Quantitative Evaluation of Vascular Density in Diabetic Retinopathy Subtypes using Optical Coherence Tomography Angiography

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Abstract

**Background:** To determine the discrepancy between quantitative measurement of retinal and choriocapillaris (CC) vascular density (VD) in diabetic retinopathy (DR) stages using spectral domain optical coherence tomography angiography (SD OCTA) and compare it with normal subjects.

**Methods:** 188 eyes of 97 participants were recruited in this cross-sectional study. Macular OCTA (3x3mm) scan was performed and VD at the level of superficial capillary plexus (SCP), deep capillary plexus (DCP) and CC were measured with the device software.

**Results:** In normal subjects, VD in SCP, DCP, and CC were higher in all subsegments. In retinal VD, all calculated parameters were reduced in the more extreme stages of DR, except for foveal VD of SCP. There was a constant pattern of decrease in VD of CC from normal cases to cases of NDR and NPDR and then a slight increase happened in the PDR stage but never touching the normal quantities. Age, fasting blood sugar, and years of diabetes mellitus were correlated with reduced VD in different segments. Multivariate linear regression analysis showed that best-corrected visual acuity (BCVA) was positively correlated with parafoveal VD at SCP and VD of foveal area at CC. VD of all subfields of macular area except foveal DCP VD showed reduced levels in diabetic macular edema (DME) patients compared to those without DME.

**Conclusions:** The findings of the study endorse retina VD changes as a potential biomarker for DR development before retinopathy becomes clinically evident. It seems that parafoveal VD of SCP and foveal VD of CC are good biomarkers to predict VA in the diabetic patients.

**Background**

Diabetic retinopathy (DR) is a degenerative neurovascular disease caused by activation of multifactorial pathologic mechanisms, which leads to microvascular abnormalities including microaneurysm development, capillary non-perfusion, vascular leakage, and neovascularization. Microvascular changes and destructions, as the loss of pericytes and endothelial cells and capillary leaks and occlusions also occur in earlier DR stages. The resulting ischemia induces upregulation of angiogenic signaling molecules, including vascular endothelial growth factor (VEGF) and erythropoietin, which increases vascular permeability and ultimately fosters proliferative diabetic retinopathy (PDR).

Fluorescein angiography (FA) and indocyanine green angiography (ICGA) are the gold standard imaging modalities for imaging of microvascular abnormalities of retina and choroid in DR for years. However, a number of limitations, including their invasive nature, the risk of allergic reactions in patients with iodine or seafood allergy or idiosyncratic reaction, the cost, the long duration of imaging and the occasional shortage of fluorescein and ICG dyes, have raised the clinical need for a shift to newer imaging techniques. For these reasons, FA and ICGA are not routinely performed in the early stages of DR for the assessment of the retinal and choroidal vasculatures.
New noninvasive imaging modalities (as OCTA) may provide valuable information about microvascular changes, the perfusion status of the retina and the likelihood of retinopathy progression during the various stages of DR. OCTA uses the motion contrast provided by flowing erythrocytes to allow dye-free and volumetric visualization of the retinal and choroidal vasculatures at micron-scale resolutions\textsuperscript{10–13}. This technology uses split-spectrum amplitude-decorrelation angiography (SSADA) algorithm to detect motion of erythrocytes in the capillaries\textsuperscript{14}. OCTA delivers depth-resolved retinal vascular structure images, making it possible to differentiate superficial and deep capillary plexuses (SCP and DCP) of retina and also choriocapillaris (CC)\textsuperscript{11,15,16}. Previous qualitative studies in DR have shown that OCTA is able to delineate retinal capillary nonperfusion with higher resolution than conventional FA\textsuperscript{17–19}.

Previous studies have shown that vascular density (VD) of parafovea in SCP and DCP decreases with significant FAZ enlargement in diabetic patients with DR, even in those with no diabetic retinopathy (NDR), compared to healthy subjects\textsuperscript{20,21}. There were no significant differences in the retinal thickness between control subjects and patients with NDR at the same time. Significantly reduced VD in the SCP and DCP in mild nonproliferative DR (NPDR) in comparison to control subjects has also been observed\textsuperscript{22}. It seems that retinal vascular alterations precede structural changes in the retina. This may highly suggest a causal role of circulatory deficit in the development of DR\textsuperscript{20}. Kim et al have detected qualitatively decreasing capillary density, branching complexity, and progressively increasing average vascular caliber in eyes at different stages of DR\textsuperscript{23}. Overall, depending on the method used and methodological differences and metabolic status of the patients studied, conflicting results of the blood flow in diabetic retinal vessels have also been reported both to decrease\textsuperscript{24–26} and to increase\textsuperscript{27–30}.

In this cohort, the aim is the quantitative measurement of VD (as a marker of retinal perfusion) using OCTA in subtypes of DR including those with NDR, NPDR, and proliferative diabetic retinopathy (PDR) as a continuum, and to compare these findings with each other and the normal population. In addition, we evaluate the correlation of VD as an independent predictor of BCVA in diabetic patients.

**Methods**

This prospective cross sectional study was performed between January 2015 and December 2019 at Farabi Eye Hospital, a tertiary university eye center in Tehran, Iran. The institutional ethics committee of Tehran University of Medical Sciences approved the research protocol and a written informed consent was obtained from the participants. The study adhered to the tenets of the Declaration of Helsinki. More than 10 eligible patients did not provide informed consent and were excluded from the study.

