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Novel imaging biomarkers in diabetic retinopathy and diabetic macular edema

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***Abstract*:** Diabetic retinopathy is one of the major microvascular complications of diabetes mellitus. The most common causes of vision loss in diabetic retinopathy are diabetic macular edema and proliferative diabetic retinopathy. Recent developments in ocular imaging have played a significant role in early diagnosis and management of these complications. Color fundus photography is an imaging modality, which is helpful for screening patients with diabetic eye disease and monitoring its progression as well as response to treatment. Fundus fluorescein angiography (FFA) is a dye-based invasive test to detect subtle neovascularization, look for areas of capillary non-perfusion, diagnose macular ischemia, and differentiate between focal and diffuse capillary bed leak in cases of macular edema. Recent advances in retinal imaging like the introduction of spectral-domain and swept source-based optical coherence tomography (OCT), fundus autofluorescence (FAF), OCT angiography, and ultrawide field imaging and FFA have helped clinicians in the detection of certain biomarkers that can identify disease at an early stage and predict response to treatment in diabetic macular edema. This article will summarize the role of different imaging biomarkers in characterizing diabetic retinopathy and their potential contribution in its management.

Keywords: biomarkers, diabetic macular edema, fluorescein angiography, optical coherence tomography, optical coherence tomography angiography, retinopathy

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Introduction

Diabetic retinopathy (DR) is one of the leading causes of blindness in the middle-age population.[1] Among patients with DR, diabetic macular edema (DME) is the leading cause of moderate visual loss.[2] In the current era, imaging modalities play an important role in deciding the treatment protocol as well as prognosticating outcome. They also have significant utility in screening patients with diabetes mellitus to rule out any features of DR at an early stage.

Recently published literature on DME has focused on several novel clinical, laboratory, and imaging biomarkers. Laboratory biomarkers that aid in determining the disease severity and response to therapy include levels of cytokines and inflammatory markers in serum, vitreous, aqueous, and tear fluid.[3] However, most of these tests are invasive, and their clinical applicability is still under investigation. Imaging biomarkers provide an in vivo assessment of the health of the retina and choroid non-invasively. These tools provide a near-histological assessment of various alterations, including retinal capillary densities, non-perfusion, vascular remodeling, and foveal avascular zone (FAZ) area. Recently, there has been an interest in determining the prognostic value of changes on optical coherence tomography (OCT) such as foveal intraretinal cysts and edema, including size and location, presence of subretinal fluid (SRF), and integrity of retinal layers in DME.[4]

In this review, we have summarized various studies that have provided insights into the utility of tools such as OCT and OCT angiography (OCTA) in DR and DME. This review provides

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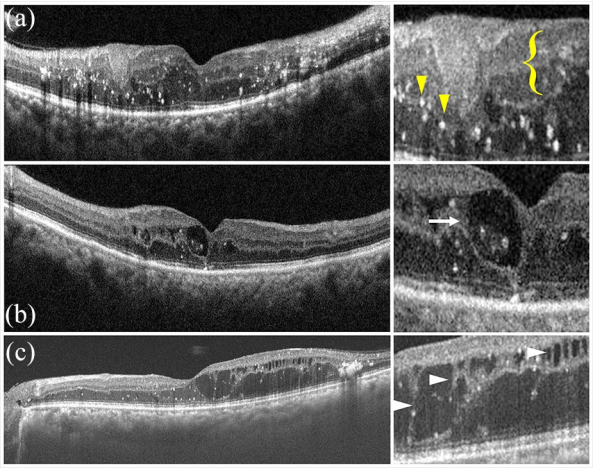

Figure 1. The optical coherence tomography (OCT) signs of diabetic macular edema (DME). In panel (a), a patient with DME and multiple hyperreflective retinal foci (HRF) is shown. The magnified image shows the presence of disorganization of retinal inner layers (DRIL; yellow bracket) with multiple HRF mainly in outer retinal layers (yellow arrowheads). OCT of another subject (b) shows the presence of few HRF in outer nuclear layer with few cystic spaces (white arrow). The OCT scan of another patient shows bridging retinal processes between the cystic cavities (white arrowheads) (c).

a comprehensive summary of various imaging techniques and novel biomarkers that help in determining the functional outcomes of subjects with DR and DME.

Since this is a narrative review, no formal search strategy was used to compile the studies from literature.

OCT

OCT is an imaging modality that provides high-resolution cross-sectional images of the neurosensory retina and choroid by processing the backscattered light. With the advancement in technology, better OCT tools are now available compared with the previously available time-domain OCT. Presently, spectral-domain (SD) and swept-source (SS) OCT are the most advanced technologies available to image the fundus. The value of OCT in DME was first established by the Prospective OCT Study With Lucentis for Neovascular AMD study, which used OCT as a tool to monitor the intraretinal and SRF fluid to decide further therapy with anti-vascular endothelial growth factor (anti-vascular endothelial growth factor [VEGF]) agents.[5] Since then, various studies have used the pro re nata (PRN) approach based on OCT imaging in the treatment of DME with anti-VEGF agents. As OCT is non-invasive, it was quickly adopted by clinicians for the assessment of patients with DME. Currently, OCT is an invaluable and indispensable tool in patients with diabetes to determine the need for treatment and prognosticate patients with DME.

