ORIGINAL ARTICLE

MACULAR VASCULAR DENSITY ANALYSIS USING ADOBE PHOTOSHOP SOFTWARE IN DIABETIC EYES: OPTICAL COHERENCE TOMOGRAPHY ANGIOGRAPHY STUDY

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Background: Optical Coherence Tomography Angiography (OCTA) is dye less microvascular visualizing technique. In study we binaries OCTA images of macular vessels in healthy and diabetic subjects without macular oedema using Adobe Photoshop CS3 extended version. **Methods:** Prospective, single centered, observational study total of 58 eyes of 108 Diabetic Retinopathy (DR) subjects and 20 eyes of 40 normal subjects with mean age of 58.3±10.5 range (40–82) were included in our study. Ten eyes with Non-Diabetic Retinopathy (NDR), twenty-nine eyes with Non-Proliferative Diabetic Retinopathy (NPDR) (mild-10, moderate-7 and severe-12) and nineteen eyes with Proliferative Diabetic Retinopathy (PDR)are studied with images obtained using OCTA between September 2016 to June 2017. Scan area of 6×6 mm was selected to find morphological changes in the superficial retinal layers and deep retinal layers. Captured OCTA images were binarized using automated thresholding algorithm. Macular Vessel Density (MVD) (%) and Foveal Avascular Zone area (mm^2) measured for superficial and deep retinal vessel arcade. For comparison, analysis of variance and Kruskal–Wallis test are applied. **Results:** Diabetic eyes were grouped according to their severity level. MVD and FAZ are compared in all groups. Results are significantly lower in all groups except in controls and NDR. Significant decrease is observed in vascular density of most layers with progress in retinopathy. **Conclusion:** Adobe Photoshop CS3 extended version is an excellent tool for image binarization. Calculating FAZ area and MVD using OCTA images agreed closely with clinical grading system. Application of this method can be helpful in monitoring disease progression.

Keywords: Diabetic retinopathy imaging; FAZ area; Macular vascular density; Optical coherence tomography angiography; Adobe Photoshop

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INTRODUCTION

Diabetic retinopathy (DR) is a preeminent cause of impaired vision and due to sedentary life-style the expected number is increasing rapidly worldwide.[1–3] Among diabetes mellitus complication, DR is common one and has high prevalence.[4] In DR, initial changes lead to microaneurysms and detoriates blood retinal barrier, thereby leading to venous dilation and beading. Based on pathological changes, staging and classification play vital role in treatment and prognosis.[5–9] The International Severity Scale is a simplified grading method has been proposed which classify DR into 5 stages based on observed retinal vascular changes with fluoresceinangiography.[10–12]

During early and middle stages, subjective symptoms of visual acuity are rarely experienced in the absence of diabetic macular oedema or vitreous haemorrhage in DM patients.[13,14] Similarly, diseases associated with vessel abnormality and pathophysiology necessitates comprehensive investigation of retinal vasculature for diagnosis and treatment. Thus, to observe vessel abnormality and pathophysiology, OCTA is one of the most recent, non-invasive, and dye-less imaging technique. It captures dynamic motion of erythrocytes to estimates the vessels morphology.[12] Consequently, it is a beneficial imaging modality tool for assessment of various ophthalmic diseases. The hallmark of DR is vascular changes involving different retinal layers. This may lead to visually devastating complications including macular oedema, macular ischemia, and neovascularization.[8]

In this study, we are using OCTA images for examining vasculature of the FAZ and MVD in the superficial and deep retinal vasculature. Automated image thresholding method is used in diabetic eyes with and without clinical retinopathy and compare it with healthy nondiabetic control eyes. No significant published material is found in literature for DR studies based on OCTA image-binarization coupled with Photoshop CS 3.

MATERIAL AND METHODS

This study abided the Declaration of Helsinki and ethics committee of Zhongshan Ophthalmic Center, Sun Yat-Sen University, Guangzhou, China approved this prospective observational study, from September 2016 to June 2017, following the tenets of the Helsinki's declaration. We prospectively collected imaging data of DR subjects at Retina practice and healthy control subjects without history of diabetes from optometry practice. All images were captured by same operator in order to reduce inter-user variability and only one eye was enrolled as subject to ovoid false-positives as both eyes have similarities in anatomical characteristics.