Naïve diabetic patients with a history of more than 10 years have been recruited and the control patients have been selected from healthy volunteers. Inclusion criteria were best-corrected visual acuity (BCVA) of 20/20 for normal cases and refractive error between −3 and +1 D spherical equivalent in all groups. The normal volunteers had to have no ocular and systemic disease. Exclusion criteria were significant media opacity preventing high-quality imaging, motion and blinking artifact on the images, poor quality images,
previous focal or panretinal laser photocoagulation, intravitreal anti-VEGF, steroid and/or potentially retinotoxic or neurotoxic drugs consumption, optic neuropathy, any previous ocular and macular disease, previous surgeries other than uncomplicated phacoemulsification (more than 3 months), any inflammatory diseases or active or recent infectious disease (ocular and/or systemic), immunosuppressive drugs or biologic therapies, pregnancy, and uncontrolled hypertension.

Demographic characteristics and relevant laboratory tests such as fasting blood sugar (FBS) and total serum cholesterol level and presence of hypertension were documented. Hemoglobin A1C levels were not checked for all the cases. Best-corrected visual acuity (BCVA) was measured on a Snellen chart and expressed as the logMAR.

Subjects underwent thorough ophthalmic exam including slit lamp biomicroscopy and fundus examination. Intraocular pressure (IOP) was measured with Goldman applanation tonometry. Diabetic patients have been diagnosed based on the criteria of the American Diabetes Association and all were under treatment for diabetes. The diabetic patients were classified into three groups ranked in ascending order of DR severity: NDR, NPDR, and PDR, based on early treatment for diabetic retinopathy study (ETDRS) classification.

**Acquisition Of The Images**

Clinical examination and SD-OCT imagings were performed at the same day between 8:00 am and 2:00 pm. Two professional image readers (FG, SB) checked and assessed all of the OCT images. AngioVue OCTA imaging (RTVue XR Avanti; Optovue, USA- version: 2016.1.0.23- beta) using SSADA algorithm was performed. This instrument performs 70000 A-scans per second (840-nm) to capture OCTA images of horizontal and vertical B-scans in transverse dimension to provide a 3 × 3 mm (304 × 304 pixels) image centered at the fovea. Scans with low quality (i.e., presence of blink or motion artifact) were repeated until good quality scans were achieved. Automated segmentation was utilized for defining SCP, DCP, and CC. The SCP was defined as area between 3 µm below to internal limiting membrane (ILM) and 15 µm below internal plexiform layer (IPL). The DCP was considered to be between 15 µm and 71 µm below IPL.

Automatic segmentation was fine-tuned manually where appropriate. Each macular OCT-A layer was subdivided into nine areas of interest—whole macula, fovea, parafovea, superior hemield, inferior hemield, temporal, superior, nasal, and inferior for quantitative measurements of the vascular density of SCP, DCP, and CC. The foveal region was outlined as a central circle with a 120-pixel (1.2 mm) diameter, and the parafoveal region was delineated as a ring, by 91 pixels wide, surrounding the foveal region. To calculate VD, the AngioVue Analytics software extracts a binary image of the blood vessels from the gray scale OCTA image, and then calculates the percentage of pixels occupied by blood vessels in the defined region. Diabetic macular edema (DME) was defined as the central macular thickness (CMT) of more than 300 µm.
Statistical Methods

All quantitative variables were reported as mean with standard deviation after confirming normality of distribution with the Kolmogorov-Smirnov test. Non-normal distributed parameters are reported by median with the range. All statistical analyses were performed using statistical software (SPSS software Version 21; SPSS, Inc., Chicago, IL, USA). Kruskal-Wallis test and one-way analysis of variance (ANOVA) were performed for nonparametric and parametric comparison. Mann Whitney U test and post-hoc analysis (dunnett’s test) were used to compare choroidal thicknesses between groups. In this study collinearity for different variables was checked. P values less than 0.05 were considered statistically significant.

Results

General characteristics

A total of 188 eyes of 97 participants with the mean age of 56.5 ± 8.9 years (range: 25–80) were analyzed. Of these 41 (42.3%) were male and 56 (57.7%) were female. BCVA was significantly lower in the patients with PDR and NPDR in comparison with NDR and normal subjects.

Mean FBS was 209.85 ± 85.6 mg/dl in the diabetic patients. Mean diabetes mellitus (DM) duration in diabetic patients was 12.7 ± 6.3 years. The control group included 40 eyes and the diabetic group comprised 148 eyes in the study. Based on the DR severity scale, the diabetic group had 39 (26.4%) eyes with NDR, 41 (27.7%) eyes with mild to moderate NPDR, 25 (16.9%) eyes with severe NPDR, 26 (17.6%) with early PDR and 17 (11.5%) eyes with high-risk characteristic PDR. Diabetic macular edema (DME) was present in 22 (34.4%) of NPDR and 16 (35.6%) of PDR patients. Table 1 presents the baseline characteristics of the participants.
Table 1
Demographic characteristics of normal subjects and subtypes of diabetic retinopathy (N = 188).