Disorganization of retinal inner layers

Disorganization of retinal inner layers (DRIL) is defined as the inability to distinguish between the ganglion cell layer–inner plexiform layer complex, inner nuclear layer, and outer plexiform layer. DRIL can be associated with or without center-involving DME.[6] DRIL is measured on OCT B-scans by looking at the central 1 mm retinal zone. Disorganization of more than 50% or >500 µm of this area is considered significant and is associated with worse visual prognosis in eyes with edema or resolved edema. DRIL has been found to be a reliable biomarker for prognosis of visual acuity in DME. Inner retinal layers consist of axons, bipolar cells, and nuclei of amacrine cells, all of which are important for transmission of visual signals from photoreceptors to the ganglion cell layer. DRIL signifies damage to these structures leading to abnormal visual processing. Sun and colleagues found that early change in DRIL predicted the visual outcomes during the course of treatment. An increase in DRIL during 4 months predicted a decline of visual acuity by one line.[6] Figure 1 depicts DRIL.

Recent studies have correlated DRIL with the amount of maculopathy present even in the absence of any DME.[7] DRIL has been correlated with macular capillary non-perfusion and size of FAZ.[8,9] DRIL, in recent studies, has been associated with increasing severity of DR, especially proliferative DR (PDR).[10] The presence of DRIL is associated with outer retinal layer disruption, specifically ellipsoid zone (EZ) and external limiting membrane (ELM).[10] Taken together, DRIL appears to be a useful OCT biomarker for visual acuity assessment, capillary perfusion, and other morphological changes in DME.

Joltikov and colleagues[11] have studied DRIL in diabetic patients with no evidence of DR or early DR to elucidate the effects of DRIL in early neuroretinal disease. It can be used as a potential clinical marker of visual function to test potential therapies for diabetic patients with early stages of neuroretinal impairment.

Hyperreflective retinal foci

Hyperreflective retinal foci (HRF) appears as intraretinal hyperreflective dots on OCT in subjects with retinal pathologies such as DME (Figure 1). HRF represent subclinical lipoproteins that extravasate after breakdown of inner blood–retinal barrier.[12,13] Subretinal HRF are associated with subfoveal hard exudates after resolution of subretinal serous detachment.[14] In contrast to other studies, Lee and colleagues[15] proposed that HRF represent activated microglial cells since they determined the presence of increased soluble CD14 in the aqueous humor. Soluble CD14 is released by activated microglial cells. HRF are initially present in the inner retinal layers and subsequently migrate to the outer retinal layers. The features of HRF include size less than 30 μm, absence of back-shadowing, and reflectivity similar to retinal nerve fiber layer.

HRF are important imaging markers of retinal inflammation.[16] The size and number of HRF may decrease after treatment with anti-VEGF and corticosteroid implants. In a recent study, corticosteroid implants were shown to have better outcomes compared with anti-VEGF agents in subjects with DME in the presence of HRF. The study showed that the initial presence of a larger number of HRF responded better to dexamethasone implants than anti-VEGF therapy.[17] Further investigations that correlate HRF with visual acuity are needed. Recent literature suggests that a higher number of HRF on SD-OCT is associated with early recurrence of DME after steroid implant. As a result, patients with an increased number of HRF on OCT should be frequently followed up for early intervention if required.[18]

Hyperreflective choroidal foci

Hyperreflective choroidal foci (HCF) is a recently described entity, which are HRF seen in choroidal layers in DME.[19] Such HRF in choroid have been described in Stargardt's disease as lipofuscin depositions. It is believed that HCF have migrated from the retina into choroidal layers with disruption of the ELM and EZ. The presence of HCF is a poor prognostic marker in terms of visual acuity. HCF were found to be present significantly more in PDR eyes than NPDR (non-proliferative DR) eyes.

Intraretinal cystoid spaces

The size and location of intraretinal cystoid spaces are relevant in the functional outcomes of subjects with DME. Elevated VEGF level in DR affects the inner blood–retinal barrier leading to increased vascular permeability resulting in a decreased osmotic gradient, extracellular fluid accumulation, and cyst formation, while the outer blood–retinal barrier is not affected significantly (Figure 1).[20,21] Cystoid spaces in DME, unlike the cyst in cystoid macular edema (CME), can cause photoreceptor damage and affect visual outcomes.[22] Large cysts (>200 μm) in the outer nuclear layer (ONL) are seen in late stage of DME and have a negative impact on macular function (seen by microperimetry) than smaller cysts or cystoid formation occurring in inner retinal layers.[23,24] This is because cysts in the ONL have shown to damage photoreceptor cells and disrupt inner segment–outer segment (IS/OS) junction, thus causing irreversible damage to visual functions.[25]

Treatment with anti-VEGF therapy leads to a decrease in the number and size of ONL cysts by decreasing the permeability of the inner blood–retinal barrier. This was associated with improvement in best-corrected visual acuity and microperimetric retinal sensitivity.[24]

Hyperreflective signals within the cyst are associated with severe disruption of the blood–retinal barrier. There is paucity of information in the literature on the contents of these intraretinal hyperreflective signals. In one study,[26] the authors hypothesize that fibrin and other inflammatory by-products may fill these spaces. Treatment with anti-VEGF agents did not seem to directly change their natural course.