Inclusion criteria for treatment naïve DR subjects ranging from non-proliferative to proliferative-DR following the International Severity Scale.[10] Control subjects were participants without ocular trauma history and surgical intervention.

Angiogram images having signal strength ≥60 from OCTA is considered in this study as a high quality. Comprehensive ophthalmology-based examination of the subject patients is done using Best-Corrected Visual Acuity (BCVA), Intra-Ocular Pressure (IOP), slit-lamp bio-microscopy and colour fundus photography. Central macular thickness (CMT) values are measured using the ETDRS foveal central subfield by macular SD-OCT.

Images were obtained using the RTVue XR AvantiOCTA instrument (Optovue Inc). This instrument uses split-spectrum amplitude-decorrelation angiography having axial scan rate of 70,000 scans per second. Further, it uses a light source centered on 840 nm with bandwidth of 45 nm. To produce 3-dimensional angiograms, 2consecutive OCT volumes (containing 304 B-scans with 304 A-lines per B-scan) were acquired in a 3×3 mm scanning area centered on the fovea and the split-spectrum amplitude-decorrelation angiography algorithm was applied. To demarcate the inner boundary of internal limiting membrane and outer boundary at Bruch Membrane OCTA is used. Therefore, the en-face OCTA image (OCT angiogram) has a full-thickness image of the retinal vasculature. Furthermore, layered images based on superficial and deep retinal vascular networks are considered. The boundaries for superficial network extended from 3 mm below the internal limiting membrane to 15 mm below the inner plexiform layer (IPL). The deep capillary network boundaries were from 15–70 mm below the IPL.[15–18]

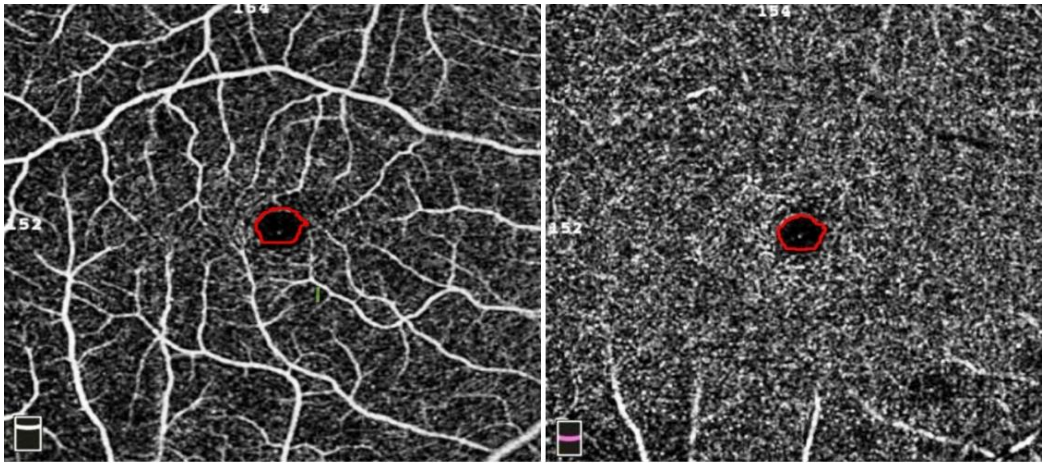
Adobe Photoshop CS3 extended version is used to process the images and perform vascular density measurements, whereas automated thresholding algorithm is used to create binary images of vascular networks. The Vascular density is considered as percentage area covers by blood vessels, while blood vessels are considered pixels having values above threshold level. This is considered for both parafoveal and perifoveal MVD. FAZ area is calculated using the product of number of pixels in contour and area of single pixel indicated in figure-1.

Parafoveal region: central circle with a 171-pixel (2.5mm) diameter. Perifoveal region: outer circle with a 376-pixel (5.5 mm) diameter. (Figure-2) The following formulae were applied in the study.

$$\text{FAZ area} = (\text{FAZ pixels} / \text{Image pixels}) \times 36\%$$

$$\text{Macular Vascular Density plexus} = \text{white pixels (retina vessel)} / \text{Image pixel} \times 100$$

