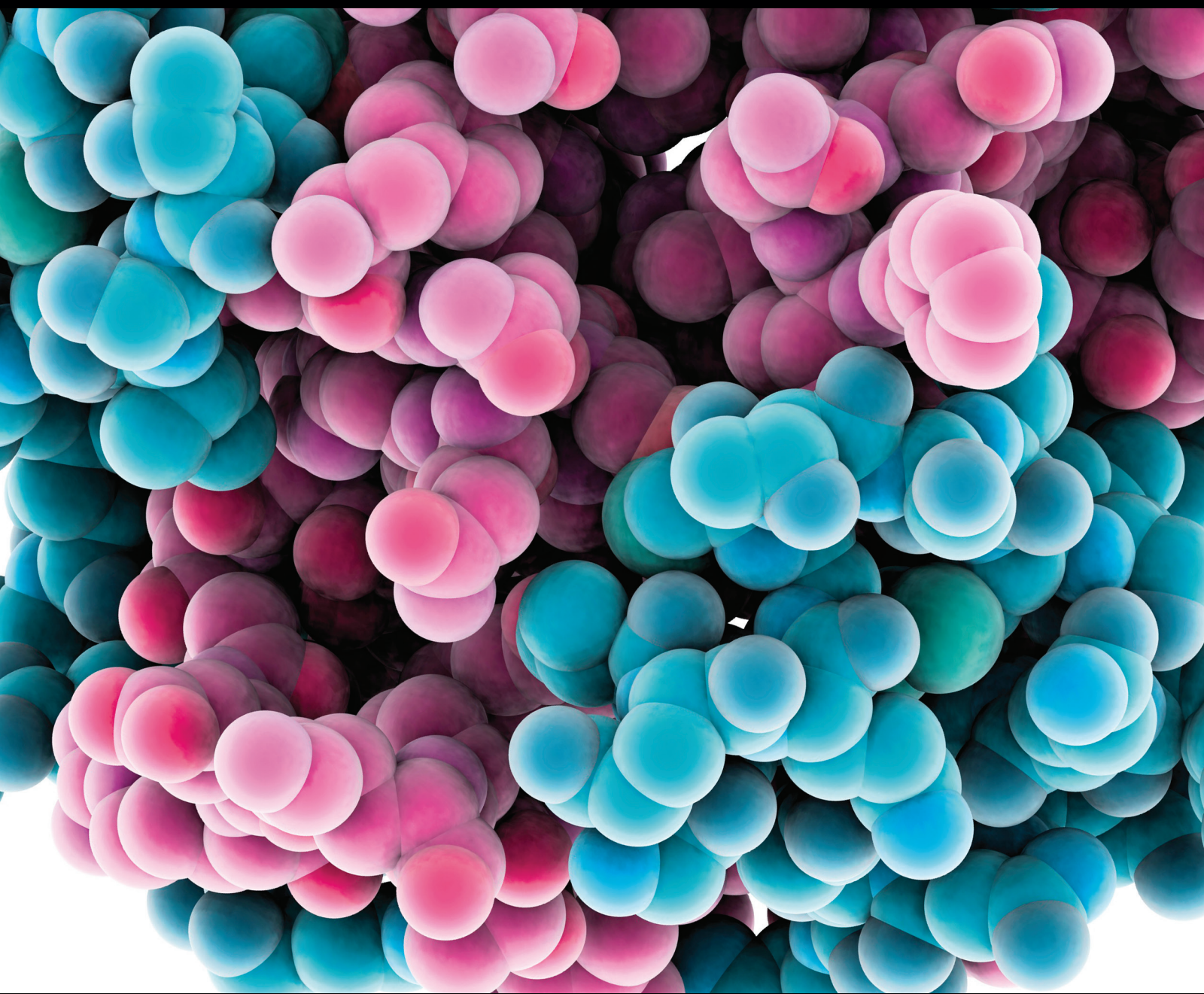
Journal of Diabetes Research

Oxidative Stress, Epigenetics, Environment, and Epidemiology of Diabetic Retinopathy

Guest Editors: Goran Petrovski, Kai Kaarniranta, and Daniel Petrovič



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Editorial Board

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Editorial

Oxidative Stress, Epigenetics, Environment, and Epidemiology of Diabetic Retinopathy

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Several environmental (external) and genetic (internal) factors are involved in the development of diabetic retinopathy (DR). Proper screening and early detection of such factors shall improve the prevention and treatment of this blinding disease. Lately, oxidative stress and factors influencing it have been recognised as important determinants of DR appearance and fulmination. Recently, beside genetical factors, epigenetic mechanisms (i.e., global acetylation of retinal histone H3) have been demonstrated to play an important role in the development and progression of DR to more severe and vision threatening stages, proliferative diabetic retinopathy and diabetic macular edema. Other internal factors affecting DR determined by exome sequencing may prove very helpful in the evaluation of diabetic retinopathy, while screening for external factors may supplement prevention or early diagnosis of the disease. Furthermore, new treatment modalities in microvascular disorders such as DR are emerging that may affect oxidative stress.

The articles contained in the present issue include both reviews and basic scientific studies focused on characterizing DR.

In the work by D. J. Eszes et al. entitled "Diabetic Retinopathy Screening Using Telemedicine Tools: Pilot Study in Hungary," the authors showed that telemedicine could be a strong method, supporting eye care professionals and allowing for faster and more comfortable DR screening. Moreover, participants found digital retinal screening to be reliable and comfortable. Thirty percent of the patients had never participated in any ophthalmological screening. From all patients examined, 25.7% had DR based upon a standard fundus camera examination and a UK-based DR grading protocol (SpectraTM software). Additionally, there was a statistically significant relationship between economic activity, education and marital status, and future interest of participation. The study represents part of a larger Diabetic Retinopathy Initiative supported by EURETINA in the pilot city, Szeged, Hungary.

In the work by S. Vujosevic and E. Midena entitled "Diabetic Retinopathy in Italy: Epidemiology Data and Telemedicine Screening Programs," the authors reviewed the available epidemiological data on DR and telematic screening realities in Italy. In Italy, the number of people living with diabetes is about 3.5 million (5.5% of the population), with an increase by about 60% in the last 20 years, and 1 person out of 3 is older than 65 years. The Italian Health Service system estimates that 10 billion euros are spent annually on caring for patients with diabetes, a figure that increases yearly. At the present time, the use of telemedicine for the screening of DR in Italy is confined to geographically limited locations. They concluded, however, that telemedicine might be helpful for establishing a national screening program in Italy.

In the work by A. Horwitz et al. entitled "Danish Nationwide Data Reveal a Link between Diabetes Mellitus, Diabetic Retinopathy, and Glaucoma," the authors showed that the

use of diabetic drugs was strongly associated with the use of antiglaucomatous drugs, while the presence of DR and/or joint complications with DR and nephropathy increases the risk of glaucoma. Moreover, concomitant antihypertensive medication was overall associated with an increased risk of glaucoma. However, the combination of β-blocker and renin-angiotensin system inhibitors appeared to have a significantly lower hazard ratio for glaucoma onset in subjects with diabetes. They concluded that a strong association between DM and treatment for glaucoma was shown in the entire Danish population.

In the work by S. Vavuli et al. entitled "Elevated Levels of Plasma IgA Autoantibodies against Oxidized LDL Found in Proliferative Diabetic Retinopathy but Not in Nonproliferative Retinopathy," the authors showed that IgA autoantibodies were increased in proliferative DR, especially in Type 2 diabetes. The high levels of IgA reflect the inflammatory process and enlighten the role of oxLDL and its autoantibodies in proliferative DR.

In the work by S. S. Pukl et al. entitled "Visual Acuity, Retinal Sensitivity, and Macular Thickness Changes in Diabetic Patients without Diabetic Retinopathy after Cataract Surgery," the authors showed that underlying diabetes has no effect upon the surgical outcome of cataract surgery in patients with diabetes and having no DR. Best corrected visual acuity (BCVA) measured by ETDRS letters improved significantly and similarly in subjects with diabetes in comparison to subjects without diabetes. However, slight thickening of wider macula and corresponding decrease in retinal sensitivity observed in patients with diabetes 6 months postoperatively might influence visual function on the long term.

In the work by P. Romero-Aroca et al. entitled "Diabetic Macular Edema Pathophysiology: Vasogenic versus Inflammatory," the authors addressed the pathogenesis of diabetic macular edema. In the paper they reviewed the data currently available, focusing on vascular endothelial growth factor (VEGF), angiogenesis, and inflammation.

Acknowledgments

We would like to express our appreciation to all the authors for their informative contributions and the reviewers for their support and constructive critiques in making this special issue possible.

Goran Petrovski
Kai Kaarniranta
Daniel Petrovič

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Clinical Study

Visual Acuity, Retinal Sensitivity, and Macular Thickness Changes in Diabetic Patients without Diabetic Retinopathy after Cataract Surgery

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Aim. Functional and morphological macular study after cataract surgery in a group of diabetics without diabetic retinopathy compared to nondiabetics to evaluate the effect of surgical oxidative stress on diabetic retina. *Methods*. Prospective, comparative study. Preoperative eye exam, best corrected visual acuity (BCVA) measured by ETDRS letters, and optical coherence tomography (OCT) were followed by standard cataract surgery. The follow-up visits at 1, 3, and 6 months postoperatively included BCVA, OCT, and microperimetry, to analyze changes within and between the groups. *Results*. The BCVA improved significantly in diabetics and controls: 64.2 to 81.0 and 61.9 to 82.1 ETDRS at 6 months, respectively. The central macula at OCT significantly thickened in both groups, while the central 5 fields, corresponding to the microperimetry area, subclinically thickened from 284.20 to 291.18 μm at 6 months only in diabetics ($p = 0.026$). A matching slight decrease in the microperimetry sensitivity from 1 to 6 months was found also only in diabetics, with mean average difference −0.75 dB ($p = 0.04$). *Conclusion*. Underlying diabetes does not influence the surgical outcome in diabetics without diabetic retinopathy. However, slight thickening of wider macula and corresponding decrease in retinal sensitivity observed in diabetics 6 months postoperatively might influence visual function on long term.

1. Introduction

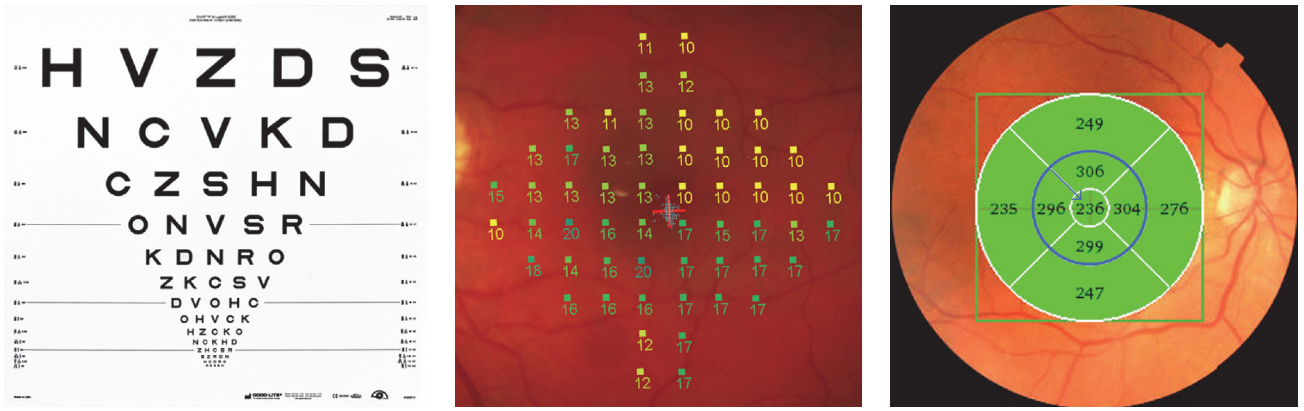
Hyperglycemia activates several biochemical pathways leading to oxidative stress, the hallmark in the pathogenesis of diabetic retinopathy [1].

The term oxidative stress refers to an imbalance between the antioxidant defense system of the cell and the intracellular amount of harmful reactive oxygen species (ROS). Oxidative stress may result from endogenously produced ROS (caused by hyperglycemia) or by external sources (caused by cataract operation). Cataract surgery influences the intraocular balance in different aspects. It is one of the well-known sources of free radicals [2–6], and it also causes a medium termed lowering of the antioxidative substances in the anterior chamber [7–11].

As a result of free radicals accumulation, the risk of developing or worsening of macular edema following cataract surgery is higher in patients with diabetes than in patients without diabetes and correlates well with the progression of diabetic retinopathy [12–17].

There are some controversies in the results of the studies reporting developed increase in central macular thickness (CMT) or macular edema after cataract surgery in patients with diabetes but no diabetic retinopathy (in diabetic patients without diabetic retinopathy). In a retrospective database study performed on more than 4500 diabetics without preoperative macular edema, the postoperative incidence of macular edema was reported 4%, higher than in the population without diabetes ($p < 0.001$) [14]. The risk for development of macular edema in diabetics without retinopathy (RR 1.80) was reported to be higher than in the population without diabetes (RR 1.17) [14].

On the contrary, a recently published meta-analysis showed no statistically significant increase in CMT values



(a) (b) (c)

Figure 1: (a) ETDRS chart for best corrected visual acuity test. (b) Macular photography with the microperimetry test area. (c) Optical coherence tomography of macula and ETDRS rings thickness measurements, blue arrow to the central subfield, encircled blue central 5-field area.

after cataract surgery in diabetic patients without diabetic retinopathy at 1, 3, and 6 months after cataract extraction [12]. On the other hand, Katsimpris et al. found increased macular thickness after uncomplicated cataract surgery in diabetics without retinopathy compared to preoperative values or to a control group of patients at all follow-ups up to twelve months after cataract surgery [18].

This clinical study was designed to compare visual function (visual acuity, retinal sensitivity) and morphologic retinal changes (macular thickness) before and after cataract surgery in diabetic patients without diabetic retinopathy in comparison to nondiabetic patients.

2. Methods

2.1. Patient Enrolment. This prospective, comparative study was designed to assess the clinical outcome of diabetic patients without diabetic retinopathy undergoing cataract surgery. Participant enrolment and treatment took place at the Eye Hospital, University Medical Center Ljubljana, Slovenia. The study was approved by the Republic of Slovenia's National Medical Ethics Committee and conducted in accordance with the Declaration of Helsinki 1964. Written and fully informed consent was voluntarily provided by all participants prior to enrolment in the clinical study.

Inclusion criteria were clinically significant age related cataract of LOCS III (lens opacities classification system) grade N3, diabetes mellitus type II for the group of diabetic patients, and no diabetes as proved by fasting blood glucose test for the control group.

Exclusion criteria were any other ocular pathology except cataract.

Eighteen eyes of diabetics without diabetic retinopathy and 10 eyes of nondiabetic patients were included in the test and the control group, respectively.

Preoperative visit evaluation was followed by a standard microinvasive cataract surgery. Postoperative follow-up visits were scheduled for day 1, 1 month, 3 months, and 6 months postoperatively.

2.2. Cataract Surgery. A standard cataract surgery with phacoemulsification was performed by one of two surgeons (NVV, VP) as follows: preoperatively, topical installation of NSAID (Naclof®, Alcon, Texas) three times in 10-minute intervals, corticosteroid and antibiotic (Maxitrol®, Alcon, Texas) three times in 10-minute intervals, topical mydriatics, a combination of two eye drops, 1% tropicamide (Mydriacyl 1%®, Alcon Pharmaceuticals, Hünenberg, Switzerland) and generic 2,5% phenylephrine, three times in 10-minute intervals, followed by topical anesthetic and iodine application performed in 5-minute intervals. After sterile preparation, a 2,2 mm clear corneal incision, intracameral lidocaine, intracameral hydroxypropyl methylcellulose (Acryvisc®, Zeiss, Oberkochen, Deutschland), and 5–5.5 mm continuous curvilinear capsulorhexis were followed by phacoemulsification performed at the same phacomachine (Millenium®, Bausch & Lomb Storz) in all patients, aspiration irrigation, and hydrophobic IOL implantation. Intracameral antibiotic (1.0 mL generic vancomycin used off-label) was administrated at the end of the surgery. Postoperatively, topical NSAID (Naclof, Alcon, Texas) to prevent cystoid macular edema [19] and corticosteroid and antibiotic (Maxitrol, Alcon, Texas), both three times a day, were prescribed for 3 weeks in both groups.

2.3. Preoperative and Postoperative Assessments and Outcome Measures. Preoperative visit evaluation in addition to routine comprehensive ophthalmological exam included best corrected visual acuity using the ETDRS charts (4-meter 2000 series revised ETDRS chart (Precision Vision®, La Salle, USA)), measurement of retina sensitivity by microperimetry (MP-1 Micro Perimeter, Nidek), and measurement of macular thickness, using optical coherence tomography (Topcon 3D OCT-1000, Tokyo, Japan) (Figure 1).

At all follow-up visits, the complete ophthalmological exam with best corrected visual acuity using the ETDRS charts and the optical coherence tomography of the macula were repeated. Additionally, microperimetry was repeated at 1, 3, and 6 months after surgery.

Table 1: Demographics of all patients included in the study with type of diabetes treatment and percentage of accompanying systemic diseases.

	Number of eyes	Age (years)	Duration of diabetes (years)	Therapy for diabetes (% of patients)	Systemic diseases (% of patients)
Diabetics without DR	18	57–83 mean 73.5 SD 7.01	1–30 mean 11.8 SD 8.6	Insulin (17%) Per os (75%) Diet (8%)	Arterial hypertension (83%) Hyperlipidemia (8%)
No diabetes	10	60–73 mean 68.8 SD 4.70	NA	NA	Arterial hypertension (40%) Hyperlipidemia (20%)
		$p = 0.167^*$			

NA: not applicable.
*Significance level $p < 0.05$.

The scanning protocol for optical coherence tomography used in this study was the Fast Macular Thickness program (Topcon 3D OCT-1000, Tokyo, Japan), which creates a retinal map algorithm consisting of six radiating cross-sectional scans, each of 6 mm length, that produces a circular plot in which the fovea is a central circular zone of 1 mm diameter. Superior, nasal, inferior, and temporal parafoveal zones represent annular bands in these respective sectors. There are other two concentric zones, the first having a diameter of 3 mm and the second one of 6 mm. The nine zones (the central zone is named field 1, the first annular ring fields 2–5, and the second annular ring fields 6–9) have been called ETDRS-type regions because of their similarity to zones of analysis of photographs by ETDRS graders (Figure 1(c)) [20, 21]. To correlate retinal thickness data accurately with retinal sensitivity data, we compared the central fields 1 to 5, and we excluded fields 6 to 9, as the microperimetry MP-1 grid covered only a limited area of these latter fields (Figures 1(b) and 1(c)).

Microperimetry was performed after pupil dilatation with automatic fundus-related perimeter (MP-1 Micro Perimeter; Nidek Technologies, Padova, Italy) (Figure 1(b)) [22]. The fundus is imaged in real time; the fixation target and stimuli are projected onto the retina. The central 10° visual field was tested with the Humphrey 10–2°16 dB56s program, fast strategy, and background illumination: 1.27 cd/m^2, stimulation time 200 ms, stimulation spot size: Goldmann III. In all, 0 dB (equivalent to 1.27 cd/m^2) represented the brightest luminance, and the stimulus intensity varied from 0 to 20 dB. The subsequent exams of the same eye were performed using the follow-up option of the software, which enables projection of the testing spots at the exact same area of the retina.

2.4. Statistical Analysis. The statistical analysis of the data was performed using SPSS (SPSS, Inc., Chicago, IL) for Windows 11.5 package program. Power analysis was performed to detect the sample size. Mean standard deviation (SD) was used to describe quantitative data. Student's t-test was used to analyze the difference between two samples and ANOVA was used for analysis of more samples together in macular sensitivity and thickness in the two groups. Threshold of statistical significance was 0.05.

3. Results

Twenty-eight eyes of twenty-eight patients, with a mean age of 71.1 ± 6.9 (SD) years, underwent cataract surgery. Participants had a clinical diagnosis of cataract and diabetes, diabetes mellitus type 2 without diabetic retinopathy ($n = 18$), mean age 73.5 ± 7.01 (SD) or cataract and no diabetes ($n = 10$), mean age 68.8 ± 4.70 (SD); the age difference was not statistically significant, $p = 0.167$. The accompanying systemic diseases are presented in Table 1.

The cataract surgery was uneventful in all eyes. The mean phacoemulsification time was 2,73 seconds, without statistically significant difference between the groups.

3.1. Visual Acuity. Table 2 and Figure 2 show the mean best corrected visual acuity (BCVA) in diabetics without DR and the no-diabetes group eyes at baseline, day 1, 1 month, 3 months, and 6 months after cataract surgery. BCVA improved in both groups.

The mean best corrected visual acuity ETDRS in the diabetics without diabetic retinopathy group eyes improved for 16.8 ETDRS letters or 26.2%, $p < 0.001$. Equivalently, in the no-diabetes group eyes, it improved for 20.2 ETDRS letters or 32.6%, $p < 0.001$, without significant difference between the groups ($p = 0.183$). All eyes achieved a BCVA of 76 or more and 75 or more ETDRS letters in the diabetic and control group, respectively.

3.2. Optical Coherence Tomography Thickness Measurements. At OCT examination, mean retinal thickness in the central field (field 1) in the diabetic group changed from 238.6 μm preoperatively to 255.2 ($p = 0.02$) 6 months after cataract surgery. In the control group, the thickness in the central field changed from 247.6 μm preoperatively to 261.7 ($p = 0.03$) 6 months after cataract operation (Table 3, Figure 3).

Observing a wider macular area, namely, the central five fields (Figure 5), the mean retinal thickness in diabetics without DR showed increased thickness from the preoperative value of 284.2 to 291.2 μm 6 months after surgery ($p = 0.03$) (Table 4). In the control group, however, the values did not show significant changes (Table 4, Figure 4).

Table 2: Mean best corrected visual acuity (BCVA) in ETDRS letters preoperatively and at all postoperative follow-ups.

	BCVA					
	Preoperative	Postoperative				The association between preoperative and postoperative (6 months) BCVA
		Day 1	1 month	3 months	6 months	
Diabetics without DR	64.2 ± 5.6 (SD)	75.5 ± 5.9 (SD)	80.7 ± 3.6 (SD)	80.9 ± 3.9 (SD)	81.0 ± 2.9 (SD)	$p < 0.001$
No diabetes	61.9 ± 8.9 (SD)	78.1 ± 3.9 (SD)	79.5 ± 3.5 (SD)	80.1 ± 2.1 (SD)	82.1 ± 3.7 (SD)	$p < 0.001$

Significance level if $p < 0.05$.

Table 3: Central macular thickness (CMT) in μm at optical coherence tomography preoperatively and at all follow-ups.

	CMT (μm)					
	Preoperative	Postoperative				The association between preoperative and postoperative (at 6 months) BCVA
		Day 1	1 month	3 months	6 months	
Diabetics without DR	238.6 ± 29.0 (SD)	233.7 ± 30.2 (SD)	244.5 ± 24.0 (SD)	251.3 ± 27.8 (SD)	255.2 ± 31.5 (SD)	$p = 0.02$
No diabetes	247.6 ± 25.0 (SD)	240.2 ± 23.0 (SD)	247.5 ± 21.0 (SD)	247.6 ± 20.0 (SD)	261.7 ± 29.0 (SD)	$p = 0.03$

Significance level if $p < 0.05$.

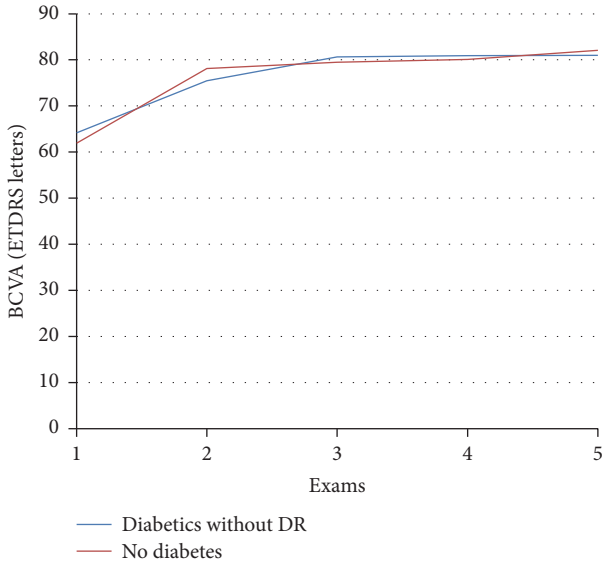


Figure 2: Best corrected visual acuity (BCVA) in ETDRS letters before (exam 1) and after cataract surgery: 1st day (exam 2), 1 month (exam 3), 3 months (exam 4), and 6 months (exam 5).

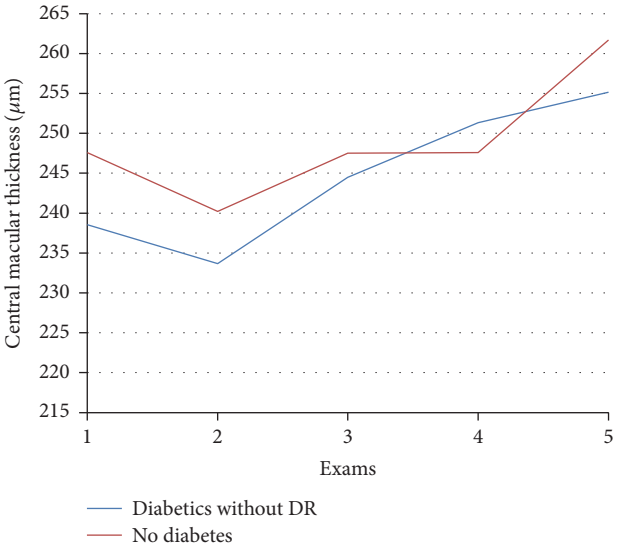


Figure 3: Central macular thickness on optical coherence tomography in μm before (exam 1) and after cataract surgery: 1st day (exam 2), 1 month (exam 3), 3 months (exam 4), and 6 months (exam 5).

3.3. Microperimetry. Mean sensitivity on microperimetry showed relatively stable improvement in the diabetic group when comparing preoperative exam to 1-, 3-, and 6-month postoperative follow-up exams; the average difference was 3.62 dB (SD 2.31), 3.69 (SD 2.39), and 3.45 (SD 2.34). Direct comparison of the mean sensitivity improvement after cataract surgery between the DM2 without DR and the control eyes groups was not done, because the absolute improvement depends mainly on the severity of the cataract, and the groups were not balanced on the basis of cataract severity. However, when the comparison was made among the postoperative follow-up exams between the groups, a decrease in the mean sensitivity was found in the DM2 without DR group eyes (Figure 5) but not in the control group eyes. In the DM2 without DR group eyes, a mean average difference of −0.75 dB (SD 2.38) from 1 to 6 months of follow-up was observed. The control group eyes, on the other hand, showed increased mean sensitivity of 1.3 dB (SD 2.13) when compared to the postoperative follow-up exams from 1 to 6 months after surgery.