Kashani and colleagues[27] have described this intraretinal reflective material as suspended scattering particles in motion (SSPiM) on OCTA. SSPiM which appears as an extravascular signal on OCTA is likely due to Brownian motion of particles within intraretinal fluid pockets. SSPiM are usually found at vascular–avascular junctions and in some cases resolve with formation of hard exudates. Extravasated lipid is believed to be involved in the formation of these SSPiM and subsequently hard exudates.

The intraretinal cystoid spaces can be categorized based on their size. The cysts have been classified as small (<100 μm), large (101–200 μm), or giant (>200 μm). Large cysts are associated with poor visual prognosis. Size of the cyst is correlated with the extent of macular ischemia. Both horizontal

and vertical diameter of cyst increases with severity of macular ischemia.[28]

Other parameters to be considered while analyzing CME include location of the cyst about the center and its lateral extension, the degree of the anatomical damage caused to the inner and outer layers by the cystoid change, any associated photoreceptor damage or retinal pigment epithelial (RPE) damage, and the presence of associated SRF.[23,29] All these characteristics may influence baseline visual acuity and visual outcomes in response to treatment.

Bridging retinal processes

Recently, a study by Farhan and colleagues[30] demonstrated that bridging retinal processes which are seen in between the cystic cavities is associated with improved visual acuity after treatment. These tissues represent residual neural elements, which connect outer and inner retina and thus help in transmitting visual impulses from the inner retinal layers to the optic nerve axons. Although exact nature of these tissues is not known, it is believed that Muller cells and bipolar cells are an important component.

In contrast to this, absence of these bridging retinal tissues between inner and outer retina have poor prognosis post treatment. These eyes are unlikely to improve despite resolution of cysts and end up with foveal atrophy and thinning.[31]

Retinal thickness

The primary cause for increased retinal thickness in DME is intra- and subretinal edema. Edema occurs as a result of breakdown of blood–retinal barrier and extravasation of lipid in the intraretinal space. Central subfield thickness (CST) can be easily measured on SD-OCT, and measurement of CST is invaluable in assessing the degree of DME. When the retinal thickness increases beyond the stretching capability limit of retina, it can lead to damage to the bipolar axons leading to decreased visual signal transmission. Thus, despite resolution of DME, the gain in vision may be suboptimal. Often, resolution of DME is accompanied by macular atrophy due to permanent damage to the photoreceptors. An important consideration is that CST is not a reliable indicator of visual acuity. CST is not prognostic or predictive of final outcomes. It is always important to look for other associated biomarkers like pattern of DME, extent of retinal involvement, and differential involvement of inner versus outer retinal tissue. Cross-sectional area of retinal tissue between the plexiform layers in CME is a better predictor of visual acuity than macular thickness (80% *versus* 14%).[31]

Subfoveal choroidal thickness

Studies suggest that baseline subfoveal choroidal thickness is a predictor of response to anti-VEGF therapy.[32] A greater baseline choroidal thickness was independently associated with better anatomical and functional response to anti-VEGF therapy. Patients with greater choroidal thickness are presumed to have an intact choriocapillaris and thus a less ischemic outer retina. This means the photoreceptor function is well preserved in these eyes, thus explaining superior visual outcomes following anti-VEGF therapy.

Choroidal thickness increases with increasing severity of DR, owing to increased VEGF production. Increased VEGF levels might result in vasodilation of choroidal vessels and subsequently increased choroidal thickness.[33]

Photoreceptor outer segment

Photoreceptor outer segment (PROS) is defined as the length between the photoreceptor inner and outer segment junction and the RPE. Studies have shown that PROS length correlated better than macular thickness at the fovea with visual acuity in patients with DME.[34] Opsin plays an important role in regulation of visual cycle. It is believed that outer segments which act as opsin reservoirs are more important than inner segments as the source of adenosine triphosphate. Therefore, PROS may be considered as an important prognostic biomarker in eyes with DME.

Ozkaya and colleagues[35] have shown that PROS length was significantly less in eyes with DR or DME compared with healthy subjects or diabetic patients with no retinopathy. More extensive studies are required to judge the true predictive power of this index in future.

Hard exudates

Hyperreflective spots which are larger than 30 μm, associated with back-shadowing, present in the outer retinal layers, having reflectivity similar to RPE–Bruch's complex are suggestive of hard

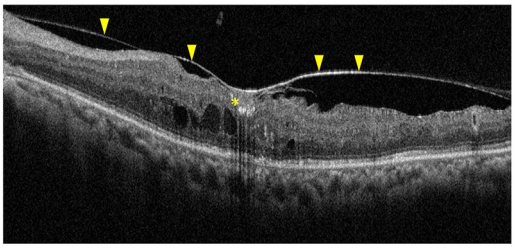

Figure 2. Optical coherence tomography (OCT) scan of a patient with diabetic macular edema shows a thick posterior hyaloid membrane (TPHM; yellow arrowheads) with increased retinal thickening. There are hard exudates in the inner retina (yellow asterisk) causing back-shadowing of OCT signal.