Two independent retinal image graders (W.J, L.H.T) from the Zhongshan Image Reading Center were asked to compare original and binarized images to examine clarity FAZ and clarity of blood vessel delineation in superficial and deep vascular plexus. Each original image was provided along the binarize image for comparison. Intraclass correlation coefficient (ICC) between graders was measured. Values ranged from 0–1 (no agreement & 1 perfect agreement) with lowest acceptable agreement (0.85). We used SPSS, Version 20 (SPSS, Inc, Chicago, Illinois, USA) for statistical analysis and graphs were prepared using Microsoft Excel (Microsoft Corp., Seattle, WA). For difference between vascular networks in our pool, t-test was used. One-way ANOVA was used to compare the demographics in study pool. Macular vascular density comparisons were made at two levels: The superficial retinal capillaries, the deep retinal capillaries. An ANOVA analysis and Post Hoc Tukey HSD tests were used to compare MVD in two layers in controls, NDR, NPDR, and PDR eyes. We analysed this trend in each layer, in 6×6-mm scans. Values of $p \leq 0.05$ were considered statistically significant.



(A) (B)

Figure-1: Mapping of FAZ withmanual bordering using Adobe Photoshop CS3 extended version software. (A.) OCTA of the macula showing the FAZ in the superficial vascular plexus (sFAZ). (B). Corresponding images of FAZ in the deep vascular plexus (dFAZ)

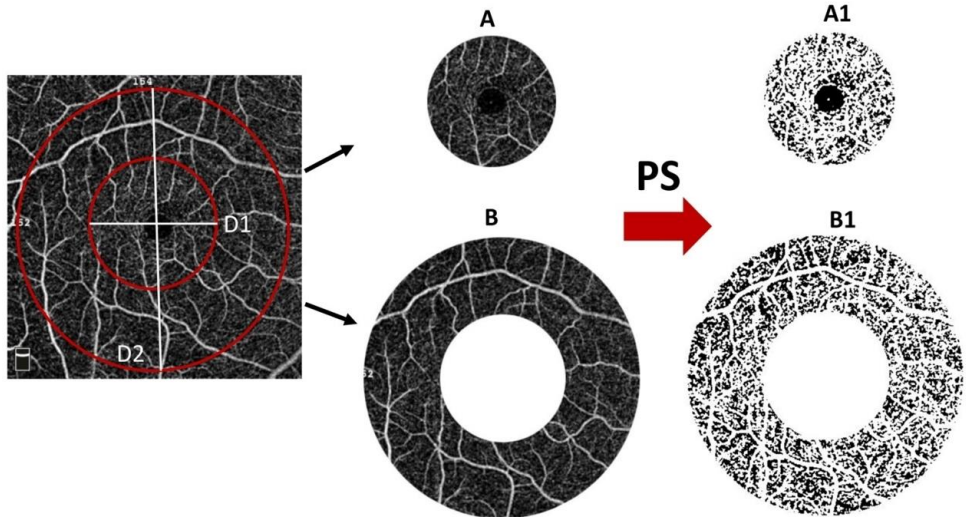


Figure-2: En face angiograms representing D1 as parafoveal and D2 as perifoveal vascular networks and converted to binary images using an automated thresholding method. Blood vessel signals were identified as white pixels.

RESULTS

A total of 58 eyes of 108 DR subjects and 20 eyes of 40 normal subjects with mean age of 58.3±10.5 range (40-82) were included in our study. Ten eyes with NDR, twenty-nine eyes with NPDR (mild-10, moderate-7 and severe-12) and nineteen eyes with PDR were included. Demographics are mentioned in table-1. We determine % MVD difference between control and study patients. In our study we observed that lower MVD in diabetic patients in superficial and deep retinal layers using 6×6-mm scans. FAZ area(mm^2) and % macular vascular density values for controls and DR subjects are presented in table-2. The mean FAZ area in the superficial layer was (0.181± 0.081) mm^2 in the control group and (0.382±0.09) mm^2in DR group whereas (0.229±0.094) and (0.428±0.139) in deep layers respectively. The FAZ area in the deep layer was also enlarged in both the groups compared with the control group.

