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MICROVASCULAR COMPLICATIONS—RETINOPATHY (DL CHAO AND G YIU, SECTION EDITORS)

Imaging and Biomarkers in Diabetic Macular Edema and Diabetic Retinopathy

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Abstract

Purpose of Review Diabetic retinopathy (DR) is the leading cause of acquired vision loss in adults across the globe. Early identification and treatment of patients with DR is paramount for vision preservation. The aim of this review paper is to outline current and new imaging techniques and biomarkers that are valuable for clinical diagnosis and management of DR.

Recent Findings Ultrawide field imaging and automated deep learning algorithms are recent advancements on traditional fundus photography and fluorescein angiography. Optical coherence tomography (OCT) and OCT angiography are techniques that image retinal anatomy and vasculature and OCT is routinely used to monitor response to treatment. Many circulating, vitreous, and genetic biomarkers have been studied to facilitate disease detection and development of new treatments.

Summary Recent advancements in retinal imaging and identification of promising new biomarkers for DR have the potential to increase detection, risk stratification, and treatment for patients with DR.

Keywords Diabetic retinopathy · Diabetic macular edema · Biomarkers · Retinal imaging

Introduction

Diabetic retinopathy (DR) is a progressive microvascular disease that is the leading cause of acquired vision loss in adults across the globe. The World Health Organization estimates that the total number of adults with diabetes mellitus will reach 366 million by 2030 [1] and the number of people with DR will reach 191 million by 2030 [2•]. In addition to vision loss, DR is linked to systemic complications of diabetes including cardiovascular events and nephropathy [3, 4].

DR is classified into two stages, non-proliferative DR (NPDR) and proliferative DR (PDR). The earlier stage, NPDR, is characterized by microaneurysms, retinal hemorrhages, and hard exudates while the more advanced stage, PDR, is defined by presence of retinal neovascularization. Diabetic macular edema (DME) can occur at any stage and is identified by retinal thickening and hard exudates. PDR is the most common type of vision-threatening lesion in type 1 diabetes while DME is the most common cause of vision loss in type 2 diabetes [5].

Though there are many well-known clinically important risk factors associated with development and progression of DR and DME, such as control of blood glucose and blood pressure [6, 7], these risk factors do not account for all of the patients who develop DR and many patients with these traditional risk factors do not develop DR. Identifying imaging findings and biomarkers capable of predicting risk of development and progression of DR is important for screening, monitoring, and treatment of patients. Early detection and proper treatment are essential for preservation of vision. This review will outline current and promising new imaging techniques and biomarkers that are valuable for clinical diagnosis and management of DR and DME.

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Imaging in Diabetic Retinopathy and Diabetic Macular Edema

There have been huge advancements in retinal imaging in the last several decades. New technologies have allowed for better visualization of the anatomy of the retina and choroid, earlier detection of retinal disease, and increased access of screening across the globe. We will discuss recent improvements in traditional imaging modalities such as fundus photography and fluorescein angiography as well as newer imaging techniques like optical coherence tomography and optical coherence tomography angiography. Table 1 is a summary of imaging modalities in DR.

Fundus Photography

Fundus photography is a rapid, non-invasive, well-tolerated, and widely available imaging modality that produces high quality retinal images. It is widely used in conjunction with clinical assessment to document extent of disease in DR. The traditional gold standard for staging DR is the Early Treatment of Diabetic Retinopathy Study (ETDRS) grading system [8] which uses seven fields of color fundus photography to evaluate vascular lesions. However, this system has limited clinical applicability due to the intensive analysis required. The Internal Clinical Diabetic Retinopathy and Diabetic Macular Edema Severity scale is a simplified scale based on the ETDRS system that was developed in 2001 that is more user friendly for clinicians [9].