| Groups                          | NL (40 eyes) | NDR (39 eyes) | NPDR (64 eyes) | PDR (45 eyes) | P-value |
|--------------------------------|--------------|---------------|----------------|---------------|---------|
|                                | (M ± SD)     | (M ± SD)      | (M ± SD)       | (M ± SD)      |         |
| Age (Y)                        | 50 ± 0.72    | 59.28 ± 1.02  | 58.31 ± 1.14   | 59.03 ± 1.68  | 0.003   |
| (%) OD                         | 20 (50)      | 19 (48.7)     | 33 (51.6)      | 25 (55.6)     | 0.929   |
| Sex-male (%)                   | 24 (60)      | 15 (38.5)     | 25 (39.1)      | 20 (44.4)     | 0.156   |
| BCVA (decimal)                 | 0.93 ± 0.01  | 0.84 ± 0.02   | 0.65 ± 0.02    | 0.62 ± 0.04   | < 0.001 |
| The last FBS (mg/dl)           | -            | 158.46 ± 5.72 | 189.78 ± 8.38  | 202.12 ± 7.30 | < 0.001 |
| Duration of diabetes mellitus (Y) | -            | 12.38 ± 1.02  | 12.96 ± 0.78   | 16.54 ± 1.1   | < 0.001 |
| Hypertension number (%)        | 2 (5%)       | 16 (41%)      | 27 (42%)       | 17 (51.5%)    | < 0.001 |
| Hyperlipidemia number (%)      | 0 (5%)       | 15 (38.5%)    | 32 (50%)       | 16 (48.5%)    | < 0.001 |

**BCVA:** Best corrected visual acuity, **DME:** Diabetic macular edema, **FBS:** Fasting blood sugar, **NDR:** No diabetic retinopathy, **NL:** Normal, **NPDR:** Nonproliferative diabetic retinopathy, **PDR:** Proliferative diabetic retinopathy

The difference in BCVA (decimal), sex and age were statistically significant. Spherical equivalent, axial length or intraocular pressure did not vary significantly in the groups (p > 0.05). Hyperlipidemia and hypertension were more prevalent in diabetic groups than the control group (p < 0.001). The hypertension was under control in all groups.

### Vascular Density

Tables 2, 3, and 4 show VD in SCP, DCP, and CC, respectively. Figure 1 illustrates the distribution of grid based VD in the SCP, DCP, and CC in a radar plot. Statistically significant differences were observed in VD at various areas among normal control subjects and different stages of DR. All the VD amounts in SCP and DCP had abnormal distribution in the Kolmogorov–Smirnov test. The trend toward lower amount of VD (median) from normal subjects to PDR patients in macular area and all various subsegments was notable in both SCP and DCP. There was continuous and significant decrease from the normal cases to the NDR group in both SCP and DCP (Tables 2 and 3). The comparative tests (Mann-Whitney U test) showed that in all studied subsegments the decrease in the amounts by stepping from one stage to the...
others was statistically significant ($P < 0.05$). The exception was the foveal VD that was not significantly changed when comparing NDR with NPDR and NPDR with PDR in both SCP and DCP.
Table 2
Vascular density in superficial capillary network (SCN) of normal subjects and subtypes of diabetic retinopathy (N = 188).

| Groups       | NL (40 eyes) | NDR (39eyes) | NPDR (64eyes) | PDR (45eyes) | P-value Kruskal –Wallis test |
|--------------|--------------|--------------|---------------|--------------|-----------------------------|
| Reference group | Median (range) (%) | Median (range) (%) | Median (range) (%) | Median (range) (%) |                             |
| Whole image  | 54.65 (47.00–50.04) | 51.90 (42.40–56.28) | 48.05 (35.07–55.78) | 45.50 (33.74–50.48) | < 0.001*                   |
| Normal       |              |              |               |              |                             |
| NDR          |              |              |               |              |                             |
| NPDR         |              |              |               |              |                             |
| PDR          |              |              |               |              |                             |
| Fovea        | 29.17 (23.94–41.86) | 26.26 (20.77–34.20) | 27.07 (16.11–38.10) | 24.00 (17.68–36.14) | 0.001                       |
| Normal       |              |              |               |              |                             |
| NDR          |              |              |               |              |                             |
| NPDR         |              |              |               |              |                             |
| Para-fovea   | 57.37 (50.09–62.24) | 54.54 (42.74–59.47) | 49.96 (36.69–57.81) | 47.24 (35.77–52.32) | < 0.001                     |
| Normal       |              |              |               |              |                             |
| NDR          |              |              |               |              |                             |
| NPDR         |              |              |               |              |                             |

NDR: No diabetic retinopathy, NL: Normal, NPDR: Nonproliferative diabetic retinopathy, PDR: Proliferative diabetic retinopathy

* Mann- Whitney test
| Groups          | NL (40 eyes) | NDR (39 eyes) | NPDR (64 eyes) | PDR (45 eyes) | P-value |
|----------------|--------------|---------------|----------------|---------------|---------|
| Superior-hemi  |              |               |                |               |         |
| Normal         | 57.25        | 54.03         | 49.92          | 46.67         | < 0.001 |
|                | (48.60–62.67)| (44.06–59.20)| (34.64–57.05)  | (33.59–53.64) |         |
|                |              |               |                |               |         |
| Normal         | 57.39        | 54.13         | 49.70          | 46.82         | < 0.001 |
|                | (49.50–62.60)| (41.44–59.73)| (38.70–58.55)  | (38.06–53.41) |         |
|                |              |               |                |               |         |
| Normal         | 55.76        | 53.57         | 48.62          | 46.17         | < 0.001 |
|                | (48.17–60.28)| (39.58–58.12)| (36.52–57.70)  | (37.79–52.46) |         |
|                |              |               |                |               |         |
| Normal         | 58.50        | 55.57         | 50.58          | 46.25         | < 0.001 |
|                | (49.62–63.98)| (45.22–59.62)| (36.96–57.50)  | (34.26–53.65) |         |
|                |              |               |                |               |         |