Table-1: Characteristics of study population

Characteristics	NDR	All- NPDR	PDR	*p*
Sex (Male/Female)	6/4	17/12	8/11	-
Age(years)	54.2±7.5	59.3±10.5	57.4±8.00	0.62‡
DM –Type (1/2)	3/7	7/20	3/16	-
HbA1c (%)	7.3±1.3	7.7±1.2	7.9±1.3	0.34†
Duration of DM	7.00±1.2	9.00±1.1	13.2±1.5	0.01†

†Student's t-test. ‡One-way ANOVA

Table-2: Comparison of FAZ (mm^2) and %MVD in Controls and Diabetic Subjects (6×6mm)

	Controls (Mean±SD)	DR (Mean±SD)	*p*
S-Faz	0.181±0.081	0.382±0.09	<0.001
D-Faz	0.229±0.094	0.428±0.139	<0.001
S-Para	72.62±3.66	56.93±10.89	<0.001
D-Para	78.06±2.51	67.58±10.62	<0.001
S-Peri	76.30±3.47	56.00±12.50	<0.001
D-Peri	82.46±6.05	67.24±10.31	<0.001

Non-paired t-test. Superficial Foveal Avascular Zone(S-Faz), Deep Foveal Avascular Zone(D-Faz), Superficial Parafoveal (S-Para), Deep Parafoveal (D-Para), Superficial Perifoveal (S-Peri), Deep Perifoveal (D-Peri)

Table-3: Mean values for % MVD for controls, normal and DR patients (6×6mm)

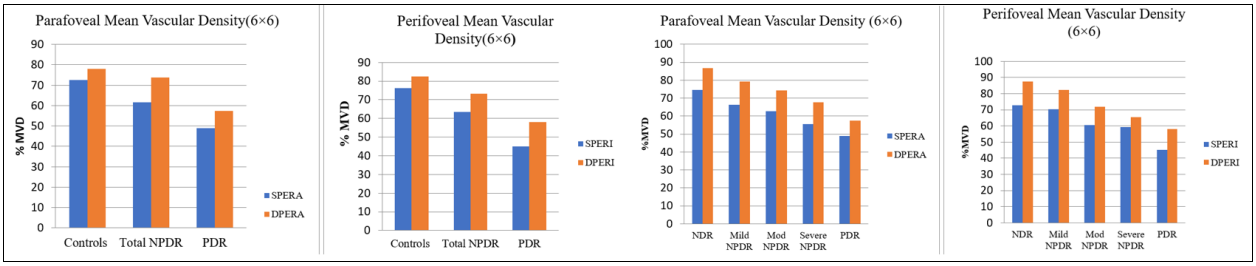
	S-Peri	S-Para	D-Peri	D-Para	S-Faz	D-Faz
Control	76.30±3.47	72.61±3.66	82.46±6.05	78.06±2.51	0.18±0.08	0.23±0.09
NDR	72.6305±9.0	74.68±9.33	86.44±4.34	87.734±4.80	0.26±0.09	0.29±0.10
MildNPDR	66.4557±5.6	70.30±7.25	79.37±4.58	82.20±6.90	0.27±0.04	0.31±0.06
Mod NPDR	60.58±6.02	62.60±8.48	71.74±4.41	74.3±5.23	0.35±0.06	0.38±0.07
Severe NPDR	55.27±14.6	59.6±8.91	65.47±6.32	67.6±8.33	0.41±0.07	0.53±0.28
PDR	45.13±7.80	49.04±8.81	57.10±6.40	58.43±5.60	0.44±0.08	0.56±0.06

Superficial Foveal Avascular Zone(S-Faz), Deep Foveal Avascular Zone (D-Faz), Superficial Parafoveal (S-Para), Deep Parafoveal (D-Para), Superficial Perifoveal (S-Peri), Deep Perifoveal (D-Peri). Non-diabetic retinopathy (NDR), Non-Proliferative diabetic retinopathy (NPDR), Proliferative diabetic retinopathy (PDR)

Table-4: Comparison of % MVD in Study population in Capillary Layers (6×6mm)

	S-Para	D-Para	S-Peri	D-Peri	D-Faz	S-Faz
Controls versus NDR	0.091	0.981	0.127	1.000	0.122	0.001
Controls versus mild NPDR	0.021	<0.001	0.476	0.017	0.001	0.264
Controls versus mod NPDR	<0.001	0.049	<0.001	<0.001	<0.001	<0.001
Controls versus severe NPDR	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
Controls versus PDR	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
NDR versus mild NPDR	0.024	0.001	0.961	0.100	0.995	0.993
NDR versus moderate NPDR	<0.001	<0.001	<0.001	<0.001	0.001	0.082
NDR versus Severe NPDR	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
NDR versus PDR	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
Mild NPDR versus Mod NPDR	0.680	0.055	0.001	<0.001	0.028	0.452
Mild NPDR versus Sev.NPDR	0.005	<0.001	0.002	<0.001	<0.001	<0.001
Mild NPDR versus PDR	<0.001	<0.001	<0.001	<0.001	<0.001	0.001
Mod NPDR versus Sev.NPDR	0.077	0.003	0.995	0.013	0.355	0.005
Moderate NPDR versus PDR	<0.001	<0.001	<0.001	<0.001	<0.001	0.096
Sev.NPDR versus PDR	0.093	<0.001	<0.001	<0.001	0.598	0.523