exudates in retinal OCT (Figure 2).[36] Hard exudates are lipo-proteinaceous (albumin and fibrin) deposits that accumulate due to breakdown of inner blood–retinal barrier. Quantitative measurement of hard exudates in patients with DME has been associated with serum lipid levels; high triglyceride levels can increase the risk of central macular involvement causing accumulation of subfoveal hard exudates.[37,38] Quantitative information may be useful to monitor progression of hard exudates and treatment response in diabetic maculopathy.[39] Intravitreal steroids (triamcinolone, dexamethasone implants) may be more effective in reducing hard exudates in patients with DME compared with anti-VEGF agents, especially when the exudates are subfoveal.[40]

Subfoveal neurosensory detachment

The prevalence of subfoveal serous retinal detachment in subjects with DME is 15–30%. Serum albumin has been shown to be a sensitive marker for the presence of SRF.[41] Hypoalbuminemia can lower the intravascular osmotic pressure. This along with increased hydrostatic pressure can cause retention of fluid in the subretinal space.

Presence of SRF in DME is an important OCT biomarker. The role of SRF in final visual and anatomical outcomes is still confusing. There are a number of studies which have shown that the presence of SRF is associated with good anatomical and functional gains. Contrary to this, there are other studies which have shown that the presence of SRF is associated with poor visual gains.[42,43] Results from RESTORE study[44] and post hoc analysis from RISE/RIDE study proved the protective role of SRF.[45] These studies showed that the presence of baseline SRF was associated with better visual gains at the end of 1 year. These studies also showed a positive impact from SRF in response to ranibizumab therapy. Furthermore, eyes with SRF were shown to have better visual gains in a study evaluating the effect of vitrectomy in diffuse DME.[46]

Post hoc analysis of VIVID-DME and VISTA-DME studies showed that visual outcomes were better with intravitreal aflibercept than laser, regardless of baseline SRF status, although greater treatment effect of intravitreal aflibercept was seen in patients with baseline SRF than those who did not.[47]

Moon and colleagues[48] have shown DME eyes with SRF to respond significantly with dexamethasone implants and advocate the use of dexamethasone implants in patients with DME with SRF. Eyes with SRF have shown increased Interleukin (IL)-6 levels, signifying active inflammation in these eyes.[49] Zun and colleagues[4] have shown that the presence of SRF is associated with a better response with dexamethasone implants.

To conclude, our understanding of the relationship between SRF status and visual outcomes is still evolving and will need further long-term studies.

Integrity of ELM and EZ

The integrity of outer retinal layers is a direct indicator of the health of the retinal photoreceptors and RPE. Studies have shown that eyes with intact IS/OS junction have better visual gains post treatment. IS/OS junction can be graded as completely continuous, partly disrupted and completely disrupted.[4] Subjects with long-standing DME may demonstrate loss of ELM and EZ focally or in a diffuse manner. Studies have revealed suboptimal visual acuity gains in patients with outer retinal disruption. Visual acuity has shown a positive correlation with the survival rate of ELM and the EZ.[14]

Taut posterior hyaloid membrane

Patients with DR demonstrate several abnormalities in the vitreous referred to as diabetic vitreopathy. The alterations in the vitreous result in abnormal posterior vitreous detachment (PVD; anomalous PVD) that is characterized by strong focal/diffuse retinal adhesions and incomplete detachment. Often, the posterior hyaloid forms a

sheet along the posterior pole resulting in tractional force and mechanical retinal distortion. This is termed as taut posterior hyaloid membrane (TPHM; Figure 2). TPHM is responsible for recalcitrant macular edema. Patients with TPHM benefit by pars plana vitrectomy and removal of the taut hyaloid.[50,51]

Choroidal vascularity index

Choroidal vascularity index (CVI) was initially described by Agrawal and colleagues[52] as a ratio of choroidal luminal area to total choroidal area. CVI, unlike choroidal thickness, is unaltered by ocular or systemic factors. CVI has been recently described as a novel OCT parameter to monitor the progression of DR.[53] Studies have shown a decrease in CVI with unaltered choroidal thickness in patients of DR. CVI has also been shown to correlate with the progression of DR. Patients with PDR have a significantly lower CVI than those with mild to moderate NPDR.[54] CVI can be altered even before the onset of DR suggesting that choroidal vasculature may be the site of primary insult in diabetic eye disease.

Fundus fluorescein angiography

Fundus fluorescein angiography (FFA) has been considered the gold standard in studying the retinal vasculature.[5] FFA can help in determining various changes such as retinal capillary nonperfusion, vascular telangiectasia, capillary dropouts, enlargement or irregularity of the FAZ, and the presence of neovascularization. Since FFA allows dye leakage and pooling, it allows rapid assessment of retinal vascular changes, some of which may be missed on routine dilated fundus examination (for instance, occult retinal neovascularization). FFA is an important tool to differentiate between intraretinal microvascular anomalies (IRMAs) and neovascularization elsewhere (NVE).[5] FFA helps to differentiate between focal leak and diffuse capillary bed leak in cases of DME.[55] Before the introduction of OCTA, FFA was used to assess the health of the macula and degree of macular ischemia.[56]

Ultrawide field imaging and angiography

Conventional fundus imaging and angiography can provide valuable information that is limited to the central 30–50 degrees of retina.[57] Imaging angles more than 50 degrees are termed as widefield imaging, and imaging angles greater than 100 degrees are termed as ultrawide field imaging.[58] With peripheral sweeps, it is possible to obtain fundus images of a reasonable quality of an additional 20–30 degrees. Since the earliest signs of DR are seen in the mid-periphery, an extended field of view is crucial and desirable in the screening, diagnosis, and treatment of DR. Currently, the ultrawide field imaging tools, which are cSLO (confocal scanning laser ophthalmoscope) based, provide a panoramic imaging of 82% of the retina (200-degree) in a single frame with no need of mydriasis or contact lens.

