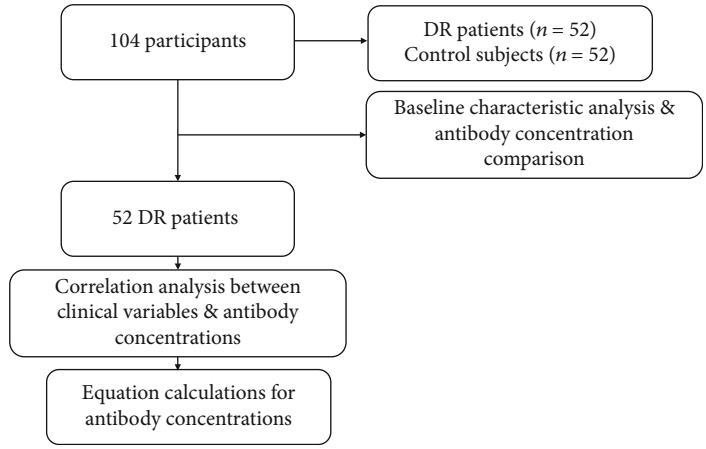

Figure 1: Flow chart of the study selection process. Abbreviation: DR: diabetic retinopathy.

epiretinal membrane of DR patients [9]. These in turn confirm the contribution of inflammatory factors in the pathogenesis of DR.

B cells play key roles in the production of cytokines and antibodies in humans and mice [10, 11] and were found to regulate inflammation in patients with DM [12–15]. Antigen-specific antibodies that are produced by activated B cells are the first-line defense against pathogens in exposed surfaces, and this is done by neutralizing antigens, facilitating phagocytosis and antigen presentation [16]. Besides, the self-reactive antibodies are involved in the destruction of self-tissues and initiation of autoimmune diseases [17]. Thus, B-cell-mediated immune response and regulation are important in immune response, and these B-cell functions might also contribute to the development of DR. However, there is limited evidence on the activation of B cells in DR patients.

In the current study, the concentrations of B-cell-produced immunoglobulin (Ig)A, IgM, and IgG subtypes in vitreous humor of T2DM patients with DR and control subjects were analyzed. Furthermore, the correlations between the concentrations of these antibodies and clinical variables of DR were investigated.

2. Materials and Methods

This prospective study was conducted from May 2018 to March 2020 in accordance with the tenets of the Declaration of Helsinki. This study obtained ethical approval from the local Research Ethics Committee of the Guangdong Provincial People's Hospital (Number: 2016232A) before conducting the study. Informed consent was obtained from all patients. A flow chart of included population and analyses is shown in Figure 1. T2DM was diagnosed by endocrinologists based on the diagnostic criteria of the American Diabetes Association [18]. Diagnosis and classification of DR were confirmed according to the international clinical diabetic retinopathy severity scales [19]. Patients who underwent vitrectomy for vitreous hemorrhage, proliferative epiretinal membrane, or tractional retinal detachment were included. The control group included patients without DM but underwent vitrectomy for idiopathic preretinal membranes, idiopathic macular holes, or rhegmatogenous retinal detachment. The primary endpoint of the study was follow-up at one month after vitrectomy surgery. The patients were regularly followed up after that. The exclusion criteria were as follows: patients (1) with other ocular conditions associated with inflammation (such as age-related macular degeneration, glaucoma, and uveitis), (2) with a history of ocular surgery or trauma, (3) who received anti-VEGF treatment, and (4) with a history of severe systemic inflammatory diseases, primary kidney diseases, or any other kidney diseases that are the cause other than DM secondarily. All subjects underwent a complete ocular examination and blood pressure, fasting blood glucose (FBG), glycated hemoglobin (HbA1c), serum creatinine (sCr), blood urea nitrogen (BUN), estimated glomerular filtration rate (eGFR), and urinary albumin to creatinine ratio (UACR) which were measured before surgery. The value of eGFR was calculated based on the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) equation according to the guidance of an experienced nephrologist (Levey [20]). All patients underwent pars plana vitrectomy in accordance with the standardized operation procedures using the 23-gauge trocar and cannula system (Alcon Laboratories, Inc. Fort Worth, Tex. the USA). About 0.2-0.4 ml of vitreous humor was aspirated into a sterile syringe before intraocular infusion. The vitreous samples were centrifuged immediately at 2500 rpm at 4°C for 10 min. The supernatants were aspirated and subsequently stored at -80°C until further analysis.

The Bio-Plex Pro™ Human Isotyping Panel, 6-plex kit (#171A3100M, control 64190954, Bio-Rad Laboratories, Inc., Hercules, CA, USA) was used to measure the concentrations of 6 human antibodies, including IgA, IgM, IgG1, IgG2, IgG3, and IgG4. The experimental procedures were conducted according to the manufacturer's instructions. Next, 40 μl of undiluted vitreous humor sample was used for the reaction and finally analyzed the fluorescence intensity from the immunoassay using the Bio-Plex™ 200 System (software version 6.1, Bio-Plex Manager, Bio-Rad Laboratories).

2.1. Statistical Methods. Statistical analyses were performed using IBM SPSS statistics version 19.0 (IBM SPSS Statistics;

Table 1: Clinical characteristics of the subjects.

	DR ($n = 52$)	Non-DR ($n = 52$)	p value
Age (y)	53.56 ± 12.05	57.17 ± 9.72	0.095
Male/female	30/22	33/19	0.552
Duration of DM (years)	7.37 ± 8.59	N/A	N/A
Duration of DR (months)	7.10 ± 9.79	N/A	N/A
SBP (mmHg)	129.19 ± 18.72	128.19 ± 18.29	0.783
DBP (mmHg)	77.90 ± 10.93	79.25 ± 10.47	0.523
FBG (mmol/l)	9.64 ± 3.44	6.30 ± 1.77	<0.001*
HbA1c (%)	7.67 ± 1.53	5.99 ± 0.77	<0.001*
sCr (μmol/l)	256.52 ± 237.52	86.86 ± 49.28	<0.001*
BUN (mmol/l)	12.37 ± 8.23	6.00 ± 2.64	<0.001*
eGFR (ml/min/1.73 m^2)	37.02 ± 22.51	85.42 ± 20.34	<0.001*
UACR (mg/g)	903.69 ± 873.52	19.91 ± 17.13	<0.001*

Abbreviation: DM: diabetes mellitus; DR: diabetic retinopathy; SBP: systolic blood pressure; DBP: diastolic blood pressure; FBG: fasting blood glucose; HbA1c: glycated hemoglobin; sCr: serum creatinine; BUN: blood urea nitrogen; eGFR: estimated glomerular filtration rate; UACR: urinary albumin to creatinine ratio. Duration of DM and DR were unavailable in the control group ($n = 52$). *p value of <0.001 by independent, two-tailed Student's t-tests was considered statistically significant. One-way ANOVA was used for evaluating the differences in sex.

