Diabetic mellitus (DM) is a chronic condition that is estimated to rise to 360 million by the year 2030, according to the World Health Organization. Patients with DM are at high risk of developing diabetic retinopathy, a progressive condition that causes alterations in the retinal microvasculature. Diabetes mellitus can lead to conditions such as retinal neovascularization, macular edema, and retinal ischemia. Optical coherence tomography angiography (OCTA) is a recent, noninvasive, dye-free imaging technique that can be used for evaluating the retinal vasculature by capturing the dynamic motion of erythrocytes. Optical coherence tomography angiography has been shown to be a useful imaging modality for evaluation of ophthalmologic diseases such as DR, artery and vein occlusions, and glaucoma.

Recent qualitative and quantitative studies on DR using OCTA have shown modifications in the foveal avascular zone (FAZ) and retinal microvasculature compared with normal eyes. This study aimed to develop an automated method for segmentation and quantification of the FAZ, vessel density, and spacing between large and small vessels in the OCTA images of the superficial and deep retinal vascular plexuses. The study used local fractal analyses to quantify the local variations in both the superficial and deep retinal vasculature of the OCTA scans. Correlations of levels of fasting blood sugar (FBS), postprandial blood sugar (PPBS), and glycosylated hemoglobin (HbA1c) with the retinal vascular parameters were also analyzed.

**METHODS**

This prospective, observational, cross-sectional study was approved by the institutional ethics committee of the Narayana Nethralaya Super Specialty Eye Hospital, Bangalore, India. The research followed the tenets of the Declaration of Helsinki. Written informed consent was obtained from all the subjects before imaging. A total of 209 eyes of 122 type 2 DM Indian patients with DR (age: 38–80 years; female-to-male ratio = 0.51) and 60 eyes of 31 normal Indian subjects (age: 24–60 years; female-to-male ratio = 0.82) were included in this study. We excluded the following patients from this study: patients with macular edema, media opacities, refractive error more than ±6 diopters, and retinal inflammation, and should therefore be regarded as equivalent authors.

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**RESULTS.** Normal eyes had a significantly lower FAZ area, higher vessel density, and lower spacing between large and small vessels compared with DR grades (P < 0.001). In the superficial layer, PDR and severe NPDR had higher spacing between large vessels than mild and moderate NPDR (P = 0.04). However, mild NPDR had higher spacing between the small vessels (P < 0.001). Spacing between the large vessels in the superficial retinal layer correlated positively with HbA1c (r = 0.25, P = 0.03); fasting (r = 0.23, P = 0.02); and postprandial (r = 0.26, P = 0.03) blood sugar. The same spacing in the deep retinal vascular plexus had the highest area under the ROC curve (0.99 ± 0.01) and was uniformly elevated in all diabetic eyes (P > 0.05).

**CONCLUSIONS.** Spacing between the large vessels in the superficial and deep retinal layers had superior diagnostic performance than overall vessel density.

Keywords: retina, blood vessels, optical coherence tomography, angiography, fractal analysis
diabetics, low vision, renal disease, hypertension, coexisting retinal diseases, large retinal nonperfusion around macula, intraocular surgery performed less than 6 weeks prior to imaging, patients undergoing intravitreal injection treatment, and those who underwent focal laser or panretinal photocoagulation laser treatment less than 6 weeks prior to imaging. Furthermore, OCTA images with significant defects (motion artifacts, projection artifacts, vessel doubling, and/or stretching defects) were also excluded. For every patient, a detailed medical and ophthalmic history was obtained, including the duration of diabetes. In each DR patient, we evaluated FBS (mg/dL) after a minimum of 8 hours overnight fasting; PPBS (mg/dL); blood pressure (mm Hg); body mass index (BMI, kg/m²); hemoglobin (Hb, gm/dL); HbA1c (%); low density lipoprotein (LDL, mg/dL); and high density lipoprotein (HDL, mg/dL).