Comparison of MVD at superficial and deep retinal capillary level in ANOVA results with Post Hoc tukey HSD. The data are all values listed as 0.05. Superficial Foveal Avascular Zone(S-Faz), Deep Foveal Avascular Zone(D-Faz), Superficial Parafoveal (S-Para), Deep Parafoveal (D-Para), Superficial Perifoveal (S-Peri), Deep Perifoveal (D-Peri). Non-diabetic retinopathy (NDR), Non-Proliferative diabetic retinopathy g(NPDR), Proliferative diabetic retinopathy (PDR)



A B C D

Figure-3: (A-D): Figure 1 and 2 focus on controls, total NPDR and PDR. Both total NPDR group and PDR group had a decrease in %MVD in all layers when compared with controls whereas figures 3 and 4 focus normal and diabetic retinopathy stages in parafoveal and perifoveal capillary layers. All graphs present decrease in % MVD as severity of retinopathy increases.

DISCUSSION

OCTA is a dye less retina imaging method, with high resolution which allows us to observe deep retinal microvascular layers. In this study we have analysed the FAZ area, macular vascular density changes in diabetic eyes after grading them according to their severity level and demonstrate the application of binarization by Adobe Photoshop CS3.We used wider field of view (6×6) mm angiograms which are helpful in detecting vascular changes where DR changes are first believed to appear. This study evaluated the FAZ area detected by OCTA in controls and diabetic eyes. The images we obtained showed that the dimensions of FAZ in diabetic patients were enlarged as comparing to the controls. In healthy subjects, the OCTA FAZ area is reported having mean of 0.358±0.084 mm^2 in superficial FAZ area and 0.584±0.15 mm^2 in deep FAZ area.[19] These reported results are analogous to this study. We also found that deep layer FAZ area has significant enlargement (Table-4). FAZ enlargement in diabetic eyes is consistent with published literature. Takase *et al*[20] have analysed OCTA changes in 20 DR and 24 NDR eyes. They found significant FAZ and deep plexus layer enlargement in diabetic eyes as compared to healthy eyes ($p<0.01$). Various other studies support the notion that deep layer FAZ area has significant enlargement due to diabetic retinopathy changes.[21] Bresnick and colleagues have analysed FA of diabetic patients and in their study they have found that enlargement of FAZ is diabetic patients have correlation with capillary nonperfusion. We have also found comparable results in our study. Previous studies also testify that FAZ enlargement can be used as a marker to stage diabetic retinopathy.[22] We also found a significant enlargement of the FAZ in NDR eyes, in addition to finding that there were no significant differences for the FAZ areas between NDR and Mild and Moderate-NPDR eyes.

We incorporated superficial and deep vascular networks for finding percentage area of MVD, occupied by blood vessels in our study sample. Previous studies demonstrate that calculating vascular densities has an interpretable, decipherable and noninvasive method to evaluate macular perfusion status quantitatively having various potential applications.[19–21] Whereas the results obtained in this study shows reduced density of deep and superficial vascular plexus of NDR, NPDR and PDR patients considering parafoveal and perifoveal layers indicating compromised circulation of blood in the layers. These results are reported in table-2. The mean vascular densities are higher in parafoveal vascular than the perifoveal vascular network as in perifoveal region less space has been occupied by vessels which produce less pixels hence lead to lesser macular vascular density values (Table-3). Technically AngioVue OCTA software uses the same number of A-scans (304×304) in detecting changes in 3×3 mm or 6×6 mm fields.[23]

The results also showed decrease in blood vessel densities in vascular plexus at superficial and deep layer when NDR patients are compared with control sample while considering parafoveal and perifoveal layers. Similarly, when patients with NDR were compared with control group, decrease in vessel density and increase in FAZ at superficial level is also observed even at deep vascular layers. In our study we have not detected significant changes among controls, normal and mild NPDR eyes (Table-4). Our finding is supported by Kim *et al*, as they do not detect significant changes between controls and mild NPDR.[24] Previous studies show that in NDR, the FAZ is enlarged in the superficial and deep retinal vascular plexus.[23]