The microperimetry testing area corresponds to the five-field retina on the OCT, so the above results were paralleled and correspondent.

Table 4: Mean retinal thickness of the macular 5 fields (RT 5 fields) at optical coherence tomography in μm preoperatively and at all follow-ups.

	RT 5 fields (μm) preoperative	RT 5 fields Day 1	RT 5 fields 1 month	RT 5 fields 3 months	RT 5 fields 6 months	The association between preoperative and postoperative (6 months) OCT RT 5 fields
Diabetics without DR	284.2 ± 19.1 (SD)	280.7 ± 19.1 (SD)	290 ± 19.4 (SD)	294 ± 16.7 (SD)	291.2 ± 16.8 (SD)	$p = 0.03$
No diabetes	284.0 ± 18.0 (SD)	280.3 ± 18.0 (SD)	286.2 ± 15.0 (SD)	283 ± 13.0 (SD)	288.1 ± 20.0 (SD)	$p = 0.32$

Significance level if $p < 0.05$.

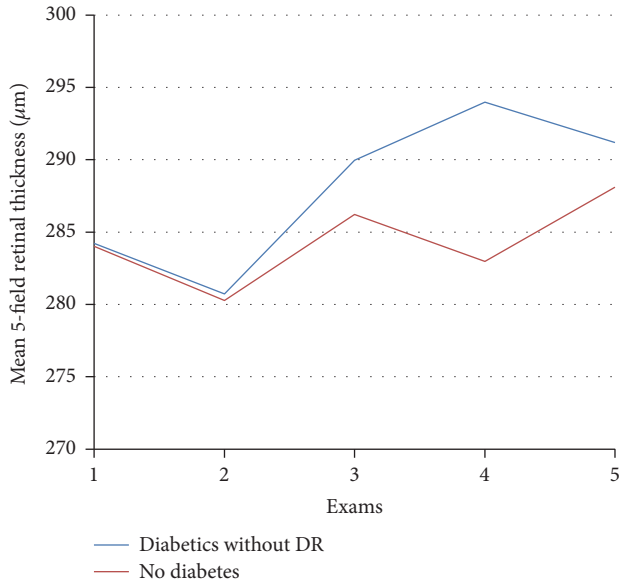


Figure 4: Mean retinal thickness of the macular 5 fields on optical coherence tomography in μm before (exam 1) and after cataract surgery: 1st day (exam 2), 1 month (exam 3), 3 months (exam 4), and 6 months (exam 5).

4. Discussion

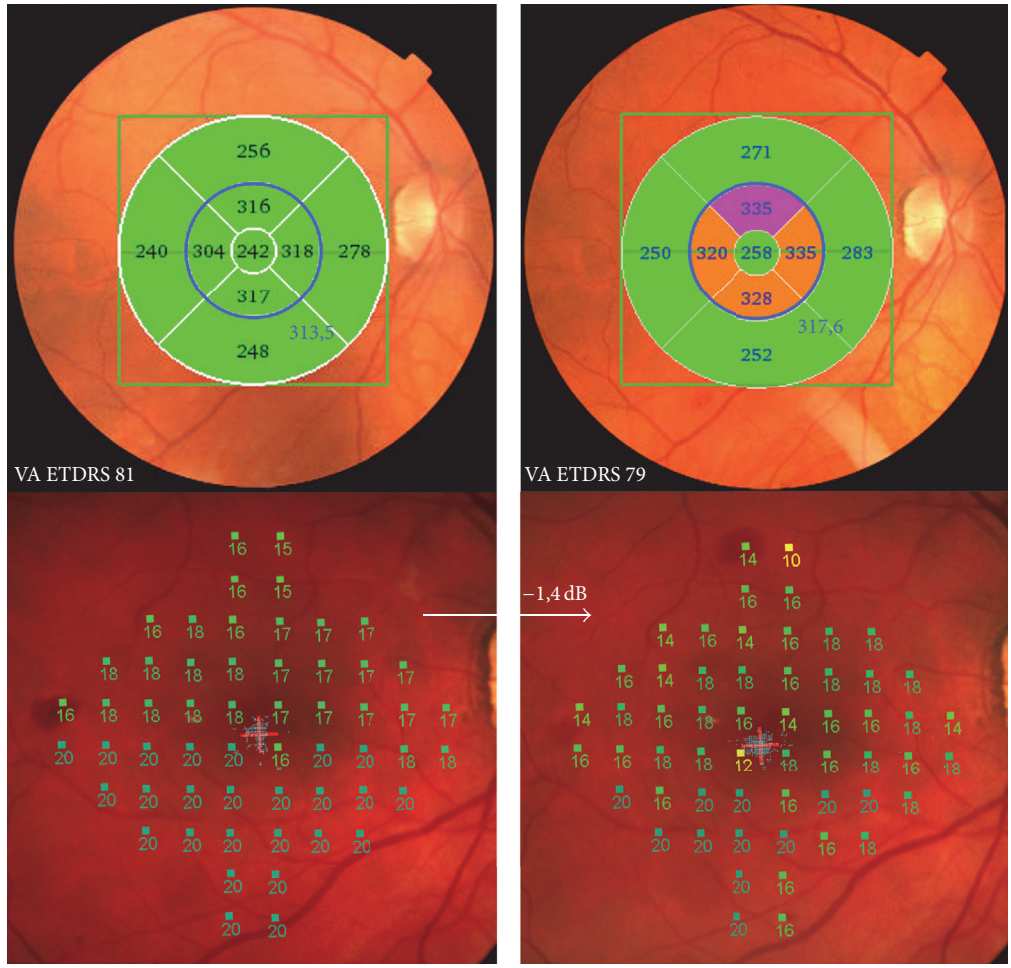
In our study, we demonstrated that cataract surgery in diabetic patients without diabetic retinopathy did not influence the good visual acuity outcome after cataract surgery. However, slight increase in paracentral macular thickness and a corresponding decrease in mean retinal sensitivity after 6 months were present in diabetics without diabetic retinopathy group eyes and not in the control group.

Our study included both functional and morphological examination methods of the macula, in order to try to find clinical and subclinical difference after cataract surgery between nondiabetic and diabetic patients without diabetic retinopathy. A wider macular area was examined additional to the central macular thickness, and macular sensitivity test was added to the visual acuity testing, which, according to our knowledge, was not analyzed in previous published studies.

In our study, both groups, diabetics and nondiabetic patients, showed equal statistically significant thickening of the central macular subfield 6 months after cataract operation, diabetics for 16.6 μm and controls for 14.1 μm after 6 months. Cataract surgery is a source of oxidative stress, which can result in macular edema, as it produces free radicals, causes medium termed lowering of antioxidative substances in the anterior chamber, and also influences normal oxygen levels in the eye on the long term by removing lens epithelial cells [7, 23]. The production of free radicals can be influenced by the cataract grade, as more phaco energy is usually used, any possible complications during the surgery, and longer operation time for any other reasons. All of these factors were controlled in the study to prove if diabetes is a factor of difference.

Additional analysis of the ETDRS subfields at OCT of the macula showed slightly more pronounced thickening in the cumulative central 5 fields in the diabetic group 6 months after surgery in comparison to the control group. The area covered by central 5 fields corresponds to the testing area of the automated microperimetry retinal sensitivity testing [24]. A matching decrease in mean retinal sensitivity at 6 months was found in the diabetic group. Microperimetry added to visual acuity and thickness measurements shows a supplementary macular function, especially of value in patients at risk for macular changes. Cataract surgery itself plays an important risk factor for macular thickening and also for occurrence of macular edema with vision deterioration by releasing prostaglandins and increasing oxidative stress [23]. Because of this additional oxidative stress caused by cataract surgery in diabetic patients, who are already exposed to higher oxidative stress due to the underlying disease, macular thickening could be expected to occur more often and to be more exaggerated.

Several authors reported increased central macular thickness in diabetic patients with diabetic retinopathy after cataract surgery. Kim et al. studied changes in central point thickness on optical coherence tomography (OCT) after uncomplicated cataract operation in diabetic patients with different status of the retina and reported thickening for more than 30% in 22% of the participants [16]. Kwon et al. reported that after cataract surgery 18% of diabetic patients with diabetic retinopathy developed thickening of more than 30% of the central subfield of the macula, which correlated to the severity of retinopathy [17]. The risk for macular thickening after cataract surgery depends on the severity of retinopathy and/or preexisting diabetic macular edema (DME) [13, 16]. However, a meta-analysis report claimed no significant difference in central macular thickness



(a) (b)

Figure 5: Investigations of a 58-year-old diabetic patient. (a) One month postoperatively: above mean thickness in the central 5-field macular area at OCT (blue circle), BCVA, and below microperimetry sensitivity. (b) Six months postoperatively: above increased mean thickness in the central 5-field macular area at OCT (blue circle), decreased number of ETDRS letters of BCVA, and below microperimetry mean sensitivity decrease (arrow).

1 and 3 months after cataract surgeries in diabetic patients without diabetic retinopathy [12]. In terms of prevention of postoperative worsening of DME and improvement of the final visual outcome, it is advisable to inject an intravitreal anti-VEGF drug during cataract operation in patients with preexistent diabetic macular edema [13, 25, 26], and there are clinical studies done proving that even patients with stable diabetic retinopathy without significant macular edema do benefit from this procedure in terms of less thickening of the macula and better visual outcome [13, 27].

Our results show an increase in the central 5-field area of macular thickness and a corresponding decrease in retinal sensitivity in diabetic patients without diabetic retinopathy 6 months after cataract surgery not observed in the control group and not reported before. Both OCT measurements and microperimetry are known to show diurnal and long term variability; thus, the results need to be interpreted carefully [28–31]. In our study, the differences between diabetic patients without retinopathy and nondiabetic patients were observable, but they were small and subclinical and could only represent a greater magnitude of variability connected to pathologic conditions [32]. On the other hand, several new and precise measures available today do offer new data, which might be proved useful and important in the future. And the changes observed in diabetic patients in the study might be important in predicting possible changes over a longer time period.

There are certain limitations of this study. There were a relatively small number of cases included, and the optical coherence tomography measurements were not repeated.

In conclusion, the combination of increased macular thickness in the central 5-field macular area and decreased retinal sensitivity in diabetics after cataract surgery in the era of premium intraocular lenses and refractive lens procedures, where a perfect status of the macula is desired, might be of interest. On the other hand, the improvement of the best corrected visual acuity and only minimal functional macular changes ease the decision to perform cataract surgery in

diabetic as in healthy patients when appropriate. However, diabetics should be treated with additional caution, the use of topical NSAID after cataract surgery should be considered, and follow-up visits could be more accurate with additional measurements of macular morphology and function.

Whether changes in macular thickness and retinal sensitivity observed in diabetic patients without diabetic retinopathy in our study could progress to clinical important consequence over a longer period of time remains to be elucidated in studies with longer follow-up and higher number of subjects enrolled.

Competing Interests

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

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Research Article

Elevated Levels of Plasma IgA Autoantibodies against Oxidized LDL Found in Proliferative Diabetic Retinopathy but Not in Nonproliferative Retinopathy

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Aims. This study investigated the association of autoantibodies binding to oxidized low-density lipoproteins (oxLDL) in diabetic retinopathy (DR). *Methods*. Plasma from 229 types 1 and 2 patients with DR including diabetic macular edema (DME) and proliferative diabetic retinopathy (PDR) was analysed with ELISA-based assay to determine IgA, IgG, and IgM autoantibody levels binding to oxLDL. The controls were 106 diabetic patients without retinopathy (NoDR) and 139 nondiabetic controls (C). *Results*. PDR group had significantly higher IgA autoantibody levels than DME or NoDR: mean 94.9 (SD 54.7) for PDR, 75.5 (41.8) for DME ($p = 0.001$), and 76.1 (48.2) for NoDR ($p = 0.008$). There were no differences in IgG, IgM, or IgA that would be specific for DR or for DME. Type 2 diabetic patients had higher levels of IgA autoantibodies than type 1 diabetic patients (86.0 and 65.5, resp., $p = 0.004$) and the highest levels in IgA were found in type 2 diabetic patients with PDR (119.1, $p > 0.001$). *Conclusions*. IgA autoantibodies were increased in PDR, especially in type 2 diabetes. The high levels of IgA in PDR, and especially in type 2 PDR patients, reflect the inflammatory process and enlighten the role of oxLDL and its autoantibodies in PDR.

1. Introduction

Diabetes and its long-term complications continue to represent a severe health problem all around the world. A number of recent studies have emphasized that diabetes carries a strong inflammatory component and the induction of vascular inflammation in diabetes involves a dysregulation of oxidation reaction [1–3]. Elevated plasma levels of circulating oxidized low-density lipoprotein (oxLDL) have been associated with obesity-related metabolic disturbances such as the metabolic syndrome and diabetes [4]. The presence of vascular oxidative stress and low-density lipoprotein (LDL) with increased susceptibility to oxidation is especially prominent in type 2 diabetes [5].

Oxidized low-density lipoproteins are immunogenic [6] and circulating autoantibodies binding to oxidized epitopes of oxLDL have been detected in human and animal plasma [7, 8]. In mouse models of atherosclerosis immunoglobulin M (IgM) type autoantibodies binding to oxLDL have exhibited putative atheroprotective properties [9]. In some studies, the concentrations of serum IgA binding to oxidized LDL have been elevated in subjects with metabolic abnormalities [8] and this phenomenon correlated with plasma levels of inflammatory mediators [10]. Furthermore, plasma IgA

autoantibody levels binding to oxLDL have been shown to be positively and IgG autoantibody levels to be negatively associated with markers of glucose metabolism and also to be independent risk factors for type 2 diabetes [8].

The levels of autoantibodies binding to oxLDL decline with age, diabetes duration, and glycated hemoglobin (HbA1c) levels [1, 11]. This phenomenon has been attributed to increased formation of oxLDL-specific immune complexes [12]. It seems that these complexes [13] as well as the oxLDL itself [14] can induce macrophages to be converted into foam cells. The autoantibodies induce the macrophages to produce cytokines [15] which in turn activate endothelial cells and trigger an inflammation cascade [13, 16]. The presence of inflammation and activation of endothelial cells are also key elements in the initiation of diabetic retinopathy (DR) [17, 18]. Previously, oxLDL has been demonstrated to play a role in the development of DR since immunostaining of apolipoprotein B (apoB) oxLDL has been detected in the retinas of type 2 diabetic patients with or without DR and an increase in oxLDL levels reflects the severity of retinopathy [19]. In another study, Fu et al. demonstrated that the oxidative stress was induced by modified LDL in DR; that is, the modified LDL exerted toxic effects on the capillary pericytes [20]. Furthermore, high levels of oxLDL in immune complexes have been shown to associate with progression of retinopathy in type 1 diabetes [21].

Plasma levels of autoantibodies binding to oxLDL might serve as a biomarker for the severity of the diabetic retinopathy, but their role is not yet well characterized. The purpose of this study was to investigate the levels of autoantibodies binding to oxLDL in the plasma of diabetic patients with and without diabetic retinopathy in a homogenous, well-characterized Finnish study population.

2. Methods

2.1. Study Subjects. This is a case-control study with the study population (Figure 1) consisting of 229 diabetic patients with clinically moderate to severe DR (DR group) from Oulu University Hospital or Helsinki University Hospital and 106 diabetic patients without signs of retinopathy (noDR group) attending fundus imaging for screening of DR in Oulu City Health Centers. The diabetic patients were matched for duration of diabetes by including only those patients with a disease duration of at least 10 years in the noDR group since diabetes' duration and hyperglycemia are the main prognostic factors for the development of DR. The diagnosis and classification of DR were made in the clinical examination and/or from fundus images by experienced ophthalmologist at the Department of Ophthalmology of Oulu and Helsinki University Hospital [22, 23]. We also examined 139 nondiabetic patients undergoing eye surgery in the Helsinki University Hospital as control group (C group).

The DR patients were divided into two groups according to their retinopathy status (Figure 1). Thus the DME group consisted of 65 patients with clinically significant diabetic macular edema (DME) and the proliferative diabetic retinopathy (PDR) group consisted of 76 patients. All subjects from Oulu University Hospital and Oulu City Health Centers filled in a questionnaire and provided blood samples. The questionnaire included questions about their diabetes, diabetic complications, other diseases and medications, and lifestyle. Subjects from the Helsinki University Hospital also provided blood samples and filled in a different type of questionnaire, but therefore some data is missing from the tables and figures. Diabetic patients who had been previously diagnosed with microalbuminuria or proteinuria were classified as having nephropathy and the diagnosis of neuropathy was based on a previous diagnosis according to the questionnaire. This study follows the guiding principles of the Declaration of Helsinki and was approved by the Ethics Committee of the Oulu University Hospital.

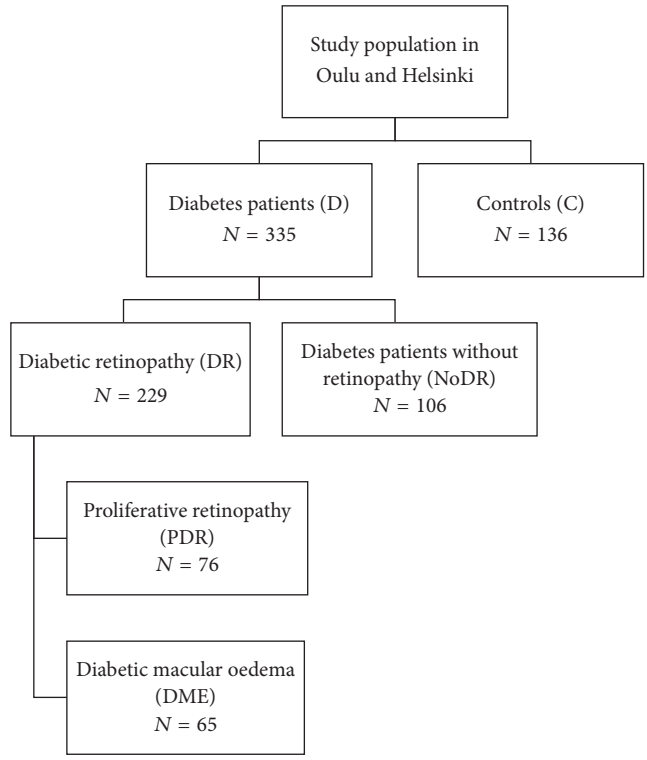

Figure 1: Study population.

2.2. Laboratory Measurements. Plasma samples were taken after an overnight fast, centrifuged, and stored −70°C. The levels of fasting plasma glucose, HbA1c, creatinine, total cholesterol, LDL, high-density lipoprotein (HDL), and triglycerides were determined from the blood samples provided by the subjects from Oulu University Hospital and Oulu City Health Centers as routine laboratory measurements in Oulu University Hospital. LDL was extracted from a human plasma sample pool of seven healthy control study subjects of both genders and purified with dialysis [24]. Purified LDL was oxidized for 25 min at +37°C with 0.5 M malondialdehyde (MDA) [6]. The MDA-solution used was produced from the MDA-base solution incubated with 4 N hydrochloric acid in +37°C for 12–15 minutes until turning yellow and neutralized with 1 N sodium hydroxide.

2.3. Autoantibody Measurements. Plasma IgG, IgM, and IgA class autoantibody titers to oxidized LDL were measured with chemiluminescence ELISA as previously described [25]. The measurement plates were coated with MDA-oxLDL and incubated overnight at +4°C. The wells were washed with a buffer solution (phosphate buffered saline with ethylenediaminetetraacetic acid (PBS-EDTA)) and postcoated with 0.5% FISH-gelatin for one hour at room temperature. After a second wash with PBS-EDTA, samples in 0.5% FISH-gelatin were added and incubated overnight at +4°C. Each plate contained also a triplicate standards of commercial purified immunoglobulins as controls (Sigma Aldrich, St. Louis, MO), a zero-sample of pure PBS-EDTA-FISH-gelatin, and two triplicate control samples ("high" and "low"), diluted to cover as wide a range of the standard as possible. After a third wash, the anti-human antibodies in 0.5% FISH-gelatin were added and incubated for one hour at room temperature. Subsequently the plates were washed with PBS-EDTA and distilled water and then they were incubated with 0.33% LUMIPHOS (Lumigen Inc. Southfield, MI) for 90 minutes and analysed in a VICTOR multilabel counter (PerkinElmer, Waltham, MA).

In order to maintain the sample luminescence counts within the standard range, the samples were diluted in FISH-gelatin. Prior to the final analysis, we performed multiple tests to determine the dilutions for each autoantibody type. The selection of the dilutions was based on the lowest coefficient of variation (CV) between measurements. The dilutions were 1 : 2000 for IgG, 1 : 1333 for IgM, and 1 : 400 (MDA-ox) or 1 : 100 (Cu-ox) for IgA. We performed triplicate measurements of each sample. The CV was calculated from each triplicate measurement. The CVs were below 20% in all samples.

An average relative light unit (RLU) value was calculated from the luminescence counts by reducing the blank value (zero-sample) from the average luminescence count of the triplicate measurements. A linear standard curve was created and the RLUs were converted into relative plasma autoantibody levels by dividing the RLUs with the standard curve slope and then multiplied by the dilution coefficient. The levels are expressed as relative units (RU).

2.4. Statistical Analyses. Statistical analysis was performed with the IBM SPSS software (IBM Corporation, Armonk, NY). The statistical significance of the autoantibody levels between two study groups was calculated with independent samples t-test and ANOVA was used for comparisons between several study groups. Crosstabs (Chi-square) was used to assess differences between categorical variables. Multiple linear regression analysis was used to explain the levels of autoantibodies and variables included in the model were selected due to correlation (Pearson correlation) with autoantibody levels (sex, age, BMI, diabetes duration and type, gHbA1c, LDL, and medications). p values less than 0.05 were considered statistically significant.

Table 1: Clinical characteristics and levels of MDA-ox LDL of diabetic patients with diabetic retinopathy (DR) and without diabetic retinopathy (noDR). The data are expressed as mean (standard deviation (SD)) or n (percent (%)).

	DR $n = 229$	NoDR $n = 106$	p
Age (years)	58.9 (14.4)	55.9 (16.3)	0.126
Gender			0.411
Women	97 (42.4%)	50 (47.2%)	
Men	132 (57.6%)	56 (52.8%)	
BMI (kg/m^2)	28.8 (5.8)	27.2 (5.0)	0.056
Diabetes			0.636
Type 1	98 (43.8%)	50 (47.2%)	
Type 2	126 (56.2%)	56 (52.8%)	
Duration	22 (11.5)	24.2 (7.8)	0.046
BP systolic (mmHg)	152.2 (23.6)	133.9 (13.5)	0.032
BP diastolic (mmHg)	83.8 (11.8)	83.6 (10.1)	0.976
Nephropathy	64 (37.2%)	16 (15.8%)	<0.001
Neuropathy	68 (40.5%)	19 (18.4%)	<0.001
Hypertension	171 (76.7%)	55 (51.9%)	<0.001
Cholesterol (mMol)	4.1 (1.1)	4.1 (0.9)	0.553
LDL (mMol)	2.3 (0.9)	2.1 (0.8)	0.122
HDL (mMol)	0.9 (0.5)	1.1 (0.5)	<0.001
Triglycerides (mMol)	1.2 (1.4)	0.6 (0.8)	<0.001
Creatinine (μMol)	102 (82.8)	72.9 (21.8)	<0.001
Glucose (mMol)	8.9 (4.0)	8.0 (2.9)	0.029
HbA1c (%)	8.5 (1.8)	7.7 (1.3)	<0.001
MDA-ox IgG	6827 (5397)	7177 (5056)	0.579
MDA-ox IgM	3316 (4489)	3536 (2933)	0.644
MDA-ox IgA	81.3 (45.4)	76.1 (48.2)	0.346

BMI: body mass index, BP: blood pressure, HbA1c: glycated hemoglobin, HDL: high-density lipoprotein, and LDL: low-density lipoprotein.

3. Results

3.1. Baseline Characteristics. The clinical characteristics of the study groups are shown in Tables 1 and 2. The DR ($n = 229$) and NoDR ($n = 106$) groups did not differ significantly in terms of age, sex, body mass index (BMI), diabetes type or LDL, and total cholesterol concentrations, but the DR group had worse diabetes control (Table 1) than the NoDR group, with the mean value of glycated hemoglobin being higher in the DR group. The DR group had poorer lipid profile having higher triglyceride and lower HDL concentration than the NoDR group (Table 1).

The mean age of the patients in the DME group was older than in the PDR group (63.6 and 55.4 years, resp., $p < 0.001$) (Table 2). As expected, the proportion of patients with type 2 diabetes was higher in the DME group than in the PDR group (72.3% and 39.5% of patients in DME and PDR, respectively ($p < 0.001$)) but there was some overlapping. There were no differences in other measured clinical characteristics between the groups (Table 2), except that more patients suffered from nephropathy (microalbuminuria) in the PDR group as compared to the DME group (42.9% versus

Table 2: Clinical characteristics of the diabetic retinopathy patients (DR) with diabetic macular edema (DME) or proliferative retinopathy (PDR). The data are expressed as mean (standard deviation (SD)) or n (percent (%)).

	DME $n = 65$	PDR $n = 76$	p
Age (years)	63.6 (10.2)	55.4 (15.1)	<0.001
Gender			0.272
Women	29 (44.6%)	27 (35.5%)	
Men	36 (55.4%)	49 (64.5%)	
BMI (kg/m^2)	30.1 (5.8)	28.2 (5.6)	0.057
Diabetes			<0.001
Type 1	18 (27.7%)	46 (60.59%)	
Type 2	47 (72.3%)	30 (39.5%)	
Duration	22.1 (9.5)	24.9 (10.2)	0.095
BP systolic (mmHg)	145.4 (24.1)	152.2 (20.6)	0.448
BP diastolic (mmHg)	78.3 (7.1)	83.9 (13.9)	0.305
Nephropathy	15 (23.8%)	30 (42.9%)	0.020
Neuropathy	21 (33.9%)	32 (46.4%)	0.145
Hypertension	54 (84.4%)	55 (78.6%)	0.389
Cholesterol (mMol)	4.6 (1.1)	4.7 (1.1)	0.496
LDL (mMol)	2.7 (0.9)	2.8 (0.9)	0.737
HDL (mMol)	1.3 (0.3)	1.3 (0.4)	0.966
Triglycerides (mMol)	1.5 (1.0)	2.0 (1.7)	0.086
Creatinine (μMol)	91.6 (64.2)	114.0 (103.7)	0.124
Glucose (mMol)	8.9 (3.9)	9.3 (3.9)	0.521
HbA1c (%)	9.2 (2.1)	9.1 (1.7)	0.657

BMI: body mass index, BP: blood pressure, HbA1c: glycated hemoglobin, HDL: high-density lipoprotein, and LDL: low-density lipoprotein.

Table 3: Percentages of diabetic patients using lipid lowering, antihypertensive, oral diabetes medication, insulin, or ASA.