IBM Corporation, Chicago, IL, USA). One-way ANOVA was performed for evaluating the sex differences between the DR group and the control group. Data normality was confirmed by Shapiro-Wilk test. Independent, two-tailed Student's t -tests were performed to compare other clinical variables and concentrations of the antibodies between the two groups. Bonferroni-corrected significance threshold ($p = 0.006$) was used for the multiplicity of measurement of antibodies between the two groups. Pearson's correlation test was used to analyze the associations between the clinical variables and the concentrations of antibodies. Furthermore, multiple linear regression analysis was used to yield equations for calculating the concentrations of antibodies according to the clinical variables that are statistically significant in Pearson's correlation analysis. Sample size calculation was performed by using a web-based simple power/sample size calculation, UCSF Biostatistics: Power and Sample Size Programs, https://www.stat.ubc.ca/~rollin/stats/ssize/, $\alpha = 0.006$ (after Bonferroni correction), power = 0.90, and two-sided test. A two-tailed $p < 0.05$ was considered to be statistically significant.

3. Results

3.1. Baseline Characteristics of the Included Subjects. Fifty-two DR patients (10 eyes with vitreous hemorrhage, 24 eyes with proliferative epiretinal membrane, and 18 eyes with tractional retinal detachment according to the primary diagnosis) and 52 non-DR subjects (17 eyes with idiopathic preretinal membranes, 17 eyes with idiopathic macular holes, and 18 eyes with rhegmatogenous retinal detachment) were recruited, including 63 males and 41 females. Clinical characteristics of the DR and the control group are presented in Table 1. The levels of FBG, HbA1c, sCr, BUN, and UACR in the DR group were significantly increased (all $p < 0.001$), while eGFR was significantly decreased ($p < 0.001$) when compared to those in the control group. There were no significant differences in other clinical characteristics including age, gender, systolic blood pressure, and diastolic blood pressure between the two groups ($p > 0.05$ for all).

3.2. B-Cell-Produced Antibodies in the Vitreous Samples. The concentrations of B-cell-produced antibodies between the DR and the control group are shown in Table 2. The concentrations of all the antibodies were within the detection limit, and the results revealed that IgA, IgM, and total antibodies in the DR group were significantly higher than those in the control group after Bonferroni correction (all $p < 0.001$). A detailed description on the levels of IgA, IgM, and total antibodies between the two groups was shown in Figure 2. The four IgG subtypes showed no significant differences between the two groups after Bonferroni correction ($p > 0.006$ for all).

3.3. Correlations. There were low correlations between clinical variables and levels of B-cell-produced antibodies (Table 3). The concentration of IgA was positively correlated with FBG (low correlation with a $r = 0.317$, $p = 0.001$) and negatively correlated with eGFR (low correlation with a $r = -0.443$, $p < 0.001$). The concentration of IgM was positively correlated with UACR (low correlation with a $r = 0.363$, $p < 0.001$) and negatively correlated with eGFR (low correlation with a $r = -0.377$, $p < 0.001$).

3.4. Calculating Equations for Antibodies in Vitreous Humor. Multiple linear regression analysis using clinical variables (such as the duration of DM and DR, eGFR, and UACR) was performed to predict IgA, IgM, and total antibody values, which yielded three equations:

$$\text{IgA (ng/ml)} = -15.805 * \text{age (years)} - 11.342 * \text{eGFR (ml/min/1.73 m}^2\text{)},$$

$r = 0.542$, $p < 0.001$, and standard error of estimate = 776.67 ng/ml.

Table 2: Concentrations of antibodies in the vitreous humor.

	DR ($n = 52$)	Non-DR ($n = 52$)	p value
IgG1 (ng/ml)	1654.05 ± 993.35	2196.35 ± 1310.44	0.019
IgG2 (ng/ml)	436.01 ± 236.54	529.7 ± 237.61	0.047
IgG3 (ng/ml)	478.6 ± 266.97	523.2 ± 212.83	0.348
IgG4 (ng/ml)	926.77 ± 888.32	1065.81 ± 589.86	0.350
IgG (ng/ml)	3495.42 ± 1682.07	4315.05 ± 1477.15	0.010
IgA (ng/ml)	2156.71 ± 1029.42	1376.49 ± 469.8	<0.001*
IgM (ng/ml)	2013.55 ± 1877.88	471.28 ± 237.1	<0.001*
Total antibodies (ng/ml)	7665.67 ± 2348.57	6162.82 ± 1480.91	<0.001*

Abbreviation: DR: diabetic retinopathy; Ig: immunoglobulin. *Statistically significant by independent, two-tailed Student's t-tests (p value < 0.006 after Bonferroni correction).

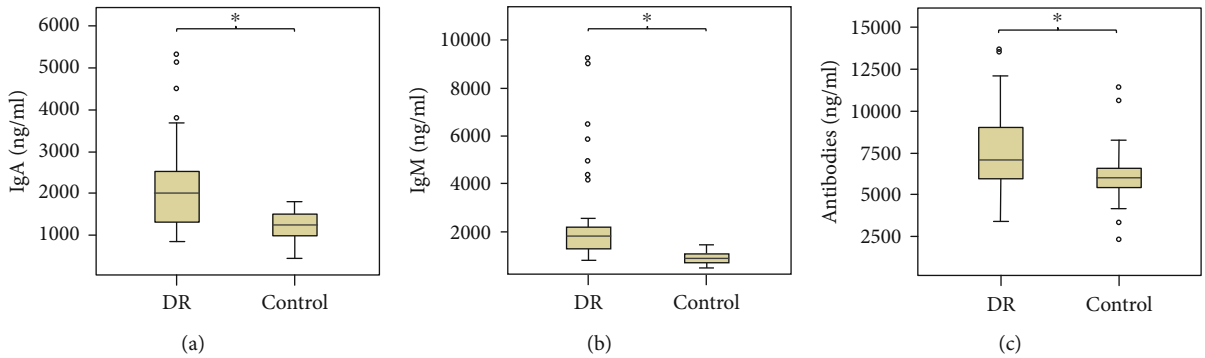

Figure 2: The concentrations of IgA, IgM, and antibodies in the vitreous humor of the two groups. Abbreviations: DR: diabetic retinopathy; Ig: immunoglobulin. (a) The concentration of IgA antibody (ng/ml) in the vitreous humor of the DR patients and the control group. (b) The concentration of IgM antibody (ng/ml) in the vitreous humor of the DR and control groups. (c) The concentration of antibodies (ng/ml) in the vitreous humor of the DR group and the control group. The levels of IgA, IgM, and total antibodies in the vitreous humor were significantly higher in the DR group than in the control group. *Statistically significant p value < 0.001 by independent, two-tailed Student's t-tests.

$$\text{IgM (ng/ml)} = -10.861 * \text{eGFR (ml/min/1.73 m}^2\text{)} + 0.447 * \text{UACR (mg/g)},\ r = 0.461,\ p < 0.001,$$ and standard error of estimate = 1394.90 ng/ml.

$$\text{Total antibodies (ng/ml)} = 6749.95 - 14.473 * \text{eGFR (ml/min/1.73 m}^2\text{)},\ r = 0.312,\ p = 0.016,$$ and standard error of estimate = 2019.53 ng/ml.

These three equations showed that the concentrations of IgA, IgM, and total antibodies in the vitreous humor of the DR patients were associated with eGFR, which is a marker for kidney damage.