A number of studies have shown the utility of ultrawide field imaging in DR.[59–61] Silva and colleagues[59] showed that additional peripheral retinal findings were detected in ultrawide field images compared with the Early Treatment Diabetic Retinopathy Study (ETDRS) standard 7-field fundus photography. This resulted in better assessment of DR and documentation of additional findings resulting in change in therapeutic decision-making in 10% eyes. In addition, studies have also correlated DME to peripheral retinal ischemia.[60,61] Wessel and colleagues[60] found a direct correlation of peripheral retinal ischemia with DME on OCT. Patients with peripheral retinal ischemia had 3.75 times more odds of developing DME as compared with the patients with no peripheral retinal ischemia. It is believed that this retinal ischemia is responsible for increased VEGF production. Patients with extensive peripheral ischemia should be followed up more closely than with no retinal ischemia. These patients can respond better with targeted retinal photocoagulation in adjunct to anti-VEGF and macular laser therapy. Studies have shown that mean decrease in central macular thickness is correlated inversely with ischemic index measured on widefield imaging. Ongoing Protocol AA aims to compare the ultrawide field fundus imaging with ETDRS seven-standard-fields imaging for the assessment of DR and predict rates for worsening of DR.

Biomarkers on FFA

The earliest signs of DR, microaneurysms, might not be evident on clinical examination.[62,63] They are highlighted in FFA as punctate areas of hyperfluorescence (Figure 3).[64] The dot and blot hemorrhages cause fluorescence block.[65] Marked aneurysm formation results in capillary closure, which is generally seen in mid-peripheral retina that gradually increases toward the periphery.[66–68]

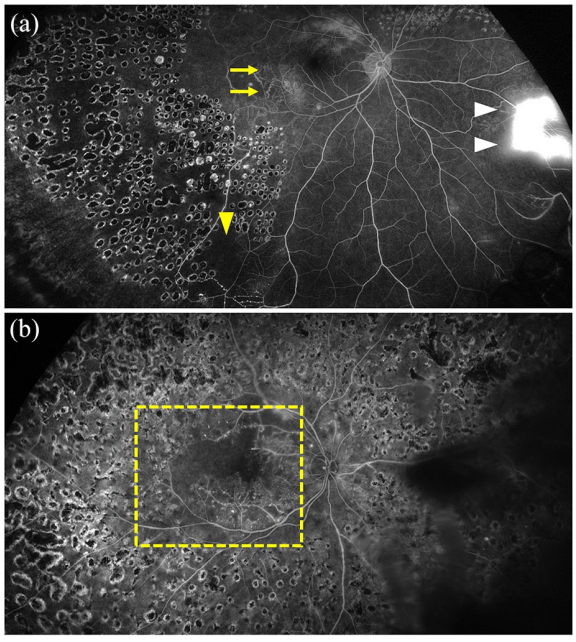

Figure 3. Ultrawide field fluorescein angiography (UWF FA) of a patient with proliferative diabetic retinopathy (PDR) (a) shows a large leak from a nasal retinal neovascularization (white arrowhead). There are peripheral capillary non-perfusion areas (yellow arrowhead). In the posterior pole, the FA shows the presence of macular capillary dropouts with vascular telangiectasia (yellow arrows) and leakage. Another patient with PDR on UWF FA (b) shows a large area of capillary non-perfusion involving the posterior pole (yellow dashed square). This patient has been treated with laser pan-retinal photocoagulation.

Non-perfusion of capillaries leads to patchy areas of hypofluorescence (Figure 3).[69]

Macular ischemia is characterized by an increase in FAZ.[64] Dilated, tortuous, shunt vessels are seen in the peripheral ischemic retina.[70] Fluorescence being 80% protein bound leaks out of incompetent vessels suggesting a breach in blood–retinal barrier.[71] Leakage (Figure 3) is seen in retinal neovascularization which is seen at the junction of perfused and non-perfused retina.[64,72,73] Optic disc neovascularization appears before retinal neovascularization suggesting a choroidal blood supply to the optic disc.[70]

DME is a very common cause of decreased vision. Diabetic maculopathy has been grouped as focal, diffuse, and ischemic maculopathy.[74] In focal maculopathy, focal leakage occurs from microaneurysms with hard exudates arranged in a circinate pattern around the leakage.[75] In diffuse maculopathy, there is a generalized breakdown of the blood–retinal barrier that causes profuse early leakage from the entire capillary bed of the posterior pole.[76]

This is accompanied by cystoid changes in the macula that can be appreciated in OCT. Ischemic maculopathy is characterized by an enlargement in the FAZ. CME is seen in a petaloid pattern due to late staining of fovea with pooling of the dye into parafoveal cyst-like spaces.[70] FFA helps in locating the leakage point in cases of macular edema, therefore assisting in focal or grid laser for treatment.[77]

Leakage pattern

Fluorescence leakage indicates breach in blood–retinal barrier.[78] Fluorescein, the fluorescent dye, helps in tracking the leak. However, in cases of neovascularization, the leakage obscures and blurs the image of a new vessel. Microaneurysms result in focal leakage, which cause focal macular edema, whereas diffuse capillary bed leak due to disruption of blood–retinal barrier causes diffuse macular edema.