	Yes		No		Missing	
	N	%	N	%	N	%
ACE/ATII	164	49.0	151	45.1	20	6.0
β-Blocker	113	33.7	210	62.7	12	3.6
ASA	104	31.0	219	65.4	12	3.6
Statin	109	32.5	210	62.7	16	4.8
Oral DM medication	121	36.1	205	61.2	9	2.7
Insulin	215	64.2	55	16.4	65	19.4

23.8%, $p = 0.020$). The medications the diabetic subjects used are shown in Table 3. The diabetic patients, according to clinical guidelines, had medications influencing blood pressure and lipid profile in addition to antidiabetic drugs and the percentage of patients having beta blocker, ACE inhibitor, and statin medications was higher in DR group than in NoDR group. No differences in insulin, oral diabetes medication, or ASA were found between DR and NoDR.

3.2. Autoantibody Levels in DR. Retinopathy did not influence the measured autoantibody levels: IgG, IgM, or IgA; autoantibody levels did not differ significantly between the DR and noDR groups ($p = 0.644$, $p = 0.579$, and $p = 0.346$, resp.) (Table 1, Figure 2). However, PDR group had significantly increased IgA autoantibody levels; that is, the mean value of IgA was 94.9 (SD 54.7) compared with 75.5 (SD 41.8) in DME ($p = 0.023$) (Figure 2) and 76.1 (SD 48.2, $p = 0.008$) in NoDR (Table 1).

3.3. Autoantibody Levels in Diabetes. We also wanted to assess the effect of diabetes on autoantibody levels. Diabetes influenced IgM autoantibody levels: diabetic patients (both DR and NoDR) had significantly lower IgM autoantibody levels against MDA-oxLDL than nondiabetic controls (3389 (SD 3998) versus 4258 (SD 3578), $p = 0.043$), but the IgG and IgA autoantibody levels did not differ significantly between the D group (DR and NoDR) and the C group. The levels for for IgM, IgG, and IgA were 3389 (SD 3998), 6944 (SD 5280), and 79.6 (SD 46.3) for D group and 4258 (SD 3578), 6874 (SD 4718), and 80.7 (SD 46.2) for C group, respectively.

3.4. Effect of Diabetes Type on Autoantibody Levels. The mean age of type 1 diabetic patients was 45.7 years (SD 13.5) and of type 2 diabetic patients was 66.8 (SD 9.6). We subdivided them according to type of diabetes, and it was found that the IgA autoantibody levels were significantly lower in type 1 diabetes than in type 2 diabetes (65.5 (SD 30.5) for type 1 and 86.0 (SD 51.3) for type 2, $p < 0.001$) (Figure 2). We further tested the effect of diabetes type in PDR group and found that the IgA levels were highest in the PDR group having type 2 diabetes (119.1 (SD 64.1) versus 77.5 (SD 38.7) in PDR type 1 population ($p = 0.002$)) (Figure 3).

3.5. Multiple Linear Regression. Multiple linear regression was run to test the main determinants of autoantibody levels. Variables in the model were sex, age, BMI, diabetes duration and type, gHbA1c, LDL, and medications. The variables that added statistically significantly to the equation are shown in Table 4. In general, IgG autoantibodies were increased by type 2 diabetes and decreased by oral diabetes medication and statin medication ($R^2 = 0.122$). High LDL concentration influenced IgM levels and they were decreased by female sex and oral diabetes medication ($R^2 = 0.161$). Furthermore, it was found that IgA autoantibody levels were increased by increasing age, gHbA1c, LDL, and ASA medication ($R^2 = 0.227$).

4. Discussion

In this study, we have investigated the autoantibody levels against MDA-OxLDL in diabetic retinopathy, an observational analysis using a cross-sectional study design, and found that the levels of IgA type autoantibodies were increased in PDR patients as compared with DME or noDR and the type 2 patients in PDR group had the highest levels.

There is evidence that autoantibodies binding to oxLDL are involved in the pathogenesis of DR and it may be that its tissue-specific function is related to immune complexes. It has been shown by Wu et al. that oxidized LDL measured as intraretinal immunofluorescence of apoB-100 (the protein component of LDL) is present in human donor diabetic

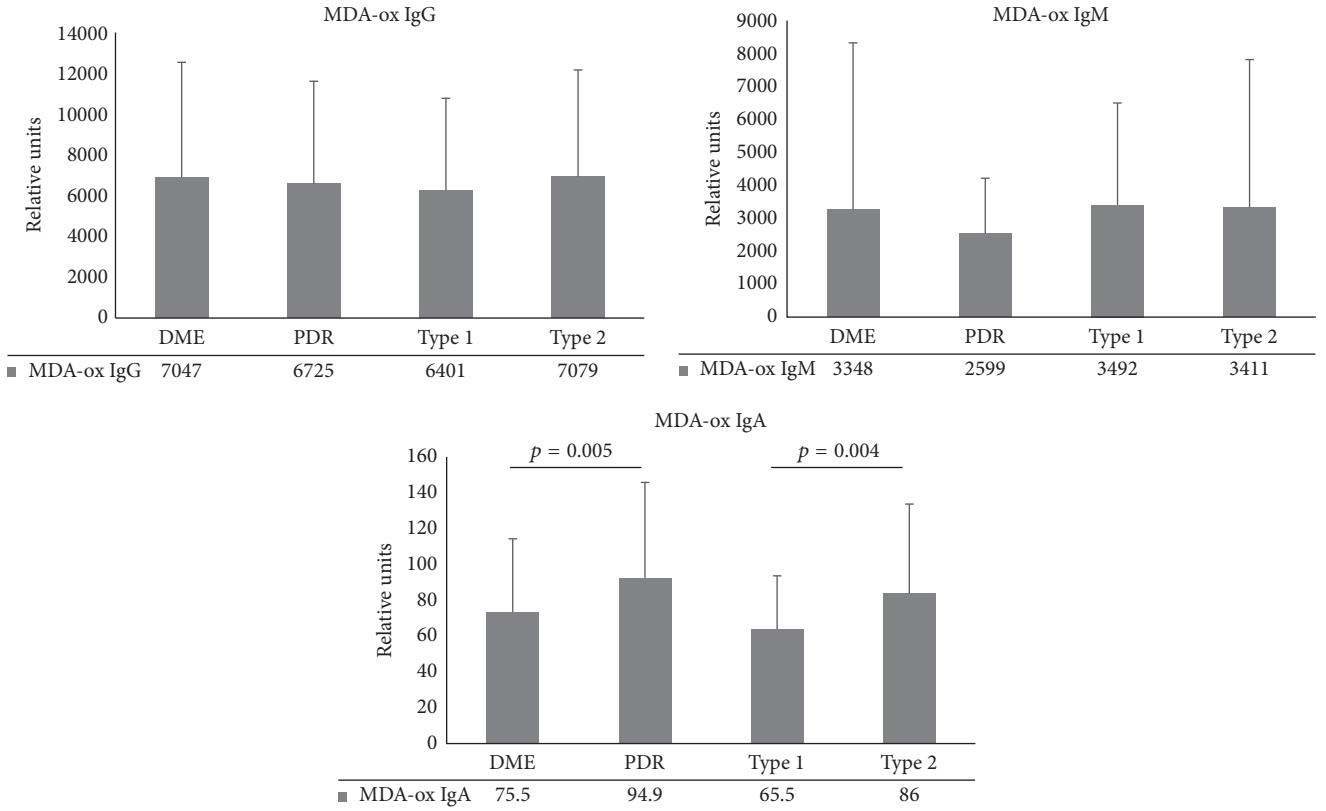

Figure 2: Autoantibody levels against MDA-oxLDL (MDA-Ox IgG, MDA-Ox IgM, and MDA-Ox IgA) in macular edema patients (DME), proliferative retinopathy (PDR), and type 1 and type 2 diabetes patients. The levels are expressed as mean relative units and standard deviation.

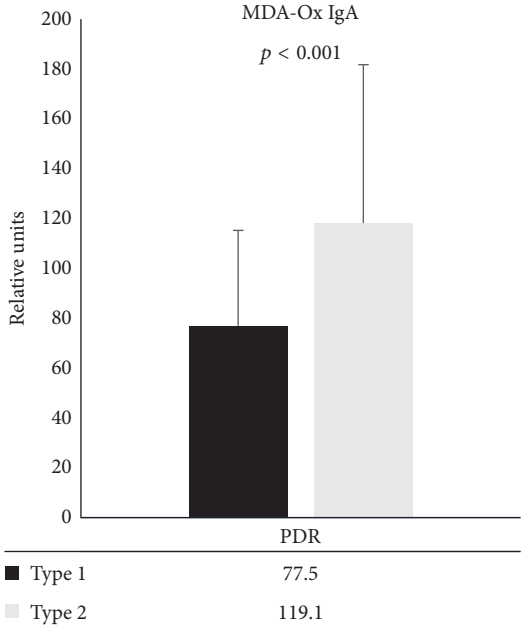

Figure 3: IgA autoantibody levels against MDA-oxLDL (MDA-Ox IgA) in macular edema patients (DME) and proliferative retinopathy (PDR) patients divided by diabetes types (type 1 and type 2). The levels are expressed as mean relative units and standard deviation.

retinas and the level of immunofluorescence increases with the severity of DR [19]. Even at the earliest stages of DR before the onset of clinical retinopathy, an aggregation of oxidized LDL has been detected in the retina. The oxidized LDL is also an effective trigger of apoptosis in retinal capillary pericytes leading to the breakdown of the blood-retina barrier, a key event in the development of DR [26]. Extravasated modified lipoproteins may play a key role in the development of DR; the phenomenon is described in human, animal, and cell culture studies [20] indicating that lipoproteins may be potential causal factors in the development of DR and that the pathogenic process occurring in DR might be somewhat parallel to that observed in atherosclerosis. Furthermore, OxLDL may either be important in the pathogenesis of DR per se or act as a trigger for inflammation in the retina and in the surrounding retinal vessels. It has been shown that modified lipoproteins activate innate and adaptive immune responses with proinflammatory signals and disturb the integrity of the microvasculature [27]. Also the study of Fu et al. supports this hypothesis, since oxLDL-immune complexes triggered apoptosis and enhanced inflammatory cytokine secretion towards retinal pericytes [28]. Furthermore, it seems that oxLDL and autoantibodies might also be important in prognosis of DR as oxLDL-immune complexes and advanced glycation end products modified low-density lipoproteins (AGE-LDL) were associated with an increased

Table 4: Multiple linear regression for autoantibody levels. The variables included in the model were sex, age, BMI, diabetes duration and type, gHbA1c, LDL, and medications. Negative values indicate inverse effect and for sex, female sex has decreasing effect.

Variable	B	95% confidence interval	p
MDAOx-IgG			
Constant	5070.0		**0.005**
Oral diabetes medication	−3857.0	−5693.9 to −2020.1	<0.001
Diabetes type	3624.4	1590.1 to 5658.0	0.001
Statin medication	−1954.9	−3495.4 to −414.3	0.013
MDAOx-IgM			
Constant	−1777.7		**<0.001**
Sex	−995.8	−1912.8 to −78.69	0.033
LDL	919.5	314.5 to 1524.4	0.003
Oral diabetes medication	−2038.5	−3415.0 to −662.0	0.004
MDAOx-IgA			
Constant	−40.93		**<0.001**
Age	0.84	0.37 to 1.32	0.001
gHbA1c	3.64	0.44 to 6.87	0.026
ASA	21.91	8.20 to 35.62	0.003
LDL	8.08	0.91 to 15.25	0.027

risk of progression to advanced retinopathy in patients with type 1 diabetes [21].

As stated, there seem to be several similarities in the role of oxLDL in the pathogenesis of DR and atherosclerosis. There is evidence in humans that the IgM antibodies binding to oxLDL might have an atheroprotective effect as shown in human and mouse studies [9, 24], although with controversy [29]. It seems that instead of being an independent risk factor in atherosclerosis, IgM autoantibodies may modulate the risk of coronary artery disease associated with elevated levels of oxidative biomarkers [30]. The role of IgA autoantibodies binding to oxLDL in atherosclerosis still remains somewhat unclear. It has been postulated that high autoantibody levels binding to oxLDL could well be useful clinical parameters of lipoprotein oxidation for detecting the presence of macrovascular disease in diabetic patients [31]. Our results suggest that determining plasma autoantibody levels against oxLDL might represent a potential indicator for diabetic retinopathy, but this deserves further studies.

Plasma levels of autoantibodies against oxLDL have also been shown to correlate with diabetes or diabetes risk. Previously, low levels of oxLDL antibodies, especially of the IgG type autoantibodies, have been associated with type 2 diabetes, since a low total oxLDL autoantibody level has been linked with the development of type 2 diabetes in women [32] and inversely correlating with markers of glucose metabolism [8]. Furthermore, a harmful role has been proposed for IgA autoantibodies, since higher levels of IgA autoantibodies increased the risk of diabetes in a population-based cohort although no association was found between the levels of IgM autoantibodies and glucose metabolism in a previous study [8]. We found that the IgA levels were highest in type 2 diabetes among PDR group, indicating that the higher levels found were not only disease-specific but also diabetes-type-specific. Previously, it has been shown that these IgA autoantibodies were associated with inflammatory markers, obesity, and type 2 diabetes [10]. In PDR the inflammation and oxidative processes are more active than in DME and it seems that, in type 2 diabetes with PDR, these processes might be further increased as a consequence to local (or general) oxidative stress. It seems that IgA autoantibodies to oxLDL might have a role in the complications encountered in type 2 diabetes, at least at the microvascular level. Other types of autoantibodies seem to be associated with other types of diabetes complications, since IgG type autoantibody was increased in type 1 diabetes [33] and may have a pathogenic role in the development of nephropathy [34].

In our case-control study, we observed a significant increase in levels of IgA autoantibodies in PDR as compared to NoDR or DME groups and this increase was most prominent in type 2 PDR patients. This is the first analysis of autoantibody levels binding to oxLDL in patients with type 2 diabetes and retinopathy. In the present study, a relatively large number of well-characterized patients including both type 1 and type 2 diabetic patients with both DME and PDR were studied. Nonetheless, since diabetes control, retinopathy screening and treatment of DR are stringently regulated in Finland, the study population is rather homogenic. Some limitation of the current study might be that the levels of free autoantibodies and the autoantibodies bound to oxLDL forming immunocomplexes [10] may vary between individuals. Our method will not measure total autoantibody levels to or reflect the total immune response to the antigen. This is a universal phenomenon in measuring the circulating antibodies in plasma samples. In addition, the plasma levels of oxLDL autoantibodies are not specific for retinal damage might also be influenced by the severity of atherosclerosis and/or renal disease or might be affected by influence of diabetes on the vascular wall [35]. There is also some contrasting evidence about using anti-oxLDL as a marker, as antibody concentrations might reflect individual immunity strength, which remains to be solved in future studies.

5. Conclusions

Our study shows that the plasma levels of IgA type autoantibodies were increased in PDR and especially the type 2 patients in PDR group had the highest levels. Our results highlight the role of oxLDL and its autoantibodies in PDR and suggest that they might have relevance as an indicator of DR. Our understanding of oxLDL in the pathogenesis of diabetic retinopathy is increasing but there are still unexplored areas. Clarifying the role of inflammation and immunity in the development of diabetic vascular complications deserves increased attention and may bring tools for DR prevention and treatment.

Competing Interests

The authors declare that they have no competing interests.

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Research Article

Diabetic Retinopathy Screening Using Telemedicine Tools: Pilot Study in Hungary

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Introduction. Diabetic retinopathy (DR) is a sight-threatening complication of diabetes. Telemedicine tools can prevent blindness. We aimed to investigate the patients' satisfaction when using such tools (fundus camera examination) and the effect of demographic and socioeconomic factors on participation in screening. *Methods.* Pilot study involving fundus camera screening and self-administered questionnaire on participants' experience during fundus examination (comfort, reliability, and future interest in participation), as well as demographic and socioeconomic factors was performed on 89 patients with known diabetes in Csongrád County, a southeastern region of Hungary. *Results.* Thirty percent of the patients had never participated in any ophthalmological screening, while 25.7% had DR of some grade based upon a standard fundus camera examination and UK-based DR grading protocol (Spectra™ software). Large majority of the patients were satisfied with the screening and found it reliable and acceptable to undertake examination under pupil dilation; 67.3% were willing to undergo nonmydriatic fundus camera examination again. There was a statistically significant relationship between economic activity, education and marital status, and future interest in participation. *Discussion.* Participants found digital retinal screening to be reliable and satisfactory. Telemedicine can be a strong tool, supporting eye care professionals and allowing for faster and more comfortable DR screening.

1. Introduction

The global incidence of diabetes mellitus (DM) among adults (age 18 years and older) was 9% worldwide in 2014 [1], while its prevalence still shows an increasing tendency due to obvious obesity epidemic and aging of the population [2–4]. In Hungary, a total of 865 069 patients (9.5% of the population) suffered from DM in the same age group in 2011 [5], and some degree of diabetic retinopathy (DR) could be observed among 19% of the patients with type 1 DM (T1DM) and 24% in those suffering from type 2 DM (T2DM) for 3 or 4 years [6]. DR is the fourth most common cause of blindness in the overall population, but it is in second place among active adults in industrialized countries [7], accounting for a significant drop in quality of life (QoF) and working ability of the patients [8, 9]. 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 [10]. 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 correlating risk factors are being investigated. Unfortunately, 50% of the people with diabetes are unaware of the characteristics of their disease and the compliance in attending screening programs is poor. The disease is determined by the outcome of the complications. Since high blood sugar and fat destroy the wall of the arteries, it is not surprising that people with diabetes have 2 to 4 times higher cardiovascular mortality rate and 2 to 4 times

higher risk of strokes than patients without diabetes. Renal failure is also a common complication with the estimated number of 30–40% of the patients with diabetes, while 60–70% of the patients develop neuropathy. This is not only an individual problem, but a societal problem as well. According to a 2009 survey, the average annual health expenditure for diabetic patients was $1205 per capita and for patients with complications this number was $2276 per capita. Half of this cost is made up of drugs, but only a quarter of the cost spent on drugs is for antidiabetics [11]. Similarly, the treating expenses doubled in Germany and America, where $174 billion was spent on the treatment of diabetes in 2007 [11]. The Hungarian data cover only the cost of the National Health Insurance Fund, while there are other economic aspects like time off from work or restricted work due to complications of the disease. DR is caused by damage to the retinal microvasculature. Proper screening for DR is an important milestone towards achieving early and efficient laser photocoagulation and/or anti-vascular endothelial growth factor (anti-VEGF) treatment for preventing visual loss [12]. Depending on the severity of DR, four stages can be distinguished in general: preretinopathy (R0), background retinopathy (R1), preproliferative retinopathy (R2), and active proliferative retinopathy (R3A) [13]. A further subclassification exists for stable proliferative retinopathy (R3S) in patients who have received panretinal laser photocoagulation (PRP) under R3A and then became "stable"; these cases are considered safe to keep in a surveillance clinic [14]. Once fundus lesions appear as a complication of DM, the patient has an apparent DR with either low, intermediate, or high risk for developing some grade of DR. Therefore, the focus should rather be on raising prevention programs and early detection, as well as successful treatment of the basic disease.

DR is usually asymptomatic before the appearance of any vision loss, but it is detectable by retinal imaging techniques objectively and by accurately taken best corrected VA measurements. Much research around the world has been focused on the use of telemedicine tools for fundus imaging and screening, the UK system standing up at the top in terms of reliability, precision, and standardized input and output. The results so far have been very promising, with each study being reported to date pointing out the high sensitivity for detecting several fundus lesions in the initial stages of DR by a standard fundus camera and a grading software [15].

The Spectra DR software is designed around the requirements of the UK National Health Service (NHS) national screening program for DR; it is highly complex and requires a high level of sophistication in the software to meet its requirements. Spectra DR enables patient appointments to be created, data entry, image capture, and grading. A generation of patient results is provided together with a report regarding the patients' screening prediction via a "plug-in" algorithm. With the use of nonmydriatic or investigational hand-held portable cameras, a quick and simple DR evaluation process will likely improve the patients' willingness to participate in future screening tests.

In 1980, Iceland began regular DR screening for T1DM patients, which resulted in the reduction of disease-related blindness from 2.4% to 0.5% [16]. The Icelandic population being used for the cohort study and development of a commonly used risk calculator (Risk Medical Solutions, Iceland) is much more homogenous when it comes to ethnic and socioeconomic differences compared to the population in Hungary. Nevertheless, with these new screening and telemedicine tools, it is realistic to expect similar results to be achieved in other European countries, including Hungary, within 5 to 10 years time [17].

The present research explores how DM patients subjectively experience the telemedicine tools and examination through participation in a free fundus camera screening program conducted in a southeastern county (Csongrád) in Hungary and obtains feedback on whether they would participate in such an examination in the future. Furthermore, demographic factors such as age, gender, economic activity, and socioeconomic status (SES) (level of education, support from family, and subjectively perceived financial status) are examined for their effect upon participation in future screening programs.

2. Methods

A free screening test was performed on a random population including 178 eyes from 89 patients with confirmed DM diagnosis. Handling of the fundus camera and the image acquisitions were performed by a qualified professional in a darkened room, which were then forwarded through a secure internet connection to a specialist doctor/ophthalmologist (A. F./M. C. M.) or ROG (G. R./P. K.) for evaluation. In 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 within 10 working days using Spectra DR software. The recordings were safely deposited and kept inaccessible to third parties for 10 years at a central server, so that later they can be used in further comparative studies on DR.

The images were acquired by an 18-megapixel Canon EOS digital camera which was connected to a Canon CR2 color, nonmydriatic, 45° retinal camera. Two pictures were taken of the participants' each eye: one with the macula and another with the optic nerve in the center—this is in line with the UK screening requirements [18]. 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./M. C. M./G. R./P. K.) classified the signs and the stages of DR and maculopathy in the standardized UK-based software Spectra DR and graded the images in alignment with the UK standard grading protocols [19]. Each image was evaluated in two stages: first, the ROG (G. R./P. R.) evaluated them, and then a supervisor/ophthalmic consultant confirmed the diagnosis (A. F./M. C. M.). At the end, an expert opinion regarding the grade of retinopathy was sent back to the screening site, that is, stage of retinopathy (R0/1/2/3A/S) and 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 was as follows:

M0: no maculopathy was detected; repeated screening was recommended one year later.

M1: there was a sight-threatening maculopathy; within one month a medical examination is required.

R0: there was no clinical anomaly; repeated screening was recommended one year later.

R1: mild nonproliferative phase, microaneurisms, dot- or blot-like hemorrhages, or exudates could be seen; control examination was recommended one year later.

R2: moderate or severe nonproliferative phase, major bleeding(s), cotton-wool spots, venous looping, and intraretinal microvascular abnormalities (IRMAs) were visible; control examination was required within one month.

R3A: active proliferative phase, neovascularization of the optic disc (NVD) or elsewhere (NVE) or preretinal bleeding(s), vitreous bleeding, preretinal fibrosis, and tractional retinal detachment could be observed; immediate medical examination was required within two weeks.

R3S: stable proliferative retinopathy; a retinal image showing stable post-PRP laser with no signs or reactivation or active referable retinopathy; only to be determined in the presence of "benchmark images" taken at the time of discharge for comparison; screening intervals may be at the discretion of the trained ROG.

Other recorded, but not reported, changes/fundus pathology included age-related macular degeneration (AMD), glaucoma changes in the optic nerve, and any other signs of eye disease.

2.1. Self-Completed Questionnaire. The self-completed questionnaire collected information about the individual's demographic status such as age, gender, economic activity (full-time, part-time, and retired), SES such as education (primary, secondary, and higher), and marital status (married or lives with a partner, single, separated or divorced, and widowed). The general part of the questionnaire was based on the European Health Interview Survey 2009 [20], and it collected data about DR associated exposure parameters and some other health connected parameters, type of DM, or presence of hypertension, as well as the type of eye diseases. Furthermore, data were collected about the frequency of measuring blood sugar levels and also about participation in screening programs, which are important for preventing retinopathy, including the frequency of attending Diabetology or Ophthalmology specialist clinics. Questions regarding the perceived reliability of results (yes/no/maybe), willingness to participate again (yes/no/maybe), comfortability (dissatisfied/satisfied/acceptable) of the tests performed, and the overall perception of the screening examinations as well as whether they would participate in a similar examination next time were being asked/collected as well. Some categories underwent merging due to missing data, for example, the intensity of blood sugar measurement (monthly/less than a month, weekly/every few days, and daily/more than once a day). If the participants could not understand or read the questionnaire for whatever reason, they received professional help accordingly.

2.2. Statistical Analysis. The analysis of the data was performed by descriptive statistical analysis on N number of participants, and percent distribution, median, and interquartile range (IQR) are being shown. The Chi-square (χ^2) and Fisher exact tests were used to test differences of the distributions of categorical variables. The relationship between two variables was considered statistically significant when $P < 0.05$. The graphs were made in GraphPad Prism 5.01 (GraphPad Software Inc., La Jolla, CA, USA). The statistical analysis of the data was performed by using Stata (Intercooled Stata 8.0, Stata Corporation, College Station, TX, USA) and Excel software (Microsoft Corporation, USA).

2.3. Ethical Issues. The Regional and Institutional Human Medical Biological Research Ethics Committee of the Albert Szent-Györgyi Health Centre, University of Szeged, approved the study protocol (number 197/2015). The research provided anonymity to the participants. Before the beginning of a test, the participants signed a written consent form in which they agreed to permit the use of data for research purposes.

3. Results

178 eyes of 89 people were examined in the study out of which 30 were men (33.7%) and 59 were women (66.3%). Table 1 shows the demographic characteristics of the patients, the median age of whom ranged between 56 and 68 years of age and had median HbA1c of 7.2% (ranging between 6.4 and 7.9%). Table 2 shows the distribution of the types of DM and the stages of DR in the screened population, based upon and compared to the UK grading system and software (Spectra DR). Twenty percent of the participants had T1DM out of which 70.8% had T1DM diagnosed by a Diabetology department, the rest being yet undiagnosed or hidden disease patients. Mild nonproliferative DR (grade R1) was detected in 23.0% of the participants, while higher (moderate/R2 and proliferative/R3) grade DR was detected in 1.4% and 1.4% of the subjects, respectively; maculopathy/M1 was present in 5.4% of the studied group (representative images from these were captured from each grade and processed in the Spectra software as shown in Figure 1). Another retinal pathology was detected in 28.4% of the participants. There was an overall left-shift in the distribution of earlier stages of DR in the UK population compared to the one represented by the Hungarian graded images and based upon the Spectra DR software, probably due to the existence of a well established screening system in the UK and early detection of the disease.

According to the self-perceived satisfaction with the classical pupil dilation versus fundus camera examination,

Table 1: Patients' demographics.

Variables	Percent (%)
Gender	
Male	33.7%
Female	66.3%
Age (median, IQR)	63 (56–68)
HbA1c (median, IQR)	7.2 (6.4–7.9)
Hypertension*	
No	19.1%
Yes	76.4%
Occupation	
Full-time	22.2%
Part-time	9.3%
Retired	68.5%
Education*	
Primary	23.6%
Secondary	52.3%
Higher	21.8%
Marital status	
Married or lives with a partner	55.6%
Single, separated, or divorced	24.1%
Widowed	20.4%
Attendance of blood sugar screening	
Monthly/less than a month	20.8%
Weekly/every few days	39.6%
Daily/more, than once a day	39.6%

IQR: interquartile range.
*The remaining percent of participants either were not aware of their disease (hypertension) or provided no response (education).