4. Discussion

Systemic inflammation is associated with the whole course of T2DM, and it plays an important role in the development and progression of DR. Activation of B cells contributed to the development of DM in recent years [21–23]. However, the immune response mediated by B cells has been rarely reported in the DR patients [24]. This study analyzed the B-cell-produced antibodies including IgA, IgM, and four IgG subtypes in the vitreous humor of T2DM patients with DR both qualitatively and quantitatively. The concentrations of IgA, IgM, and total antibodies were significantly increased in the DR group when compared to the control group. Besides, there were low negative correlations between levels of antibodies (IgA, IgM) and eGFR. These results might shed light on novel insights regarding the role of B cells in the development and progression of DR.

In the present study, the concentrations of IgA, IgM, and total antibodies, but none of the IgG subtypes, were increased in the vitreous humor of patients with DR. The reason for the increase of IgA might be due to this type of antibody being "spared" by the phagocytes in the eye, as it mainly possesses receptors for Fc fragments of other antibodies, e.g., IgG [24]. On the other hand, increase in IgM might be caused by the destruction of the blood vessel-retinal tissue barrier in DR and is followed by a large number of antigens and inflammatory cells entering the retina and vitreous humor to trigger an acute inflammatory response [25]. In the future, the above hypotheses and the functional roles of antibodies in the pathogenesis of DR require further investigation. With regard to the concentration of IgG, no significant difference between DR patients and control subjects was detected. These findings were similar to the results of a previous study [26]. Taken

Table 3: Correlations between clinical characteristics, serum metrics, and levels of antibodies.

Clinical characteristics	IgA (ng/ml)		IgM (ng/ml)		Antibodies (ng/ml)	
	r value	*p* value	*r* value	*p* value	*r* value	*p* value
Age (y)	-0.186	0.029*	-0.123	0.108	-0.097	0.164
Sex	0.119	0.114	-0.082	0.204	-0.053	0.298
Duration of DM (years)	0.128	0.099	0.260	0.004*	0.146	0.070
Duration of DR (months)	0.178	0.036*	0.069	0.242	0.026	0.395
SBP (mmHg)	0.121	0.110	-0.027	0.392	-0.005	0.480
DBP (mmHg)	0.203	0.020*	0.027	0.393	-0.008	0.468
FBG (mmol/l)	*0.317*	*0.001* *	0.132	0.091	-0.005	0.479
HbA1c (%)	0.296	0.001*	0.276	0.002*	0.201	0.020*
sCr (μmol/l)	0.276	0.002*	0.147	0.068	0.094	0.171
BUN (mmol/l)	0.287	0.002*	0.14	0.078	0.119	0.115
eGFR (ml/min/1.73 m^2)	*-0.443*	*<0.001* **	*-0.377*	*<0.001* **	-0.289	0.001*
UACR (mg/g)	0.110	0.133	*0.363*	*<0.001* **	0.185	0.030*

Abbreviation: DM: diabetes mellitus; Ig: immunoglobulin; SBP: systolic blood pressure; DBP: diastolic blood pressure; FBG: fasting blood glucose; HbA1c: glycated hemoglobin; sCr: serum creatinine; BUN: blood urea nitrogen; eGFR: estimated glomerular filtration rate; UACR: urinary albumin to creatinine ratio. Italicized letters: low correlations were found between B-cell-produced antibodies and clinical variables of DM. *Statistically significant p value of <0.05 by Pearson's correlation analysis. **Statistically significant p value of <0.001 by Pearson's correlation analysis.

together, these results suggested that IgG might not play a significant role in the pathogenesis of DR. As known, the first Ig that is synthesized during the early phase of humoral immune response is IgM, which acts as a first-line defense. The humoral immune system switches to the production of IgG that serves as the subsequent defense and is responsible for immune memory. The increased levels of IgM in the DR patients of our study indicate that the development of DR might be associated with an acute ocular humoral immune response. However, similar IgG levels in DR patients and control subjects suggest that long-term immune memories of triggering factors might not be developed in eyes with DR.

Correlation analysis revealed low negative correlations between levels of antibodies (IgA, IgM) and eGFR, a marker of kidney damage. These negative correlations suggested that kidney damage tended to be more severe in patients with stronger B-cell-mediated immune responses. Moreover, multiple linear regression yielded three equations for predicting the concentrations of IgA, IgM, and total antibodies in the vitreous humor according to eGFR, suggesting possible associations between retinopathy and nephropathy during the development of DM [27–29]. The mechanisms of the associations between ocular B-cell-produced antibodies and eGFR in T2DM still remain unknown. Considering that diabetic nephropathy and diabetic retinopathy are microvascular complications of T2DM, a potential "common pathway" might exist in their underlying mechanisms. A previous study has demonstrated that persistent hyperglycemia and insulin resistance could lead to the progression of vascular inflammation and dysfunction of endothelial cells [30]. When this process occurs in kidneys, it causes glomerular filtration dysfunction, resulting in diabetic nephropathy [31]. When the similar process affects eyes, it led to progressive breakdown of hematoocular barrier and occurrence of DR [32]. If microvascular complications occur, then the activated B cells reach the damaged sites through the blood vessel wall and produce antibodies. In addition, the antibodies produced by the activated circular B cells can also be carried to the damaged sites by the blood flow. These pathophysiological processes might be the mechanisms that underlie the increased concentrations of antibodies in the vitreous humor of T2DM patients with DR, although further studies are warranted to figure out the exact mechanisms. Moreover, based on the above results with regard to the correlation analysis and multiple linear regression analysis, the extent of ocular B-cell activation in the vitreous humor of DR patients showed correlation with the levels of certain serum metrics. These findings remind us of the necessity of cooperation of ophthalmologists with endocrinologists and renal physicians to monitor microvascular damage in DM [33, 34].

The present study for the first time reported that local humoral immune response was involved in the pathogenesis of DR in T2DM, suggesting that the B cells might play an important role in the development and progression of DR. However, there were several limitations that should be acknowledged in the current study. Firstly, the sample size is not large and the study is not randomized which may lead to selection bias. Although significant differences have been observed using the included number of samples, population-based randomized studies with large sample sizes are needed to validate the results of the present study. Secondly, it would be more convincing if a group of diabetic patients without DR was added. However, during patient recruitment, very few T2DM patients without DR who met the inclusion criteria were referred to our clinic. Thirdly, the concentrations of antibodies in the serum samples were not analyzed. Although the previous studies have proven that the levels of plasma antibodies were increased in DR [14, 35], it would be better if the serum antibodies were measured in our patients.

5. Conclusions

This study showed that higher levels of IgA, IgM, and total antibodies produced by B cells were detected in the vitreous humor of T2DM patients with DR. There were low negative correlations between concentrations of IgA and IgM in the vitreous humor and eGFR, indicating a potential relationship between retinopathy and nephropathy in T2DM. Further investigations are needed to verify the functional roles of B cells in DR and other microvascular complications of diabetes.

Data Availability

The data used during the current study are available from the corresponding author on reasonable request.

Ethical Approval

This study was performed in accordance with the tenets of the Declaration of Helsinki and approved by the local Research Ethics Committee of the Guangdong Provincial People's Hospital (Number: 2016232A).

Consent

Informed consent was obtained from the subjects.

Disclosure

The sponsors or funding organizations had no role in the design or conduct of this research.

Conflicts of Interest

The authors declare that they have no competing interests.

Authors' Contributions

Baoyi Liu, Yijun Hu, and Qiaowei Wu are co-first authors.