All normal and DR subjects underwent imaging on a spectral-domain OCTA system (AngioVue; Optovue, Inc., Fremont, CA, USA) by a single operator using the accompanying software (Optovue, Inc.). The optical coherence tomography device had a high scan speed of 70,000 A-scans per second. Analyses were performed on a scan area of 3 × 3 mm generated from the superficial and deep retinal vascular plexuses around the fovea for all eyes. In addition to OCTA imaging, all eyes underwent fundus photography to grade the eyes according to the severity of the disease. The diabetic retinopathy eyes were classified as mild nonproliferative diabetic retinopathy (NPDR), moderate NPDR, severe NPDR, and proliferative diabetic retinopathy (PDR) using the Early Treatment Diabetic Retinopathy Study classification.10 To analyze the local variations and complexity in the vasculature of the OCTA scans generated from the superficial and deep retinal vascular plexuses, local fractal analysis was applied.11,12 The box counting method was used in Equation 1 to calculate the fractal dimension:

\[
\text{Fractal Dimension} = \frac{\log(N_p)}{\log(p)} \tag{1}
\]

where \( N_p \) was the number of boxes of magnification \( p \) required to include a structure in the image. A modification was made to the box counting method by using a moving window of size \((2w+1) \times (2w+1)\) pixels. The moving window was used to calculate the local fractal dimension of each pixel in the OCTA image of the superficial and deep retinal vascular plexuses using Equation 2:

\[
N(i,j) = \text{Local Fractal Dimension}([i + k, j + k]; -w < k < w) \tag{2}
\]

where \( I \) was the original OCTA image and \( N \) was the new image formed after substituting the center pixel of each window by the computed fractal dimension of the window. A window size of \( 3 \times 3 \) pixels was used to calculate the local fractal dimension, as it was observed to be the most accurate for measurement of the FAZ area.12 To assess the FAZ area, the OCTA image was generated by projecting the entire inner retina into one enface angiogram. Then the area of the FAZ was segmented by binarizing the image, followed by boundary detection and connected component labeling (MathWorks, Inc., Natick, MA, USA). Using Equation 2, each pixel in the OCTA image was assigned a fractal dimension value. However, the magnitude of fractal dimension of each pixel varied with the distribution of the vascular network around the pixel. Thus, a pixel in a larger vessel had a higher fractal dimension than a pixel in a smaller vessel or a nonvessel region. Thus, a normalized fractal dimension ratio was computed for each pixel by taking the ratio of its local fractal dimension with the maximum computed local fractal dimension in the image (\( N \)).12 The normalized ratio was represented as a two-dimensional contour map and represented the probability of presence of a given pixel in the OCTA image within a vessel or a nonvessel region.12 A normalized ratio closer to 1 indicated a vessel and a ratio closer to 0 indicated a nonvessel region. A scoring system was developed based on visual comparison of the normalized ratio map with the OCTA image.12 The pixels within the large vessels had a normalized ratio of 0.9 to 1.0 while the pixels within the small vessels had a ratio between 0.7 and 0.9.12 Pixels in regions which were devoid of or had minimal vascular features had a normalized ratio between 0.0 and 0.3.12 By visual examination, these regions were observed to occur between or around the large vessels. In some cases, these regions were also observed between sparsely packed small vessels. In general, these were termed as “spaces between large vessels.” The only exception to this classification was the FAZ, which was common to all the images. Pixels in regions around closely packed small vessels, which may be branching out from a large vessel or surrounding small vessels, had a normalized ratio between 0.3 and 0.7.12 These were termed as “spaces between small vessels.” The vessel density was computed as a percentage by counting all the pixels with a normalized ratio between 0.7 and 1.0 and then dividing by the total number of pixels in the OCTA image.12 Similarly, the spacing between large vessels and the spacing between the small vessels were expressed as the percentage of the total number of pixels in the OCTA image.12 Figure 1 shows the description of the scoring system. Vessel density, and spacing between the large and small vessels were computed for OCTA images of the superficial and deep retinal vascular plexuses. Figures 2A and 2B show examples of OCTA images of the superficial and deep retinal vascular plexuses in moderate NPDR. Figures 2C and 2D show their respective normalized ratio contour maps. Figures 3A through 3D show the examples of OCTA images of the superficial retinal vascular plexus in NPDR (mild, moderate, severe) and PDR eyes. Figures 3E through 3H show their respective normalized ratio contour maps. All the above methods were implemented using a computing environment (MATLAB v7.10; MathWorks, Inc.).