FAZ

FAZ is polygonal in normal eyes and it reflects microcapillary foveal circulation. FAZ is increased in DR eyes which is the result of perifoveal capillary occlusion.[79] FAZ borders are seen to be irregular in DR eyes. However, FAZ dimensions do not significantly correlate to fluorescence leakage, and it has not been correlated independently to the staging of retinopathy.[80,81]

OCTA

OCTA is a novel exciting tool that provides detailed information of the retinochoroidal microvasculature. The information provided by OCTA supplements the information obtained using FFA, including precise areas of capillary non-perfusion, presence of collaterals or retinal/optic nerve head neovascularization, and abnormalities of the FAZ.[82] OCTA is advantageous and unique because it can separately analyze each of the three retinal capillary plexi which is important for understanding the pathophysiologic changes in DR. OCTA produces static images of flow in the retinal circulation as compared with FFA, which produces dynamic images. OCTA images are three-dimensional (3D) and can be

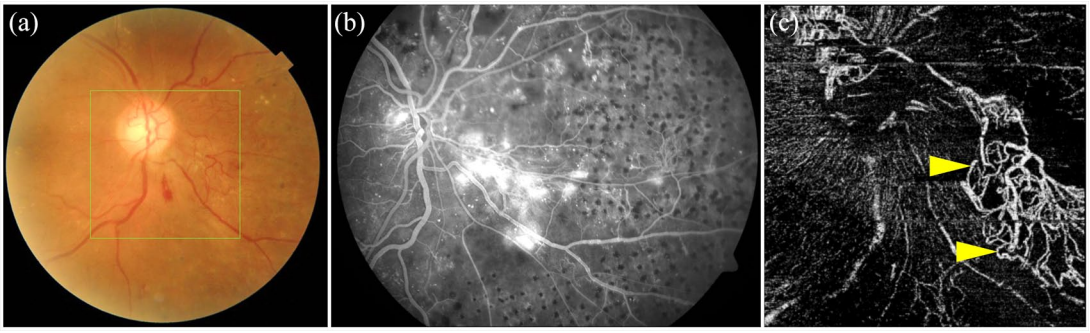

Figure 4. Fundus photograph (50-degree view) of a patient with diabetic retinopathy shows a large neovascular frond nasal to the optic disc (a). This is seen on the corresponding fluorescein angiography as leakage from the neovascularization (b). Optical coherence tomography angiography shows the presence of the large neovascularization (yellow arrowheads) (c).

manually segmented and co-registered with the accompanying structural OCT images. With the advent of widefield OCTA and flow overlay, one can differentiate between NVE and IRMA, both of which signify an advanced stage of DR.[17,83]

OCTA can identify microaneurysms, IRMAs, capillary non-perfusion area, and neovascularization even before they are appreciated clinically or on fundus photography.[84] Thompson and colleagues[85] detected microaneurysms on OCTA even before they were seen on dilated clinical examination. Although FFA has higher sensitivity than OCTA on detecting microaneurysms, studies have shown that OCTA can even detect microaneurysms, which are not picked up on FFA.[85,86]

IRMAs on en face images appear as dilated or looping vessels near the areas of non-perfusion. OCTA has higher detection rates of IRMAs as compared with color fundus photography. Other features which favor IRMAs include presence of intraretinal hyperreflective dots and outpouching of the internal limiting membrane (ILM). IRMA appears as focal areas of increased intraretinal blood flow within the superficial capillary slab on OCTA.[87]

Standard OCT does not provide information about IRMAs and their flow status. Structural OCT B scan with a flow overlay will help in differentiating between IRMA and NVEs. IRMA will have flow signals within the retina under the ILM, whereas NVEs appear as flow voids above the ILM and will be seen in preretinal space. OCTA has emerged as an effective tool to differentiate between IRMAs and NVs.[82] Different morphological patterns of neovascularization at disc (NVD) have been described on OCTA which can help us to detect the response to treatment.[88] Figure 4 shows NVD and NVE detected on OCTA.

Widefield OCTA has emerged as a promising tool with a potential to replicate or replace FFA in diagnosing or monitoring the progression of the disease. Widefield OCTA has been compared with ultrawide field FFA in patients with DR. Recent observational studies have shown widefield OCTA to be comparable with ultrawide field FFA in detecting NVs. It also allows to detect subtle changes in NVs after treatment, thus predicting the future course of these lesions.[89] Couturier et al. showed that the detection rate of capillary non-perfusion areas was higher with widefield OCTA than ultrawide field FFA.[90] This is attributed to the fact that OCTA, unlike FFA, is not affected by leakage and can clearly delineate the abnormal vasculature and capillary non-perfusion areas. These studies suggest that widefield OCTA has the potential to replace ultrawide field FFA in the future, in diagnosing and monitoring the changes in DR.