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Clinical Study

Restoration of Foveal Bulge after Resolution of Diabetic Macular Edema with Coexisting Serous Retinal Detachment

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Purpose. To evaluate the impact of restoration of foveal bulge (FB) in optical coherence tomography (OCT) images on visual acuity after resolution of diabetic macular edema with coexisting serous retinal detachment (SRD-DME). *Methods*. A total of 52 eyes with resolved SRD-DME and an intact ellipsoid zone at the central fovea were included. All eyes underwent best-corrected visual acuity (BCVA) examination and OCT scanning at baseline and follow-up visits (1, 3, and 6 months). The eyes were divided into two groups according to the presence of FB at 6 months. BCVA, central foveal thickness (CFT), height of SRD (SRDH), outer nuclear layer (ONL) thickness, photoreceptor inner segment (PIS), and outer segment (POS) length were compared between the two groups. *Results*. A FB was found in 25 of 52 (48%) eyes at 6 months. The FB (+) group had lower SRDH at baseline, and better BCVA, longer POS length at 6 months (all $P < 0.05$). There was no significant difference in the CFT, ONL thickness, and PIS length at 6 months between the two groups (all $P > 0.05$). More eyes in the FB (+) group had complete SRD resolution at 1 month ($P = 0.009$) and 3 months ($P = 0.012$). Eyes with complete SRD resolution at 1 month ($P = 0.009$) or 3 months ($P = 0.012$) were more likely to have a FB at 6 months. *Conclusions*. The Presence of the FB is associated with better BCVA after resolution of SRD-DME. Eyes with lower baseline SRDH or faster SRD resolution are more likely to have a FB at 6 months.

1. Introduction

Retinal detachment (RD) refers to a clinical situation where the neurosensory retina is detached from the underlying retinal pigment epithelium (RPE) [1]. Separation of the neurosensory retina from the RPE leads to deprivation of nutrition and oxygen supplies to the outer retina which in turn causes photoreceptor apoptosis and visual loss [1–3]. For instance, disruption of the ellipsoid zone (EZ), which represents the junction of the inner and outer photoreceptor segments, has been observed in patients with rhegmatogenous RD (RRD) using optical coherence tomography (OCT) [4, 5]. Successful reattachment of the neurosensory retina is essential for vision recovery in RD patients. Retinal reattachment allows restoration of blood supply to the outer retina and regeneration of the photoreceptors, and the patients' visual acuity is recovered accordingly. Better visual recovery is usually closely correlated to an intact EZ at the fovea after retinal reattachment [6, 7]. However, visual acuity in some RRD patients after successful retinal reattachment is still

unsatisfactory despite the presence of an intact EZ at the fovea [8, 9]. In these patients, the absence of a foveal bulge (FB) is considered to be a reason for the incomplete visual recovery after retinal reattachment [8, 9].

Serous RD (SRD) is a subgroup of RD commonly seen in central serous chorioretinopathy (CSC) and diabetic macular edema (DME) [10, 11]. DME with coexisting SRD (SRD-DME) can be observed in the OCT images of DME patients [10]. Photoreceptor damage can also be seen in eyes with SRD-DME and the photoreceptors can be restored after resolution of the DME [12–14]. However, the visual acuity of some DME patients cannot be fully restored despite the complete edema resolution and presence of an intact EZ at the fovea. In light of the previous studies of RRD, we suppose the FB may have an impact on the visual acuity of these DME patients. In the present study, we aimed at determining whether the presence of a FB is correlated with a better visual acuity after the resolution of SRD-DME.

2. Methods

2.1. Subjects. In this retrospective study, 43 patients (52 eyes) with resolved SRD-DME were recruited from the Department of Ophthalmology at Guangdong Provincial People's Hospital (GPPH) between January 1, 2017, and March 1, 2019. All the patients received comprehensive baseline ophthalmologic examinations including best-corrected visual acuity (BCVA) with decimal chart which was converted to the logarithm of minimal angle of resolution (LogMAR) and the Snellen visual acuity, slit-lamp biomicroscope anterior segment and fundus examination, intraocular pressure (IOP) measurement, and baseline SD-OCT scanning (Spectralis; Heidelberg Engineering, Heidelberg, Germany). All patients underwent BCVA measurement, fundus examination, and SD-OCT scanning at 1, 3, and 6 months after 3 monthly consecutive intravitreal injections of ranibizumab (IVR) treatment. The study was conducted according to the 1964 Helsinki declaration and was approved by the Institutional Review Board of GPPH. Informed consent was obtained from all the patients after explanation of the nature of the study.

The inclusion criteria were SRD-DME secondary to type 2 diabetes mellitus (DM) and involving the fovea at baseline, SRD-DME resolution with an intact EZ at the central fovea at 6 months, treatment-naive eyes or eyes that received previous anti-VEGF or retinal photocoagulation no less than 6 months ago, BCVA between 0.3-1.0 LogMAR (≈20/200 - 20/40), and central foveal thickness (CFT) more than 275 μm before treatment [15, 16]. We excluded eyes with macular edema or SRD secondary to other causes such as age-related macular degeneration, polypoidal choroidal vasculopathy, retinal artery/vein occlusion, CSC, rhegmatogenous retinal detachment, eyes with macular ischemia, glaucoma, IOP > 21 mmHg, severe cataracts, refractive error greater than 6 diopters (D), a history of vitrectomy or macular grid photocoagulation, or DME previously treated with intravitreal or periocular injection or retinal photocoagulation within 6 months. Eyes that could not be scanned using SD-OCT due to poor patient cooperation were also excluded.

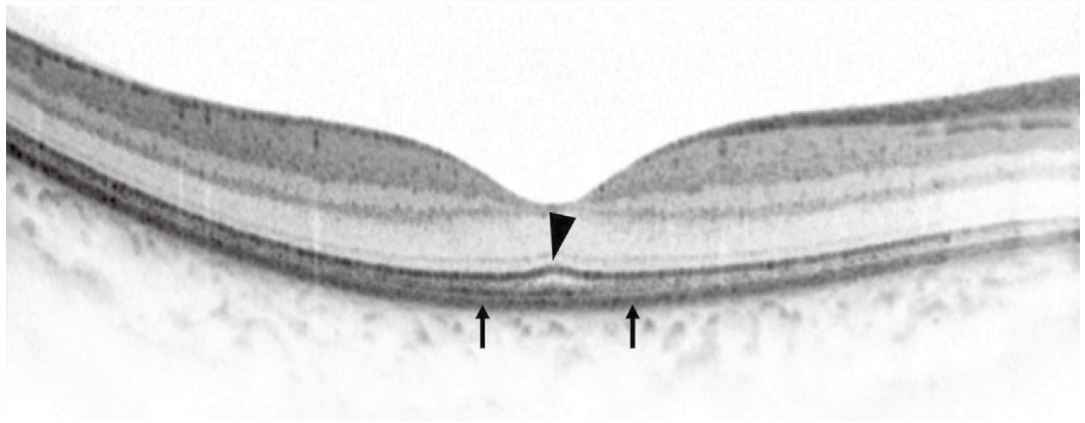
2.2. Intravitreal Injection of Ranibizumab. All patients received 3 monthly consecutive 0.5 mg IVR. After the loading treatment, patients received an additional injection if they met any of the following criteria: (a) BCVA decrease of ≥0.1 LogMAR; (b) CFT increase of ≥100 μm; or (c) BCVA decrease due to newly formed or enlargement of previous intraretinal cyst or SRD, as decided by the surgeons. Treatment was suspended if one of the following criteria were met: (a) stable vision over 3 consecutive visits, including the current visit evaluation, specifically no further BCVA improvement attributed to treatment at the 2 last consecutive visits; or (b) BCVA ≤ 0.0 LogMAR observed at the 2 last consecutive visits [17].