20.4% versus 83.6% of the participants expressed satisfaction, respectively, while 37.0% versus 9.1% were unsatisfied, and 42.6% versus 7.3% could not decide. The classical pupil dilation versus fundus camera examination was found to be definitely reliable by 75.5% versus 72.0%, possibly reliable by 18.4% versus 16.0%, and unreliable by 6.1% versus 12.0%, respectively. The willingness to participate in a classical pupil dilation versus fundus camera examination was found to be positive by 78.2% versus 67.3%, while 9.1% versus 10.9% responded that they would not participate, and 12.7% versus 21.8% responded as maybe doing it. There was no significant difference between the satisfaction from the examination ($P = 0.9$) and reliability ($P = 0.3$), although the willingness to participate significantly differed between the classical versus fundus camera examination ($P = 0.01$) (Table 3).

The economic activity significantly affected the participation in a blood sugar screening ($P = 0.001$). Sixty percent of those employed in a part-time job had attended blood sugar screening more than once a day or daily, 20% weekly/every few days or monthly/less than a month. The daily/more than once a day attendance was 33.3% among retired, while the weekly/every few days screening was 55.6%, and the monthly/more than a month was 11.1% in this age/patient group. Among the full-time workers, the daily/more than once a day and monthly/less than a month screening was 45.5% versus 54.5% (Figure 2(a)). Similarly, marital status (being married or living with a partner) significantly impacted the likeliness to attend blood sugar screening ($P = 0.04$); this population had a higher daily/more than once a day blood sugar screening attendance, with a frequency of 50% compared to those living alone (single, separated, or divorced: 30.8%; widowed: 18.2%); the latter two populations had otherwise the highest weekly/every few days attendance (single, separated, or divorced: 53.9%; widowed: 72.7%). The least frequent or monthly/less than a month screening attendance was the highest among married or living with a partner population (28.6%), while it was the smallest among widowed participants (9.1%) (Figure 2(b)).

The willingness to participate in the annual fundus camera screening was the highest among the full-time workers (91.7%) and the lowest among part-time workers (20.0%) Those who reported maybe versus no attendance were higher among part-time workers (40.0% versus 40.0%, resp.), while the willingness to participate differed significantly between the analyzed economical groups ($P = 0.003$) (Figure 3(a)). The satisfaction with the fundus camera screening also increased significantly with the level of education (primary (69.2%) and secondary (82.8%); higher (100%), $P = 0.003$) (Figure 3(b)).

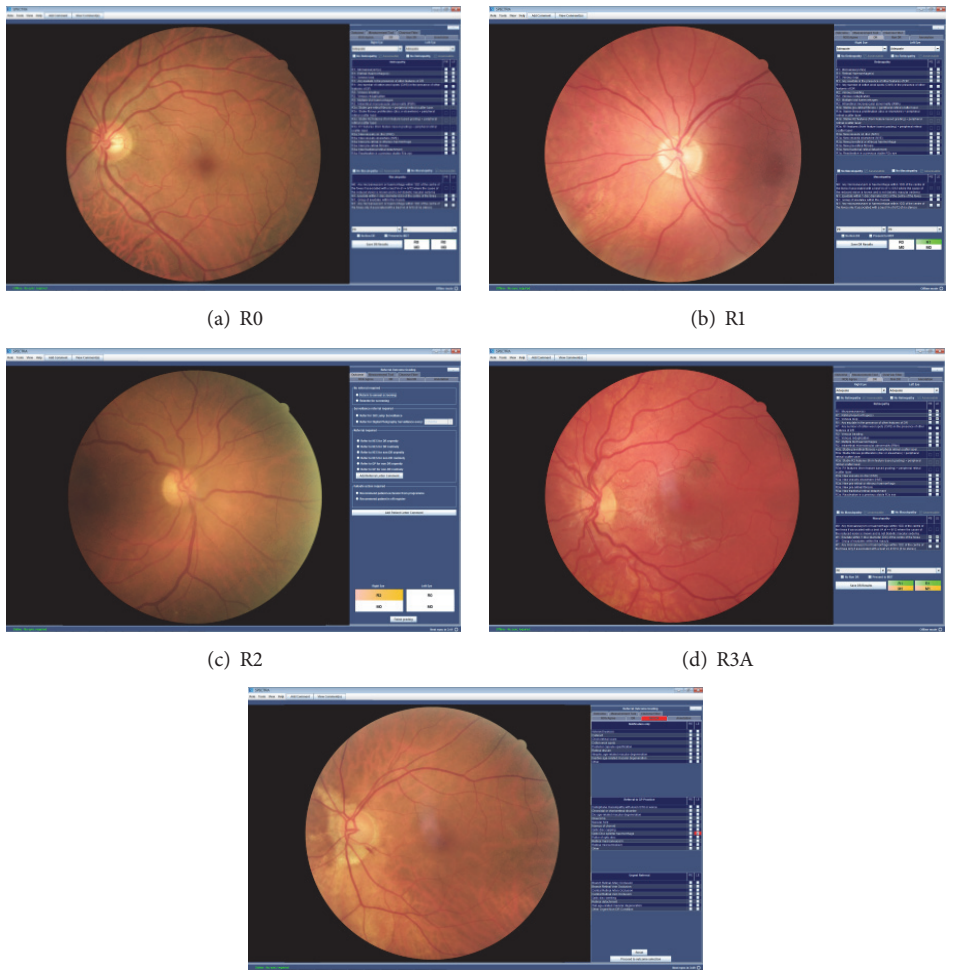
Among participants with secondary or higher education, the most common argument used against the classical fundus exam was "I cannot see clearly after." The participants with primary school level education had significantly higher rate of stating dissatisfaction of the pupil dilation examination. This reason was not stated among higher educated patients, although the "I cannot drive after" reason seemed to appear more often in this group of patients.

4. Discussion

The present study aimed to investigate the patients' experience with the use of telemedicinal tools for screening of DR and the ability to collect the parameters needed to calculate DR risk (age, gender, type and duration of DM, HbA1c, hypertension, and fundus image grading) in a southeastern county (Csongrád) in Hungary. The justification for using health care tools aimed at screening DR is high, due to the great availability of tools for DR prevention and avoidance of late complications such as STR. The population of Csongrád County is very plausible for initiating such a study, since it has a known higher prevalence of DM compared to other counties in Hungary [5]. In addition, the study followed the progressive trend of DM worldwide and examined the willingness to participate in screening tests, the attitude towards screening examination, and the influence of demographics and socioeconomic factors like education, financial, and marital status. Regarding the risk factors, the SES has been already shown to have a very significant impact on the attendance in screening examinations, while occupation has been related to a greater impact on nonattendance in screenings [21]. The screening frequency for blood sugar levels in full-time workers was indeed significantly lower in our study, but the willingness to participate in fundus screening examination was higher in that subpopulation.

Table 2: Distribution of the DM types and DR grade in the studied population in relation to the UK-based grading system implemented by the Spectra™ analysis.

	T1DM	T2DM	R0	R1	R2	R3	M1
Csongrád County, Hungary	20.2%	79.8%	73.0%	23.0%	1.4%	1.4%	5.4%
East Anglia, UK	15.0%	85.0%	68.7%	27.5%	0.6%	0.3%	3.2%



(a) R0 (b) R1

(c) R2 (d) R3A

(e) M1

Figure 1: Spectra DR based grading of the DR retinopathy. Representative images of the different grading stages are shown in the studied population.

From the standpoint of DR formation and progression, it is 76.4% of the patients who had high blood pressure which, by itself or as a codisease, gives poorer prognosis for the DM patients due to a predisposition for premature vascular sclerosis. The occurrence of DR in the studied sample population was 25.5%, which is higher than any previous results in Hungary [22], although somewhat expected in Csongrád County.

Although the Diabetology guidelines recommend blood glucose levels to be checked several times a day, only a little over a quarter of the participants performed it accordingly. Strikingly, 60% of the study participants performed blood glucose testing every few days, if not more rarely. Upon diagnosis with DM, the Diabetologist or the General Practitioner informed the patient of the possible complications from the disease and recommended an annual eye screening test. Our results coincide with the International Diabetes Federation's (IDF's) observation that 50% of the people with DM are not aware of the characteristics of their disease [23]. In Hungary, the number of known patients with diabetes makes nearly 10% of the total population. It would take 100 ophthalmologists (from the total of 968 practicing) working full-time if they want to carry out only the annual screenings by using traditional tools on such a sized population. This may change by using the telemedicine system [17]. Introducing a new screening program always faces challenges, but previous studies from other countries show promising results. DR could be detected at early stages by digital imaging even in

Table 3: Reliability, satisfaction, and willingness to participate again in a classical or fundus camera examination for DR screening.

Variables	Pupil dilation $N = 89$ (%)	Fundus camera $N = 89$ (%)	P^*
Reliability of the examination			
Yes	75.5%	72.0%	
No	6.1%	12.0%	0.3
Maybe	18.4%	16.0%	
Willingness to participate again			
Yes	78.2%	67.3%	
No	9.1%	10.9%	0.01*
Maybe	12.7%	21.8%	
Satisfaction with the comfort of the screening			
Dissatisfied	37.0%	9.1%	
Satisfied	20.4%	83.6%	0.9
Acceptable	42.6%	7.3%	

* $P < 0.05$.

rural areas [24]. Diabetes causing vision loss is successfully confined in countries like Iceland, where regular screening was implemented.

In our study, only a third of the participants had not visited an ophthalmologist, while 12.4% of them have been diagnosed with DM within a year; only 56.2% of the participants complied with the one-year recommendation. In the UK, patients compliance in attending traditional screening was 45% and 50% in fundus camera screening in the first year [25]. After using a mobile fundus camera screening unit to reach more patients, the compliance elevated to 80% in the fifth year [26]. Compliance is a highly influential factor of cost effectiveness because of the fixed costs (digital imaging camera, computer system, etc.) [25]. Patient satisfaction affects the attendance rate of the screening. The response to the subjective experiences perceived during fundus examination did produce satisfactory results: more than three-quarters of the participants were satisfied with the fundus camera examination and one out of five with the traditional method. In both cases, three-quarters of the participants considered the results of the study to be reliable, a significant difference being found between the two screening procedures. There were fewer problems than expected (e.g., subjects being not able to drive after pupil dilation), but it can be a factor which is most likely related to older age of the sampled population. It is interesting to note, however, that during the procedure of pupil dilation, one quarter of the subjects found administering eye drops being irritating or uncomfortable, in particular, those who had lower education.

There is a level of contradiction in the assessment of reliability and satisfaction in the study, since significantly more people were willing to participate in the traditional retinal screening method than in the fundus camera test (78.2% versus 67.3%). A possible weakness of the study is the size of the sample. 83.6% of the participants were dissatisfied with the examination, which raises the suspicion they could have chosen "Other" for their response to having no other comments, and this could have been done out of necessity. Among the inconvenience experienced during the test with pupil dilation, the "Other" category was chosen by only 4.1% in which no mention of any reasons for the selection made was stated whatsoever.

During the analysis, the economic activity and education appeared to pose an effect on the individual's willingness to participate in the screening test. The fundus camera test was preferred mostly by the full-time employees, with whom it was presumably important to see well after the test in order to be able to continue their work during the same day. Based on the level of education, the few subjects that evaluated the fundus camera test as satisfactory were those who found eye drops to be the most uncomfortable in the traditional test. These data are somewhat contradictory, as mydriatic drops are always required in traditional testing. People with higher education found only the driving restriction and the bad sight after the examination as a negative aspect of the screening; in this context, they were 100% satisfied with the fundus camera test.

The telemedicine part of the study also concerns data safety and patient anonymity preservation which are now guided by an EU law contained in the Charter of Fundamental Rights of the European Union, Article 8 (2000/C 364/01) [27], as well as the need to safely store and make backup files for high resolution fundus images acquired from the patients and their retention; these rules were followed in the present study entirely. The issue of having decentralized and near the patient DR screening and fundus imaging services and centralized image reading remains to be evidenced in future telemedicinal studies for screening DR in Hungary, the UK grading system being the golden standard for achieving the task properly.

In conclusion, the analyzed demographic and socioeconomic factors showed a significant relationship with the future participation in the fundus camera screening for DR. The participants' age or gender appears not to affect the experience (satisfaction) of the examination (e.g., fundus examination under pupil dilation). However, the level of

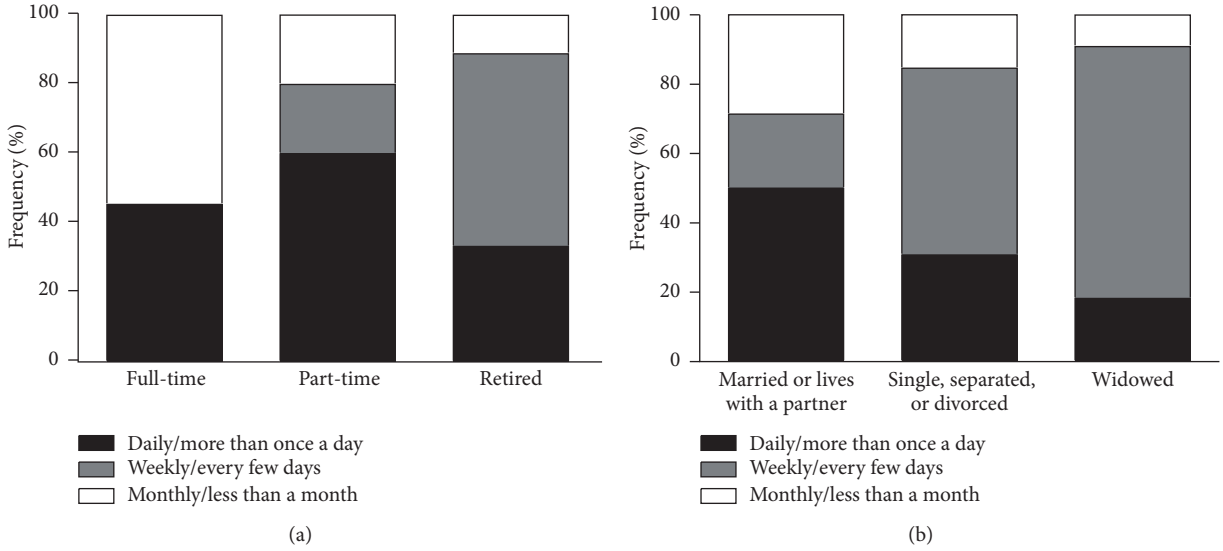


Figure 2: Effect of economic activity (a) and marital status (b) upon the blood sugar screening frequency.

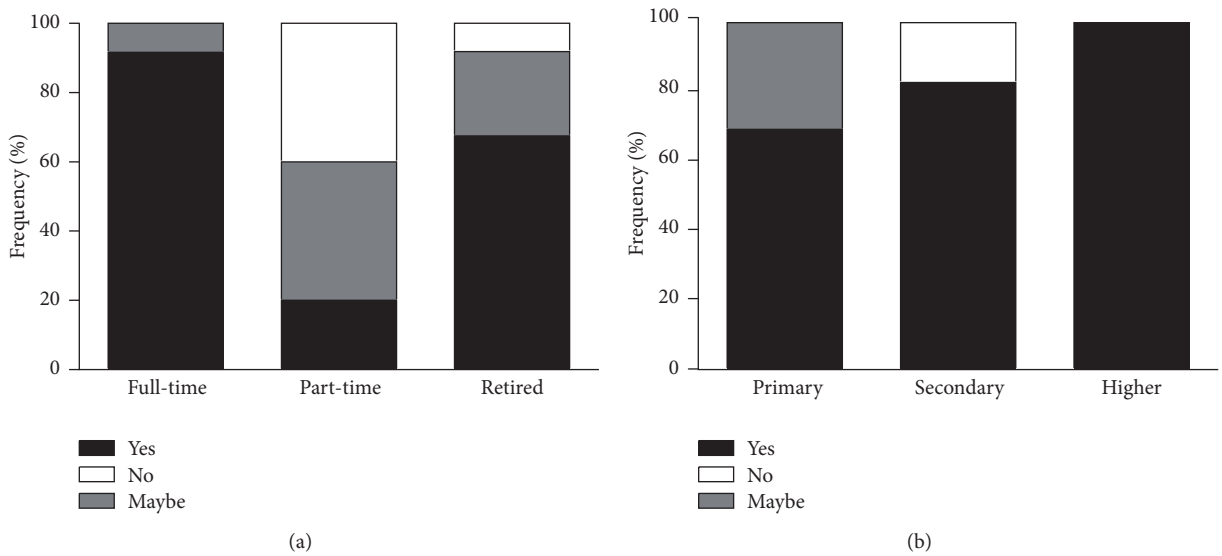


Figure 3: Economic activity in relation to willingness (a) and educational level in relation to satisfaction (b) in participating in fundus screening examination.

education appears to have an important role: higher educated patients were more likely to participate in pupil dilation examination using an ophthalmoscope. This is in contradiction to the fact that only slightly more than half of the participants in this group took part in such screening examination within a period of one year. It was also not confirmed that the distribution of DR grades in this study is similar to the results of previous national studies [17, 22], as Csongrád County is not a representative population comparable to other parts of Hungary where the prevalence of DM and DR is lower. Further research is therefore needed on a larger or more representative sample from different counties in Hungary where the percent of distribution of patients diagnosed with DM varies.

In general, the treatment of DM patients is an interdisciplinary task of primary care physicians, diabetologists/dietologists, ophthalmologists/optometrists, and public health specialists. These professionals are responsible for giving lifestyle advice and for directing patients towards more appropriate screening tests. Ophthalmic monitoring is required every year after the diagnosis of diabetes and every other year for patients with excellent glycemic control without retinopathy at the previous examination but annually if there are risk factors [28]. Furthermore, if retinopathy is manifested to some degree, the screening time should be reduced to half a year (in the case of nonproliferative retinopathy) and three months (for preproliferative retinopathy). In case of proliferative retinopathy patients should go

immediately to an ophthalmologist, in order to initiate laser treatment in time and thus save the eye from STR. The present state, unfortunately, seems to involve lack of realistic assessment or judgment of the risk from complications by the patients, and therefore a neglect to participate in the recommended screening tests. Constant maintenance of normal blood sugar levels is indispensable. Fast, easy, and accurate fundus camera examination is an alternative to the traditional, time-consuming, and "unsatisfactory" fundus test under pupil dilation. The patients, who tried this method, agreed that this new way is more satisfaction than the one they got used to, while they appreciated its reliability in the same way. Indeed, in the UK, due to the systematic screening implemented, DR is no longer the leading course of blindness in the working age population [29].

Competing Interests

The authors declare that they have no competing interests.

Acknowledgments

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Review Article

Diabetic Retinopathy in Italy: Epidemiology Data and Telemedicine Screening Programs

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In Italy, the number of people living with diabetes is about 3.5 million (5.5% of the population), with an increase by about 60% in the last 20 years and with 1 person out of 3 older than 65 years. The Italian Health Service system estimates that 10 billion euros is spent annually on caring for patients with diabetes, a figure that increases yearly. No national data on prevalence and incidence of legal blindness in patients with diabetes and no national registry of patients with diabetic retinopathy (DR) are currently available. However, the available epidemiological data (in several locations throughout the country) are consistent with those reported in other European countries. The use of telemedicine for the screening of DR in Italy is confined to geographically limited locations. The available data in the literature on implementation and use of telematic screening proved to be successful from patient, caregiver, and authorities point of view. This review addresses the available epidemiological data on DR and telematic screening realities in Italy and thus may help in establishing a national screening program.

1. Diabetes Mellitus: The Italian Scenario

Diabetes mellitus (DM) is considered a global epidemic of the 21st century with currently 382 million people affected worldwide and with a projection of doubling this number (592 million) by 2035, as estimated by the World Health Organization [1]. In Italy, the number of patients with DM has increased by about 60% in the last 20 years, from 3.4% in 1993 to 5.5% (thus 3.5 million people) [2–4]. Recent epidemiologic data from the ARNO observatory (a partnership between the Italian Society of Diabetology and the Inter-University Consortium ARNO Cineca) reported that 1 person out of 3 affected by DM is older than 65 years, and of these, 1 out of 4 is older than 75 years of age [2]. Less than 1% are younger than 20 years and 3% are younger than 35 years [2]. The prevalence of DM is 6.1% in men and 5.5.% in women with a consistent difference of 10% across all age groups >35 years [2]. Currently 67% of patients are treated with oral hypoglycemic drugs, 10% of them with a combination with insulin and 11% with insulin alone [2]. It is estimated that patients with type 1 diabetes mellitus (T1DM) represent approximately 2-3%, whereas patients with type 2 diabetes mellitus (T2DM) represent more than 90% of all patients with known DM in Italy [5]. The Bruneck study (long-term, prospective, population-based study in the town of Bruneck located at the very north of Italy) reported an incidence rate of 7.6 per 1,000 person-years of T2DM in individuals aged 40–79 years and independent risk factors for incident DM as follows: impaired fasting glucose, overweight/obesity, insulin resistance, and impaired insulin response to oral glucose [6]. In the province of Torino, the incidence rate of T1DM in the age group of 30–49 years was 7.3 (6.2–8.6) per 100,000 person-years, being at least as high as that in the age group of 15–19 years (6.8, 6.3–7.4) [7]. Men had two-fold higher risk for developing T1DM than women in all age groups [7]. The incidence of known T2DM was 50.7 per 100,000 person-years in the age group of 30–49 years, representing the great majority of new cases of DM [7]. The risk for developing T2DM increased markedly with age, being seven-fold higher in the age group of 40–49 years than in the age group of 30–34 years, irrespective of sex [7]. The incidence of T1DM has progressively increased in Italy with 3-4 times higher rates in

Sardinia than in other parts of Italy [8, 9]. The Italian Health Service system estimates that 10 billion euros is the annual cost for the care of patients with DM, and these costs are increasing over time [10, 11].

2. Diabetic Retinopathy: Global and Italian Epidemiology Data

Diabetic retinopathy (DR) is the leading cause of legal blindness among the working aged adults [12]. Nearly all the patients with T1DM and the majority of those with T2DM are affected by some form of DR after 20 years of disease duration and 50% may develop sight-threatening DR [13–15]. The main risk factors associated with an early onset and rapid evolution of DR are duration of DM, poor glycemic control, and presence of concomitant arterial hypertension [5]. The Wisconsin Epidemiologic Study of Diabetic Retinopathy (WESDR) reported that the incidence of diabetic macular edema (DME) is 29% in T1DM over a period of 25 years and 25.4% among those with T2DM requiring insulin [13, 14, 16]. A pooled analysis from 35 studies worldwide (from 1980 to 2008) evaluating more than 20000 people with DM reported an overall prevalence of any DR of 34.6% (95% confidence interval) (CI, 34.5–34.8), proliferative DR (PDR) of 6.96% (CI, 6.87–7.04), DME of 6.81% (CI, 6.74–6.89), and sight-threatening DR of 10.2% (CI, 10.1–10.3) [17].

In Italy, there are no national data about prevalence and incidence of legal blindness due to DR, and there is no national registry of patients with DM [11]. However, several studies reported the prevalence and incidence of DR from geographically limited population-based studies [18–20]. In one of these studies, 1321 patients with DM were examined for DR in the Veneto Region (northeast of Italy). DR prevalence was 26.2% (24.4% background DR and 1.8% PDR) as reported in 1991 [18]. The prevalence of DR was significantly related ($p < 0.01$) to the duration of DM (17.3% for <5 years; 60.8% for >20 years) [18]. In the province of Torino (northwest of Italy), DR was the second most common cause of bilateral blindness (13.1%) in 4549 residents who were certified blind between 1967 and 1991 [19]. Of the 6857 consecutive patients seen between 1992 and 2003, the prevalence of DR was 39% (19% mild nonproliferative DR (NPDR), 11% moderate NPDR, and more severe in the remaining cases) [21]. Furthermore, data collected by general practitioners and diabetes specialists in Italy reported in 1997 that 13% of patients with diabetes had PDR and 2% suffered from blindness [20].

In the province of Viterbo (located in the Lazio Region, Central Italy) in 2002, DR was the fourth most frequent cause of blindness accounting for 15% of cases [22]. When DM is diagnosed after 30 years of age, the prevalence of DR is about 20% after 5 years, 40–50% after 10 years, and >90% after 20 years of disease [19]. The cumulative incidence of DR ranged from 34% to 59% during a four-year period, depending on the age of patient and severity of disease [18, 19]. As a whole, DR was responsible for 13% of cases of severe visual impairment in Italy [18, 19].

Therefore, screening for DR remains crucial for early diagnosis of the disease and preventing blindness and is recommended in all patients with DM [23–25]. The *"Associazione Medici Diabetologi" (AMD)*, *"Società Oftalmologica Italiana," "Società Italiana della Retina," "Società Italiana di Diabetologia,"* and other organizations have jointly published a guideline for the screening, diagnosis, and treatment of DR in Italy, the *"Linee-Guida Retinopatia Diabetica"* [26].

However, screening for DR is delivered to only approximately half of all patients with DM (as reported in the United States), where the annual fundus examination was recommended as the annual screening for DR [27, 28]. As a consequence, the access to the treatment has been also limited for these patients. The use of retinal photography with an overall sensitivity of approximately 85% is considered currently the standard method to be used in a screening setting, especially as it allows for implementation of telemedicine programs [29–32].

3. Telemedicine Screening Programs in Italy

Data about the use of telemedicine for DR screening in Italy are very limited in the literature. Vujosevic et al. underlined the reliability of nonmydriatic techniques used in screening and grading settings and confirmed the importance of digital images over ophthalmoscopic examination in screening and grading of DR [32]. These authors evaluated 3 nonmydriatic field color fundus photos covering 45 degrees consisting in field 1 (central), centered on the macula; field 2 (nasal), centered on the nasal margin of the optic disc; and field 3 (temporal), centered superiorly and temporally from the macula and compared to just one central fundus color photo and to 7 standard stereoscopic 30-degree photos (ETDRS fields) in detecting referable DR, defined as severe NPDR and PDR and DME [32]. Sensitivity and specificity for detecting referable DR were 82% and 92% and 83% and 97% for referable DME for 3 nonmydriatic fields fundus photos and significantly lower (below the requested target of the British Diabetic Association necessary for an effective screening, set at 80%) for one field fundus photo [32, 33].