There is level 1 evidence that fundus photography interpreted by a trained reader is a very effective screening tool for DR [10]. Telemedicine initiatives based on digital fundus photography have been shown to have high accuracy and are being implemented across the globe to expand access to DR screening [11–13]. The development of automated algorithms to detect DR is another advancement that may confer many advantages such as consistency of reporting, instantaneous results, and further increased accessibility of screening. Machine algorithms have been shown to be effective for screening of DR [14]. Using a data set of 9963 images and an operating point selected for high sensitivity, a recently tested algorithm based on deep learning had 97.5% sensitivity and 93.4% specificity for detecting DR and DME on retinal fundus photographs [15]. A study by Tufail et al. identified

Table 1 Summary of retinal imaging modalities in diabetic retinopathy

Imaging modality	Advantages	Disadvantages	Key findings in DR
Fundus photography	• Widely available and non-invasive • Gold standard for diagnosis and staging along with clinical exam	• Two-dimensional image • Qualitative rather than quantitative assessment	• Microaneurysm • Intraretinal hemorrhage • Cotton-wool spot • Venous beading • Intraretinal microvascular abnormalities • Optic nerve or retinal neovascularization
Fluorescein angiography (FA)	• Gold standard for visualizing vasculature • Better sensitivity for low flow vascular lesions than OCTA • Able to capture peripheral lesions	• Invasive and time-consuming • Contrast dye has potential adverse reactions • Leakage of dye can obscure details of vascular structures	• Microaneurysm • Vascular leakage • Non-perfusion • Optic nerve or retinal neovascularization
Optical coherence tomography (OCT)	• Non-invasive method of obtaining cross-sectional images • Gold standard for diagnosis of DME and monitoring of treatment response	• Cannot visualize vascular changes • Requires subject fixation	• Macular edema • Epiretinal membrane • Retinal thinning • Vitreomacular adhesion • Intraretinal cysts • Disorganized retinal inner layers (DRIL)
Optical coherence tomography angiography (OCTA)	• Non-invasive imaging of vasculature • Able to delineate capillary plexuses • Lack of staining allows for visualization of vascular details • Allows for quantification of non-perfusion and vessel density	• Good fixation needed for high-resolution images • Limited view of periphery • Susceptible to projection artifact from anterior structures • Not widely available	• Microaneurysms • Enlarged foveal avascular zone • Areas of non-perfusion • Abnormal vascular loops • Optic nerve or retinal neovascularization

two automated DR imaging assessment systems (ARIAS), EyeArt and Retmarker, as having high sensitivity (93.8% and 97.9%, respectively) for identifying referable retinopathy and suggested these systems as a cost-effective screening tool [16•]. A subsequent study by Eyenuk, Inc. demonstrated that the EyeArt system has high sensitivity and specificity (95.8% and 80.2%, respectively) for detecting any DR even when applied to smartphone fundus photography [17].

The American Food and Drug Administration (FDA) recently approved the first ever autonomous artificial intelligence diagnostic system based on the results of the iDx-DR clinical trial [18•]. The iDX-DR trial involved 900 patients across 10 sites and was the first to prospectively analyze the performance of an automated DR identification system. The system exceeded pre-specified superiority endpoints with a sensitivity of 87.2% (95% CI, 81.8–91.2%) and specificity of 90.7% (95% CI, 88.3–92.7%) for identifying more than mild DR and DME using the DTDRS severity scale as a standard. The introduction of autonomous artificial intelligence systems into healthcare is uncharted territory; however, there is great potential to increase accessibility of DR screening and decrease costs.

Ultra-widefield (UWF) fundus photography is based on a technology that captures up to 200° of field in a single image compared to standard fundus photography which captures between 30 to 55° of field in a single image. A recent study demonstrated that UWF imaging not only has good agreement with the ETDRS standard for grading diabetic retinopathy [19] but also confers several additional advantages such as decreased image acquisition time. Additionally, UWF imaging reduces rate of ungradable images by 71% and identified DR 17% more frequently than single field fundus photography [20, 21]. There is also potential prognostic significance since UFW imaging can detect peripheral lesions and eyes with predominantly peripheral lesions have been shown to have a 4.7-fold increased risk for progression to PDR compared to eyes without predominantly peripheral lesions [22].

Fluorescein Angiography

Fluorescein Angiography (FA) is a technique that uses fluorescent dye to examine retinal vasculature in vivo. The major advantage of FA is that it provides information on vascular flow and vessel permeability over time [23]. The dye allows high contrast visualization of lesions such as microaneurysms and areas of non-perfusion which can assist with staging and monitoring of DR. Leakage of the dye seen on FA can identify the source of vessel breakdown or neovascularization for targeted laser treatments.