The results noted in Table. 4 identify increase in FAZ in patients having retinal vascular layer NDR when compared to control subjects having significant difference with superficial vascular plexus. A study based on perifoveal vascular network observes and demonstrates variation at deep layers with having larger capillary density in comparison with superficial layers.[25] The same increase is supported by our studies when deep and superficial retinal vascular plexus are compared.[19] Metabolic demand varies between the two retinal plexuses like neuronal demand in the human cerebral cortex.[26,27]

In our study some limitations exist. We have obtained the data from 6×6 mm field where we measured parafoveal region with central circle with a 171pixel (2.5mm) diameter and perifoveal region as outer circle with a 376-pixel (5.5mm) diameter. Data obtained and compared with 3×3mmOCT angiograms would produce effective results in detecting microvascular changes and FAZ delineation. In previous studies vascular densities in DR population have been measured by integrated computer softwares.[15–19,21] In our study we have measured vascular changes with binary images created by using an automated thresholding algorithm. For such measurement no "gold standard" exists in diabetic patients with whom we can compare our results. Measurement variability in FAZ and deep vascular network can be another limitation as the deep vessels approach less closely to the center.[28]

CONCLUSION

In conclusion, we demonstrated a method for manual measurement of FAZ area and retinal vascular plexus. Adobe Photoshop is accessible and potential for analysing image parameters. Our study suggests that impairment of microcirculation in macula exhibits before retinopathy enhances in diabetic subjects and OCTA is a useful tool to detect those microcirculatory changes.

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AUTHORS CONTRIBUTION

SNAS: Conceived the study design, supervised the study and write up; LY and MI did proof reading. UF: Statistical analysis. MI, MUK, TMS and ZA: Literature review. JYP, LJ, LRY image analysis.