2.3. OCT Measurement and Classification of DME. A custom 20°x 20° volume acquisition protocol was used to obtain a set of high-speed scans from each eye. With this protocol, 25 horizontal and central vertical cross-sectional B-scan images were obtained, each composed of 512 A-scans [18]. The horizontal image through the fovea as determined by simultaneous evaluation of the red-free image on the computer monitor of the OCT scanner [19] was exported for manual measurement of the CFT, height of SRD (SRDH), outer nuclear layer (ONL) thickness, photoreceptor inner segment (PIS), and outer segment (POS) length (Figure 1). OCT images were read and measured independently by 2 Chinese board-certified ophthalmologists (QW, BL) in a masked manner. If there were discordance between the 2 ophthalmologists, arbitration was performed by a retinal specialist (HY) to generate the final decision.

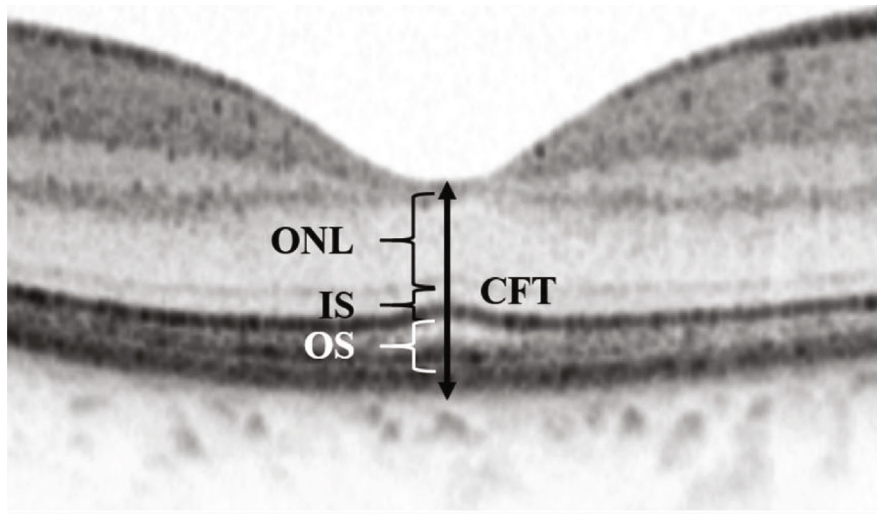
SRD-DME was defined as DME with an elevation of the neurosensory retina and an optically clear space between the retina and RPE, with possible coexistence of intraretinal swelling or cysts in the macular area [17]. The eyes included were divided into two groups, the FB (+) group and the FB (-) group, based on the presence of FB at 6 months after IVR. The presence of the FB was defined as the POS length at the central fovea being 10 μm longer than the average POS length at 250 μm temporal and nasal from the central fovea [8, 9]. Typical OCT images of the FB (+) group and the FB (-) group are shown in Figures 2 and 3.

The CFT, SRDH, ONL thickness, PIS length, and POS length were manually measured at the central fovea. The CFT was defined as the distance between the surface of the internal limiting membrane (ILM) and the outer border of the RPE. The SRDH was defined as the vertical distance between the first signal from the top of the SRD and the signal from the anterior boundary of the RPE-choriocapillaris region. The ONL thickness was measured as the distance between the outer border of the ILM and the outer border of the external limiting membrane (ELM). The PIS length was the distance between the outer border of the ELM and the outer border of the PIS/POS line. The POS length was the distance between the outer border of the PIS/POS line and the inner border of the RPE [8, 9].

2.4. Statistical Analysis. The data are presented as mean ± standard deviation. All statistical analyses were performed using the SPSS 20.0 (SPSS. Inc., Chicago, IL, USA). To



(a)



(b)

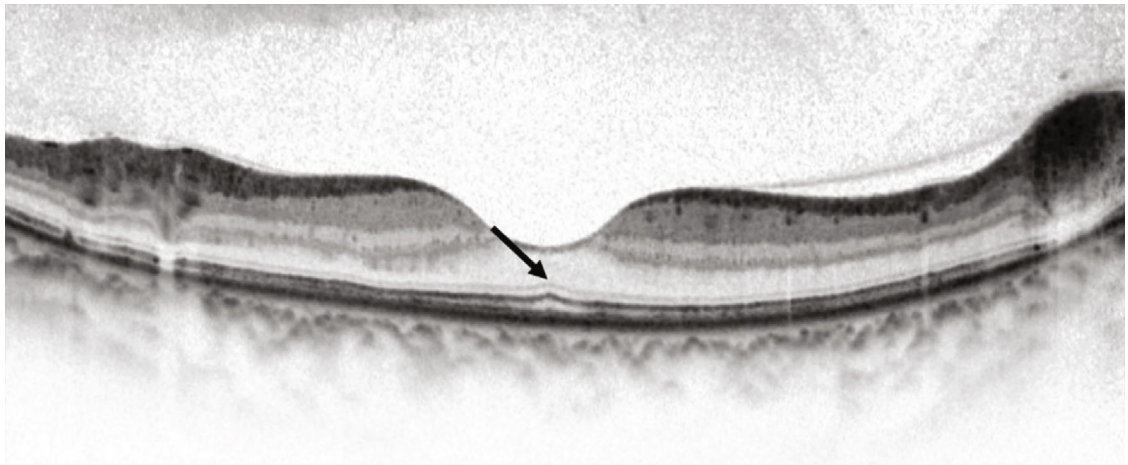
Figure 1: Illustration of spectral-domain optical coherence tomography (SD-OCT) image. (a) A horizontal 30° scan through the central fovea was obtained. The SD-OCT image shows that the photoreceptor inner segment/outer segment (PIS/POS) line has a bulge at the central fovea, named a foveal bulge (arrowhead). The foveal bulge is defined by the POS length at the central fovea being 10 μm longer than the average POS length at 250 μm temporal and nasal from the central fovea (arrows). (b) Magnified view. The CFT is the distance between the surface of the internal limiting membrane (ILM) and the outer border of the retinal pigment epithelium (RPE) at the central fovea. The thickness of the outer nuclear layer (ONL) is the distance between the outer border of the ILM and the outer border of the external limiting membrane (ELM). The length of the PIS is the distance between the outer border of the ELM and the outer border of the PIS/POS line. The length of the POS is the distance between the outer border of the PIS/POS line and the inner border of the RPE.

validate the agreement between the two ophthalmologists (QW, BL), the intraclass coefficient (ICC) was calculated. Statistical differences in the parameters between the FB (+) group and the FB (-) group were assessed using the unpaired Mann-Whitney test, Chi-square test, or Fisher's exact test after confirming the data normality. For all the tests, $P < 0.05$ was considered statistically significant.