NDR: No diabetic retinopathy, NL: Normal, NPDR: Nonproliferative diabetic retinopathy, PDR: Proliferative diabetic retinopathy

* Mann-Whitney test
| Groups         | NDR (39eyes)     | NL (40 eyes)     | NPDR (64eyes) | PDR (45eyes) | P-value       |
|---------------|------------------|------------------|---------------|--------------|---------------|
|               | Median (range)   | Median (range)   | Median (range)| Median (range)| Kruskal –Wallis test |
| Nasal Normal  | 53.44 (49.50–62.64) | 57.13 (49.50–62.64) | 49.31 (33.37–57.84) | 46.34 (31.09–52.93) | < 0.001*       |
| NDR NPDR      | < 0.001*         | < 0.001*         | < 0.001*      | 0.002*       |
| Inferior Normal| 55.06 (48.20–63.75) | 57.87 (48.20–63.75) | 50.35 (39.90–59.36) | 47.34 (38.26–54.97) | 0.043          |
| NDR NPDR      | < 0.001*         | < 0.001*         | < 0.001*      | < 0.001*     |
|               |                  |                  | < 0.001*      | < 0.001*     |

NDR: No diabetic retinopathy, NL: Normal, NPDR: Nonproliferative diabetic retinopathy, PDR: Proliferative diabetic retinopathy

* Mann-Whitney test
Table 3
Vascular density in deep capillary network (DCN) in normal subjects and subtypes of diabetic retinopathy (N = 188).

| Groups       | NL (40 eyes) | NDR (39 eyes) | NPDR (64 eyes) | PDR (33 eyes) | P-value Kruskal –Wallis test |
|--------------|--------------|---------------|----------------|---------------|-----------------------------|
| VD/Reference group | Median (range) (%) | Median (range) (%) | Median (range) (%) | Median (range) (%) |                            |
| Whole image  | 60.42 (47.00–64.55) | 58.05 (51.34–60.64) | 53.84 (41.34–60.67) | 51.24 (42.50–57.58) | < 0.001*                   |
| Normal       | < 0.001*     | < 0.001*      | < 0.001*        | < 0.001*       |                             |
| NDR          |              |               |                |               |                             |
| NPDR         |              |               |                |               |                             |
| Fovea        | 31.24 (20.30–42.32) | 27.72 (20.28–35.46) | 27.14 (15.48–40.50) | 24.94 (12.19–39.30) | < 0.001*                   |
| Normal       |              |               |                |               |                             |
| NDR          | 0.001*       | < 0.001*      | < 0.001*        | < 0.001*       |                             |
| NPDR         |              |               |                |               |                             |
| Para-fovea   | 62.89 (50.10–67.22) | 60.77 (52.90–63.51) | 56.49 (42.94–62.75) | 54.16 (42.88–59.89) | < 0.001*                   |
| Normal       |              |               |                |               |                             |
| NDR          | < 0.001*     | < 0.001*      | < 0.001*        | < 0.001*       |                             |
| NPDR         |              |               |                |               |                             |

NDR: No diabetic retinopathy, NL: Normal, NPDR: Nonproliferative diabetic retinopathy, PDR: Proliferative diabetic retinopathy

*Mann-Whitney test
| Groups          | NDR (39 eyes) | NPDR (64 eyes) | PDR (33 eyes) | P-value Kruskal –Wallis test |
|-----------------|---------------|----------------|---------------|-----------------------------|
| Superior hemi   | 61.02         | 56.14          | 55.33         | < 0.001*                   |
| Normal          | (52.40–64.34) | (44.02–62.61)  | (41.19–61.91) |                             |
| NDR             | < 0.001*      | < 0.001*       | < 0.001*      |                             |
| NPDR            | < 0.001*      | < 0.001*       | < 0.001*      |                             |
|                 |               |                | < 0.001*      |                             |
| Inferior hemi   | 60.51         | 55.96          | 54.07         | < 0.001*                   |
| Normal          | (52.17–63.94) | (40.75–63.36)  | (43.65–60.35) |                             |
| NDR             | < 0.001*      | < 0.001*       | < 0.001*      |                             |
| NPDR            | 0.001*        | < 0.001*       | < 0.001*      |                             |
|                 |               |                | < 0.001*      |                             |
| Temporal        | 61.04         | 53.33          | 53.31         | < 0.001*                   |
| Normal          | (49.90–63.36) | (32.32–61.74)  | (42.70–60.56) |                             |
| NDR             | 0.011*        | < 0.001*       | < 0.001*      |                             |
| NPDR            | < 0.001*      | < 0.001*       | < 0.001*      |                             |
|                 |               |                | 0.004*        |                             |
| Superior        | 62.09         | 58.62          | 55.60         | < 0.001*                   |
| Normal          | (53.30–65.34) | (45.35–63.41)  | (42.14–60.70) |                             |
| NDR             | < 0.001*      | < 0.001*       | < 0.001*      |                             |
| NPDR            | < 0.001*      | < 0.001*       | < 0.001*      |                             |
|                 |               |                | 0.005*        |                             |