Like fundus photography, UWF technology and automated software analysis have also recently been applied to FA. In a study of 218 eyes of diabetic patients, UWF FA was able to detect retinal pathology not evident on traditional imaging in 10% of eyes [24]. Increased peripheral ischemia on UWF FA imaging has been associated with presence of DME and automated calculation of ischemia index using UWF FA has demonstrated larger areas of non-perfusion in patients with recalcitrant DME that required extensive treatment. Eyes with the highest ischemic index required 5.7 photocoagulation treatments compared with 2.3 treatments for eyes with the lowest ischemic index [25]. Additionally, a recent study reported a fully automated algorithm capable of quantifying area of leakage in FA that is 86% accurate when compared with expert manual segmentation [26].

The major disadvantage to FA is that it is an invasive and time-consuming procedure. The contrast dye used in FA can have many potential side effects such as nausea, vomiting, urticaria, seizures, anaphylaxis, and even death [27, 28]. The advantages of FA must be weighed against the possible risks and alternatives of using less invasive imaging procedures.

Optical Coherence Tomography

Optical coherence tomography (OCT) is a rapid, non-invasive imaging modality that uses light waves to render a cross-sectional view of the retina in vivo. It creates high-resolution images of retinal morphology allowing volumetric quantification of retinal and choroidal thickness and macular edema. OCT allows clinicians to detect macular edema in patients before retinal thickening is visible on slit lamp microscopy [29], is more sensitive than fundus photography, and is the gold standard for diagnosis of DME [30]. Additionally, OCT can be used to distinguish between subtypes of DME, identify presence of macular traction, and localize edema to specific layers of the retina [31, 32]. Disorganization of retinal inner layers (DRIL) on OCT is a proposed biomarker that is not only associated with visual acuity but can also predict future changes in vision in patients with DME [33, 34]. DRIL is defined as the loss of boundaries between the ganglion cell, inner plexiform, and outer nuclear plexiform layers of the retina on OCT, measured as the transverse extent. DRIL has a greater correlation ($p < 0.0001$) with visual acuity than other common OCT parameters such as epiretinal membrane ($p = 0.5307$), ellipsoid zone disruption ($p = 0.4046$), and presence of large cysts ($p = 0.2250$) [34]. Furthermore, a 300 μm increase in disorganization at 4 months is associated with a one-line decrease in visual acuity at 8 months [33]. Further studies are needed to confirm results, create a consensus, and establish guidelines before DRIL can be a useful tool in clinical practice. Recent advances in enhanced depth imaging OCT have allowed better visualization of the choroid, which is a subject of controversy in DR and DME [35]. The current evidence is conflicting regarding whether the choroid is thicker or thinner in diabetic eye disease [36, 37].

OCT is the gold standard for monitoring treatment response in DME and is widely used in both clinical and research

settings [38, 39]. However, a major limitation of OCT compared with FA is that it is unable to provide functional information since it only captures images at one point in time.

Optical Coherence Tomography Angiography

OCT angiography (OCTA) is a recent addition to OCT that allows visualization of retinal and choroidal vasculature without the use of contrast dye. OCTA generates images though analysis of light reflecting off red blood cells as they move through vessels in the retina. Thus, OCTA is not only able to create a highly detailed three dimensional image of retinal and choroidal vasculature but also able to provide information about vascular flow at a given time point. This allows for identification of both areas of high flow and ischemia, which are important aspects of DR.

OCTA has many advantages when compared with traditional forms of imaging. It provides similar information as FA, such as areas of non-perfusion and microaneurysms, without the use of contrast dye [40] and is better able to visualize neovascularization [29]. OCTA is unique because it is able to distinctly analyze each of the three retinal capillary plexuses [41•, 42] which is important for understanding the pathophysiologic changes in DR. For example, a recent study found that increased FAZ and microaneurysms in the deep capillary plexus (DCP), but not superficial capillary plexus (SCP), were predictive of response to anti-VEGF treatment in DME [43]. Further investigation into the middle capillary plexus (MCP), a distinct plexus that has previously been segmented with the SCP and DCP, may provide additional insights into the pathological ischemic state of DR [41•]. Figure 1 shows SCP, MCP, and DCP segmentation as well as common DR/DME findings on OCTA.