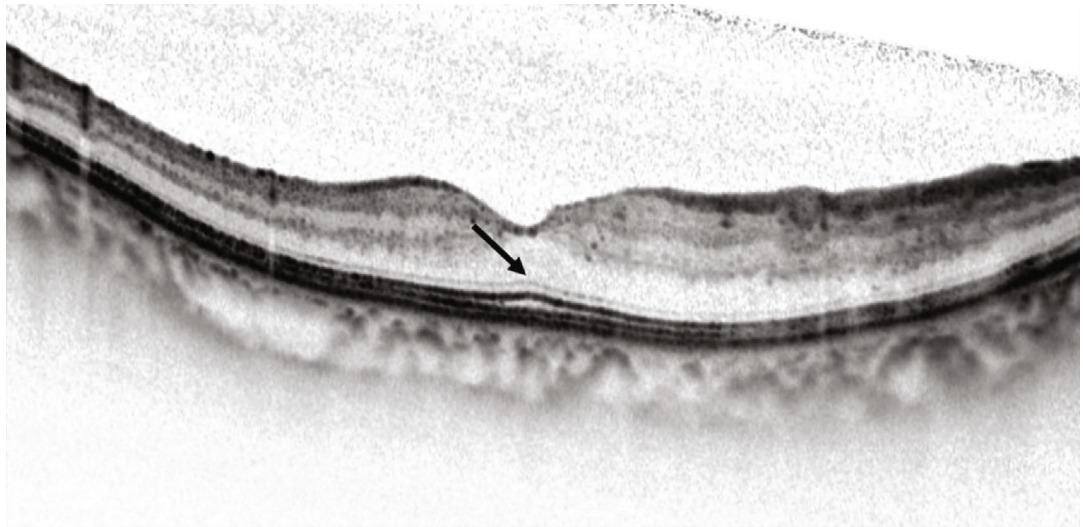
3. Results

3.1. Basic Characteristics. A total of 52 eyes with completely resolved SRD-DME and an intact ellipsoid zone at the central fovea were included. A FB was found in 25 eyes (48%) at 6 months. Basic characteristics were not significantly different between the FB (+) group and the FB (-) group (Table 1). Regarding the reproducibility of OCT measurements, the interobserver ICC was 0.958 for ONL thickness, 0.847 of PIS length, and 0.910 for POS length, suggesting good reproducibility for OCT measurements between the two ophthalmologists (QW, BL).

3.2. BCVA and OCT Measurements at 6 Months. The FB (+) group had better BCVA and longer POS length at 6 months compared to the FB (-) group (Table 2). At 6 months, the BCVA was 0.19 ± 0.18 in the FB (+) group and 0.35 ± 0.18 in the FB (-) group ($P = 0.004$, unpaired Mann-Whitney test). There were 9 eyes with a BCVA ≥20/20, and 7 of the 9 had a FB and 2 of the 9 had no FB. In the FB (+) group, there were 7 of 25 eyes with a BCVA ≥20/20, compared to 2 of 27 eyes in the FB (-) group ($P = 0.071$, Chi-square test). The CFT at 6 months was 187.68 ± 27.00 μm in the FB (+) group and 196.37 ± 29.54 μm in the FB (-) group ($P = 0.314$).



(a)



(b)

Figure 2: Spectral-domain optical coherence tomography (SD-OCT) images of eyes with diabetic macular edema. (a) The SD-OCT image of a 42-year-old woman 6 months after 3 monthly consecutive intravitreal injections of ranibizumab (IVR) treatment shows a complete resolution of DME with coexisting serous retinal detachment (SRD-DME). The SD-OCT image shows longer foveal photoreceptor outer segment (POS) length and the presence of a foveal bulge (arrow). The BCVA was 20/20. (b) The SD-OCT image of a 49-year-old man 6 months after 3 monthly consecutive IVR treatment shows a complete resolution of SRD-DME. The SD-OCT image also shows a longer foveal POS length and the presence of a foveal bulge (arrow). The BCVA was 20/25.

The ONL thickness at 6 months was $94.80 \pm 15.53\,\mu$m in the FB (+) group and $101.67 \pm 18.17\,\mu$m in the FB (-) group ($P = 0.203$). The POS length at 6 months was $41.48 \pm 3.39\,\mu$m in the FB (+) group and $31.44 \pm 3.24\,\mu$m in the FB (-) group ($P < 0.001$). The PIS length at 6 months was $31.28 \pm 2.76\,\mu$m in the FB (+) group and $30.70 \pm 3.45\,\mu$m in the FB (-) group ($P = 0.289$).

3.3. Factors Associated with FB Formation. Mean SRDH was $214.96 \pm 85.01\,\mu$m in the FB (+) group and $308.11 \pm 186.27\,\mu$m in the FB (-) group ($P = 0.040$). In the FB (+) group, there was 84.0% of the eyes having complete SRD resolution at 1 month, compared to 48.1% of the eyes in the FB (-) group ($P = 0.009$, Fisher's exact test). At 3 months, 96.0% of eyes in the FB (+) group had SRD resolution, compared to 66.7% of eyes in the FB (-) group ($P = 0.012$) (Table 2). On the other hand, 61.8% of the eyes with complete SRD resolution at 1 month had a FB at 6 months, and 22.2% of the eyes with residual subretinal fluid at 1 month had a FB at 6 months ($P = 0.009$). Moreover, 57.1% of eyes with complete SRD resolution at 3 months had a FB at 6 months, and 10.0% of eyes with residual subretinal fluid at 3 months had a FB at 6 months ($P = 0.012$) (Table 3).

4. Discussion

In the present study, a FB was found in 48.1% of eyes with resolved SRD-DME at 6 months after IVR. Eyes in the FB (+) group had faster SRD resolution at 1 and 3 months and better BCVA and longer POS length at 6 months compared to the FB (-) group. The results of our study were consistent with previous studies showing better BCVA and longer POS