NDR: No diabetic retinopathy, NL: Normal, NPDR: Nonproliferative diabetic retinopathy, PDR: Proliferative diabetic retinopathy

*Mann-Whitney test
| Groups VD/ Reference group | NL (40 eyes) Median (range) (%) | NDR (39 eyes) Median (range) (%) | NPDR (64 eyes) Median (range) (%) | PDR (33 eyes) Median (range) (%) | P-value Kruskal –Wallis test |
|---------------------------|--------------------------------|---------------------------------|---------------------------------|---------------------------------|-----------------------------|
| Nasal                     | 62.56 (52.90–67.02)            | 59.17 (52.40–62.53)             | 55.64 (35.39–63.44)             | 53.48 (39.92–59.70)             | < 0.001*                    |
| Normal                    |                                |                                 |                                 |                                 |                             |
| NDR                        | < 0.001*                       | < 0.001*                        | < 0.001*                        |                                 |                             |
| NPDR                       |                                 | 0.001*                          |                                 |                                 |                             |
|                            |                                 |                                 |                                 |                                 |                             |
| Inferior                  | 63.76 (48.20–67.86)            | 62.09 (52.23–65.59)             | 56.76 (43.45–64.21)             | 54.06 (44.73–61.92)             | < 0.001*                    |
| Normal                    |                                |                                 |                                 |                                 |                             |
| NDR                        | < 0.001*                       | < 0.001*                        | < 0.001*                        |                                 |                             |
| NPDR                       |                                 | 0.013*                          |                                 |                                 |                             |
|                            |                                 |                                 |                                 |                                 |                             |

NDR: No diabetic retinopathy, NL: Normal, NPDR: Nonproliferative diabetic retinopathy, PDR: Proliferative diabetic retinopathy

*Mann- Whitney test
Table 4
Choriocapillaris (CC) vascular density in different study groups (ANOVA) normal participants and diabetic patients. Post Hoc analysis of subfields choroidal thickness of the studied participants (N = 188).

| Groups              | VD/ reference group | NDR (39 eyes) (M ± SD) (%) | NPDR (64 eyes) (M ± SD) (%) | PDR (33 eyes) (M ± SD) (%) | P-value ANOVA |
|---------------------|---------------------|-----------------------------|-----------------------------|-----------------------------|---------------|
| Whole image Normal  | 69.82 ± 4.02        | 70.01 ± 2.90                | 67.06 ± 5.52                | 67.25 ± 4.66                | < 0.001       |
| Normal NDR          | < 0.001*            | 0.949*                      | < 0.001*                    | < 0.001*                    |               |
| NPDR                | 0.949*              |                            |                             |                             |               |
| Fovea Normal        | 71.39 ± 3.97        | 71.50 ± 3.80                | 68.32 ± 5.16                | 68.98 ± 5.29                | < 0.001       |
| Normal NDR NPDR     | 0.996               | < 0.001*                    | 0.001*                      | 0.003*                      |               |
| Normal              | 0.996               | < 0.001*                    |                             |                             |               |
| NPDR                | 0.950               |                             |                             |                             |               |
| Para-fovea Normal   | 69.36 ± 4.54        | 69.52 ± 2.97                | 66.37 ± 5.89                | 67.05 ± 4.88                | < 0.001       |
| Normal NDR NPDR     | 0.980*              | < 0.001*                    | 0.001*                      | 0.008*                      |               |
| Normal              | 0.980*              | < 0.001*                    |                             |                             |               |
| NPDR                | 0.798*              |                             |                             |                             |               |
| Superior hemi Normal| 69.92 ± 4.12        | 69.72 ± 2.94                | 66.74 ± 5.74                | 67.39 ± 4.25                | < 0.001       |
| Normal NDR NPDR     | 0.914*              | < 0.001*                    | 0.011*                      | 0.074*                      |               |
| Normal              | 0.914*              | < 0.001*                    |                             |                             |               |
| NPDR                | 0.437*              |                             |                             |                             |               |
| Inferior hemi Normal| 69.70 ± 4.06        | 70.28 ± 3.12                | 67.16 ± 5.58                | 67.70 ± 5.18                | < 0.001       |
| Normal NDR NPDR     | 0.993*              | < 0.001*                    | 0.004*                      | 0.002*                      |               |
| Normal              | 0.993*              | < 0.001*                    |                             |                             |               |
| NPDR                | 0.991*              |                             |                             |                             |               |

NDR: No diabetic retinopathy, NL: Normal, NPDR: Nonproliferative diabetic retinopathy, PDR: Proliferative diabetic retinopathy

*Tuckey HSD test
| Groups | NL (40 eyes) | NDR (39 eyes) | NPDR (64 eyes) | PDR (33 eyes) | P-value |
|--------|--------------|---------------|----------------|---------------|---------|
| VD/    | (M ± SD) (%) | (M ± SD) (%)  | (M ± SD) (%)   | (M ± SD) (%)  | ANOVA   |
| reference group | | | | | |
| Temporal | 69.25 ± 5.79 | 69.66 ± 3.46 | 66.66 ± 6.23 | 67.88 ± 6.13 | < 0.001 |
| Normal | | 1.000* | 0.002* | 0.020* | |
| NDR | | 0.002* | 0.018* | | |
| NPDR | | | 0.964* | | |
| Superior | 69.19 ± 4.52 | 69.19 ± 3.15 | 66.66 ± 6.14 | 66.92 ± 4.91 | < 0.001 |
| Normal | | 0.984* | < 0.001* | 0.042* | |
| NDR | | 0.001* | 0.107* | | |
| NPDR | | | 0.488* | | |
| Nasal | 69.34 ± 4.56 | 68.78 ± 3.63 | 65.84 ± 7.10 | 66.92 ± 4.33 | < 0.001 |
| Normal | | 0.785* | < 0.001* | 0.025* | |
| NDR | | 0.001* | 0.236* | | |
| NPDR | | | 0.382* | | |
| Inferior | 69.54 ± 4.32 | 70.90 ± 3.01 | 66.78 ± 6.38 | 67.33 ± 5.34 | < 0.001 |
| Normal | | 0.933* | 0.003* | 0.010* | |
| NDR | | 0.001* | < 0.001* | | |
| NPDR | | | 0.999* | | |