Additionally, OCTA can detect changes associated with DR even before clinical signs of DR are evident [44, 45]. A recent study found that SCP foveal avascular zone (FAZ), DCP vessel density, and acircularity of the fovea were the best parameters to distinguish DR severity groups. Receiver operating characteristic curves using these parameters for no DR versus any DR showed an area under the curve of 0.946 ($p < 0.001$) with sensitivity of 89% and specificity of 96% [46]. Other parameters, such as blood flow index, vessel branching complexity, and vessel caliber, have been shown to correlate with severity of DR [47•, 48] and could allow for an objective, quantitative way to distinguish between patients with different severities of DR [49, 50] that may augment clinical judgment. However, there is currently no consensus in the literature regarding the best parameters to use. Furthermore, a wide variety of algorithms are used to segment OCTA images and different nomenclature is used by different researchers which creates difficulties in communicating results within the field [51].

Because OCTA is still a new technology, large, prospective studies are still needed to evaluate and validate these measures. There are several different OCTA devices that are commercially available, including AngioVue™ (Optovue Inc., Fremont, Calif., USA), AngioPlex™ (Carl Zeiss Meditec Inc., Dublin, Calif., USA), and Spectralis® OCTA (Heidelberg Engineering, Heidelberg, Germany) to name a few. Detailed comparisons of each device, specific algorithms and characteristics in terms of image quality (vessel visibility, number of artifacts), and data acquisition time and reliability can be found elsewhere [52, 53]. Limitations of OCTA include small field of view (typically a 3 mm × 3 mm or 6 mm × 6 mm parafoveal field), inability to show leakage, lack of general consensus and proclivity to artifact [51, 54].

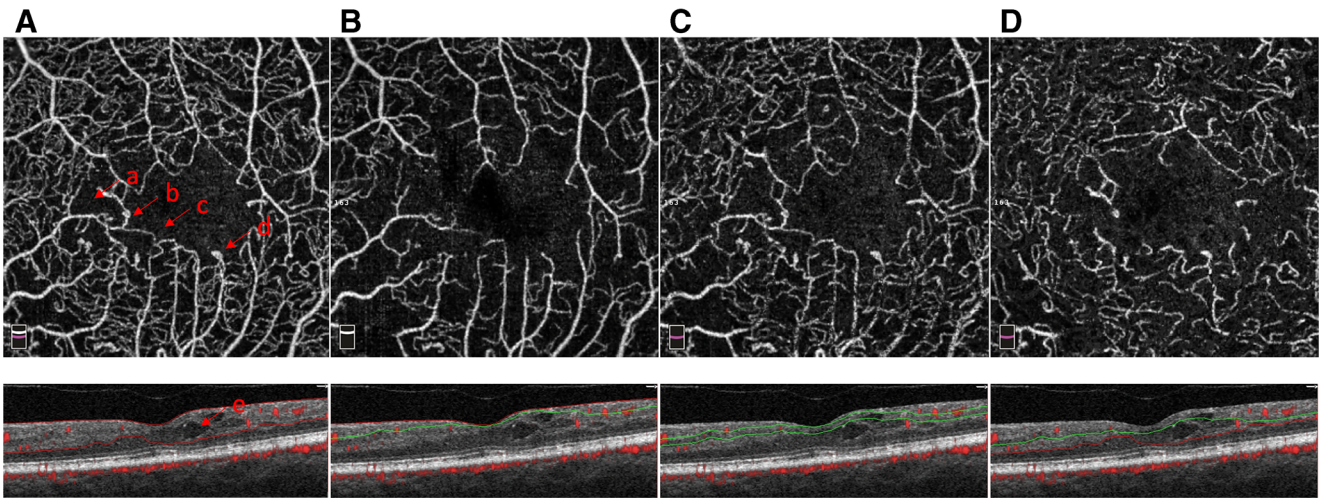

Fig. 1 OCTA of Diabetic Macular Edema. OCTA of (A) full retina, (B) superficial capillary plexus (SCP), (C) middle capillary plexus (MCP), and (D) deep capillary plexus (DCP) with cross-sectional OCTA below including *red* flow overlay and *red* and *green* segmentation boundaries. Common features of diabetic retinopathy: (a) area of non-perfusion, (b) microaneurysm, (c) enlarged foveal avascular zone, (d) abnormal vascular loop, (e) intraretinal cyst

Artifacts in OCTA, due to image acquisition, eye motion, intrinsic ocular characteristics, image processing, and display strategies, can lead to inaccurate image interpretation and methods to address these issues are constantly under development [55]. For example, projection artifacts, where vascular structures of superficial layers are incorrectly displayed in deep layers, occur in virtually all OCTA images [56]. New projection-resolved OCTA (PR-OCTA) algorithms attempt to minimize these artifacts and preserve the image integrity of deeper layers [57]. Despite these limitations, OCTA is nonetheless a very promising imaging technology that has the potential to change clinical practice.