**Statistical Analysis**

All analyzed variables were reported as mean ± standard error of the mean. The analyzed variables were FAZ area (mm²); vessel density (%); spacing between large vessels (%); and spacing between small vessels (%). One-way ANOVA was performed for each analyzed variable between the normal eyes and DR grades. Also, the analyzed vascular parameters were correlated with all the systemic indicators. Area under the curve, sensitivity, and specificity of the vascular parameters to differentiate DR eyes from normal eyes was performed with receiver operating characteristics (ROC) curve. A value of \( P < 0.05 \) was considered statistically significant and was Bonferroni-corrected for multiple group comparisons. All statistical analyses were performed using statistical software (MedCalc v15.8; MedCalc, Inc., Ostend, Belgium).

**RESULTS**

The number of eyes was 60, 35, 95, 57, and 22 in normal, mild NPDR, moderate NPDR, severe NPDR, and PDR groups, respectively. Table 1 shows the clinically significant systemic characteristics of all the DR subjects included in the study. Duration of DM \( (P = 0.01) \); HbA1c \( (P = 0.03) \); FBS \( (P = 0.02) \); and PPBS \( (P = 0.03) \) were significantly altered among the...
FIGURE 1. Contour map of normalized fractal dimension ratio of OCTA image of the superficial retinal vascular plexus of an area 3 × 3 mm of a moderate NPDR eye showing the FAZ. Examples of large vessels; small vessels; pixels in regions with no or minimal vascular features (referred to as “spacing between large vessels” in the text); and pixels in regions around small vessels (referred to as “spacing between small vessels” in the text).

FIGURE 2. (A, B) Optical coherence tomography angiography image of the superficial and deep retinal vascular plexuses, respectively, of an area 3 × 3 mm of a moderate NPDR eye. (C, D) Corresponding contour maps created using the normalized fractal dimension ratio.
grades of DR (Table 1). Table 2 shows the FAZ area, vessel density, and spacing between large and small vessels in the superficial and deep retinal layers between the normal eyes and the DR grades. Normal eyes had a lower FAZ area ($P < 0.001$); higher vessel density ($P < 0.001$); and lower spacing between large ($P < 0.001$) and small vessels ($P < 0.001$) compared with DR grades (Table 2).

Among the DR grades, vessel density was similar ($P > 0.05$) in both superficial and deep retinal layers (Table 2). Also, the FAZ area did not significantly change ($P = 0.82$) among the DR grades. In the superficial layer and among the DR grades (Table 2), PDR (24.1 ± 1.21) and severe NPDR (24.1 ± 0.9) had a significantly higher spacing ($P = 0.04$) between the large vessels compared with mild (21.1 ± 1.01) and moderate NPDR (21.9 ± 0.56). However, mild NPDR (39.6 ± 0.56) had a higher spacing between small vessels ($P = 0.001$; Table 2) compared with moderate NPDR (37.9 ± 0.21); severe NPDR (37.3 ± 0.44); and PDR (36.8 ± 0.52). Among the DR grades (Table 2), the spacing between the large vessels ($P = 0.34$) and the spacing between the small vessels ($P = 0.19$) were similar in the OCTA scans of the deep retinal vascular plexus.