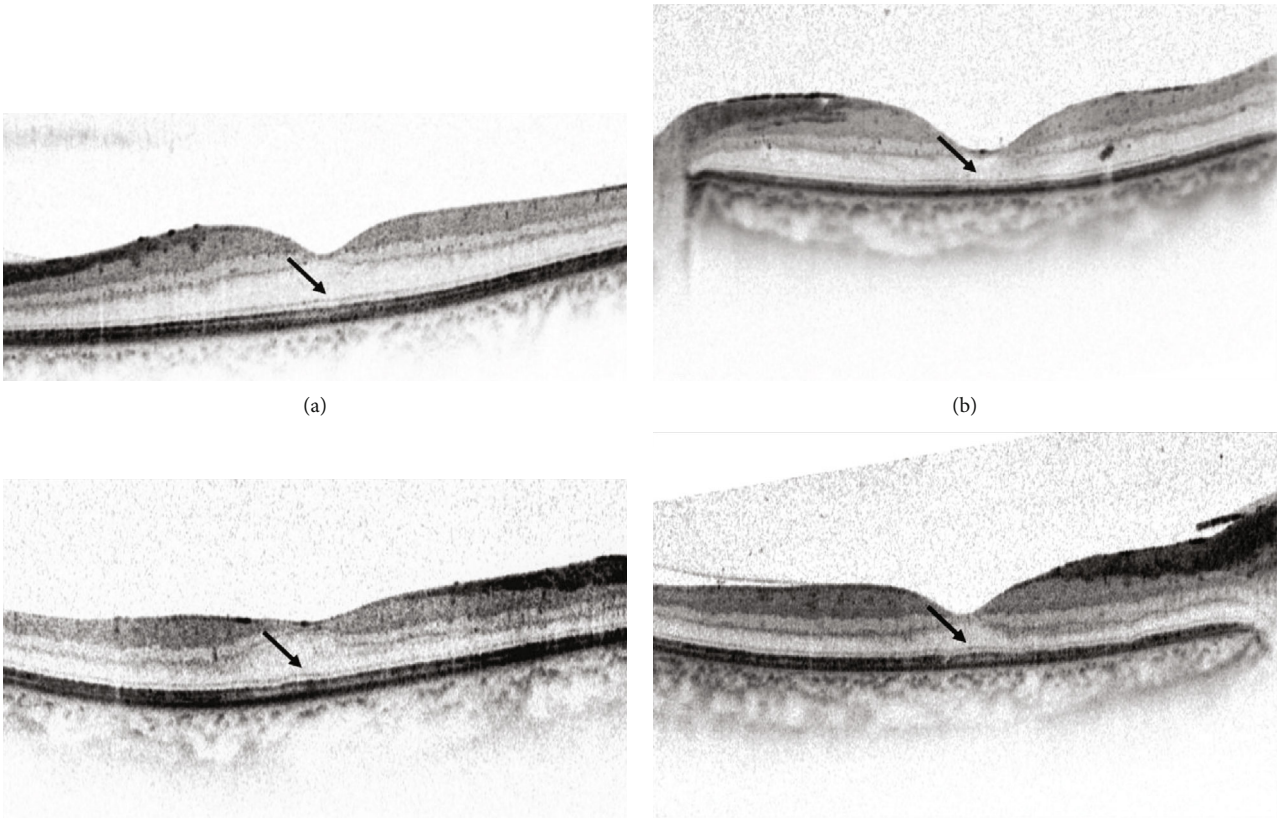


Figure 3: Spectral-domain optical coherence tomography (SD-OCT) images of resolved diabetic macular edema eyes without a foveal bulge (arrow). (a) The BCVA was 20/40. (b) The BCVA was 20/63. (c) The BCVA was 20/40. (d) The BCVA was 20/32.

Table 1: Baseline characteristics of diabetic macular edema eyes with coexisting serous retinal detachment in foveal bulge (+) group and foveal bulge (-) group.

	Foveal bulge (+) ($n = 25$)	Foveal bulge (−) ($n = 27$)	P
Mean age (SD) (years)	54.64 (8.83)	56.67 (13.50)	0.134†
Mean IOP (SD) (mmHg)	14.12 (2.19)	15.07 (2.73)	0.132†
Mean time since diagnosis of DM (SD) (years)	7.72 (3.71)	9.04 (5.42)	0.568†
Mean duration of DME before IVR (SD) (months)	7.99 (6.43)	10.50 (7.43)	0.161†
Mean HbA1C (SD) (%)	8.09 (1.30)	8.66 (2.60)	0.761†
Diabetic retinopathy severity (n (%))			0.746‡
NPDR	15 (60.0%)	15 (55.6%)	
PDR	10 (40.0%)	12 (44.4%)	
Photocoagulation treatment (n (%))	13 (52.0%)	14 (51.9%)	0.991‡
Mean baseline BCVA (SD) (logMAR/Snellen VA)	0.46 (0.24)/≈20/57.7	0.52 (0.15)/≈20/66.2	0.135†
Mean baseline CFT (SD) (μm)	533.84 (178.69)	587.37 (235.75)	0.589†
Mean baseline SRDH (SD) (μm)	214.96 (85.01)	308.11 (186.27)	0.040†

†Unpaired Mann-Whitney test; ‡Chi-square test. IOP: intraocular pressure; DM: diabetic mellitus; IVR: intravitreal injection of ranibizumab; HbA1c: glycosylated hemoglobin; NPDR: nonproliferative diabetic retinopathy; PDR: proliferative diabetic retinopathy; BCVA: best-corrected visual acuity; Snellen VA: Snellen visual acuity; CFT: central foveal thickness; SRDH: height of serous retinal detachment; SD standard deviation.

length in eyes with a FB after successful RRD repair or resolution of macular edema associated with branch retinal vein occlusion (BRVO) [8, 20]. Based on our best knowledge, there has been no previous study about FB formation in eyes with resolved SRD-DME. Therefore, our study may shed light to further investigations about prognostic factors of visual outcomes after DME treatment. In our study, the BCVA in eyes with resolved SRD-DME and an intact EZ at the fovea varied from 20/100-20/16, and 82.7% of the eyes had a BCVA<20/20. These findings suggest that an intact

Table 2: Comparison of posttreatment optical coherence tomography measurements in diabetic macular edema eyes with coexisting serous retinal detachment in foveal bulge (+) group and foveal bulge (-) group.

	Foveal bulge (+) ($n = 25$)	Foveal bulge (-) ($n = 27$)	P
IVR (SD) (n)	4.00 (1.29)	4.19 (1.18)	0.460†
Mean 6M BCVA (SD) (LogMAR/Snellen VA)	0.19 (0.18)/≈20/31.0	0.35 (0.18)/≈20/44.8	0.004†
Mean 6M CFT (SD) (μm)	187.68 (27.00)	196.37 (29.54)	0.314†
Mean ONL thickness (SD) (μm)	94.80 (15.53)	101.67 (18.17)	0.203†
Mean photoreceptor IS length (SD) (μm)	31.28 (2.76)	30.70 (3.45)	0.289†
Mean photoreceptor OS length (SD) (μm)	41.48 (3.39)	31.44 (3.24)	<0.001†
SRD complete resolution (n (%))			
1 M	21 (84.0%)	13 (48.1%)	0.009‡
3 M	24 (96.0%)	18 (66.7%)	0.012‡

†Unpaired Mann-Whitney test; ‡Fisher's exact test. IVR: intravitreal injection of ranibizumab; 6 M: 6 months after 3 monthly consecutive intravitreal injections of ranibizumab treatment; BCVA: best-corrected visual acuity; Snellen VA: Snellen visual acuity; CFT: central foveal thickness; ONL: outer nuclear layer; IS: inner segment; OS: outer segment; SD: standard deviation; SRD: serous retinal detachment.

Table 3: Presence of a foveal bulge at 6 months in diabetic macular edema with coexisting serous retinal detachment eyes with complete serous retinal detachment resolution of at 1 or 3 months.

1 M	SRD complete resolution (+) ($n = 34$)	SRD complete resolution (-) ($n = 18$)	P
FB (+) (n (%))	21 (61.8%)	4 (22.2%)	0.009†
3 M	SRD complete resolution (+) ($n = 42$)	SRD complete resolution (-) ($n = 10$)	P
FB (+) (n (%))	24 (57.1%)	1 (10.0%)	0.012†

†Fisher's exact test. SRD: serous retinal detachment; 1 M: 1 month after 3 monthly consecutive intravitreal injections of ranibizumab treatment; 3 M: 3 months after 3 monthly consecutive intravitreal injections of ranibizumab treatment; FB: presence of foveal bulge at 6 months after 3 monthly consecutive intravitreal injections of ranibizumab treatment.