Retinal vascular density

Vessel density (VD) is defined as the ratio of blood vessel area to the total measured area.[91] It decreases in both superficial capillary plexus (SCP) and deep capillary plexus (DCP) in patients with DR (Figure 5).[92] VD has also been shown to decrease in diabetic patients without DR.[93] This is attributed to the fact that parafoveal capillary non-perfusion in DCP may be an early sign of DR. VD has been unaltered post

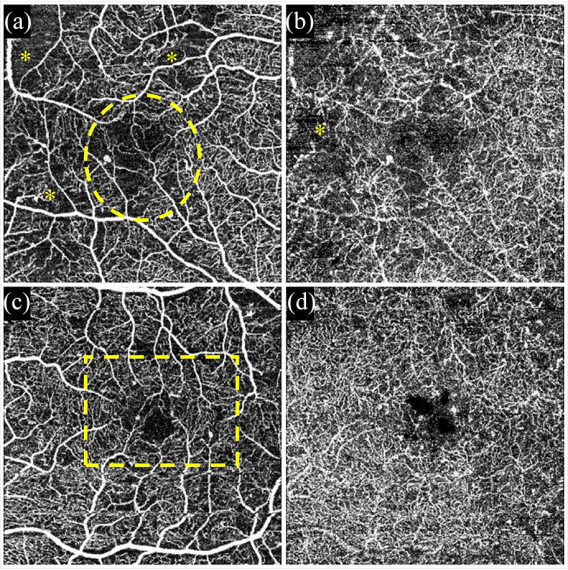

Figure 5. Optical coherence tomography angiography (OCTA; 3 mm × 3 mm scans) of two subjects with diabetic macular edema is shown. Patient #1 shows areas of capillary dropouts (yellow asterisks) in the superficial retinal plexus (a) along with increased intercapillary spacing and an irregular foveal avascular zone (FAZ; yellow dashed circle). On the deep retinal capillary plexus (b), these areas of capillary dropouts are better visualized. Patient #2 shows increased intercapillary spacing involving the central macula (yellow dashed square) in the superficial capillary plexus (c) and multiple microaneurysms in the en face scan of the deep capillary plexus (d).

dexamethasone injection in both SCP and DCP. However, VD in choriocapillaris tended to increase post treatment.[94]

Intercapillary spacing

Studies have shown intercapillary spacing as more sensitive parameter than VD and FAZ to detect early capillary dropouts or areas of non-perfusion.[95]

FAZ

Quantitative measurements of FAZ on OCTA can be done using various indices like axis ratio, FAZ area, acircularity index, perimeter, and area.[96] Larger FAZs (both acircularity index and axis) have been seen on deep plexus slab in OCTA scans of patients with diabetes compared with normal irrespective of DR (Figure 5).[97] Also significantly larger FAZ has been documented in eyes with DRIL as compared with eyes with no DRIL.[98]

Fractal dimension

Fractal analysis analyzes the microvascular alterations and the geometric alterations of the retinal vasculature. Fractal dimension in both the SCP and DCP has shown a significant decrease in diabetic eyes compared with normal control subjects. However, the metric does not vary among different severity levels of DR.[99]

There are some limitations with use of OCTA. The field of view with OCTA is smaller as compared with available FFA platforms. Even with the introduction of widefield OCTA of 12 mm × 12 mm scans, they are still not comparable with ultrawide field FFA/indocyanine green angiography (ICGA).[83] Although this issue has been overcome with montage OCTA using 12 mm × 12 mm scan, issues such as increased acquisition time and misalignment of images are still a problem. Second, limitation with OCTA is that it is unable to assess the dynamic characteristics of flow velocity. Third, problems like motion and projection artifacts are commonly encountered while analyzing the images.[100]

3D OCTA analysis

Borrelli and colleagues[101] have recently described in vivo rotational 3D analysis of microaneurysms using OCTA. Both OCT and OCTA provide structural cross-sectional and en face imaging of the retina, respectively. This two-dimensional (2D) visualization cannot provide information about the origin, orientation, and location of microaneurysms within the retina. This shortcoming can be overcome by using 3D analysis of microaneurysms on OCTA. Overlapping anatomy and vessel foreshortening can be tackled by rotating these images on their three axes. Using 3D visualization, it has been shown that microaneurysms are associated with two vessels and not at the vascular junctions. Besides, different morphological varieties of microaneurysms can be seen using 3D visualization.