In the superficial layer, the spacing between the large vessels correlated positively with Hba1c ($r = 0.25, P = 0.03$); FBS ($r = 0.23, P = 0.02$); and PPBS ($r = 0.26, P = 0.03$). Also, the vessel density correlated negatively with Hba1c ($r = -0.28, P = 0.006$); FBS ($r = -0.27, P = 0.009$); and PPBS ($r = -0.28, P = 0.009$). However, vessel density and spacing between the large vessels showed no correlation with other systemic indicators ($P > 0.05$). The foveal avascular zone area and spacing between the small vessels showed no correlation with systemic indicators ($P > 0.05$). In the deep layer, the systemic indicators showed no correlation with retinal vascular parameters ($P > 0.05$). Table 3 summarizes the results of the ROC analyses. Area of the FAZ (Table 3) had the lowest sensitivity and specificity among all the vascular parameters in the superficial and deep retinal vascular plexuses. Vessel density had a relatively higher sensitivity (94.7%) and specificity (85.5%), particularly when the deep retinal plexus was used as a differentiator (Table 3). Interestingly, spacing between large vessels achieved the highest sensitivity and specificity in both the superficial (92.7% and 92.9%, respectively) and deep (96% and 92.7%, respectively) retinal vascular plexuses. The area under the ROC curve of spacing between the large vessels also was significantly better than the same for vessel density in both superficial ($P = 0.001$) and deep retinal plexuses ($P = 0.01$).

**DISCUSSION**

Optical coherence tomography angiography can demonstrate clinically relevant changes in the retinal vasculature of DR patients qualitatively such as distortion and enlargement of foveal avascular zone, retinal capillary dropouts, and microaneurysms comparable with fundus fluorescein angiography (FFA).\textsuperscript{13–15} In this study, a local fractal-based method was used to quantify the retinal vascular parameters in the OCTA images.

<table>
<thead>
<tr>
<th>Table 1.</th>
<th>Systemic Characteristics in Subject Eyes</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mild NPDR, $n = 35$</td>
</tr>
<tr>
<td>Age, y</td>
<td>64.3 ± 2.16</td>
</tr>
<tr>
<td>IOP, mm Hg</td>
<td>14.2 ± 0.88</td>
</tr>
<tr>
<td>Duration of DM, y</td>
<td>11.0 ± 2.31</td>
</tr>
<tr>
<td>Hba1c, %</td>
<td>8.1 ± 0.53</td>
</tr>
<tr>
<td>FBS, mg/dL</td>
<td>141.3 ± 16.32</td>
</tr>
<tr>
<td>PPBS, mg/dL</td>
<td>215.8 ± 17.99</td>
</tr>
</tbody>
</table>

IOP, intraocular pressure.

* Indicates statistically significant difference between the groups. A value of $P < 0.05$ was considered statistically significant.
of superficial and deep retinal layers and were correlated with systemic indicators to explore the possibility of using these vascular parameters as metrics to gauge severity of DR. In addition to vessel density, the study defined “spaces between large vessels” and “spaces between small vessels.”

The superficial and deep retinal vascular plexuses merged at the edge of FAZ in the OCTA images (Fig. 2). Thus, segregating the FAZ into the superficial and deep retinal vascular plexuses could be anatomically misleading. So, the FAZ was assessed by projecting the entire inner retina into one enface angiogram. In a recent study that showed lower capillary density in DR compared with normal eyes. Similar findings were reported as baseline, then the increase in spacing in diseased eyes compared with normal eyes indicated loss of capillaries. This in turn indicated a decrease in capillary perfusion. A previous study using the same technique and definition of ranges for normalized local fractal dimension was validated with quantitative numbers of vessel density in living animal eyes. However, new insights into macular capillary changes may result in modification of the defined range in the future.