In Torino the use of nonmydriatic fundus photos in the screening program was introduced in 2000 [34]. The fundus photos were taken in the diabetes center by trained nurses or medical personnel and consist in 2 nonmydriatic 45-degree color fundus photos, one centered on the macula/central field and the other centered on the optic disc (nasal field) as proposed by EURODIAB procedure [34, 35]. Grading was performed by diabetes specialists, after specific training, according to the European Working Party recommendations [34, 36]. Patients were assessed at retinal photography and formally graded later. Feedback on referrals was by direct discussion with the consultant ophthalmologists working in the DR Centre. The authors evaluated the 6-year cumulative incidence of referable DR and reported 10.5% (95% CI, 9.4–11.8) [34]. Referable DR was considered in case of moderate NPDR or worse (preproliferative DR, PDR, photocoagulated DR, and advanced diabetic eye disease with or without macular involvement), equivalent to Early Treatment of Diabetic Retinopathy Study (ETDRS) level >35 [37], whereas patients with mild NPDR, equivalent to an ETDRS level ≤35, did not require referral and were given rescreening appointments.

Retinopathy progressed within 3 years to referable severity in 6.9% (95% CI, 4.3–11.0) of patients with age at onset ≥30 years, who were on insulin treatment and had a known disease duration of 10 years or longer. The other patients, especially those with age at onset <30 years, on insulin and with a duration <10 years, progressed more slowly [34]. The authors concluded that screening can be repeated safely at 2-year intervals in any patient without DR [34].

The telematic screening program for DR in Padova area (northeast of Italy) was systematically implemented for those with DM in 2005 and since then a total of 17344 screening exams of 9347 patients with DM have been performed (data reported up to 2015). Color fundus photos of patients with DM are acquired in two remote diabetes centers by qualified staff (nurses or technicians) and thereafter sent by dedicated intranet link to the Reading Centre, at the Department of Ophthalmology of the University of Padova. Images are acquired with the use of nonmydriatic fundus cameras. The grading of images is performed by certified medical personnel and confirmed by the responsible medical retina specialist. In order to minimize errors, all evaluations of images are performed in double grading fashion and in case of discordance all adjudications are performed by the experienced grader. The final grading report with the results of DR grading is sent back electronically to the referring Diabetes Clinic. Grading of images is based on the International Diabetic Retinopathy and Macular Edema Severity Scale [38]. The National Guidelines for Screening of DR are adopted for determining the follow-up of patients [26]. Patients without DR or with mild NPDR are recommended a reevaluation within 12 months in the screening service, while patients with moderate NPDR are rescreened within 6–10 months and patients with severe NPDR or proliferative DR or with any maculopathy are referred to the DR Clinic for a complete ophthalmologic examination with possibility to perform optical coherence tomography and fluorescein angiography, if necessary. If grading is not certain or not possible due to poor quality of images, a recommendation to repeat either the screening examination or the ophthalmologic evaluation is given (in 1.3% of cases). From 2005 to 2015, the overall prevalence of DR in the city of Padova was 27.6% consisting in 12.5% mild NPDR, 11.3% moderate NPDR, 2.9% severe NPDR, and 0.9% proliferative DR (PDR) *(manuscript submitted)*. The overall prevalence of maculopathy was 5.7% consisting in 2.8% mild, 2.2% moderate, and 0.7% severe maculopathy. The 10-year incidence of sight-threating DR (STDR) was 0.6% in patients with no DR, 5.5% in patients with mild NPDR, and 21.1% in patients with moderate NPDR at the first examination. The 10-year incidence of maculopathy was 2.1% mild, 1.7% moderate, and 0.2% severe maculopathy in patients with no maculopathy at the first examination. When evaluating type and duration of DM together, patients were divided into low risk, medium risk, and high risk as follows: T2DM and duration lower than 10 years—low risk patients; T1DM and duration lower than 10 years—medium risk patients; and duration higher than 10 years and either T1DM or T2DM—high risk patients. The best sensitivity/specificity ratio (94.4%/32.4%) was found at 2.5 years for low risk patients with no DR at first examination. Therefore, the authors concluded that screening for DR can be safely repeated in a two-and-a-half-year period in those patients with diabetes who were deemed to be low risk diabetics. However, in case of presence of risk factors, a more frequent follow-up regime is warranted *(manuscript submitted)*.

Another pilot screening program was recently performed (2012) in Ponzano, a part of the Local Health Authorities of Veneto Region, Treviso (northeast Italy), with participation of a multidisciplinary group including general practitioners, diabetes experts, administrative staff, nurses, epidemiologists and ophthalmologists, and the reading centre [39]. This project aimed to assess the feasibility of a future larger application, in comparison with the "no prevention" strategy. Screening for DR was based on 3 nonmydriatic, 45° field, color fundus photos, obtained according to a previously validated technique [32]. All photographs were obtained by trained paramedical staff. All images were electronically transmitted to the reading centre and stored in an on-line secured database for the second step examination by certified and expert graders from the Reading Centre, University of Padova. Retinal images were graded for DR and DME according to the International Classification proposed by the American Academy of Ophthalmology [38]. When the quality of the images was "inadequate" for the clinical evaluation and when fundus photographs were graded as "positive," these patients were referred for further ophthalmologic examination. "Positive" findings included retinal changes that required ophthalmologic management: moderate and severe NPDR, proliferative DR, DME, or any other retinal abnormality. A report with the results of the screening and the correct follow-up timetable for the "negative" screened population was sent to the patient's general practitioner within 1 month after the screening. The authors reported that a total of 498 patients with diabetes were identified among the larger sample and were invited for screening, with an attendance rate of approximately 80%. Of these, 115 patients (33.82%) were referred to an ophthalmologist, including patients with ungradable images and cases with any abnormal retinal findings, other than DR. Moreover, 9 cases required prompt treatment for either PDR or DME. Significant importance of screening program, also from an economic point of view, was found, leading to a substantial saving in comparison with the "no prevention" strategy, including the costs that avoid blindness, in terms of the validity of the intervention and the direct costs absorbed (efficiency) by the Regional Healthcare Service (Veneto Region) [39].

In Milano, a recent observational study reported a 1-year (2012-2013), single-center, remote evaluation of semiautomatic fundus photography for DR screening performed at the Endocrinology Unit, during routine systemic visits for patients with T2DM [40]. A total of 1281 adults with T2DM underwent fundus photos consisting in three 30-degree fields color fundus photos (central, nasal, and temporal) obtained before and after pupil dilation and thereafter assessed by 2 expert ophthalmologists who were blinded to the results of the slit-lamp fundus examination. After pupil dilation 240 patients (18.74%) had ungradable images; approximately two thirds of patients (823 patients) did not have DR, and 218 (17.01%) were diagnosed with DR. Consequently, a total of

458 (35.75%) patients (240 ungradable and 218 with DR) were referred to the ophthalmologist. The authors evaluated also the economic impact of the telematic screening and reported a significant cost saving compared to slit-lamp fundus examination (the evaluated costs included reading centre staff evaluating images, fundus camera, and the cost of the standard funduscopic examination) [40].

Although initially annual screening for DR was recommended by many professional societies and was practiced in many countries, currently there is an increasing evidence of cost-effectiveness studies that suggest that screening could be safely extended beyond one year in patients with T2DM at low risk of progression to DR (considered to be patients with well controlled DM on dietary treatment, with low HbA1c and no DR), without increased risk of vision loss [41]. Biennial screening showed long safety record in Iceland and Sweden [42, 43]. Moreover, adopting biennial screening approach, a reduction in approximately 25% of screening costs can be obtained without increased risk to the patient [44]. Two recent studies reported cost-effectiveness of adopting risk-stratified approaches to extended screening intervals in the national DR screening programs in England and Scotland [45, 46]. Scanlon et al. conducted a modelling study and reported that for patients without DR on two consecutive screening examinations the adoption of 2-year screening intervals would save on average 225000 pounds per QUALY (quality-adjusted life years) lost compared with annual screening in England [45]. Scotland et al. reported similar results for patients with T2DM and lower increment in cost-effectiveness ratios for patients with T1DM (85000 pounds) per QUALY gained [46].

4. Conclusions

DR is a relevant and significant complication of DM and affects a large number of patients, with significant costs for the Health System. Prevention of DR through reducing risk factors and screening (early diagnosis) results in preventing visual impairment. Telematic screening for DR has been implemented with success in several local health entities in Italy, demonstrating good interdisciplinary collaboration and patient satisfaction. Moreover, with recent reports [45–49] on possibility to effectively increase screening intervals in patients with no DR and at low risk for developing sight-threating DR, the screening program becomes even more cost-effective procedure with appropriate use of resources and safe care delivered for patients. The preliminary data already present in the literature together with already available local experience in DR screening may become a basis for developing a national screening program.

Disclosure

The authors alone are responsible for the content and writing of the paper.

Competing Interests

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

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Research Article

Danish Nationwide Data Reveal a Link between Diabetes Mellitus, Diabetic Retinopathy, and Glaucoma

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Aims. To determine the association between treatment against diabetes mellitus (DM) and treatment with antiglaucomatous drugs in the entire Danish population and to investigate the comorbidity between DM and its complications with antiglaucomatous treatment. *Methods*. Retrospective nationwide cohort study with data over a 16-year follow-up period. The National Danish Registry of Medicinal Products Statistics was used to identify all claimed prescriptions for antiglaucomatous medication and DM drugs. ICD-10 classifications were furthermore used to identify comorbidities between antiglaucomatous medication and the DM complications, diabetic retinopathy (DR), and nephropathy. *Results*. A total of 6,343,747 individuals in the period between 1996 and 2012 were analyzed. The overall incidence rate of new-onset glaucoma patients was 0.07 per 1000 person-years for the reference population compared to 36 per 1000 person-years for all diagnosed DM cases. Patients treated with DM drugs had about two times higher relative risk of glaucoma, when adjusting for a range of factors. The presence of DR alone or in combination with nephropathy increased the risk of glaucoma. *Conclusions*. The present study reports a strong association between DM and onset of glaucoma treatment in the entire Danish population.

1. Introduction

Diabetic retinopathy (DR) is the most common late complication of diabetes mellitus (DM) in the working-age population and one of the leading causes of blindness in the elderly, accounting for a significant drop in quality of life (QoL) and working ability for the patients [1–3]. Nonproliferative DR presents clinically as superficial retinal hemorrhages, cotton wool spots, or microvascular abnormalities [4–6]. Even so, proliferative diabetic retinopathy (PDR) can remain asymptomatic for a very long time [6], and in that light, patients with DM in Denmark are therefore monitored closely by ophthalmologists annually.

The lack of oxygen in the retina causes fragile blood vessels to grow into the vitreous body and along the retina, causing an eminent risk of bleeding and formation of fibrovascular/proliferative membranes, leading consequently to tractional retinal detachment. These new blood vessels can furthermore grow into the angle of the anterior chamber and cause neovascular glaucoma [5].

To date, numerous screening studies have addressed the question of whether DM is a risk factor for primary open angle glaucoma (POAG); however, no converging or uniform conclusions exist to date. Some studies state that POAG is more prevalent in diabetic than in nondiabetic populations [7–12], while others found no statistically increased correlation between the two, although being based on small population sizes and yet raising the main reasonable question concerning patient referral and bias associated with it [9, 13–15]. A recent cohort study from the Tayside region of Scotland included a population of 175,211 participants over a 2-year period and found a relative risk of 1.57 for POAG and 1.38 for

elevated eye pressure compared to the rest of the population, although the results were not statistically significant [16].

The aim of the present study was to use data from the entire Danish population using redeemed prescription on antidiabetic and antiglaucoma drugs over a 16-year period and check whether DM is a risk factor for developing glaucoma after adjusting for diabetic complications and several other demographic factors. Furthermore, we wanted to investigate whether any difference exists in glaucoma with regard to medication type, diabetic complications, or concomitant medications such as antihypertensive drugs in patients having DM.

To the best of our knowledge, only one previous cohort study on the topic has been carried out to date, and no study has addressed the association between the medication used within the group of DM patients and patients treated with antiglaucomatous medication. Moreover, the large dataset, amounting to a 16-year follow-up of more than 6 million individuals, constitutes a comprehensive source of data for investigating comorbidities between DM and glaucoma.

2. Methods

2.1. Registers and Study Population. The study population comprised all individuals living in Denmark in the period between 1996 and 2012 and without previously diagnosed DM or glaucoma, amounting to 6,343,747 individuals. Data from the National Danish Civil Registration System contain information on vital status of all individuals born in, or migrating to, Denmark [17, 18]. In a subpart of the analysis, the sample was restricted to individuals only prescribed antidiabetic medication(s) and, within that group, patients aged 40 to 100 years, thus focusing on age-dependent rather than congenital disorders. The healthcare system in Denmark is fully tax financed and equally available to all inhabitants independent of social and financial status, and the Danish government is responsible for collecting this high-quality, nationwide healthcare data.

The information contained in the database contains dates of redemption of antidiabetic or antiglaucomatous medication (if any) for each individual and furthermore data on patients diagnosed with diabetic complications such as DR and/or nephropathy. The diabetic complications were identified based on the diagnostic codes, according to the International Statistical Classification of Diseases and Related Health Problems (ICD-10), and were retrieved from the register accordingly. On the other hand, the incident glaucoma and DM were based upon the pharmacotherapy used, and glaucoma and DM information was retrieved from the Danish Register of Medicinal Product Statistics, which holds data on all prescriptions dispensed in Denmark that are classified according to the Anatomical Therapeutic Classification system; this register is directly linked to the government for reimbursement purposes and is therefore very accurate. All pharmacies in Denmark are required by the government-financed Danish healthcare system to register all redeemed prescriptions at the individual level by the Danish Personal Identification number (the so-called CPR-number). We hereby identify individuals taking antidiabetic therapy through the National Danish Registry of Medicinal Products Statistics. Drugs administered during a hospital admission are, however, not included. Our data contain the dates of all redeemed antidiabetic treatments as well as all antiglaucomatous treatments, excluding those from before 1995, when mixed data on new and old prescriptions was combined in the register. To assure that the data only contain new prescriptions, all prescriptions registered in the database after January 1, 1996, were therefore included in the study.

2.2. Definition of Pharmacotherapy and Comorbidity. *DR* was identified using ICD-10 and diagnosed as DH36, DH368, DH360H, DH360J, DH360K, DH368D, DH368D1, and DH368D2.

Diabetic nephropathy was identified using ICD-10 and diagnosed as DE103, DE112, DE132, DE142, DN083, DN083, DN251, and DN.

Glaucoma, for the present study, was identified according to the following ACT codes for glaucoma medication: use of β-blockers (S01ED01-05); prostaglandin analogues (S01EE); α2-adrenergic agonists (S01EA); parasympathomimetic drugs (S01EB); carbon anhydrase inhibitors (S01EC); fixed combination drugs (S01EA51, S01EB51, and S01ED51). Patients were defined as having glaucoma if they received at least 2 prescriptions within 90 days for at least one type of antiglaucomatous medication.

Hypertension as comorbidity was used in a subset of the analysis. Hypertension is most often managed by patients' primary physicians, and so the in-hospital ICD-10 diagnosis for essential hypertension (ICD-10I10) is used irregularly. Therefore, identification of hypertensive patients in the studied population was validated by an algorithm (with a positive predictive value of 80.0% and sensitivity of 94.7%), based upon the use of at least 2 classes of antihypertensive drugs (β-blockers (BB), renin-angiotensin system (RAS) inhibitors, calcium antagonists (Ccb), or diuretics (DD) and vasoprotectives (VP)) [19]. Patients treated with all drugs types were excluded from the study due to lack of relevant controls.

The ATC codes for antihypertensive drugs used in the analyses are listed here by classes of origin: BB are in the group of C07; RAS inhibitors are in the group of C09; DD are in the group of C03; Ccb are in the group of C08D, and vasoprotectives are in the group of C02. To investigate the effect of pharmacotherapy, the focus was placed on RAS and BB, compared to the rest.

Patients were classified as incident with glaucoma (having their onset of glaucoma) by their first redemption of an antiglaucoma medication and incident with hypertension (having their onset of hypertension) by their first redemption of a second antihypertensive drug.

The selection process for the study population is illustrated in Figure 1.

2.3. Rationale for Definitions. DM and glaucoma are most often managed and diagnosed by patients' primary physicians and an out-of-hospital specialist in ophthalmology, respectively. Therefore, the in-hospital ICD-10 diagnoses for the two diseases are not relevant. Instead, we identify the population of glaucoma patients and those with DM and hypertension

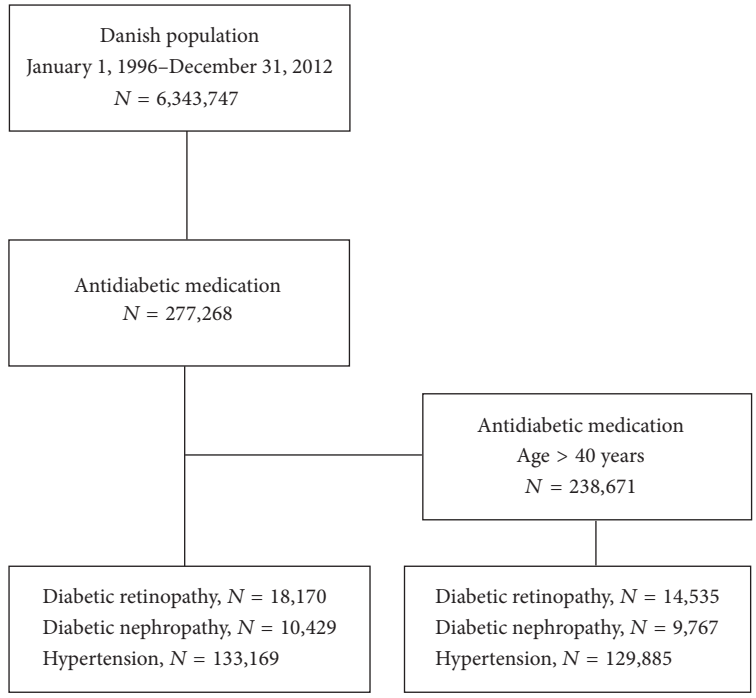

Figure 1: Flowchart of diabetes mellitus and glaucoma in the Danish population in the period from 1996 to 2012. The National Danish Registry of Medicinal Products Statistics was used to identify all individuals who were treated with glaucoma medication and/or antidiabetic drugs.

using the National Danish Registry of Medicinal Products Statistics as descripted above. With this approach, we are able to identify patients with a redeemed prescription for DM and/or glaucoma. Furthermore, this procedure allows us to identify the onset of the condition.

In patients who redeemed antidiabetic medication, comorbidity with hypertension was then identified using a validated algorithm based on the use of at least two classes of antihypertensive drugs. This algorithm is shown to have a tremendous sensitivity of 94.7% and a positive predictive value of 80.0% [19], meaning that our validation algorithm precisely identifies individuals with hypertension.

The Danish Civil Registration System contains information about dates of birth and death of all Danish citizens since 1972 [18], while the Danish Registry of Medicinal Products Statistics contains data on all prescriptions dispensed in Denmark since 1995, including information about size of doses, quantity dispensed, and dispensing date. Prescriptions are classified according to the Anatomical Therapeutic Chemical (ATC) System [17].

2.4. Statistical Analysis. To describe the evolution of the incidence of DM, DR, and glaucoma, the incidence rates were calculated in 5-year age strata, as a function of time, and then summarized as events per 1,000 person-years at risk. Furthermore, duration analysis models, based on the Poisson distribution, were employed to investigate the associations between DM treatments and DR, as well as the risk of developing glaucoma, adjusted for a range of potentially confounding factors.

The baseline characteristics are presented as means with standard deviations or frequencies and percentages accordingly. DM was considered to be a time-dependent variable and thus subjects who developed DM contributed risk time in the reference group until the time of diagnosis. Comorbidity was updated continuously throughout the follow-ups. Hazard ratios (HRs) for the study endpoint were estimated using Cox proportional hazards models adjusted for confounding factors including age, sex, comorbidity with DR, hypertension (concomitant medications with antihypertensive drug(s)), and diabetic nephropathy.

The primary analysis was not adjusted for medications used for the treatment of hypertension. An additional analysis was carried out with inclusion of antihypertensives when investigating all patients redeemed with antidiabetic medication to estimate its impact on the HRs of glaucoma. All statistical analyses were performed using SAS 9.4. Heteroscedasticity-robust standard errors were used in the duration model and cluster-robust standard errors, clustered on the individual level, were used in the regression discontinuity models. A significance level of 0.05 was applied, meaning that estimated coefficients with $p < 0.05$ were considered statistically significant and 95% confidence intervals were also reported.

2.5. Definitions. The *incidence* in a given year is defined as the number of new cases in that year divided by the number of individuals living in that year. The *incidence rate* is the number of new cases over the 16-year study period per population at risk (measured in 100 person-years). The *relative risk*

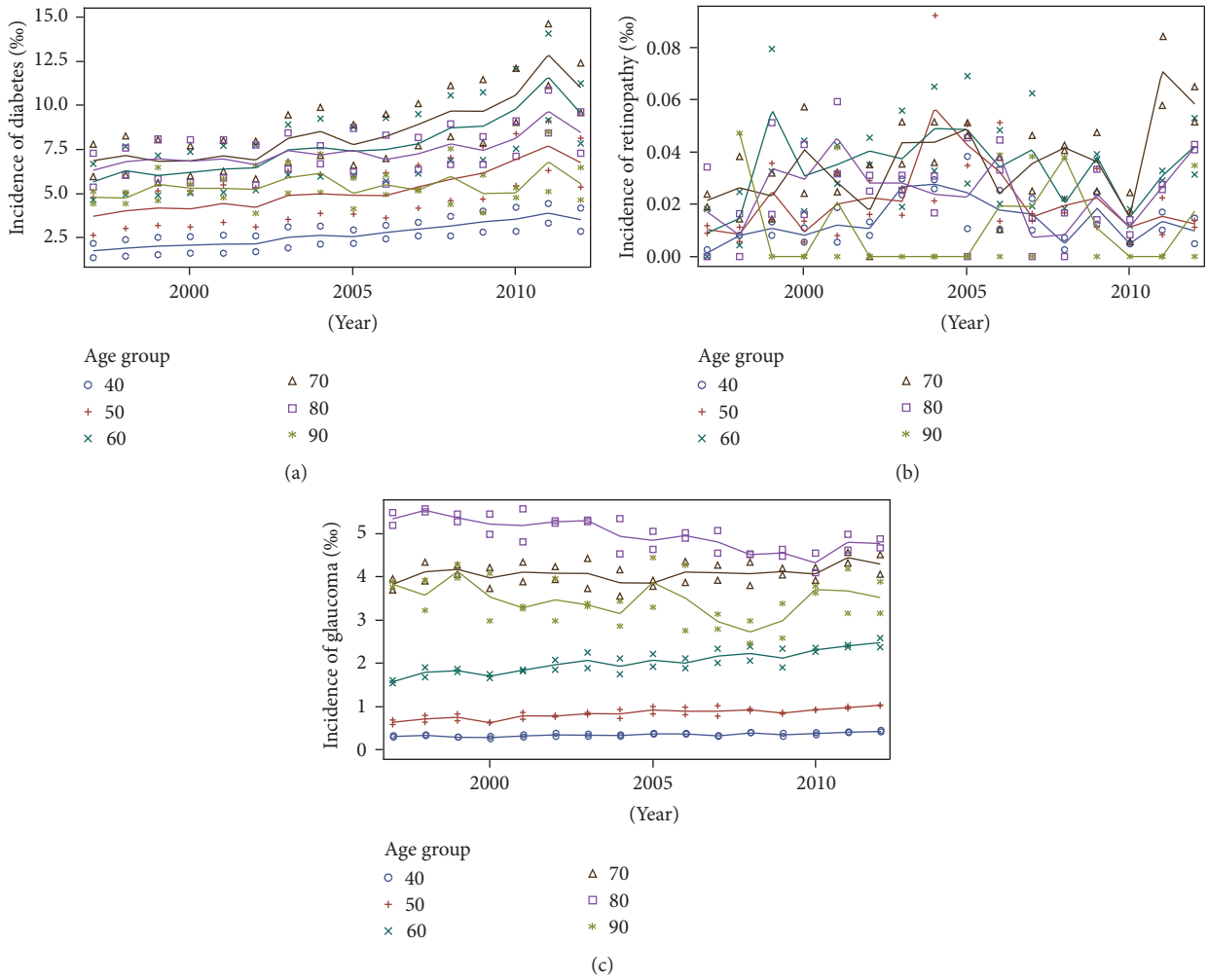

FIGURE 2: Incidence of diabetes mellitus, diabetic retinopathy, and glaucoma in the Danish population, in the period from 1996 to 2012, per 1000 individuals (‰). (a) Diabetes mellitus incidence. (b) Diabetic retinopathy incidence. (c) Glaucoma incidence.

(RR) of developing glaucoma is the probability of developing glaucoma for a certain group (e.g., males or individuals with DM) divided by the probability of developing glaucoma for the converse group. The estimates of the duration analysis are converted to RR estimates by calculating their antilogarithm.

2.6. Outcome. The primary outcome in the present study was glaucoma (as inferred by antiglaucomatous drug prescriptions used).

2.7. Ethical Aspects. The Danish Data Protection Agency approved the study (2007-58-0015, int. ref: GEH-2010-001). Retrospective register-based studies do not require ethical approval in Denmark.

3. Results

3.1. Baseline Characteristics of the Studied Population. The study comprised a total of 6,343,747 subjects within a sixteen-year follow-up. During the study period, 275,078 subjects with incident DM, 75,022 subjects with incident glaucoma, and 18,170 subjects with DR were identified, as shown in the flowchart of the study population selection (Figure 1). The average age at onset for DM was 59.19 years (range: 1.42 to 109.57 years), for DR 56.87 years (range: 4.99 to 98.74 years), and for glaucoma 69.31 years (range: 2.01 to 105.07 years). Median follow-up time was 15.66 (SD 3.08) years and 15.86 (SD 3.33) years for the reference population and DM, respectively. The mean duration from diagnosis of DM to incidence of glaucoma was 4.1 (SD 3.51) years.

3.2. Incidence of DM, DR, and Glaucoma. The incidence of DM, DR, and glaucoma in the Danish population over the period from 1996 to 2012 is depicted in Figure 2. A constant number of new glaucoma cases per year were identified in the total period, whereas the amount of new DM cases per year appeared to increase in the same period.