Biomarkers in Diabetic Retinopathy

A biomarker is a measurable, objective indication of a normal or pathologic biological process. Biomarkers can be used to detect early disease, identify patients most at risk for progression of disease, and develop new treatments. For DR and DME, there have been many potential biomarkers suggested in the literature and we will outline the most relevant circulating, ocular, and genetic biomarkers.

Circulating Biomarkers

Many systemic biomarkers have been linked to DR, but glycated hemoglobin (HbA1c) is the only validated marker routinely measured in clinical practice. The Diabetes Control and Complications Trial showed that reduction in A1c via intensive glycemic control is associated with a 35–76% reduction in early stages of microvascular disease, including DR [58]. Several other large clinical trials results have replicated these results [59, 60].

It is also well known that inflammation plays a large role in DR and many markers of inflammation are linked to DR [61, 62]. C-reactive protein (CRP), an acute phase protein that is widely used as marker of inflammation, has the strongest evidence for association with DR. It is increased in patients with DR compared with healthy subjects (standardized mean difference (SMD) = 0.22, 95% confidence interval (CI), 0.11–0.34) and increased in PDR compared to NPDR (SMD = 0.50, 95% CI 0.30–0.70) [63]. Furthermore, the association between CRP and DR is stronger in the obese population. The odds ratio for increased CRP in all patients with vision-threatening diabetic retinopathy was 1.26 while the odds ratio for patients with vision-threatening diabetic retinopathy and BMI > 30 kg/m^2 was 2.9 [62]. Obesity is a recognized risk factor for DR and is associated with a chronic subacute inflammatory state. However, further studies are needed to confirm these results and investigate the role of CRP as a biomarker for severity of DR.

Advanced glycation end products (AGE) are the result of non-enzymatic glycation of proteins and are of interest because they are directly related to the pathology of diabetes. AGEs are both directly toxic to body tissues and cause damage through activation of specific AGE receptors (RAGE) [64]. Several AGEs including, N-epsilon-carboxymethyl lysine (N-ε-CML) and pentosidine, have been suggested as potential biomarkers for detecting early DR and for predicting progression [65]. Serum levels of both AGEs were found to be significantly increased in patients with diabetes compared with controls ($p < 0.001$) as well as patients with PDR compared to those with NPDR ($p < 0.001$) [65]. Additionally, a recent study demonstrated that RAGE knockout mice are protected against DR [66] and proposed inhibition of RAGE as a therapeutic option to prevent DR. RAGEs are implicated in a variety of diabetic complications and represent a promising avenue for research and RAGE targeting therapeutics.

Ocular Biomarkers

Vitreous Biomarkers

Vascular endothelial growth factor (VEGF) is the most well-established vitreous biomarker for DR and plays a crucial role in the pathogenesis of DR through stimulation of neovascularization and vascular leakage [67]. Identification of VEGF and development of anti-VEGF therapy have revolutionized DR therapy. However, not all patients respond to anti-VEGF and the treatments have side effects as well as a short effective periods which necessitates repeat injections [68]. Thus, there is an ongoing search for other clinically relevant biomarkers that have potential to be therapeutic targets. Currently, a wide range of molecules from inflammatory mediators to growth factors have been suggested as possible biomarkers. However, there is considerable variability in results due to small sample sizes and variation in methods. Table 2 is a summary of vitreous biomarkers proposed in recent studies [69–75].