Zhang and colleagues[102] have described an automated 3D shape modeling framework to obtain high-quality 3D vessel representation using OCTA. The authors have used advanced surface reconstruction models to visualize retinal microvasculature. It is possible to have a smooth representation of vessel tortuosity and allows clear delineation of large and small caliber vessels in a 3D view. Such analyses provide a very precise measurement capabilities. Furthermore, Borrelli

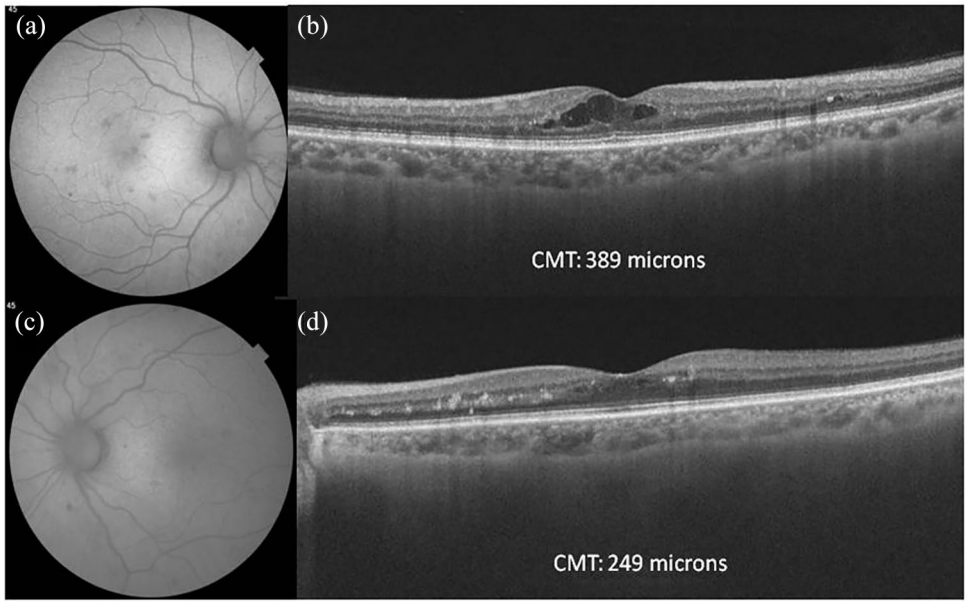

Figure 6. Fundus autofluorescence (FAF) findings in a patient with diabetic maculopathy are depicted in the figure. High signal of autofluorescence in panel (a) corresponds to diabetic macular edema (b), compared with lesser autofluorescence (c) with no diabetic macular edema in the fellow eye (d).

and colleagues have quantified DME using 3D vascular volume and perfusion densities in patients with DR. The authors observed highest area under the receiver operating characteristic curve using 3D perfusion density, and concluded that such an analysis can be reliable and efficacious.[103]

Fundus autofluorescence biomarkers

Fundus autofluorescence (FAF) is a rapid, non-invasive imaging technique that may give new insights into the evaluation of DME. Short-wavelength FAF derives its signal mainly from lipofuscin in the RPE.[104] Long wavelength autofluorescence or near-infrared (NIR) FAF derives its signal from melanin, which is present in RPE and choroid.[105] Melanin accumulates in the apical parts of the RPE cells and is thought to be protective of the RPE.

In DR, local ocular inflammation and oxidative stress lead to increased amount of lipofuscin and decreased amount of lutein and zeaxanthin in the macula.[106,107] This is responsible for increased FAF signal in subjects with DME. In addition, activation of microglia in diabetes could cause oxidation of proteins and lipids.[108] Histologic studies have found lipofuscin to accumulate in microglia. Also, intraretinal cysts in DME unmask the underlying RPE by displacing the luteal pigment in the fovea centralis. This prevents the normal blockage of foveal FAF signal at the level of retinal intraretinal cysts (Figure 6).[109,110] Significant FAF alterations have been described in diabetic patients without retinopathy.[109] FAF has been shown to have sensitivity and specificity of 81% and 69%, respectively, in detecting CME.[110]

A number of studies using short wavelength FAF have reported increased FAF signal in patients with DME.[106,111,112] Various patterns of FAF have been described, such as a single cyst of increased FAF, multi-cystic FAF, or combined single- and multi-cystic FAF. FAF was found to correlate better with OCT patterns and central field microperimetry rather than with visual acuity.[106] An increased FAF signal (hyper-autofluorescence) is associated with worsening visual acuity and an increase in the macular thickness on OCT.[113] In patients with DME, the pretreatment baseline degree of foveal FAF signal might be helpful in predicting macular cube average thickness in patients undergoing treatment with intravitreal anti-VEGF in the short term.[112] Thus, apart from assessing the health of the RPE, FAF may have several applications in subjects with DR and DME.

Summary and conclusion

Recent advancements in imaging techniques have allowed a multimodal approach in diagnosis and management of various retinal diseases. With the advent of SD and SS OCT, microstructural details of retina and choroid can be easily appreciated. CVI is a recently introduced imaging biomarker to study the choroidal vascularity on OCT. Introduction of widefield FFA allows to examine peripheral vascular abnormalities in DR. Peripheral capillary non-perfusion areas can be selectively targeted in cases of recalcitrant DME. OCTA being a non-invasive, dyeless procedure clearly delineates the abnormal retinal vasculature and non-perfusion areas. Introduction of wider scans helps detecting vascular abnormalities involving the peripheral retina. Finally, FAF has been studied recently in DME and may help to prognosticate and monitor the disease progression and response to treatment. These imaging techniques may help in the detection of subclinical disease and retinal vascular changes, even before clinically detectable changes, or development of visual symptoms.

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