3.3. Incidence Rates for DM and Glaucoma. The results showed an association between DM and the increased risk of

Groups	Subgroups	N	Events	HR	95% LCL	95% UCL	p value
Gender	Female	103,129	2.382 (2.3%)	0.99	0.94	1.05	ns
	Male	135,542	2.950 (2.2%)	1.00	.	.	
HT	Yes	129,886	3.421 (2.6%)	1.06	1.00	1.12	ns
	No	108,785	1.911 (1.8%)	1.00	.	.	
DR	Yes	14,535	684 (4.7%)	1.40	1.24	1.55	***
	No	224,136	4.648 (2.1%)	1.00	.	.	
DM drug	Sulph.	78,574	2.697 (3.4%)	1.34	1.04	1.65	ns
	Megli.	2,349	76 (3.2%)	1.31	0.93	1.69	ns
	Ins. S + R	4,477	115 (2.6%)	1.49	1.14	1.84	*
	Ins., S	649	7 (1.1%)	1.74	0.93	2.54	ns
	Ins., I	6,558	116 (1.8%)	1.00	0.65	1.36	ns
	Glita.	84	5 (6.0%)	3.73	2.80	4.65	**
	GLP, DDP-inh.	459	4 (0.9%)	0.95	−0.07	1.98	ns
	Comp	646	12 (1.9%)	1.91	1.26	2.55	*
	Bigua.	141,068	2.242 (1.6%)	1.18	0.87	1.49	ns
	AGI	418	16 (3.8%)	1.24	0.66	1.81	ns
	Ins., R	3,389	42 (1.2%)	1.00	.	.	
DR × HT	Yes	9,164	498 (5.4%)	1.10	0.92	1.28	ns
	No	229,507	4.834 (2.1%)	1.00	.	.	
DR × DN	Yes	1,694	130 (7.7%)	1.40	1.17	1.63	**
	No	236,977	5.202 (2.2%)	1.00	.	.	
DN	Yes	9,767	396 (4.1%)	0.98	0.71	1.25	ns
	No	228,904	4.936 (2.2%)	1.00	.	.	
DN × HT	Yes	7,899	339 (4.3%)	1.05	0.76	1.34	ns
	No	230,772	4.993 (2.2%)	1.00	.	.	
DM age	80<	23,731	474 (2.0%)	3.03	2.89	3.17	***
	70–79	49,354	1.418 (2.9%)	3.16	3.05	3.27	***
	60–69	71,210	1.811 (2.5%)	2.46	2.35	2.57	***
	50–59	60,588	1.213 (2.0%)	1.62	1.51	1.73	***
	40–49	33,788	416 (1.2%)	1.00	.	.	
Others	Total population	238,671		1.00	.	.	

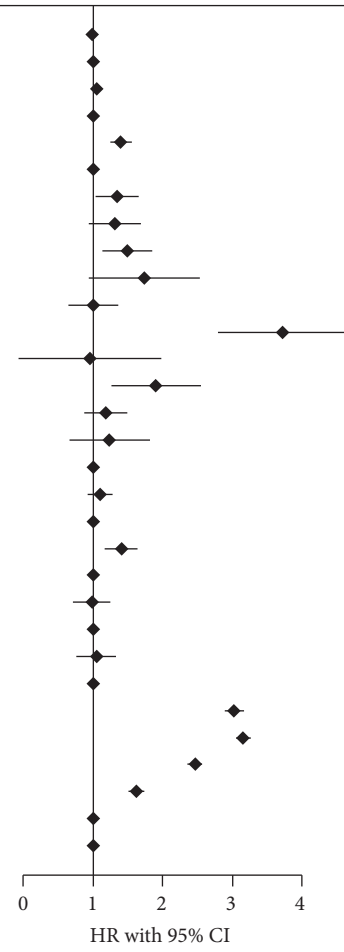

Figure 3: Hazard ratios for glaucoma development in patients treated with antidiabetic drugs. A range of confounding factors, comorbidity, concomitant medications factors, age, and gender are being adjusted for. The underlying data represents patients ≥ 40 years of age. For data on the total diabetic population, see Table 2. HR: hazard ratio; N: number of individuals; events: number of patients with glaucoma; CL: confidence limit. *Significant on the 5% level. **Significant on the 1% level. ***Significant at the 0.1% level.

new-onset glaucoma (Table 2). The overall incidence rates per 100 person-years were 0.070 (95% CI 0.069–0.071) and 0.36 (95% CI 0.35–0.37) for the reference population and patients with DM, respectively. However, a common association with age or other confounding factors may be the cause of such an association. In particular, the risks of developing either condition increase with age (Figure 3), which can potentially explain this correlation. Therefore, we account for potentially confounding factors in a duration model, presented in the next subsection.

3.4. Duration Analysis. To exclude that increased incidence of glaucoma among patients treated with antidiabetic medication is simply caused by a common association with age or other potentially confounding factors, a duration model was implemented.

Table 1 shows a series of duration models accounting for various sets of potential covariates, namely, sex, age, and calendar year fixed effects. The duration models estimate the RR for developing glaucoma in patients treated with antidiabetic drugs in the Danish population in the period from 1996 to 2012. Column 1 presents the unconditional association between DM and glaucoma. It establishes that patients treated with antidiabetic drugs have a significantly higher risk of glaucoma compared to people who never redeemed prescriptions of antidiabetic drugs (RR = 5.13, $p < 0.0001$). Column 2 establishes that patients having DR have a significantly higher risk of glaucoma compared to people who do not have DR (RR = 4.69, $p < 0.0001$). Column 3 indeed shows that use of antidiabetic drugs (RR = 5.11, $p < 0.0001$) and DR (RR = 1.93, $p < 0.0001$) have an increased risk for glaucoma. The model further accounts in column 4 for the gender of the individuals and establishes that the RR of glaucoma in the DM and DR patients is still above unity (RR = 5.16, $p < 0.0001$ and RR = 1.54, $p < 0.0001$, resp.), with a significantly higher risk of glaucoma in

Table 1: Duration analysis. The table shows the relative risk for developing glaucoma in patients treated with antidiabetic drugs in the Danish population (1996–2012). The model controls for complication with DR and age as well as calendar year fixed effects (omitted from the table). RR: relative risk; CI: confidence interval; anti-DM drug: antidiabetic drug.

Model	1 RR (95% CI)	2 RR (95% CI)	3 RR (95% CI)	4 RR (95% CI)	5 RR (95% CI)	6 RR (95% CI)
Anti-DM drug *(Reference: no anti-DM drugs)*	**5.13*** (4.99–5.28)		**5.11*** (4.97–5.25)	**5.16*** (5.02–5.31)	**1.81*** (1.76–1.86)	**2.05*** (1.57–2.68)
DR *(Reference: no DR)*		**4.69*** (3.67–6.00)	**1.93*** (1.51–2.46)	**1.97*** (1.54–2.52)	**1.86*** (1.46–2.38)	**1.82*** (1.42–2.33)
Gender *(Reference: males)*				**1.35*** (1.33–1.37)	**1.17*** (1.15–1.18)	**1.16*** (1.15–1.18)
Age	No	No	No	No	Yes	Yes
Calendar year	No	No	No	No	No	Yes

* $p < 0.001$.

women (RR = 1.35, $p < 0.0001$). Column 5 establishes that treatment with antidiabetic drugs is still associated with an increased risk of glaucoma while accounting for age (as five-year age group fixed effects) in addition to sex (RR = 1.81, $p < 0.0001$) and diagnosed DR (RR = 1.86, $p < 0.0001$). Finally, column 6 establishes that antidiabetic drugs are still significantly associated with glaucoma while accounting for calendar year fixed effects in addition to the other control variables (RR = 2.05, $p < 0.0001$).

3.5. Hazard Ratios of New-Onset Glaucoma in Patients with DM. Using multivariate Cox regression model analyses, all individuals treated with antidiabetic drugs were investigated for the HR and adjusted for age, sex, comorbidity, concomitant medications, all being factors which can potentially affect the risk of glaucoma.

In Table 2, the HR for a range of factors is shown for all individuals, as well as for patients ≥ 40 years of age. The latter regression results are illustrated in Figure 3. Overall, an increased HR for glaucoma is found in patients having DR, concomitant DR, and diabetic nephropathy and hypertension. Furthermore, we confirm the above reported substantial age-dependence, whereas the observed gender difference is not statistically significant in this specification. The results also show that patients treated with sulfonylureas, glitazone, and slow-acting insulin in combination with rapid-acting insulin have a significantly higher HR for developing glaucoma compared to patients treated with rapid-acting insulin analogues (Table 2). Following up on the reported increased HR in patients having concomitant antihypertensive medication, the differences in the type of antihypertensive medication in patients with DM were investigated (Table 2, columns 2 and 4). Indeed, the type of antihypertensive medication used has a significant effect, while a combination of BB with RAS analogues seems to have a proactive effect. Furthermore, RAS and other antihypertensive drugs have a significant increased effect, while BBs in combination with one other antihypertensive medicament have a tendency to lower the risk of glaucoma, although not significantly from that of having no hypertension at all.

4. Discussion

To the best of our knowledge, the present study is one of the largest studies investigating the association between glaucoma and DM, using data for an entire population over a sixteen-year period. We find an overall increased risk of glaucoma among patients with DM. This association remains evident when controlling for age, gender, retinopathy, and year-specific fixed effects.

Furthermore, we find that, in patients with DM, comorbidity with hypertension as well as presence of DR and/or joint complications with DR and nephropathy increases the risk of glaucoma. However, treatment with the antihypertensive combination of β-blockers and renin-angiotensin system inhibitors appears to be associated with a significantly lower hazard ratio for glaucoma onset in DM patients.

Other large population-based studies have also demonstrated an association between glaucoma and DM [10–12, 20, 21], whereas the largest case-control study and cohort study failed to find any association, explaining this lack of association by a possible referral bias [9, 22].

Among studies which supported the association, the Wisconsin study [12] found an odds ratio (OR) of 1.84 (95% CI, 1.09–3.11), while the study from Australia [10] found an OR of 2.12 (95% CI, 1.18–3.79), and the study from Rotterdam [11] found an OR of 3.11 (95% CI, 1.12–8.66). An independent study from Baltimore found no significant correlation with an OR of 1.03 (95% CI, 0.85–1.25) [9]. A recent cohort study from Scotland further was lacking to find an association between DM and glaucoma, when adjusting for age [16].

A number of studies have pointed to the possibility that DM may affect the vascular autoregulation of retina and the optic nerve and thereby promote the risk of DR and glaucoma [20, 23, 24]. The present study reports a significant correlation between DM and glaucoma, showing a higher risk of glaucoma in patients treated with antidiabetic medication. Adjusting for age and gender, the correlation is still significant. In addition, adjusting for all year-specific fixed factors, such as changes in public health policies or medical innovations that may affect the treatment of both diseases, does not change much the correlation between the two conditions.

Table 2: Multivariable Cox regression model analyses showing the hazard ratios for glaucoma by antidiabetic drugs used in patients with DM. The effect of complications such as diabetic retinopathy and nephropathy was investigated, as well as the concomitant medications used, such as antihypertensive drugs. RAS: renin-angiotensin system; PE: parameter estimate; SE: standard error; HR: hazard ratio.

Model	Age ≥ 40 years				All ages			
	1		2		3		4	
	PE (SE)	HR	PE (SE)	HR	PE (SE)	HR	PE (SE)	HR
DR *(Reference: no DR)*	**0.33***** (0.08)	**1.4**	**0.45***** (0.04)	**1.57**	**0.34***** (0.07)	**1.4**	**0.44***** (0.04)	**1.55**
Diabetic nephropathy	−0.02 (0.14)	0.98	**0.11*** (0.05)	**1.12**	0.03 (0.13)	1.03	**0.13**** (0.05)	**1.14**
Hypertension *(Reference: no antihypertensives)*	0.06 (0.03)	1.06			**0.11**** (0.03)	**1.12**		
Gender *(Reference: males)*	−0.01 (0.03)	0.99	−0.01 (0.03)	0.99	0 (0.03)	1	0 (0.03)	1
Nephropathy × DR	0.34** (0.12)	**1.4**			**0.34**** (0.11)	**1.41**		
Nephropathy × hypertension	0.05 (0.15)	1.05			0.01 (0.14)	1.01		
DR × hypertension	0.1 (0.09)	1.1			0.08 (0.09)	1.08		
Biguanides	0.17 (0.16)	1.18	0.17 (0.16)	1.19	0.24 (0.14)	1.27	0.24 (0.14)	1.28
Combination	**0.64*** (0.33)	**1.91**	**0.66*** (0.33)	**1.93**	**0.71*** (0.32)	**2.04**	**0.73*** (0.32)	**2.07**
GLP-1-analogues and DDP-IV-inhibitors	−0.05 (0.52)	0.95	−0.05 (0.52)	0.96	0.26 (0.47)	1.3	0.27 (0.47)	1.31
Glitazones	**1.32**** (0.47)	**3.73**	**1.31**** (0.47)	**3.72**	**1.36**** (0.47)	**3.88**	**1.36**** (0.47)	**3.89**
Insulin and insulin analogues (slow)	0.55 (0.41)	1.74	0.57 (0.41)	1.77	0.47 (0.4)	1.59	0.48 (0.4)	1.62
Insulin and insulin analogues (slow combined with rapid)	**0.4*** (0.18)	**1.49**	**0.4*** (0.18)	**1.49**	**0.47**** (0.16)	**1.59**	**0.47**** (0.16)	**1.6**
Insulin and insulin analogues (intermediate)	0 (0.18)	1	0.01 (0.18)	1.01	0.12 (0.16)	1.13	0.13 (0.16)	1.14
Meglitinides	0.27 (0.19)	1.31	0.27 (0.19)	1.31	**0.35*** (0.18)	**1.43**	**0.36*** (0.18)	**1.43**
Sulphonylurea	0.29 (0.16)	1.34	0.3 (0.16)	1.35	**0.4**** (0.14)	**1.49**	**0.4**** (0.14)	**1.5**
α-Glucosidase inhibitors	0.21 (0.29)	1.24	0.21 (0.29)	1.24	0.33 (0.28)	1.4	0.34 (0.28)	1.4
RAS + β-blocker			**−0.14**** (0.06)	**0.87**			−0.09 (0.06)	0.92
RAS + antiadrenergic			**0.52**** (0.18)	**1.68**			**0.56**** (0.18)	**1.75**
RAS + Ccb			**0.19***** (0.05)	**1.21**			**0.23***** (0.05)	**1.25**
RAS + Diu			**0.16***** (0.04)	**1.18**			**0.21***** (0.04)	**1.24**
RAS + Vas			0.23 (0.13)	1.26			**0.24*** (0.13)	**1.28**
RAS β-blocker + 1 other			−0.03 (0.07)	0.97			0.01 (0.07)	1.01
RAS + (other ≥ 2)			0.11 (0.09)	1.12			**0.17*** (0.08)	**1.18**
β-Blocker + other antihyp. drugs ≥ 1			−0.03 (0.05)	0.97			0.03 (0.05)	1.03
Other antihyp. drugs ≥ 2			0.09 (0.05)	1.09			**0.15**** (0.05)	1.16

Table 2: Continued.

Model	Age ≥ 40 years				All ages			
	1		2		3		4	
	PE (SE)	HR	PE (SE)	HR	PE (SE)	HR	PE (SE)	HR
Age 50–59 years *(ref 40–49)*	**0.48***** (0.06)	**1.62**	**0.49***** (0.06)	**1.63**				
Age 60–69 years *(ref 40–49)*	**0.9***** (0.06)	**2.46**	**0.91***** (0.06)	**2.48**				
Age 70–79 years *(ref 40–49)*	**1.15***** (0.06)	**3.16**	**1.16***** (0.06)	**3.19**				
Age > 80 years *(ref 40–49)*	**1.11***** (0.07)	**3.03**	**1.12***** (0.07)	**3.06**				
Age 21–40 years *(ref < 21)*					**1.07**** (0.3)	**2.91**	**1.08**** (0.3)	**2.95**
Age 41–60 years *(ref < 21)*					**2.33***** (0.3)	**10.25**	**2.34***** (0.3)	**10.39**
Age 61–80 years *(ref < 21)*					**2.96***** (0.3)	**19.25**	**2.98***** (0.3)	**19.6**
Age > 80 years *(ref < 21)*					**3.04***** (0.3)	**20.8**	**3.06***** (0.3)	**21.23**
Number of individuals		238,671	238,671		277,266		277,266	

$^{*}p < 0.05$, $^{**}p < 0.01$, and $^{***}p < 0.001$.

Although our results indicate a strong association between use of DM drugs and the use of antiglaucomatous drugs, we cannot rule out the possibility that the observed association is affected by the fact that patients with DM may get eye diseases detected more often by routine clinical care compared to healthy individuals.

As an additional finding, the present study finds an increased risk of glaucoma among DM patients with DR and/or joint complications with DR and nephropathy. The mechanisms behind this association could simply be that these patients suffer from a more severe DM disease. Both DR and nephropathy are serious conditions that need intensive treatment. Furthermore, diabetic nephropathy is often treated with ACE inhibitor as well as lipid lowering treatment and aspirin.

A possible increased risk of glaucoma in patients with concomitant hypertension and DM was further investigated. Overall, we show that antihypertensive medication is associated with an increased risk of glaucoma in patients with DM. However, the combination of β-blocker and renin-angiotensin system inhibitors appears to lower the hazard ratio for glaucoma onset in DM patients. In general, inhibitors of the renin-angiotensin system (RAS) are a commonly used medicament in treatment of hypertension. In addition to regulate blood pressure, RAS is an active local system in the eye [25–28] (1–7) and ACE is found to be significantly higher in glaucomatous eyes [25]. The literature highlights the importance of particularly angiotensin II in the etiology of eye diseases. Some studies have shown that ACE inhibitor reduces the IOP and have a protective effect against glaucoma [29–31], but it has also been shown that the peptide angiotensin II is a modulator or transmitter in retinal neurophysiology. Thereby, an inhibition of ACE results in a decrease in angiotensin II that might cause disturbance of retinal neuronal function [28, 32]. Furthermore, a counterbalancing interaction between ACE II products and ACE-I has been suggested to be important [27, 33]. Our study indicates that inhibition of the RAS either increases the risk of glaucoma or reflects a more severe form of DM. However, we find that patients receiving a combination of RAS and BB have a significantly lower risk of glaucoma compared to DM patients without comorbidity with hypertension. One explanation for the decreased risk in patients treated with BB could be the intraocular pressure-lowering effect of this drug [34]. In our study, we reveal that RAS is generally positively associated with glaucoma, except in the combination with BB. We believe that this difference in associations can be due to either a preventive effect of BB or a synergistic effect of RAS and BB. Further studies are needed to disentangle these possible mechanisms. If this observed association is proven, it may suggest that RAS treated patients with DM would need further attention for diagnosis or treatment of glaucoma.

The main strength of our study is the use of comprehensive data resources covering a large population base, namely, the entire Danish population followed over 16 years. In particular, the National Danish Registry records 100% of all dispensed prescriptions in all pharmacies in Denmark, and, furthermore, all births, deaths, emigrations, and immigrations in Denmark. However, a limitation of the study is that we use prescriptions as the indicator of glaucoma and DM. In this matter, we are not able to conclude anything concerning the etiology or severity of the conditions.

In conclusion, this study reports an increased risk of glaucoma among patients treated with antidiabetic drugs. Furthermore, comorbidity with DR and the joint comorbidity with DR and/or diabetic nephropathy increase the hazard of getting glaucoma. Concomitant medications such as antihypertensive drugs can also increase the hazard for developing

glaucoma. However, particular treatment with BB decreases the risk of glaucoma, while combination of BB and RAS conversely increases the risk.

Disclosure

Anna Horwitz had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

Competing Interests

No conflicting relationship exists for any author.

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Review Article

Diabetic Macular Edema Pathophysiology: Vasogenic versus Inflammatory

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Diabetic macular edema (DME) can cause blindness in diabetic patients suffering from diabetic retinopathy (DR). DM parameters controls (glycemia, arterial tension, and lipids) are the gold standard for preventing DR and DME. Although the vascular endothelial growth factor (VEGF) is known to play a role in the development of DME, the pathological processes leading to the onset of this disease are highly complex and the exact sequence in which they occur is still not completely understood. Angiogenesis and inflammation have been shown to be involved in the pathogenesis of this disease. However, it still remains to be clarified whether angiogenesis following VEGF overexpression is a cause or a consequence of inflammation. This paper provides a review of the data currently available, focusing on VEGF, angiogenesis, and inflammation. Our analysis suggests that angiogenesis and inflammation act interdependently during the development of DME. Knowledge of DME etiology seems to be important in treatments with anti-VEGF or anti-inflammatory drugs. Current diagnostic techniques do not permit us to differentiate between both etiologies. In the future, diagnosing the physiopathology of each patient with DME will help us to select the most effective drug.

1. Introduction

Diabetes mellitus (DM* all acronyms are given in a summary list at the end of the text) is a worldwide pandemic disease. As of 2010, more than 200 million people had been diagnosed with diabetes, and this number is predicted to increase by 62% by 2025 [1]. This increase is due to an increase in obesity together with the increased life expectancy of the world population. DM complications include macroangiopathy (myocardial infarction or vasculocerebral stroke) and microangiopathy (diabetic nephropathy, neuropathy, and retinopathy).

Diabetic retinopathy (DR) is the most common cause of blindness in Europe [2], affecting 1.9% of patients with DM [3]. Furthermore, 2.64% of diabetic patients have visual sight-threatening diabetic retinopathy (STDR). The major cause of visual impairment in DM patients is diabetic macular edema (DME), with an annual incidence of 2.19%. DME is a consequence of DR in the macular area and is secondary to retinal barrier rupture, which is in turn secondary to a range of metabolic changes brought about by hyperglycemia [4]. The most important molecule in retinal barrier rupture is the vascular endothelial growth factor (VEGF). The introduction of anti-VEGF and steroid drugs for treating DME has changed our knowledge of pathophysiology [5]. But the uses of anti-VEGF drugs have revealed that around 30% of patients are resistant to intravitreal treatment [6].

The fact that so many patients are proving to be resistant to treatment would suggest that other physiological mechanisms must be involved. A low-grade inflammatory process has been shown to be a possible cause, which would explain the improvement in DME after steroid intravitreal injections have been administered. Angiogenesis and inflammation have been shown to be involved in the pathogenesis of this

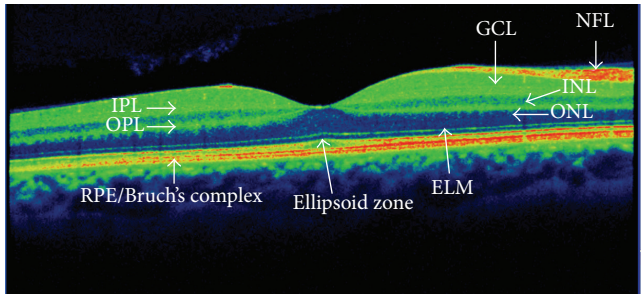


Figure 1: Optical coherence tomography of the macular area, description of different layers: RPE/Bruch's complex: retinal pigment epithelium and Bruch's layer; ELM: external limiting membrane; ellipsoid zone: inner segments/outer segments of photoreceptors (ellipsoid layer); ONL: outer nuclear layer (photoreceptors); OPL: outer plexiform layer; INL: inner nuclear layer (bipolar cell); IPL: inner plexiform layer; GCL: ganglion cell layer; NFL: nerve fiber layer.

disease, but it still needs to be clarified whether angiogenesis following an overexpression of VEGF is a cause or a consequence of inflammation. This review aims to update our knowledge on the etiology of DME, which will help us to understand the differences between the vasogenic and inflammatory etiology of DME.

In a series of questions and answers, we describe the current knowledge about DME and its etiology.

2. What Is the Tissue Target of Diabetic Macular Edema?

The retinal macular zone is the target of diabetic macular edema (DME). In order to understand the anatomic changes in DME. We describe the retinal anatomy with reference to optical coherence tomography (OCT), a technique used currently in the diagnosis and follow-up of DME, which allows us to see the multiple layers of the retina and choroid. We describe these different layers according to the classification laid down by the international panel of experts in vitreoretinal diseases [7] (Figure 1).

2.1. Retinal Anatomy. The retina is a complex structure of neural tissue made up of different cell types. The retina is divided into the *neurosensory retina (NR)* and the *retinal pigment epithelium (RPE)*. The NR includes all layers from the photoreceptors to ganglion cells, and the RPE is a monolayer formed by a single cell type located in the outermost part of the retina (Figure 1).

2.1.1. Nonsensory Layer of the Retina

(i) The *retinal pigment epithelium (RPE)* is a monolayer of cells characterised by a large presence of melanin pigment in the cytoplasm. This allows it to absorb light, which then reaches the retina. RPE is a multifunctional layer. The apical cell of the RPE is closely linked to the photoreceptors, which form a true functional unit. In the other side, the RPE forms a complex with Bruch's membrane seen at optical coherence tomography (OCT) denominated as RPE/Bruch's complex.

2.1.2. Neurosensory Retinal Layers

(α) The *photoreceptor layer* is formed by rods and cones in its internal and outer segments (IS/OS) and includes two layers observable with OCT, both of which are important in visual acuity impairment:

(i) *Ellipsoid Zone*. This is located just above the RPE and is formed by the union of the IS/OS of the photoreceptors. In the OCT, the sagittal slice of the retina is shown as a hyperreflective line. In several functional studies of the macula, a link has been found between the integrity of this layer and resulting visual acuity.

(ii) *External Limiting Membrane Layer (ELM)*. This is seen as a discrete line, located just above the ellipsoid zone, which separates the photoreceptor nucleus from its internal segments. It comprises the apical processes of Müller cells and is similar to the ellipsoid zone; there is a link between the integrity of this layer and visual acuity.

(β) The *outer nuclear layer (ONL)* is formed by the cell nuclei of rods and cones. The cones are responsible for visual acuity and colour perception.

(χ) The *outer plexiform layer (OPL)* is established by the synapses of bipolar cells between photoreceptors. In addition, it includes horizontal interneuron cells that adjust vision in extreme environmental light conditions.

(δ) The *inner nuclear layer (INL)* contains the nuclei of bipolar cells. Bipolar cells are the first-neuron cell to process the electrical stimulus coming from the photoreceptors before transmitting it to the ganglion cells. These cells are responsible for the electrical response of the retina as objectified in multifocal electroretinography.

(ε) The *inner plexiform layer (IPL)* is composed of synapses between the bipolar, ganglion, and amacrine cells (responsible for adjusting the retinal image).

(ϕ) The *ganglion cell layer (GCL)* contains ganglion cells which are the second-neuron cell in visual via and comprises cells that transmit impulses from the photoreceptors through their long axons to the thalamus.

(γ) The *nerve fiber layer (NFL)* is formed by the ganglion cell axons.

The edema is intracellular at the beginning, with Müller cells swelling as the first affected cells. Its progression induces its apoptosis. Other cells such as bipolar cells, ganglion cells, and photoreceptors undergo presynaptic elongation and a

reduction of its prolongation until the edema is reversible if metabolic status ameliorates. After this phase, the liquid passes through the cell membrane and accumulates in the interstitial space, forming cysts. In DME, cyst formation appears in the inner layers with little cysts that progress to the external layers forming much larger cysts, which will become visible under retinal biomicroscopy and fluorescein angiography. At the external plexiform layer, the edema allows lipid deposition as hard exudate. The rupture of retinal pigment epithelium allows liquid accumulation under the neurosensory retina and its detachment, which is a form of edema described in the classification of optical coherence tomography.

3. What Is the Role of Retinal Barriers in Diabetic Macular Edema?

The eye, like the brain, has mechanisms that hinder the passage of certain substances or microorganisms, thereby reducing the risk of inflammation. This is achieved by different barriers, which include

(i) the retinal pigment epithelium (RPE), which acts as an external-blood retinal barrier (e-BRB);

(ii) the retina vascular plexuses, which acts as an inner-blood retinal barrier (i-BRB);

(iii) the endothelial cells of capillaries and pigmented epithelium of the iris, forming the anterior hematoaqueous barrier;

(iv) the nonpigmented epithelium of ciliary processes, that forms the posterior hematoaqueous barrier.