NDR: No diabetic retinopathy, NL: Normal, NPDR: Nonproliferative diabetic retinopathy, PDR: Proliferative diabetic retinopathy

*Tuckey HSD test

This means that there is a pattern of fluctuation in the VD of SCP and DCN in the whole picture and foveal region but a steady slight decrease from NL to the PDR level is obvious, which is more pronounced in the NPDR stage in the parafoveal region. (Fig. 2, Table 5).
Table 5
Choriocapillaris thickness changes per percentage compared to the previous stage.

| Percentage of changes          | NDR–NL/NL (%) | NPDR-NDR /NDR (%) | PDR-NPDR/NPDR (%) |
|-------------------------------|---------------|-------------------|-------------------|
| SCP Whole image               | -5.1          | -7.3              | -5.4              |
| SCP Fovea                     | -9.9          | 3.0               | -11.4             |
| SCP Para-fovea                | -5.1          | -8.3              | -5.6              |
| DCP Whole image               | -3.8          | 1.2               | -12.9             |
| DCP Fovea                     | -11.2         | -2.2              | -8.1              |
| DCP Para-fovea                | -3.3          | -7.1              | -4.1              |
| CC Whole image                | 0.3           | -4.1              | 0.3               |
| CC Fovea                      | 0.1           | -4.5              | 1.0               |
| CC Para-fovea                 | 0.1           | -4.5              | 1.1               |

NDR: No diabetic retinopathy, NL: Normal, NPDR: Nonproliferative diabetic retinopathy, PDR: Proliferative diabetic retinopathy

In the CC the amounts of VD were normally distributed (Kolmogorov–Smirnov test) and the amount of the changes were statistically significant (ANOVA) (Table 4). Post-hoc analysis (Tuckey test) revealed that no statistically significant changes occur between normal cases and the patients with NDR (P > 0.05). The VD of CC under fovea and all studied subsegments of increased in NDR cases and then in NPDR and PDR cases decreased to less than the according amounts in normal cases. This comparison with NDR showed significant changes in the NPDR group in all subsegments. In the PDR group, all segments showed significant changes compared to NDR except at the nasal and the superior subsegments. While the VD of CC increased in all subsegments of PDR compared with NPDR cases, they were not statistically significant (P > 0.05).

This entire means that there is minor increase (0.3%) in VD of CC from normal cases to cases of NDR and then a pattern of decrease (-4.1%) in NPDR and then a slight increase (0.3%) happens again in the PDR stage but never touching the normal quantities (Fig. 2, Table 5).

Correlations Of Vd

In order to determine the association of VD and other variables univariate analysis was performed for diabetic patients. Age was only inversely correlated with parafoveal at SCP (r: -0.189, P = 0.015).

Systolic blood pressure was inversely weakly correlated with *whole image VD* in CC (r: -0.155, P = 0.034) but not with VD in other segments. FBS was inversely correlated with whole image VD in SCP, DCP (r: -0.50, P < 0.001; r: -0.460, P < 0.001; and r: -0.222, P = 0.002; respectively) and foveal VD in SCP, DCP (r:
-0.264, P < 0.001; r: -0.371, P < 0.001; respectively) but had marginally significant negative correlation with foveal VD at CC layer (r: -0.141, P = 0.056).

DM duration in patients was also a significant factor in association with VD. It had inverse strong correlation with whole image VD in SCP, DCP, and CC (r: -0.354, P < 0.001; r: -0.352, P < 0.001; r: -0.106, P = 0.002; respectively). It was slightly correlated only with foveal VD in DCP (r: -0.263, P < 0.001).

Partial correlation after adjusting for the effect of age and sex showed that in diabetic patient BCVA was not correlated with the foveal VD at SCP. There was a positive correlation between BCVA and whole image VD in SCP, DCP, and CC (r: 0.497, P < 0.001; r: 0.505, P < 0.001, r: 0.233, P = 0.0021; respectively), foveal VD in DCP, and CC (r: 0.171, P = 0.023; r: 0.354, P < 0.001; respectively) and parafoveal VD in SCP, DCP, and CC (r: 0.542, P < 0.001; r: 0.520, P < 0.001, r: 0.354, P < 0.001; respectively).