Normal eyes had a significantly lower FAZ area compared with DR patients in both the superficial and deep retinal layers. These findings were consistent with previous studies on DR using OCTA and FFA. Also, DR eyes had lower vessel density in both the superficial and deep retinal layers compared with normal eyes. Similar findings were reported in a recent study that showed lower capillary density in DR eyes compared with normal eyes. The vessel density across the DR grades was similar (P > 0.05). However, PDR and severe NPDR had a higher spacing between the large vessels compared with mild and moderate NPDR in the superficial layer. Moreover, mild NPDR had higher spacing between the small vessels compared with moderate NPDR, severe NPDR, and PDR. However, the vascular parameters in the deep layer were found to be similar among the DR grades. The perifoveal intercapillary area was increased in DR compared with normal eyes. Also, in previous studies, DR eyes showed changes in the retinal vasculature and thereby an increase in retinal nonperfusion areas. Histologic studies on diabetic retinas have shown localized regions of nonperfusion and progressive loss of retinal capillary cells. These give a possible explanation of the progressive increase in the spacing between the large vessels with increase in the severity of DR.

### Table 2. Mean ± SEM of Retinal Vascular Parameters in the Superficial and Deep Vascular Plexus OCTA Images of Subject Eyes

<table>
<thead>
<tr>
<th></th>
<th>Normals, n = 60</th>
<th>Mild NPDR, n = 35</th>
<th>Moderate NPDR, n = 95</th>
<th>Severe NPDR, n = 57</th>
<th>PDR, n = 22</th>
<th>P Value*</th>
<th>P Value †</th>
</tr>
</thead>
<tbody>
<tr>
<td>FAZ, mm²</td>
<td>0.38 ± 0.01</td>
<td>0.46 ± 0.03</td>
<td>0.45 ± 0.01</td>
<td>0.46 ± 0.02</td>
<td>0.47 ± 0.02</td>
<td>0.001‡</td>
<td>0.82</td>
</tr>
<tr>
<td>Superficial retinal vascular plexus</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vessel density, %</td>
<td>49.7 ± 0.55</td>
<td>39.2 ± 1.21</td>
<td>40.1 ± 0.58</td>
<td>38.5 ± 0.76</td>
<td>38.9 ± 1.38</td>
<td>&lt;0.001‡</td>
<td>0.11</td>
</tr>
<tr>
<td>Spacing between large vessels, %</td>
<td>14.8 ± 0.37</td>
<td>21.1 ± 1.01</td>
<td>21.9 ± 0.56</td>
<td>24.1 ± 0.9</td>
<td>24.1 ± 1.21</td>
<td>&lt;0.001‡</td>
<td>0.04‡</td>
</tr>
<tr>
<td>Spacing between small vessels, %</td>
<td>34.9 ± 0.22</td>
<td>39.6 ± 0.56</td>
<td>37.9 ± 0.21</td>
<td>37.3 ± 0.44</td>
<td>36.8 ± 0.52</td>
<td>&lt;0.001‡</td>
<td>&lt;0.001‡</td>
</tr>
<tr>
<td>Deep retinal vascular plexus</td>
<td></td>
<td></td>
<td></td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vessel density, %</td>
<td>55.1 ± 0.73</td>
<td>39.7 ± 1.57</td>
<td>40.2 ± 0.53</td>
<td>39.4 ± 0.68</td>
<td>39.2 ± 0.94</td>
<td>&lt;0.001‡</td>
<td>0.35</td>
</tr>
<tr>
<td>Spacing between large vessels, %</td>
<td>15.9 ± 0.32</td>
<td>22.1 ± 1.38</td>
<td>22.9 ± 0.59</td>
<td>24.1 ± 0.78</td>
<td>24.3 ± 1.06</td>
<td>&lt;0.001‡</td>
<td>0.34</td>
</tr>
<tr>
<td>Spacing between small vessels, %</td>
<td>32.9 ± 0.35</td>
<td>38.1 ± 0.72</td>
<td>36.8 ± 0.28</td>
<td>36.5 ± 0.51</td>
<td>36.5 ± 0.51</td>
<td>&lt;0.001‡</td>
<td>0.19</td>
</tr>
</tbody>
</table>

Foveal avascular zone area was calculated for the inner retina only.
* Indicates difference between normal eyes and DR grades.
† Indicates difference among the DR grades.
‡ Indicates statistically significant difference between the groups. A value of P < 0.05 was considered statistically significant.