The last two barriers form anterior segment barrier influencing the composition of the aqueous humour, with special characteristics different from retina interstitial liquid. Since, in this review, we do not analyse the anterior segment barriers, a description of the epithelium of ciliary processes or the endothelial cells of capillaries of the iris and ciliary muscle has been excluded. Rather, our discussion focuses on the posterior segment barriers: the external-blood retinal barrier (e-BRB) and the inner-blood retinal barrier (i-BRB). The blood-retinal barrier keeps the retina isolated from intravascular molecules, and the RPE acts as an external barrier via its tight cell junctions.

3.1. External-Blood Retinal Barrier (e-BRB) and Retinal Pigment Epithelium (RPE). The RPE is the external retinal layer whose functions include the following:

(i) Regeneration of all-trans-retinol to 11-cis-retinal is a process essential for vision, which supplies vitamin A and glucose to the photoreceptors.

(ii) Phagocytosis of the external disc and of the old photoreceptors is degraded during the visual cycle.

(iii) Nutrition functions allow oxygen and nutrients to pass from the choriocapillaris to external segments of the retina.

(iv) The RPE is in contact with the choriocapillaris layer of the choroid, which allows the diffusion of molecules into the RPE. The cells of the RPE have strong intercellular junctions between them that act as an external-blood retinal barrier (e-BRB), determining the exchange of controlled substances between the neurosensory retina and choriocapillaris. The RPE is important for pumping fluid into the choriocapillaris, preventing the formation of a macular edema [8].

Despite RPE appearing to act as an independent external barrier, such a separation from i-BRB is not clear. In fact, most of the molecules secreted by the two barrier cell components act simultaneously. The RPE synthesizes various molecules, such as the vascular endothelial growth factor (VEGF) and pigmented epithelium-derived factor (PEDF). The VEGF causes increased vascular permeability alongside its known angiogenic effect, while the PEDF has an antagonistic effect.

3.2. Inner-Blood Retinal Barrier (i-BRB). The capillaries of the central retinal artery provide a blood barrier that protects the retina from potentially harmful molecules. The retinal capillaries have two different plexuses: the superficial capillary plexus located within the ganglion cell layer (GCL) and the capillary plexus located within the inner nuclear layer (INL), adjacent to inner plexiform layer (IPL) and synaptic portion of the outer plexiform layer (OPL).

The two capillary plexus endothelial cells of the retina are strongly joined by tight and adherents junctions [9], each of which is composed of different molecules (Table 1).

The transport of molecules requires two routes:

(1) *Paracellular route* includes passage through the endothelial unions, which vary during paracellular transport, opening and closing according to the demands of the tissues of small molecules and solutes.

(2) *Transcellular route* includes a vesicular transporter which exists across the endothelial cells and is selective and regulated by membrane cell transporters.

i-BRB also includes different structural cells around the endothelial cells, which aids barriers, such as pericytes, macroglial cells like astrocytes, and Müller and microglial cells. In addition, the pericytes and endothelial cells are surrounded by a basal cell membrane that contributes to i-BRB.

In diabetes, the two barriers suffer a metabolic disruption due to different molecules being formed in a hyperglycemic environment [10].

4. What Are the Metabolic Clues in Diabetic Retinopathy and Macular Edema?

The most important finding in diabetic retinopathy (DR) is the presence of hyperglycemia, which acts on different molecular pathways and damages the blood-retinal barrier, overpowering the cells that make up these structures, like endothelial cells and pericytes.

Table 1: Blood-retina barrier components, types of union, and the molecules located in retinal endothelial junctions.

Type of union	Molecule/component/cell
Tight junctions	Claudins (numbers: 1, 2, 5 and 12) Endothelial cell-specific adhesion molecule F11 receptor (jam-1) Junctional adhesions molecules (numbers: 2 and 3) Occludin Zonula occludens (numbers: 1 and 2)
Adherents junctions	β-Caterin VE-Cadherin N-Cadherin
Basal membrane	Basal membrane
Cells	Astrocytes Endothelial cells Microglia Müller cells Pericytes

4.1. Glutamate. Is being an important pathway in the development of DR and DME the function of glutamate, a molecule that is present in high levels in diabetes? Glutamate (Glu) is the most important excitatory neurotransmitter in the brain and retina. Various studies have found high levels of extracellular retinal glutamate linked to DR. This excess causes an activation of the sodium-calcium intraneuronal receptor, initiating the mechanisms of apoptosis [11]. Under certain physiological conditions, Müller cells are responsible for regulating the level of glutamate in the extracellular and intracellular compartments. Thus, when producing excess level of extracellular glutamate, the Müller cells are internalised, facilitating its homeostasis. In diabetes, this mechanism is weaker due to its excess, producing cell toxicity.

4.2. Hyperglycemia. Hyperglycemia has been considered in recent decades as the main cause of the onset and progression of DME and DR. In this regard, two epidemiological studies, the Diabetes Control and Complications Trial (DCCT) and the United Kingdom Prospective Diabetic Study (UKPDS), have reported that intensive control of glycemia is linked to a lower risk of the onset and progression of DR in both type 1 diabetes and type 2 diabetes patients [12, 13]. However, hyperglycemia does not fully explain the wide range of functional and cellular changes that appear over the course of DR [13]. Clinical experience has shown that there is a group of diabetes patients who are unable to prevent the onset or progression of DR and DME despite achieving good metabolic control of the disease. Hyperglycemia maintained over time produces an enzymatic glycation of proteins or advanced glycation end-products (AGEs). All AGEs are highly prevalent in the retinal vasculature of diabetes patients and are involved in microvascular and macrovascular complications [14, 15]. AGEs provoke changes in the extracellular matrix (ECM) and an increase in vascular stiffness. Moreover, and more importantly, they stimulate the endothelial membrane receptors of advanced glycation end-products (RAGE), setting into motion various metabolic pathways (signalling pathways) that eventually increase the expression of molecules such as inflammatory intercellular adhesion molecule-1 (ICAM-1) and VEGF, as well as a synergistic decrease of nitric oxide (NO), causing oxidative stress in pericytes and leading to their apoptosis. The result of this process is capillary vasoconstriction, increased leukocyte adhesion resulting in hypoxia, and retinal capillary hyperpermeability with DME [16].

4.3. Results of Hyperglycemia in Diabetic Patients. Hyperglycemia induces different, imbricated metabolic pathways, initiating the development of a cascade that culminates in the development and progression of diabetic retinopathy (Figure 2).

(a) Increase in polyol production is an implicated pathway in retinal neurodegeneration. In the first step, glucose is converted into sorbitol by the action of aldose reductase; in the second step, sorbitol is converted into fructose. The sorbitol remains in the intracellular space, inducing cellular damage by an unknown pathway. In parallel, the activation of the aldose-reductase enzyme via excess of glucose induces the downregulation of glutathione, which is an antioxidant, subsequently increasing oxidative stress. The two mechanisms (sorbitol and aldose-reductase enzyme pathways) are produced into the mitochondria and they increase the oxidative stress induced by the polyol pathway, which in turn damages retinal cells and induces DR.

(b) The formation of AGEs alters the transmembrane proteins of i-BRB and initiates the inflammatory cascade. AGEs are the result of glucose-membrane cell protein unions, especially the union of fructose and membrane cell protein, and are named as glycation process. Glycation with fructose occurs at a higher rate than with glucose alone; in diabetic patients, excess of fructose permits the formation of a great number of AGE molecules. AGEs produce cell damage through their union with RAGE, and oxidative stress increases its expression, increasing the effect of AGEs. The AGE-RAGE complex induces various diabetic vascular complications, including proinflammatory responses.

(c) The activation of protein kinase C (PKC) is an important pathway in the disruption of i-BRB. PKC is one of the members of the kinase family, which is implicated in phosphorylation reactions. Overaction of PKC occurs in oxidative stress via the formation of excessive diacylglycerol (DAG), which upregulates PKC activation. PKC is important in different intracellular functions, including immunoresponses, cellular growth and development, and guiding transcription at the membrane cell level. PKC overaction causes

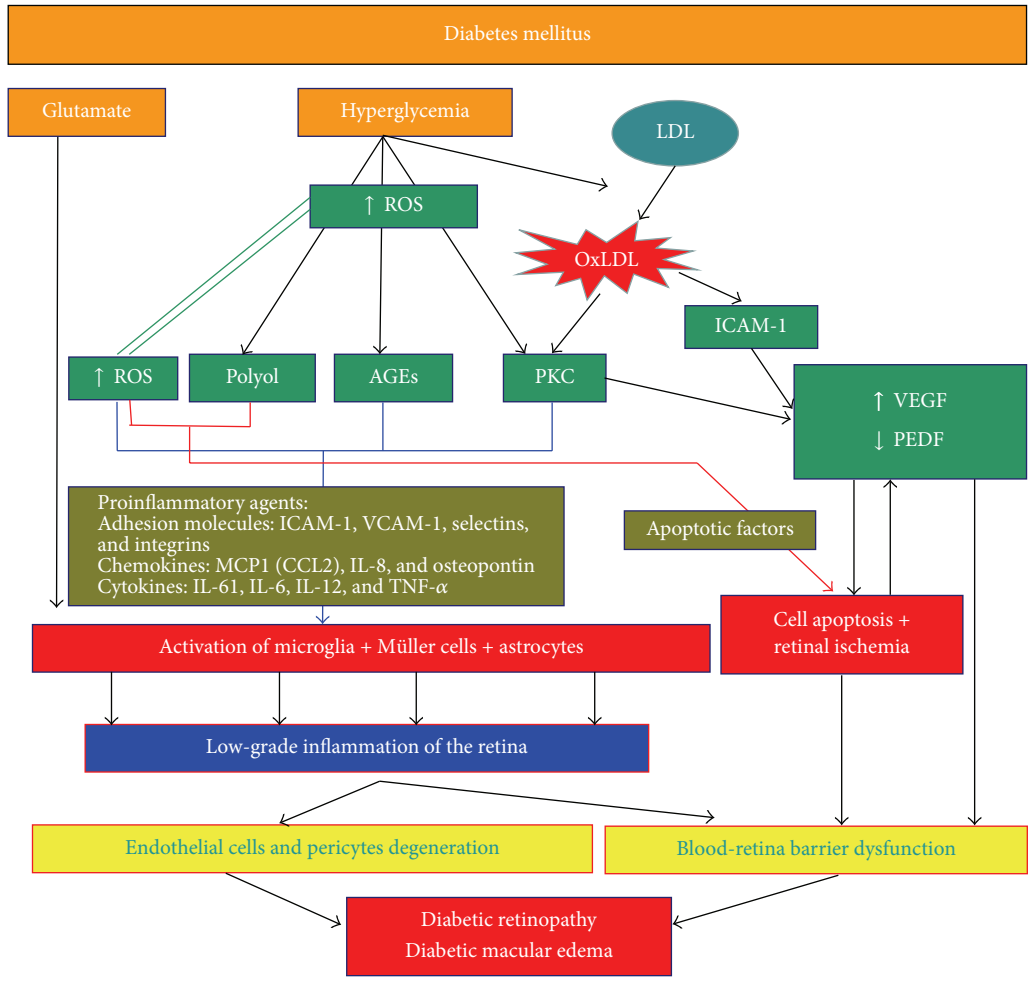


Figure 2: The different pathways in the development of diabetic macular edema.

an upregulation of these activities and initiates cell growth inducing angiogenesis. Other events induced directly by PKC or by increased expression of different factors such as VEGF or transforming growth factor-beta 1 (TGFB1) include the accumulation of extracellular matrix, fibrinolysis, and inflammatory responses.

(d) Oxidative stress, secondary to the accumulation of free radicals in the form of reactive oxidative species (ROS), is linked to histopathological changes such as the thickening of the basement membrane and the loss of endothelial cells and pericytes. The accumulation of any advanced glycation end-products (AGEs) increases the production of ROS. The polyol pathway decreases the production of the antioxidant glutathione, which inhibits ROS. Finally, ROS increases the activity of protein kinase C (PKC). The interaction between ROS and the three previously described pathways under the double route of ROS pathway activation defines ROS as playing a key role in DR development, which is difficult to control. Mitochondrial dysfunction, the source of oxidative stress, can be a potential target for DR treatment.

4.4. Protective Retinal Metabolites. The retina is a neural tissue which, like the brain, synthesizes a series of neurotrophic factors necessary for homeostasis. Its role is largely to neutralise the increased oxidative stress that occurs in certain circumstances; for example, the brain-derived neurotrophic factor (BDNF) is synthesized in both neurons and glial cells of the retina. Some studies have shown that it protects the retina and optic nerve from ischemia. BDNF has been shown to stimulate glutamate uptake by Müller cells [17] and the factor derived from the pigment epithelium-derived factor (PEDF) to protect cells from excess of glutamate [18]. DM disturbs the relationship between the neuroprotective, antiangiogenic, and proangiogenic factors. Among neuroprotective factors, a decrease in the BDNF and ciliary neurotrophic factor (CNTF) has been reported, as well as a decrease in the factor derived from the PEDF. At the same time, there is an observed increase in VEGF, which plays an important role in the onset and progression of DR and DME and protects retinal

ganglion cells. This shows that there is a dual effect where retinal ganglion cells are protected and i-BRB is disrupted.

5. What Are the Clues to the Rupture of the Inner-Blood Retinal Barrier?

In DR and DME, there is an imbalance between proangiogenic and antiangiogenic factors in favour of the former, with higher levels of VEGF, platelet-derived growth factor (PDGF), angiopoietin-2 (Ang-2), osteopontin (OPN), and erythropoietin (EPO). At the same time, a decrease in PEDF, endostatin (ES), and angiostatin (AS) has also been observed.

The most important factor in angiogenesis in retinal tissue is VEGF, which is involved in the genesis of proliferative DR and DME. VEGF is a molecule present in the vitreous of patients with RD and DME, with its levels being proportional to the severity of the diseases. On average, the level of VEGF in patients with DME or DR is 10 times higher than in diabetes patients, without affecting the retina. Various cells produce and synthesize VEGF:

(i) Müller cells

(ii) Lymph nodes

(iii) Glial cells

(iv) Retinal pigment epithelium

(v) Endothelial cells

(vi) Pericytes

High levels of VEGF increase the expression of the inflammatory intercellular adhesion molecule-1 (ICAM-1), leading to retinal capillary leukostasis and important pathogenic factors of diabetic microangiopathy [19–22].

PEDF is a glycoprotein produced by the RPE that inhibits angiogenesis. Funatsu et al. [23] found lower levels of this factor in the vitreous of patients with DME. It seems that the balance between VEGF and PEDF is essential for the maintenance and integrity of i-BRB, thus regulating its permeability [24]. In addition, the PEDF blocks the binding of VEGF to its receptor, reducing its action.

Another factor implicated in DR is the platelet-derived growth factor (PDGF), which is secreted by endothelial cells and is responsible for the function of pericytes [25]; a reduction in its function produces and increases the secretion of α-tumour necrosis factor (α-TNF), which in turn increases ICAM-1 expression.

The most accepted hypothesis to explain the increased expression of VEGF in the vitreous of patients with DR and DME is that retinal hypoxia is caused by an obstruction and loss of retinal capillaries. The capillary blockage is due to the adhesion of leukocytes and endothelial cells, which is facilitated by an increased presence of the ICAM-1 on the endothelial surface [21]. It is believed that the increased expression of the ICAM-1 protein is produced in response to the increased exposure to VEGF receptor activation via AGE protein induction.

The hypothesis that retinal ischemia leads to increased VEGF is congruent with the argument that increased expression of VEGF is regulated by hypoxia-induced factor 1α (HIF-1α). In hypoxic conditions, there is a reduction in HIF-1α, which results in the activation of genes that in turn produce proangiogenic factors (VEGF, Ang-2, etc.), [26, 27]. Studies have found high levels of HIF-1α and VEGF in the vitreous of patients with proliferative DR and DME [28].

In summary, VEGF, which is higher in the vitreous of DR patients, is the most important molecule in the development of changes in the vascular bed, the rupturing of the blood-retinal barrier, and the induction of angiogenesis [29].

6. What is the Pathogenesis of Diabetic Macular Edema?

6.1. Cytotoxic and Vasogenic Edema. In macular edema, liquid accumulation can occur in intracellular or extracellular spaces. Edema-induced accumulation of liquid within the intracellular space is defined as cytotoxic, while accumulation of liquid in the extracellular space is defined as vasogenic edema. These two different edemas can appear in different pathologies: in arterial occlusion by emboli, the edema is located in the intracellular space and is visible in OCT as hyperreflectivity in retinal nuclear layers; in venous occlusions, the vasogenic edema is the most important form.

In patients with DME, the two forms of edema appear: cytotoxic form at the beginning and a vasogenic form later on.

Cytotoxic form can result from increased sorbitol, lactate, and phosphates in the intracellular space, secondary to hyperglycemia.

Vasogenic form can be produced by many of the molecules described previously, including VEGF, nitrous oxide, and free radicals, which produce a rupture of i-BRB. The amount of accumulated extracellular fluid is determined by the difference between the osmotic and hydrostatic pressure in the retinal veins and arterioles against the extracellular environment [7, 30]. The result of vasogenic edema is an accumulation of fluid, mainly in the extracellular layer of the external plexiform and the inner and outer nuclear retinal layers. In some patients, a detachment of the neurosensory retina occurs.

The presence of cytotoxic and vasogenic lesions in DM patients induces the reduction of pericytes, Müller cells, and astrocytes, with an increase of basal membrane capillaries and a decrease in the number of endothelial cells, which in turn induces the hyperpermeability of retinal vessels due to the rupture of i-BRB. This rupture must occur before we can observe clinical signs of DR.

6.2. The Importance of Müller Cells. Müller cells are the most important glial cells in the retina. Located alongside the layers of the neurosensory retina, Müller cells' nuclei are in the internal nuclear layer, and their extensions (axons and dendrites) contact all nuclei of retinal cells. Müller cells permit contact between retinal cells and different compartments: vitreous, retinal vessels, and the subretinal space. They also

have ionic channels, transmembrane proteins, and different enzymes. An important characteristic of Müller cells is their great conductance for potassium (K^+).

The functions of Müller cells are

(i) elimination of extracellular liquid into the retinal vessels;

(ii) regulation of blood flux;

(iii) relationship with glucose metabolism of retinal neurons;

(iv) maintenance of retina pH by aqueous homeostasis;

(v) production of glutamate, which is implicated in neuronal transmission.

Macular edema appears as a consequence of an imbalance between the liquid passing from retinal vessels to the extracellular space and the reabsorption of the liquid from the extracellular space into the vessels. Müller cells are responsible for the active transport of liquid into retinal vessels; in DM, Müller cell metabolism is disturbed, and a dysfunction of interstitial liquid homeostasis and potassium exists, which permits the intracellular accumulation of liquid with increased difficulty discharging the liquid into the vessels; in the first step, cellular swelling occurs, leading to cell rupture and an increase of liquid in the extracellular space, which developed cysts spaces. This finding is in accordance with the steroid effect over these channels, which restores its metabolic functions, helps restore potassium, and promotes aqueous drainage from the retina into the vessels.

6.3. Diabetic Retinopathy Is Secondary to Microangiopathy: Is There a Concomitant Neuropathy? DR and DME are currently considered manifestations of parallel vascular and neuronal degenerative processes. Several studies support this view, suggesting that the existence of subclinical neuronal impairment prior to the appearance of vascular lesions is typical of DR.

Tests that evaluate the function of the retina, such as multifocal electroretinograms [31], contrast sensitivity tests, or automated perimetries, have highlighted the vulnerability of retinal cells. The result is an increase in retinal neuronal apoptosis [32] together with changes in retinal vasculature that culminate in the compromising of vision in diabetes patients.

In physiological conditions, a close relationship exists between the cellular and vascular component of the retina in order to maintain the homeostasis necessary for normal operation. This interaction causes neurovascular damage in the course of retinal degeneration via changes that induce early microvascular changes, events that involve the breaking of the blood-retinal barrier, which is the stage prior to the onset of DME [33].

It is not known what causes neuroinflammation in the retina. However, various nociceptive stimuli are involved, such as hyperglycemia, glutamate, deregulation of neurotrophic retinal factors, oxidative stress, AGEs, and stress-level endothelial reticulum [34]. There are those who consider DR to be a manifestation of a systemic inflammatory condition of DM.

Regardless of which factors cause inflammation of the retina, it seems that it is caused by the activation of the immune system of the retina itself, especially the microglial cells.

Microglial cells, derived from monocytes as perivascular macrophages, modulate the immune response, but, in situations of chronic stress, their inflammatory capacity increases. Under normal conditions, microglial cells are located at the two plexiform layers and are responsible for the control of the extracellular compartment of the retina, maintaining tissue homeostasis and playing a role more similar to neurons, judging by the highly branched projections they present [35, 36]. Thus, microglial activation is necessary to eliminate pathogens and apoptotic neurons. However, in DR, its activation generates anatomical and functional changes in the retinal cells.

Under conditions of high activation, microglial cells acquire a more amoeboid phenotype with increased motility, and their location is displaced from plexiform layers to the subretinal space. In addition, microglial cells synthesize and remove cytokines, proteases, nitrous oxide, and ROS in the extracellular medium, which causes neuronal death [37]. The subretinal space is an immunoprivileged space that can be altered by the presence of microglial cells and the production of cytokines. In a recent study, porous holes were observed between the retinal pigment layer and the choroid, which facilitate the passage of cellules between both layers. In the same study, Graeber et al. [38] observed, in a murine diabetic model, that, in the case of diabetes, the number of holes in epithelium pigment retina layer (EPR) increased in order to permit the transport of more inflammatory cellules to the choroid space; they also observed an increase of ICAM-1 and caviling-1 (CAV-1), both which are implicated in leukostasis, with the number of holes diminishing after one year. The microglia participate in DR inflammatory response by increasing their number around the vessels and expressing the chemokine C-C motif ligand 2 (CCL2), which induces the recruitment of macrophages to the retina. In the first step, the microglial cells pass into the subretinal space and then migrate to the choroid; but when the EPR layer becomes damaged and cannot permit the passage of more cells, the microglia accumulate at the subretinal space, increasing the inflammatory response.

In sum, it seems that the microglial cells, along with Müller cells and astrocytes, initiate inflammatory processes in the retina, and reactive glial cells amplify this response [39]; and a low-grade inflammation is maintained by the production of cytokines such as interleukin 6 (IL-6) and interleukin 8 (IL-8) or C-C motif ligand 2 [40]. IL-6 alters the function of astrocytes, which give structural support to the capillaries in the retina, thus breaking i-BRB. At the same time, the retina's ability to internalise glutamate from Müller cells is reduced. IL-8 and CCL2 act on neutrophils and monocytes, respectively. Little is known about the migration of white blood cells into the retina, but the role of IL-8 and CCL2 is analogous to the brain in that they attract leukocytes.

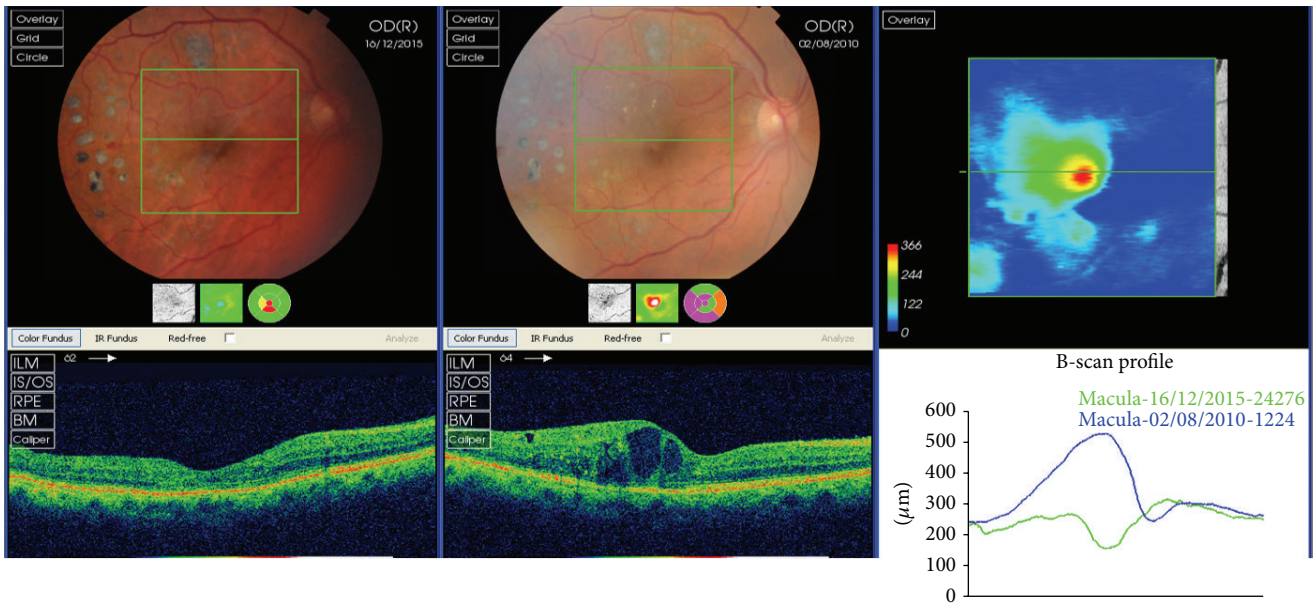


Figure 3: Evolution of a patient with diabetic macular edema, from 2012 to 2015. The patient did not respond to anti-VEGF but to steroids. Perhaps, the hyperreflective retinal spots, seen in the first tomography in the outer plexiform layer temporal to the fovea, can be a predictive sign of a positive response to steroids.

These cytokines have been consistently shown to be elevated in patients with DR and DME, correlating positively with severity. Differences in expression profile of cytokines between diabetics, without and with mild DR, and controls have been documented [41]. Also the histology of patients with DR provides evidence of the perivascular infiltration of monocytes into the most affected retinal areas.

7. What Is the Current Treatment of Diabetic Macular Edema?

As we described previously, the rupture of i-BRB involves many molecules, the most important of which, VEGF, is found in the vitreous of DM patients and acts on endothelial cells through various mechanisms:

(i) By increasing vascular permeability due to the formation of fenestration in retinal vessels

(ii) By increasing angiogenesis directly as a potent mitogen of endothelial cells and indirectly by stimulating metalloprotease production and inhibiting metalloprotease inhibitors

(iii) By acting as a proinflammatory agent

Pathologically, VEGF production is altered during DR and DME, secondary to hyperglycemia, PKC activation, and AGE protein production. Currently, we use therapies based on VEGF inhibitors (blocking or inhibiting VEGF) in the management of DME (Figure 3), which helps prevent i-BRB disruption [42–44].

Since 2010, anti-VEGF treatments have been highly effective against DME, reducing central macular thickness (CMT) in patients with edema. Despite no direct relationship having been observed between a decrease in CMT and the recovery of visual acuity, all ophthalmology guidelines recommend a decrease in CMT as a target for the resolution of DME.