In linear regression analysis, it seems that in diabetic patients after adjusting for sex, age, duration of DM, presence of DME, blood pressure and FBS in the model, among foveal VD in 3 layers, only VD of CC was significantly correlated with BCVA (B: 0.40, P < 0.001). This test for whole image VD in all 3 layers showed correlation with VD in whole VD of SCP (B: 0.26, P = 0.052). In the same model, of parafoveal VD, only SCP showed the correlation (B: 0.37, P = 0.007). Applying two significant factors, considering the collinearity, the test showed that irrespective to the staging of the DR, superficial parafoveal VD (B: 0.35, P < 0.001) and VD of foveal CC (B: 0.26, P = 0.001) had the most effect on the BCVA. Adding the staging of the DR (B: -0.25, P = 0.007), showed similar results by the parameters described above.

**DME**

Within diabetes groups, we found differences in VD between DME and non-DME patients in all subfields except VD of foveal DCP (P = 0.716). All subfields had less amounts in DME patients (all P < 0.05). These were far less than the amounts in the healthy control eyes (p < 0.05).

**Discussion**

This report, using commercially available OCTA system and integrated automated techniques, showed lower amounts of VD in macular area and all various subsegments of SCP and DCP in diabetic patients compared with normal controls. There was a constant and significant decrease from the normal cases to NDR, NDR to NPDR, and NPDR to PDR groups in both SCP and DCP, with the exception of SCP foveal VD and DCN complete VD, which increased moderately. In the CC, the VD decreased insignificantly from normal to NDR and then decreased significantly in NPDR stage and then rose marginally (not statistically significant) in the PDR stage but never reached the normal quantities. BCVA was correlated mainly with parafoveal VD in SCP and foveal VD in CC. VD of all macular area subfields except the VD of foveal DCP showed lesser levels in DME patients compared with those without DME.

**VD in retinal microvessels**
The present study showed lower amount of VD in macular area and all various subsegments of SCP and DCP in diabetic patients compared with normal controls. Although structurally different, previous studies including ours have revealed reduced VD parafoveal VD in DR without considering the staging of the DR. But our results for the central 1mm and whole image VD in SCP and DCP differs with their results. Some qualitative or quantitative studies of microvascular changes showed increased non-perfused areas from normal cases to the higher stages of the DR.

After adjusting for hypertension, age, sex, and duration of DM our research showed that VD in SCP and DCP healthy subjects were higher than that in NDR group. Nesper et al adjusted their data to the hypertension and obtained the same result as ours. This finding supports VD changes being a potential biomarker of DR before retinopathy becomes clinically apparent. Previous studies showed increased blood flow in the early stages of DR, but the flow decreases in the advanced stages of the disease. A study that evaluated retinal blood circulation, using video fluorescein angiography, has described decreased mean circulation time in diabetic patients without DR. This incongruence could be due to special retinal microvascular blood flow autoregulation and nonlinear correlation between large vessels flow and capillary flows in the retina.

On the other hand, it has been shown that early retinal vascular dysregulation occurs in DR. By Doppler method, the maximum or centerline velocity of RBCs was shown to be slower than normal in the eyes with DR. In Grunwald’s study, the maximum velocity of red blood cells was significantly lower than normal in eyes with diabetic retinopathy. Calculated volumetric blood flow rate, however, was not significantly different from normal in eyes with NDR, NPDR, and PDR, and was significantly decreased from normal in patients with PDR who had been treated by panretinal photocoagulation. Although hard to be contemplated, the slow rate of RBC movements could explain the less detectability of the microvessels by OCTA. It is known that acute elevation of fibrinogen, haptoglobin, and other globulin may increase thixotropy and viscoelasticity of the blood. There are diabetic retina changes that may trigger hypoxia at some point, diabetes, including leukostasis and sluggish circulation, excitotoxic damage to glial cells, which could contribute to impaired neurovascular coupling and hypoperfusion, and endothelial cell and pericyte loss that destroys capillaries. This indicates that slower capillary blood flow below the lowest detection level for retinal vasculature will result in undetectable vessels or lower VD on OCTA at least in the earlier phase of diabetic retinopathy or best to say NDR.

Unlike our result, Nesper et al. showed the strongest correlation between the DCP vessels density and with DR severity not the full retina, SCP, and choriocapillaris VD. Unlike our quantitative results of uniform decreasing of VD in both SCP and DCP, some qualitative studies showed more defective VD of DCP in NDR. Previous reports have found microaneurysms to be present in a larger extent in DCP than in the superficial plexus.

The contradictory findings found in the studies may be due to different imaging and measurement techniques, the various phases of the disease being investigated and the varying length of DM in the
studies, and animal rather than human researches.

**VD in CC**

Consistent with our series, Forte et al showed that in CC, the VD was not more different in Type 1 and Type 2 DM with NDR than controls in any of the evaluated quadrants.\textsuperscript{36,50}

Some studies described diabetic choroidopathy in histology, electron microscopy, and FA and ICGA studies in the earlier stages of DR.\textsuperscript{51-55} Our results fit with other studies that showed significantly decreased posterior ciliary arteries, using color Doppler imaging, in patients with background DR.\textsuperscript{20}

In addition, the severity of VD changes in SCP and DCP and CC alteration were evidently correlated except for foveal VD in both retinal layers (P>0.05). This study provides new information on CC alterations that occur in the course of DR stages from NDR to PDR. Which of the retina VD or choroid VD has more impacts on DR remains to be determined in larger studies.

Although there are many studies,\textsuperscript{51-55} with conflicting findings on the role of CC in DR, a meta-analysis is required to arrive at a definitive result in this period.