EZ may not be the only indicator of good visual recovery after SRD-DME resolution. According to our results, the presence of a FB and longer POS at the fovea were also possible indicators of better visual outcomes in eyes with an intact EZ after SRD-DME resolution.

Previous studies have shown that an intact EZ is associated with better visual recovery after RRD surgery [21–23]. However, visual acuity may be still unsatisfactory in some eyes despite an intact EZ after retinal reattachment [8, 24]. Hasegawa et al. proposed that the presence of a FB at the fovea was associated with better visual acuity after successful RRD repair. They observed a FB in all the eyes with macula-on RRD and only in 28.6% of eyes with macula-off RRD. BCVA was significantly better in eyes with a FB after successful RRD surgery. The authors proposed a mechanism of the FB formation in normal eyes which involved POS thinning, elongation, and density increase during the development of the fovea and the difference in width between the POS and PIS. They supposed that the absence of a FB after RRD repair was due to length shortening and density decrease of the POS [8].

SRD was present in 21.7%-38.5% of eyes with DME and was suggested to be caused by a breakdown of the outer blood-retinal barriers [17, 19, 25–27]. Photoreceptor damages such as POS shortening and EZ disruption have been observed in eyes with SRD-DME [17, 26]. It is very likely that the photoreceptors and EZ undergo a recovery process after SRD-DME resolution similar to the one after RRD repair. With elongation and increased density of the POS after SRD-DME resolution, a normal FB is formed at the fovea. Thus, the formation of a FB would be a sign of better anatomical recovery of the photoreceptors at the fovea after SRD-DME resolution. A better anatomical fovea in turn leads to more favorable visual outcomes. Therefore, the FB can be considered as an indicator of better anatomical recovery and a prognostic factor of better functional recovery in eyes with resolved SRD-DME. This theory could be verified by the results of our study. In our study, eyes in the FB (+) group had significantly longer POS length at 6 months after IVR than the FB (-) group, indicating better POS regeneration in the FB (+) eyes. Accordingly, the BCVA at 6 months in the FB (+) group was significantly better than the FB (-) group, suggesting more favorable visual outcomes in the FB (+) eyes. Since the mean CFT at 6 months were not significantly different between the two groups and an intact EZ was present in all of the eyes, it seemed that eyes in the FB (+) group had underwent better POS regeneration and elongation, leading to a FB formation and better visual acuity after SRD-DME resolution. It is noteworthy that the presence of a FB is associated with a higher likelihood of having better BCVA, but not a guarantee or requirement of having ≥20/20 vision. In the present study, 22.2% of eyes with a BCVA ≥20/20 did not have a FB and 72% of eyes with a FB had a BCVA<20/20.

In our study, we also aimed at finding out factors associated with the FB formation. We observed a lower mean

baseline SRDH in the FB (+) group compared to the FB (-) group. It was possible that baseline photoreceptor damage was more severe in the FB (-) group. Previous studies have shown that photoreceptor damage is more severe in DME eyes with higher SRD [26]. With the increased distance between the photoreceptors and the RPE, deprivation of nutrition and oxygen supplies to the photoreceptors is more severe which may cause more photoreceptor damages. Moreover, the speed of subretinal fluid resolution after treatment may be faster in eyes with lower SRDH. A previous study has found that the speed of SRD resolution after the intravitreal injection of dexamethasone implant is negatively correlated with the baseline SRDH [28]. In our study, the proportions of eyes with complete SRD resolution at 1 month or 3 months were significantly higher in the FB (+) group than the FB (-) group. Basic research has also demonstrated that the photoreceptors begin to recover only after the retina is reattached [29, 30]. Taken together, eyes with a lower SRDH may experience faster photoreceptor regeneration to heal the less severe EZ damage in a shorter period after DME treatment.

In the present study, the POS length was significantly longer in the FB (+) group. However, the ONL thickness or PIS length was not significantly different between the two groups. Similar findings were also reported in previous studies about FB formation after successful RRD repair or resolution of SRD associated with BRVO [8, 20]. In Hasegawa et al.'s theory, POS elongation is critical for FB formation after RD reattachment [8, 20]. On the other hand, the ONL and PIS seem not associated with the FB formation. This is reasonable since the POS loss is one of the first and primary damages caused by RD [29–31]. These findings indicate the necessity of reducing POS damage and promoting POS recovery in the treatment of RD, including SRD-DME. Previously, two clinical studies demonstrated that postoperative POS length was correlated with postoperative BCVA in DME patients treated with vitrectomy [32] and in idiopathic epiretinal membrane (ERM) patients underwent vitrectomy surgery [33]. These findings are consistent with the result of our study showing better BCVA and longer POS length in the FB (+) group. Moreover, preoperative POS length was shown to be predictive of postoperative BCVA, indicating that preoperative POS length was a potential predictor of visual outcome after vitrectomy surgery in patients with DME or ERM [32, 33]. However, the preoperative POS length was difficult to obtain in our study due to the presence of SRD in the patients. Further investigation is needed to reveal the predictive value of preoperative POS length for postoperative BCVA after complete SRD-DME resolution.

There was a substantial number of eyes with a BCVA < 20/20 after SRD-DME resolution in our study, some of the eyes even with a FB. Similar findings were also observed in a previous study about resolved macular edema associated with BRVO [20]. This might be due to the delayed functional recovery of the retina after anatomical recovery in eyes with DME [34]. The results also indicate that there are other unknown prognostic factors associated with visual outcomes of DME that need to be further investigated. The proportion of eyes with a BCVA < 20/20 seemed to be higher in our study compared to Hasegawa et al. [20]. This was because all of the eyes in our study had SRD at baseline, compared to 67.7% of eyes in Hasegawa et al.'s study [20]. The presence of SRD at baseline has been known to affect the visual acuity after DME treatment [17, 19, 27].

Our study had some limitations. Firstly, the conclusions of our study could only be applied to SRD-DME eyes treated with IVR, rather than eyes treated with intravitreal injection of other anti-VEGF medications or steroids. Although similar findings may be observed after other DME treatments, the exact course of SRD resolution may vary among different treatments. Secondly, we did not investigate the molecular mechanism of the FB formation in eyes with resolved SRD-DME. Further prospective studies may reveal the molecular pathogenesis of the photoreceptor recovery and FB formation. Thirdly, poor DM control in some of the patients might be one of the reasons for the unsatisfactory visual outcomes after treatment in these patients. Moreover, further studies with a larger number of treatment-naïve eyes and longer follow-up period are needed to validate the results of our study.

In conclusion, we have found that the presence of the FB is associated with better BCVA after the resolution of SRD-DME. SRD-DME eyes with lower baseline SRDH or faster SRD resolution are more likely to have a FB at 6 months.

Data Availability

The data used during the current study are available from the corresponding author on reasonable request.

Disclosure

The sponsors or funding organizations had no role in the design or conduct of this research.

Conflicts of Interest

The authors declare that they have no competing interests.

Authors' Contributions

Yijun Hu, Qiaowei Wu, and Baoyi Liu contributed equally to this work, so they are considered to be co-first authors.

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