The first two multicentre studies, RESOLVE [45] and RESTORE [46, 47], have reported that anti-VEGF treatments are superior to laser treatments. In the RESOLVE study, the safety and efficacy of ranibizumab in DME were investigated in a 12-month, multicentre, controlled, double-masked study that randomized patients into three groups: two groups with different concentrations of intravitreal ranibizumab and a group with a placebo injection at twelve months. Mean visual acuity increased in the two ranibizumab groups, with no significant differences between them, and decreased in the control group, concluding that ranibizumab is effective and safe in DME treatment. For DME in the RESTORE study, ranibizumab as a monotherapy or combined with laser versus monotherapy with laser was administered during also a 12-month, randomized, double-masked, multicentre phase III study, but the study included photocoagulation laser as an alternative treatment. Patients were classified into three groups: ranibizumab and laser, or placebo injections and laser. The authors conclude that ranibizumab, both as a monotherapy or combined with laser, improves visual acuity more than standard laser does in patients with visual impairment due to DME. In the RESTORE study at three years, 47 patients were eligible to receive individualized ranibizumab treatment as of month 12, guided by visual acuity and disease progression criteria at the investigators discretion. The study

concludes that ranibizumab is effective in improving and maintaining visual acuity and central retinal thickness with a progressively declining number of injections over 3 years of individualized dosing.

Results showed that ranibizumab was effective in improving and maintaining visual acuity (VA) and CMT outcomes, with a progressively declining number of injections needed over a 3-year treatment regimen. Intravitreal injections were generally well tolerated, with no safety concerns being reported over the three-year study period apart from frequently reported cataracts in 16.3% of patients [47].

Since the Diabetic Retinopathy Clinical Research (DRCR) network study [48], we have the possibility of using steroids. The study compared 4 mg and 8 mg intravitreal doses of triamcinolone acetonide (IVTA) to focal/grid laser photocoagulation. The 4 mg IVTA group had better visual acuity after three years than the 8 mg IVTA group, but the laser group had better visual acuity than either IVTA group, probably due to the fewer number of complications, cataracts, and glaucoma, which decreased visual acuity in the groups treated by IVTA.

In a second randomized controlled trial by the DRCR network, the focal/grid laser method alone was compared to a 4 mg dose of IVTA and laser. Similar to the previous study, the IVTA and laser performed better than the laser alone in terms of visual acuity but also had increased rates of cataracts and higher intraocular pressure (IOP). In the subgroup analysis of patients who were pseudophakic at baseline, the IVTA and laser group performed better than the laser treatment group, equivalent to the ranibizumab group [49].

Other steroids have also been studied in the treatment of DME, with dexamethasone implants into the vitreous demonstrating effectiveness in resolving DME in refractory cases [50, 51]. Also, fluocinolone acetonide, has also been effective [52] but with a large number of patients still developing cataracts (91% underwent a cataract extraction by the fourth year). In addition, IOP increased by up to 30 mmHg in 61.4% of implanted eyes and 33.8% required surgery for ocular hypertension [53].

7.1. When Treating DME, Should We Use Anti-VEGF Drugs or Steroids Drugs? Despite the effectiveness of anti-VEGF treatment (Figure 3), if the published data is observed carefully, and different conclusions can be reached. In the most recent studies, the RIDE-RISE trials using ranibizumab [54, 55] and the VISTA-VIVD trials using aflibercept [56], visual acuity increased following intravitreal ranibizumab and aflibercept. RIDE-RISE and VIVID-VISTA are two studies that used ranibizumab and aflibercept, respectively. In the first study with ranibizumab, patients were randomized to monthly intravitreal injections of 0.3 or 0.5 mg or placebo. At 24 months, visual acuity had increased and central retinal thickness had decreased in two ranibizumab doses compared to the control group, concluding that ranibizumab is effective. In the second study with aflibercept, patients received 2 mg every 4 weeks, 2 mg every 8 weeks, or a laser control. Results showed that the 52-week visual and anatomic superiority of aflibercept over laser continued through to week 100, with similar efficacy in both aflibercept groups.

Despite the good results in both studies, we observe that only about 38% of patients achieved the targeted increase of 15 or more letters (equivalent to three lines in the optotypes) after treatment; and, in all studies, about 30% of patients were nonresponsive. The DRCR network is funded by the National Eye Institute, and, with the collaboration of 177 clinical sites in the United States and Canada, it has conducted multiple studies about DME treatments. Properly defined, the DRCR network is a collaborative effort dedicated to facilitating multicentre clinical research on DR, DME, and associated conditions.

The DRCR network compared multiple different treatments for DME, including anti-VEGF drugs alone or in combination with lasers or steroids; as we have said, patients with DME were classified as either chronic or nonchronic DME, attending the response to anti-VEGF treatment. Nonresponders or chronic DME is diagnosed if there is a failure in the treatment, which is defined as having no increase in visual acuity over five letters or no 10% reduction in central retinal thickness. These changes must take place after four anti-VEGF intravitreal injections or after six months of treatment with the same responses.

It is interesting to note the latest results published in Protocol T [57, 58]; these studies, published after one- and two-year follow-ups of a clinical series of DME patients, demonstrated that if the initial response to anti-VEGF drugs at three months is good, final visual acuity will be good. These studies divided patients into three groups:

(1) Patients with an initial response of no fewer than five letters in the optotypes at three months

(2) Patients with an initial response of 5–9 letters

(3) Patients with an initial response of more than 10 letters

The first group included about 39.7% of patients in the sample, the second group included 23.2%, and the third group included 37.1%. All three groups retained increased visual acuity along the next 12 months.

The COCHRANE study, which reviewed the effectiveness and safety of intraocular steroids in treating DME (triamcinolone acetonide, fluocinolone acetonide implants, and dexamethasone drug delivery systems), concluded that intravitreal steroid injections may improve visual outcomes in eyes with persistent or refractory DME but with a large number of complications. Since the studies in the review focused on chronic or refractory DME, the question arises as to whether intravitreal steroid therapy could be of value at other stages of DME, especially at earlier stages, either as a standalone therapy or in combination with other therapies, such as laser photocoagulation [59].

8. What Are the Examination Techniques of Diabetic Macular Edema?

There are many different techniques for examining the macular area, including biomicroscopy, fluorescein angiography, and optical coherence tomography (OCT).

Biomicroscopy using a noncontact fundus lens is routinely used in clinical practice as the first type of examination of patients with DR, after screening by retinography. However, this technique does not allow us to retain the images and cannot determine how large a retinal lesion is.

The two principal techniques currently used in the diagnosis and follow-up of patients with DME are optical coherence tomography (OCT) and fluorescein angiography (FA). The latter is important for determining the presence of ischemia in the retinal tissue, but it is an invasive technique with possible severe adverse effects as serious angioneurotic reaction in 2% of patients; thus, in clinical practice, OCT is the most used technique in DME follow-up and for determining responses to treatment.

In the two next sections, we explain how to apply these techniques in DME and whether it is possible to determine its vasogenic or inflammatory origin.

8.1. Optical Coherence Tomography and the Different Types of DME. The challenge was to determine whether there is a marker in OCT that would help us in the diagnosis of a predominance of vasogenic or inflammatory changes in the macular area. What do we know about the signs of DME in OCT?

Over the last 15 years, the introduction of OCT has revolutionised macular examination. The image of the macula and its layers allows us to diagnose much of its pathology (Figure 1). The follow-up for DME treatment currently includes OCT, and, depending on the results, treatment is either continued or discontinued (Figure 3). Therefore, it is very important to determine which of the changes in the macular OCT are important for continuing treatment.

The different layers of the retina can be seen in the OCT, even though the images are not exactly anatomically equivalent. Figure 1 shows the different layers in a normal OCT [7] as well as the two different vascular plexuses that originate in the central artery [60].

The macular edema presents an increase in fluid accumulation in the central retinal layers, both intracellular and extracellular. The presence of an intracellular edema is difficult to observe in DME patients, and only in an edema that is secondary to acute central retinal artery occlusion can we see an increase in the width of ganglion cells and bipolar cell layers (Figure 3).

Depending on the location of extracellular fluid in the macular area, we can classify the macular edema. The standard clinical classification by Otani et al. [61] defines DME as follows:

(1) Nontractional DME

Spongiform-like
Cystoid edema
Serous retinal detachment

(2) Tractional DME

Tractional DME
Epiretinal membrane DME

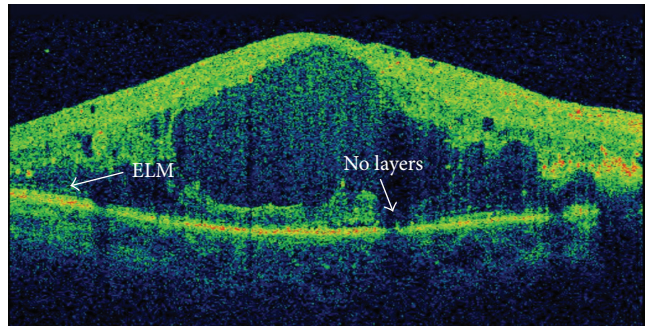


Figure 4: Absence of ellipsoid (ellipsoid zone) layer and external limiting membrane (ELM).

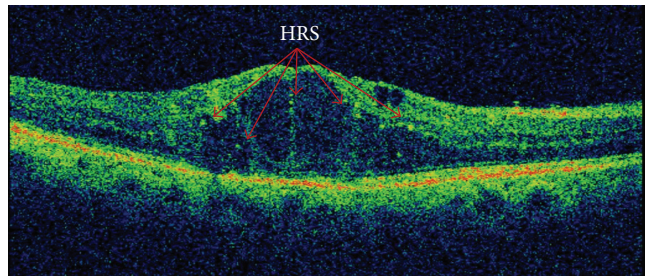


Figure 5: Cystoid diabetic macular edema with hyperreflective retinal spots.

Although the retinal thickness is visible and, after treatment, this width improves and visual acuity increases, there is no direct relationship between retinal thickness and visual acuity [62]. In patients with DME, there is an increase in central retinal thickness, secondary to extracellular fluid, and a rupture in several retinal layers occurs, the most important of which being the ellipsoid layer and the ELM layer (Figure 4).

Visual function, determined by visual acuity and multifocal electroretinogram response, seems more related to the presence or absence of the different retinal layers. In particular, the presence or absence at the beginning of treatment of the ellipsoid layer and the ELM layer is highly related to final visual function (Figure 2), a finding which has been reported by authors of previous study [63].

Can We Determine Inflammatory Changes Using the OCT? A possible relationship between hyperreflective retinal spots (HRS) and inflammation in DME has recently been published [64]. Hyperreflective retinal spots (Figure 5) have been reported to predispose different lesions. Coscas et al. [65, 66] were the first to describe HRS as hyperreflective dots or foci, located in all retinal layers but with a predominant location in the outer retinal layers around the intraretinal cystoid spaces, and they suggested that this might be secondary to the activation of microglial cells [66]. Other possible explanations include that HRS are lipoprotein extravasations prior to hard exudates [67] or a degeneration of the photoreceptors or macrophages which engulf them [68]. An interesting study by Framme et al. [69] described the disappearance of HRS after anti-VEGF treatment in patients with DME and hypothesised

that HRS represent a clinical marker of an inflammatory response, a finding supported by Coscas' observation that HRS represent clusters of microglia. Recently, Vujosevic et al. [70] in a case-control series in 20 eyes of 20 diabetic patients demonstrated that the decrease in HRS correlates with functional parameters, specifically retinal sensitivity.

Lastly, Vujosevic and Midena [71] demonstrated that HRS increase in patients with diabetes if they have DR. In the early stages, HRS are located in the same inner layers as the microglia. Later, the HRS are displaced to the outer layers, as happens with microglia when DR includes the outer retinal layers [72, 73]. An important finding of the study is that after anti-VEGF treatment, the baseline number of outer retinal HRS was linked to final visual acuity as well as ellipsoid zone disruption length and external limiting membrane disruption length [74]. In view of these similar results, more studies on the relationship between HRS, microglia, and DME are needed.

Another important image available from OCT is the presence of serious retinal detachment. This form of DME implies a detachment of the central foveal zone and is related to cystoid DME, which appears in 15% of cases of DME. The relationship with visual function is currently being debated, as some authors have not found a relationship with visual acuity [75]; others, however, have reported serious retinal detachment and large outer nuclear layer cysts as the two morphological changes that have the greatest negative impact on retinal function [76]. Retinal detachment can appear in the eyes as increased vascular permeability in the macula, which may be an important finding in terms of the possible existence of a diffused macular lesion. However, its presence is inversely related to the presence of ischemia, and patients with serious macular detachment do not present macular ischemia [77]. Curiously, serious macular detachment responds to intravitreal triamcinolone acetonide in direct proportion to the height of the serious macular detachment [78]—so, could this be a sign of inflammation? We can suppose that its appearance might be produced by a rupture in the external BRB due to a failure in the RPE pump function.

We can say, then, that the vasogenic or inflammatory etiology of DME is very difficult to distinguish from OCT. If we bear in mind that vasogenic changes appear at the beginning of the disease, then the spongiform-like macular edema might be due to a vasogenic cause and, in more advanced forms such as cystic or serious retinal detachment, inflammatory factors would be more likely. We do not know at the moment what is the pathophysiology of DME based on OCT.

In the future, the diagnosis of vasogenic or inflammatory etiology in DME will become increasingly important if we are to individualize treatments with either anti-VEGF or anti-inflammatory drugs or a combination of the two.

8.2. Fluorescein Angiography. Fluorescein angiography (FA) was first reported by Novonty and Alvis in 1961 [79], based on an injection of sodium fluorescein in aqueous solution into the vein. With a retinography, we can stimulate fluorescein with a source of white light passing through a filter that emits light at 480 nanometres, which is the wave that excites the molecules in the fluorescein. Then, we can use a barrier filter at 530 nanometres to visualise the fluorescence of the vascular bed. An important characteristic of fluorescein is that it binds with albumin, and the complex fluorescein-albumin cannot cross the blood-retinal barrier except in cases where the barrier has been ruptured, after which fluorescence in the retina is visible outside the vessels.

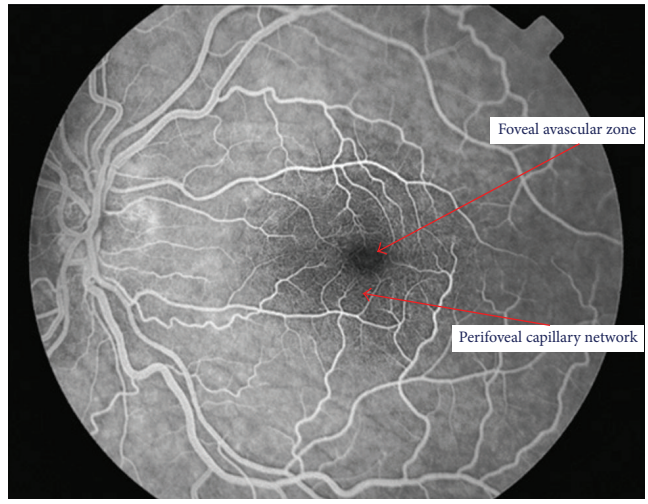


Figure 6: Normal fluorescein angiography showing the avascular foveal area and the perifoveal capillary network.

FA allows us to observe the retinal vasculature as well as how fluorescein crosses the arterial, capillary, and venous vascular phases (Figure 6). FA is an essential technique for determining a rupture in the inner-blood retinal barrier. In diabetes patients, we can see microaneurysms as dots of hyperfluorescence located at the capillary layer. The extravasation of fluorescein into the interstitial space and subsequent retinal edema helps us to diagnose DME. It also allows us to determine its origin in the vascular bed because of the incompetence of microaneurysms (focal macular edema) or as a diffuse extravasation in cases of diffuse macular edema (Figures 7 and 8). FA can also determine areas where there is no perfusion or retinal ischemia, which indicates a possible origin in cases of low visual acuity in patients with a normal macular area after treatment for DME. It is an important technique for determining the disruption of the internal blood-retinal barrier but does not allow us to determine the vasogenic or inflammatory etiology of DME [80, 81].

One problem with FA is that we have to inject fluorescein into the vein, which can have adverse effects, such as an allergic angioneurotic reaction in about 2% of cases and causing patient mortality (albeit extremely rare, occurring in only about 1 out of 220,000 angiographies).

A new technique has recently been added to the ophthalmic arsenal: OCT angiography (OCTA), which combines an image of the i-BRB vascular bed and an OCT of the macular area.

OCTA gives us the opportunity to observe the vascular structure of the retinal vessels without an injection of fluorescein. OCTA can also determine the structure of the two

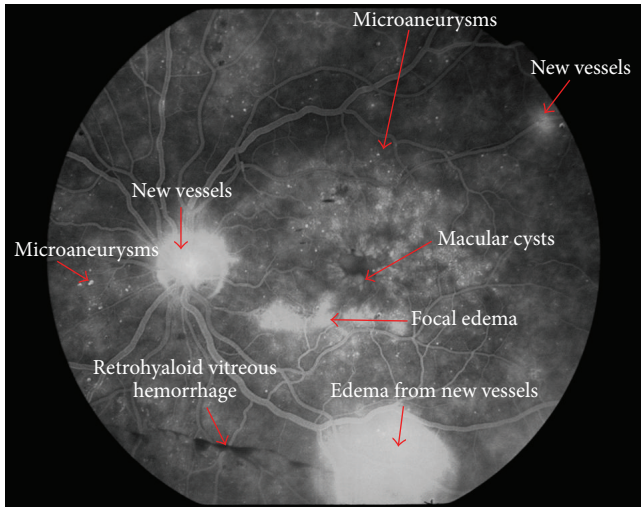


Figure 7: Fluorescein angiography of a patient with proliferative diabetic retinopathy. New vessels are visible in the optic disc and temporal (superior and inferior) arcades, and macular cysts and microaneurysms can be observed.

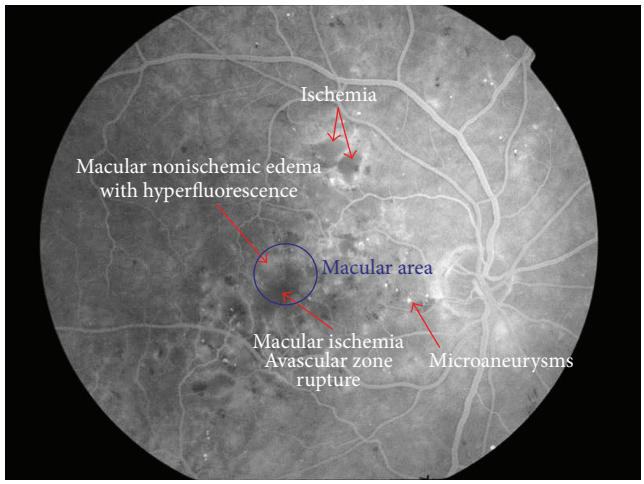


Figure 8: Fluorescein angiography of a patient with severe diabetic retinopathy and macular ischemia. We observe the rupture of avascular zone by ischemia and also hyperfluorescence area in macula at temporal side.

vascular layers, or plexuses: the superficial or internal plexus layer, located between the optic nerve fiber layer and the ganglion cell layer; and the external or deep plexus, located in the inner plexiform layer (Figure 9).

OCTA allows us to observe macular vascularisation with special attention to the foveal avascular zone and the perivascular capillary bed. It is an important technique for determining the presence of ischemia in DR.

In patients with diabetes but without DR, it is important to observe an increase in the size of avascular foveal zone, which is a sign of the possible formation of microaneurysms: a stage which can be reversible if glycemic values are controlled. Also in the deep plexus, there are small areas of ischemia without DR, with a central enlargement of the avascular zone [82, 83].

In patients with developed DR, OCTA gives us an image of nonperfused areas, an increase in the anastomosis between the two plexuses, and larger capillary loops in the inner plexus [84, 85]. Initial neovascularisation is imaged as the presence of irregular thickened vessels that emerge from the surface of the retina or the optic disk.

One limitation of OCTA is that it is not a true fluorescein angiography because its hyperreflectivity is not equal to the hyperfluorescence of FA. In addition, OCTA images are limited to the macular area, so we cannot look at the vascular bed outside of the vascular temporal arcades. Finally, errors in the technique, such as using an 8×8 field, gives poor resolution, and surface artefacts or a mirroring effect in RPE can distort the images.

In the future, OCTA will help us to determine what happens in DR and allow us to treat more incipient lesions, but the technique needs more study and further development.

We can conclude that no examination technique helps us in the diagnosis of an inflammatory origin of DME; despite some orientation that can be extracted from the images, the presence of hyperreflective dots in OCT can be a sign of inflammatory changes and increased areas of hyperperfusion around the macula in FA lead us to think a vascular origin is present. Moreover, FA helps us to determine the hypoperfused areas by occlusions of the retinal vessels that can permit laser treatment of ischemic areas (if they are away from the macular area); if they are present in the macular area, we can determine a possible negative prognosis for visual recovery.

9. Conclusions

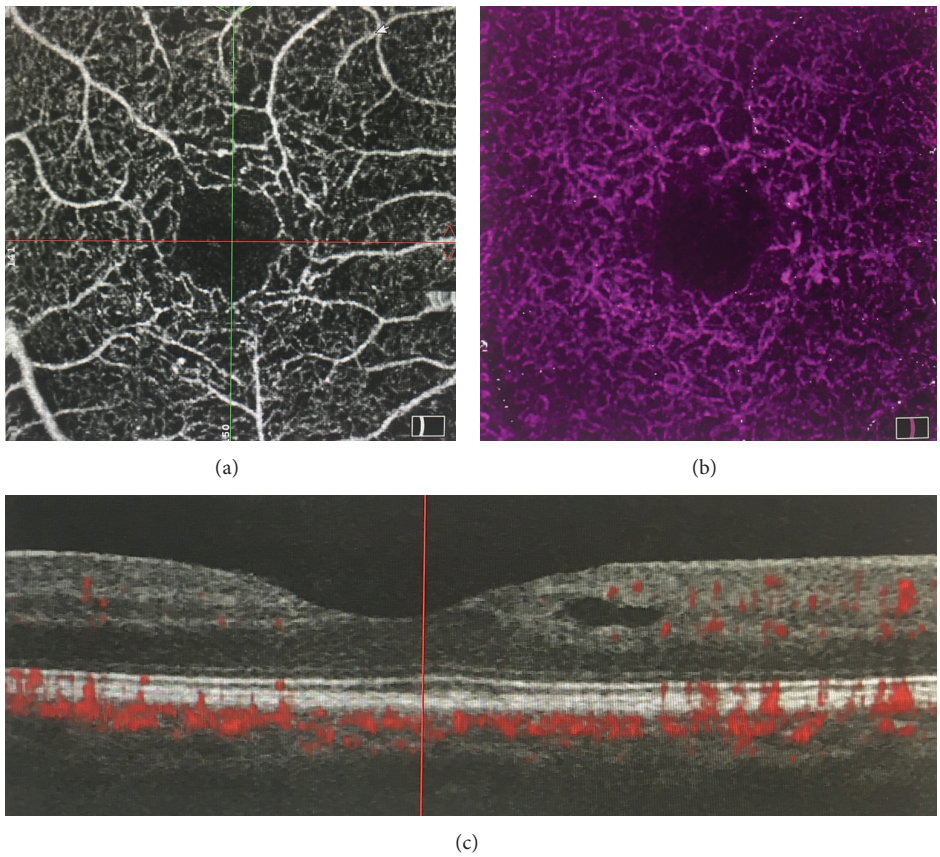
Diabetic macular edema (DME) is the leading cause of blindness in diabetic patients. Diagnosis is easy by exploring the retina under biomicroscopy and confirming it by OCT. Due to the efficacy of current treatments, it is essential to determine which etiology of macular edema is predominant.

Vasogenic changes secondary to hyperglycemia induce a rupture in the blood-retinal barrier (BRB), which begins the cascade of macular edema formation. However, the activation of a low-grade inflammation simultaneous to vasogenic changes will induce serious retinal damage and macular changes will become chronic.

Currently, DME is resistant to treatment in 30% of cases. The current anti-VEGF and steroid treatments are useful, but their use should be personalised. Prior knowledge of the predominant type of DME, vasogenic or inflammatory, is essential for determining the more effective type of drug.

10. Method of Literature Research

A PubMed and Web of Science search was conducted for the terms diabetic retinopathy, diabetic macular edema, oxidative stress, inflammation, blood-retina barrier, and optical coherence tomography in any publications between January 2000 and January 2015. A review of the abstracts



(a) (b)

(c)

Figure 9: OCT angiography of a patient. (a) Superficial plexus, (b) inner plexus, and (c) optical coherence tomography.

identified relevant articles, which were later confirmed. The citations from these articles were also used to identify articles not found with the above search terms. Any non-English references were excluded.

List of Abbreviations

AGEs:	Advanced glycation end-products
BDNF:	Brain-derived neurotrophic factor
CAV-1:	Caveolin-1
CCL2:	C-C motif ligand 2 (anteriorly named as monocyte chemoattractant protein-1 or MCP-1)
CRA:	Central retina artery
DCCT:	Diabetes Control and Complications Trial
DM:	Diabetes mellitus
DR:	Diabetic retinopathy
DRCR-net:	Diabetic Retinopathy Clinical Research Network study
e-BRB:	External-blood retina barrier
ELM:	External limiting membrane layer
FasL:	Fas-ligand
GCL:	Ganglion cell layer
HIF-1α:	Hypoxia-induced factor 1 α
i-BRB:	Inner-blood retina barrier
ICAM-1:	Inflammatory intercellular adhesion molecule-1
IL-6:	Interleukin 6
INL:	Inner nuclear layer
IOP:	Intraocular pressure
IPL:	Inner plexiform layer
IS/OS:	Inner segment/outer segment layer, currently named ellipsoid zone
IVTA:	Intravitreal doses of triamcinolone acetonide
MCP-1/CCL2:	Monocytes quimio-attract 1, currently named as C-C motif ligand 2 (CCL2)
NFL:	Nerve fiber layer
OCT:	Optical coherence tomography
ONL:	Outer nuclear layer
OPL:	Outer plexiform layer
PDGF:	Factor derived from platelets
PEDF:	Pigmented epithelial derived factor
PKC:	Protein kinase C
PRN:	Pro re nata
RAGE:	Receptors of advanced glycation end-products
RIDE/RISE:	A study of ranibizumab injection in subjects with clinically significant macular edema (me) with centre involvement secondary to diabetes mellitus

RESOLVE:	Safety and efficacy of ranibizumab in diabetic macular edema. A 12-month randomized, controlled, double-masked, multicentre phase II study.
RESTORE:	A study of ranibizumab monotherapy or combined with laser monotherapy for diabetic macular edema
RN:	Neurosensory retina
RPE:	Retina pigment epithelium
STDR:	Sight-threatening diabetic retinopathy
UKPDS:	United Kingdom Prospective Diabetic Study
VA:	Visual acuity
VEGF:	Vascular endothelial growth factor
VIVID/VISTA:	Intravitreal aflibercept for diabetic macular edema.

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Competing Interests

The authors declare that there are no competing interests regarding the publication of this paper.

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