**Correlation of VD and visual acuity**

After adjusting to several conflicting variables, this study found that vision was directly associated with parafoveal VD at SCP and subfoveal VD at CC. The result was independent of the stage of the DR. Dissimilar to our result; previous researches showed that VD of DCP has more impact on the VA than SCN.\textsuperscript{56}

In diabetic patients, the reasons for reduced VA include macular ischemia, photoreceptor dysfunction, and accumulated subfoveal hard exudates.\textsuperscript{59} There is a correlation between retinal nonperfusion and the integrity of the photoreceptors.\textsuperscript{35,60} There was a strong correlation between external limiting membrane (ELM) and ellipsoid zone (EZ) integrity for DME patients.\textsuperscript{61} On the other hand, a recent study reported similar correlations between the photoreceptor layer integrity and VA among patients with DME and implied that the extent of macular edema does not match the severity of visual dysfunction.\textsuperscript{61,62}

Using oxygen-sensitive microelectrodes and oximetry, Linsenmeier and Zhang discovered that the choroidal and retinal circulation could supply the metabolic demand of photoreceptors in various proportions under both dark and light conditions.\textsuperscript{63} It is important to know that, while most of the outer retina including photoreceptor is nourished with choroidal circulation, the contribution of the retinal circulation to photoreceptor metabolism is modest.\textsuperscript{63} They calculated that the photoreceptor demand, met by the retinal circulation, was different in dark and lighted conditions (10 to 18% in darkness).\textsuperscript{63}

In a cohort of DR patients using adaptive optics and OCTA, Nesper et al. observed changes in capillary dropout areas in the DCP, indicating that DCP integrity is required for the metabolism of the photoreceptor
Incongruously, the VD of parafoveal SCP not DCP in our patient only had some effect on the vision. Recently, by using spectral-domain OCT (SD-OCT), the concept of disorganization of retinal inner layers (DRIL) has been described to characterize retinal thinning with the loss of identifiable borders between retinal cellular layers in the background of capillary non-perfusion. Areas of DRIL were significantly correlated with disturbance of the photoreceptor (P=0.035) relative to adjacent DRIL-free areas. 

The decrease in VD in the SCP was significantly associated with the decrease in inner retinal thickness in DR, a notorious finding in mild DR, which may contribute to DRIL in the case of more serious capillary dropout. On the contrary, there was no association between the decrease in VD in the DCP and the thickness of the inner nuclear / outer plexiform layer (INL / OPL). The explanation for this disparity, also seen in healthy eyes, is unknown. May be the SCP is much important for the inner layer nourishment.

Onishi et al reported that DRIL is due to a defect in DCP with involvement of the MCP, SCP, or both in 28 cases of well developed DR (NPDR and PDR cases), although it is also reported in early stages of DR cases. Moeini et al reported capillary flow impairment in the SCP on OCTA may be a predictor of visual acuity in patients with DRIL. It is believed that the SCP, whose vascular supply is mainly arterial, has a standardized measurement, and is better to reveal ischemia data than the DCP. Other researchers measured parafoveal VD as our study. It also decreased in both the SCP and the DCP in their analysis.

DME

Macular edema can develop at any stage of DR. We found that in all subfields, the patients with DME had less VD than the patients without DME, except for the foveal VD at DCP. Inconsistent with our findings, Tang et al. did not observe any statistically significant association between OCTA metrics and the presence of DME on SCP. Lee et al. recently demonstrated that eyes with DME have lower VD only at DCP, compared with eyes without DME.

A limitation of this study was a modest age difference between control group and other groups, although in all stages of the analytic part of the study, the age matching was performed. The small field of OCTA imaging and the use of both eyes in the analysis were other limitations. The automated programmed algorithm in the AngioVue system only segments two retinal capillary plexuses: the SCP and the DCP, and the middle capillary plexus (MCP) that is defined by swept source OCT could not be calculated.

Despite previous similar study, the advantage of present study was the inclusion of treatment naïve cases and an almost large sample size as well as the use of statistical models with adjustments for covariates.
In assumption, our study showed that VD in OCTA is correlated significantly and linearly with disease severity in eyes with DR. The results support VD of retinal vessels and CC to be a potential surrogate for DR before clinical signs development. We introduced parafoveal VD of SCP and foveal VD of CC as biomarkers to predict the VA loss in the diabetic patient. As a noninvasive and rapidly acquired technique, OCTA may be a tool for early detection of microvascular abnormalities in the retina and choroid, elucidating the pathogenesis of retinopathy, and treatment response monitoring in patients with diabetes.

**Abbreviations**

CC: choriocapillaris, CMT: central macular thickness, DR: diabetic retinopathy, DME: Diabetic macular edema, MCP: middle capillary plexus, NPDR: nonproliferative diabetic retinopathy, OCTA: optical coherence tomography angiography, PDR: proliferative diabetic retinopathy, SD OCTA: spectral domain optical coherence tomography angiography, DCP: deep capillary plexus, SCP: superficial capillary plexus, VD: vascular density, VEGF: vascular endothelial growth factor

**Declarations**

**Ethics approval and consent to participate**

Ethics approval and consent were obtained from the Tehran University of Medical Sciences Institutional Review Boards.

**Consent for publication**

Not applicable.

**Availability of data and materials**

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

**Competing interests**

The authors declare that they have no competing interests.

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**Authors’ contributions**

AB, SB, AG, and FG collected the data.

FG and SS analyzed and interpreted the data.
FG and KF were major contributors in writing the manuscript.

All authors read, commented and approved the final manuscript.

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