A recent systemic review identified 11 statistically significant vitreous biomarkers for PDR and 1 for DME [76•]. Of these, several are considered currently viable therapeutic candidates. First, there are increased levels of platelet-derived growth factor BB chain (PDGF-BB), a molecule known to induce expression of VEGF, in diabetic eyes [77]. Animal studies have shown that anti-PDGF-BB in conjunction with anti-VEGF is more effective at inhibiting neovascularization than anti-VEGF alone [72, 78, 79]. Second, pigment epithelium-derived factor (PEDF) is an inhibitor of angiogenesis that is found in high concentrations in the vitreous humor of the eye [80]. PEDF is decreased under hypoxic conditions such as DR and studies using mouse models have shown that intravitreal injections of PEDF can decrease neovascularization two fold and reduce retinal vascular leakage by 60% [81, 82]. Third, ischemia stimulates erythropoietin (EPO), which

Table 2 Summary of recent studies of vitreous biomarkers in DR

Author, year	Sample characteristics	Results
Funastsu 2009 [71]	53 DME vs 15 controls	Increased: VEGF, ICAM-1, IL-6, MCP-1 Decreased: PEDF
Praidou 2009 [72]	31 PDR vs 15 controls	Increased: PDGF-AA, PDGF-AB, PDGF-BB, VEGF
Adamiec-Mroczek 2010 [69]	19 PDR vs 15 controls	Increased: ET-1, TNF-α, IL-6, vWF, sE-selectin
Suzuki 2011 [73]	76 PDR/DME vs 23 controls	Increased: IL-6, IL-8, IL-10, IL-13, IP-10, MCP-1, MIP-1β, PDGF, VEGF No difference: IL-1β, IL-1ra, IL-4, IL-5, IL-7, IL-9, IL-12, IL-15, IL-17, exotaxin, b-FGF, G-CSF, GM-CSF, IFN-γ, MIP-1α, RANTES, TNF-α
Zhou 2012 [75]	62 PDR vs 15 controls	Increased: IL-1B, IL-6, IL-8, CCL2, EDN1, VEGF, and TNF No difference: IL-10
Chernykh 2015 [70]	32 PDR vs 25 controls	Increased: VEGF, PEDF, IL-17A, IL-4, IL-6, IL-8, sIgA No difference: IL-10, MCP-1
Tsai 2018 [74]	17 DR vs 17 controls	Increased: VEGF, VEGF-A, PGF, IL-1B, IFN-γ No difference: IL-2, IL-4, IL-6, IL-13

ET-1, endothelin-1; *TNF-α*, tumor necrosis factor alpha; *IL*, interleukin; *vWF*, von Willebrand factor; *sE-selectin*, soluble E-selectin; *VEGF*, vascular endothelial growth factor; *PEDF*, pigment epithelium-derived factor; *sIgA*, secretory immunoglobulin A; *MCP-1*, monocyte chemotactic protein 1; *ICAM-1*, intercellular adhesion molecule 1; *PDGF*, platelet-derived growth factor; *IP-10*, interferon-inducible 10-kDa protein; *MIP*, macrophage inflammatory protein; *b-FGF*, basic fibroblast growth factor; *G-CSF*, granulocyte colony-stimulating factor; *GM-CSF*, granulocyte/macrophage colony-stimulating factor; *IFN-γ*, interferon gamma; *RANTES*, regulated upon activation, normal T cell expressed and presumably secreted; *CCL2*, chemokine c-c motif ligand 2; *EDN1*, endothelin 1

in turn activates VEGF and contributes to neovascularization. EPO modulation has been shown to decrease VEGF-A expression by 48% and is hypothesized as a novel treatment for early DR due to its potential neuroprotective effect on the retina [83].

Aqueous Biomarkers

Analysis of aqueous fluid samples from diabetic patients compared with controls has revealed several inflammatory and angiogenic factors that are significantly elevated in DR [84]. As in the vitreous, VEGF is significantly elevated in the aqueous humor of eyes with DR and is correlated with severity of DR [67, 85]. A recent study investigated 45 different cytokines, chemokines, and growth factors in patients with DR compared with non-diabetic patients and identified several that were significantly different [86]. In particular, IL-6, a proinflammatory and angiogenic factor, was found to increase with DR stage (PDR 47.68 vs NPDR 29.68 pg/ml; $p < 0.001$). Additionally, aqueous levels of VEGF and IL-6 have been shown to correspond significantly with vitreous levels ($p = 0.793$, $p < 0.0001$; $p = 0.737$, $p < 0.0001$, respectively) [85]. This is promising because aqueous fluid is easier and less risky to obtain though vitreous fluid is thought to be a better index for DR since it is a posterior segment fluid and DR is a posterior segment pathology.