REFERENCES

1. Klein R, Klein BE, Moss SE, Davis MD, DeMets DL. The Wisconsin Epidemiologic Study of Diabetic Retinopathy: III. Prevalence and risk of diabetic retinopathy when age at diagnosis is 30 or more years. Arch Ophthalmol 1984;102(4):527–32.
2. Stefansson E, Bek T, Porta M, Larsen N, Kristinsson JK, Agardh E. Screening and prevention of diabetic blindness. Acta Ophthalmol Scand 2000;78(4):374–85.
3. Prokofyeva E, Zrenner E. Epidemiology of major eye diseases leading to blindness in Europe: a literature review. Ophthalmic Res 2012;47(4):171–88.
4. Saaddine JB, Honeycutt AA, Narayan KV, Zhang X, Klein R, Boyle JP. Projection of diabetic retinopathy and other major eye diseases among people with diabetes mellitus: United States, 2005-2050. Arch Ophthalmol 2008;126(12):1740–7.
5. Dimitrova G, Kato S, Yamashita H, Tamaki Y, Nagahara M, Fukushima H, *et al*. Relation between retrobulbar circulation and progression of diabetic retinopathy. Br J Ophthalmol 2003;87(5):622–5.
6. Arend O, Wolf S, Jung F, Bertram B, Postgens H, Toonen H, *et al*. Retinal microcirculation in patients with diabetes mellitus: dynamic and morphological analysis of perifoveal capillary network. Br J Ophthalmol 1991;75(9):514–8.
7. Cogan DG, Toussaint D, Kuwabara T. Retinal vascular patterns. IV. Diabetic retinopathy. Arch Ophthalmol 1961;66(3):366–78.
8. Klein R. Hyperglycemie and microvascular and macrovascular disease in diabetes. Diabetes care 1995;18(2):258–68.
9. Wu L, Fernandez-Loaiza P, Sauma J, Hernandez-Bogantes E, Masis M. Classification of diabetic retinopathy and diabetic macular edema. World J Diabetes 2013;4(6):290–4.
10. Grading diabetic retinopathy from stereoscopic color fundus photographs—an extension of the modified Airlie House classification: ETDRS report number 10. Early Teatment Diabetic Retionoparhy study research group. Ophthalmology 1991;98(5):786–806.
11. Wilkinson C, Ferris 3rd F, Klein RE, Lee PP, Agardh CD, Davis M, *et al*. Global Diabetic Retinopathy Project Group. Proposed international clinical diabetic retinopathy and diabetic macular edema disease severity scales. Ophthalmology 2003;110(9):1677–82.
12. Spaide RF, Klancnik JM Jr., Cooney MJ. Retinal vascular layers imaged by fluorescein angiography and optical coherence tomography angiography. JAMA Ophthalmol 2015;133(1):45–50.
13. Nathan DM, Genuth S, Lachin J, Cleary P, Crofford O, Davis M, *et al*. The effect of intensive treatment of diabetes on the development and progression of long-term complications in insulin-dependent diabetes mellitus. N Engl J Med 1993;329(14):977–86.
14. Control D, Group CTR. Progression of retinopathy with intensive versus conventional treatment in the Diabetes Control and Complications Trial. Diabetees control and complications trial research group. Ophthalmology 1995;102(4):647–61.
15. Ishibazawa A, Nagaoka T, Takahashi A, Omae T, Tani T, Sogawa K, *et al*. Optical Coherence Tomography Angiography in Diabetic Retinopathy: A Prospective Pilot Study. Am J Ophthalmol 2015;160(1):35–44.e1.
16. Jia Y, Bailey ST, Hwang TS, McClintic SM, Gao SS, Pennesi ME, *et al*. Quantitative optical coherence tomography angiography of vascular abnormalities in the living human eye. Proc Natl Acad Sci U S A 2015;112(18):E2395–402.
17. Jia Y, Bailey ST, Wilson DJ, Tan O, Klein ML, Flaxel CJ, *et al*. Quantitative optical coherence tomography angiography of choroidal neovascularization in age-related macular degeneration. Ophthalmology 2014;121(7):1435–44.
18. Jia Y, Tan O, Tokayer J, Potsaid B, Wang Y, Liu JJ, *et al*. Split-spectrum amplitude-decorrelation angiography with optical coherence tomography. Opt Express 2012;20(4):4710–25.
19. Gadde SG, Anegondi N, Bhanushali D, Chidambara L, Yadav NK, Khurana A, *et al*. Quantification of vessel density in retinal optical coherence tomography angiography images using local fractal dimension. Invest Ophthalmol Vis Sci 2016;57(1):246–52.
20. Takase N, Nozaki M, Kato A, Ozeki H, Yoshida M, Ogura Y. Enlargement of Foveal Avascular Zone in Diabetic Eyes Evaluated by En Face Optical Coherence Tomography Angiography. Retina 2015;35(11):2377–83.
21. Talisa E, Chin AT, Bonini Filho MA, Adhi M, Branchini L, Salz DA, *et al*. Detection of microvascular changes in eyes of patients with diabetes but not clinical diabetic retinopathy using optical coherence tomography angiography. Retina 2015;35(11):2364–70.
22. Bresnick GH, De Venecia G, Myers FL, Harris JA, Davis MD. Retinal ischemia in diabetic retinopathy. Arch Ophthalmol 1975;93(12):1300–10.
23. Agemy SA, Scripsema NK, Shah CM, Chui T, Garcia PM, Lee JG, *et al*. Retinal vascular perfusion density mapping using optical coherence tomography angiography in normals and diabetic retinopathy patients. Retina 2015;35(11):2353–63.
24. Kim AY, Chu Z, Shahidzadeh A, Wang RK, Puliafito CA, Kashani AH. Quantifying microvascular density and morphology in diabetic retinopathy using spectral-domain optical coherence tomography angiography. Invest Ophthalmol Vis Sci 2016;57(9):OCT362–70.
25. Chan G, Balaratnasingam C, Yu PK, Morgan WH, McAllister IL, Cringle SJ, *et al*. Quantitative morphometry of perifoveal capillary networks in the human retina. Invest Ophthalmol Vis Sci 2012;53(9):5502–14.
26. Craigie EH. The vascularity of the cerebral cortex of the albino rat. J Comp Neurol 1921;33(3):193–212.
27. Motti ED, Imhof HG, Yasargil MG. The terminal vascular bed in the superficial cortex of the rat. An SEM study of corrosion casts. J Neurosurg 1986;65(6):834–46.
28. Shahlaee A, Samara WA, Hsu J, Say EAT, Khan MA, Sridhar J, *et al*. In vivo assessment of macular vascular density in healthy human eyes using optical coherence tomography angiography. Am J Ophthalmol 2016;165:39–46.

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