Another study found that markers of retinal glial cell activation, such as glial fibrillary acidic protein (GFAP), aquaporin 1 (AQP1), and aquaporin 4 (AQP4), are elevated ($p < 0.05$ for all) in diabetic patients with no or early NPDR compared with controls [87]. Glial cell dysfunction is an important part of the pathogenesis of DR [88] and these results represent potential biomarkers for early detection of DR. However, this was a small study and large population studies are needed to confirm these results before a robust biomarker can be developed.

Intercellular adhesion molecule 1 (ICAM-1) has also been identified as a crucial biomarker in DME, correlating with greater macular volume on OCT [89]. A subsequent study investigated the relationship between baseline aqueous biomarkers and the response to intravitreal ranibizumab treatment in DME. Of the 9 biomarkers studied, these authors found that higher ICAM-1 and lower VEGF at baseline were the only biomarkers associated with a favorable anatomic response to three monthly injections of ranibizumab as measured by

macular volume on OCT ($p = 0.01$ and $p = 0.02$, respectively) [90]. This study highlights the potential of ocular biomarkers not only to identify new therapeutic targets but also to predict response to treatment and guide treatment algorithms.

Genetic Biomarkers

Heritability estimates for DR range from 27 to 52% [91, 92], which is consistent with a substantial genetic component. Genome-wide association studies have implicated variants at many loci but that may be linked to DR. Some potential candidates include *GRB2*, a gene involved with angiogenesis [93], *AKR1B1*, a gene which encodes aldose reductase [94], and *VEGFA* [95], the gene that encodes VEGF. However, these studies have not been consistently replicated and there are currently no alleles that are widely accepted to have association with risk for DR. In addition to nuclear genes, epigenetic modifications, mitochondrial DNA, and RNA biomarkers have also been explored.

There is evidence that chronic hyperglycemia leads to metabolic memory via epigenetic modifications, which in turn plays a critical role in DR pathogenesis and has potential for novel therapeutics [96]. For example, hyperglycemia increases expression of TXNIP which induces Cox2 expression through epigenetic modifications in the histone code of the Cox2 promotor [97]. This process is known to contribute to development of DR since inhibition of TXNIP has been shown to block DR progression [98, 99]. More generally, enhanced histone acetylation is linked to progression of DR and inhibition of histone acetyl-transferases blocks these epigenetic modifications and DR progression [100].

Altered mitochondrial DNA (mtDNA) is also implicated in the pathogenesis of DR. Hyperglycemia damages mtNDA and is linked to retinal capillary cell degeneration and overproduction of retinal reactive oxygen species [101, 102]. Increased serum levels of mtDNA and d-loop damage of mtDNA are significantly ($p < 0.05$) increased in patients with diabetic retinopathy when compared with patients with diabetes without retinopathy, which indicates a potential for mtDNA to serve as a biomarker [103]. Finally, MicroRNAs are small, non-coding RNAs that have been proposed as possible diagnostic biomarkers as well as drug targets for DR [104]. A recent study found 3 key serum miRNAs that modulate angiogenesis and were significantly different between NPDR and PDR patients [105]. However, these findings have yet to be translated into clinical practice.

Though promising, more research is needed in order to validate these genetic biomarkers across populations and to develop time and cost-efficient analytical techniques that will allow for wide spread clinical use.

Conclusions

Recent advancements in retinal imaging, such as UWF imaging and deep learning algorithms, have the potential to improve access to screening and lead to faster and more accurate detection and staging of DR [15, 21]. New technologies like OCTA are able to detect pathological changes before clinical signs of DR are evident as well as visualize abnormalities in each of the three macular capillary plexuses and give new insights into the diseased state [42, 45]. Identification of novel systemic, ocular, and genetic biomarkers may help detection and prognostication of disease as well as lead to new treatment targets.

Compliance with Ethical Standards

Conflict of Interest The authors declare that they have no conflict of interest.

Human and Animal Rights and Informed Consent All reported studies/experiments with human or animal subjects performed by the authors have been previously published and complied with all applicable ethical standards including the Helsinki declaration and its amendments, institutional/national research committee standards, and international/national/institutional guidelines.

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