### Table 3. Receiver Operator Characteristic of the Retinal Vascular Parameters Between Normal Eyes and DR Grades

<table>
<thead>
<tr>
<th>Retinal Vascular Parameters</th>
<th>Area Under Curve</th>
<th>Sensitivity, %</th>
<th>Specificity, %</th>
<th>Cutoff</th>
</tr>
</thead>
<tbody>
<tr>
<td>Superficial retinal vascular plexus</td>
<td></td>
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</tr>
<tr>
<td>Vessel density, %</td>
<td>0.85 ± 0.03</td>
<td>76.8</td>
<td>87.8</td>
<td>44.97</td>
</tr>
<tr>
<td>Spacing between large vessels, %</td>
<td>0.96 ± 0.01</td>
<td>92.7</td>
<td>92.9</td>
<td>16.78</td>
</tr>
<tr>
<td>Spacing between small vessels, %</td>
<td>0.84 ± 0.03</td>
<td>77.8</td>
<td>79.6</td>
<td>56.17</td>
</tr>
<tr>
<td>Deep retinal vascular plexus</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vessel density, %</td>
<td>0.94 ± 0.02</td>
<td>94.7</td>
<td>85.5</td>
<td>48.83</td>
</tr>
<tr>
<td>Spacing between large vessels, %</td>
<td>0.99 ± 0.01</td>
<td>96.0</td>
<td>92.7</td>
<td>15.58</td>
</tr>
<tr>
<td>Spacing between small vessels, %</td>
<td>0.85 ± 0.05</td>
<td>86.8</td>
<td>74.2</td>
<td>53.54</td>
</tr>
</tbody>
</table>
With progression, a few smaller capillary-free zones may become enlarged due to vessel dropout and may get converted to large vacant spaces as a result. As shown in Table 2, a decrease in spacing between small vessels was accompanied by a corresponding increase in spacing between large vessels with increasing DR severity. This may explain why patients with NPDR had higher spacing between small vessels than the more severe grades. In a recent paper, a similar increase was observed in total avascular area in diseased eyes and was termed as “capillary non-perfusion.” The analyses of ROC confirmed that spacing between large vessels (maximum area under the curve = 0.99 ± 0.01 in the deep retinal vascular plexus; Table 3) could be a better indicator of adverse vascular changes in DR and changes in the vasculature following treatment instead of just vessel density (maximum area under the ROC curve = 0.94 ± 0.02 in the deep retinal vascular plexus; Table 3).

Glycosylated hemoglobin is used for the diagnosis of DM and for gauging progression of DR. Previous studies showed a positive correlation among HbA1c, FBS, and PPBS, thereby indicating that individuals with lower HbA1c levels have a lower chance of developing DR. In this study, spacing between large vessels and vessel density in the superficial layer showed a significant correlation with HbA1c, FBS, and PPBS levels. Levels of HbA1c were elevated (>9%) while FBS and PPBS were higher in severe NPDR and PDR compared with mild and moderate NPDR (Table 1). Systemic data was included in this study to assess their correlations with the OCTA parameters. Every ophthalmologist may not have access to OCTA, but they do have access to the systemic data of DR patients. Therefore, we assessed if levels of OCTA parameters such as vessel density were predictive of commonly used DM biomarkers. From the analyses, systemic data correlated with the OCTA parameters, but the correlation was not strong enough (low r value) for use as a predictive tool. Although the correlations were weak, longitudinal assessment of progression of disease in the same eye or newer biomarkers may lead to stronger correlations.

The study had a few limitations. In this study, normal subjects (mean age: 38.7 ± 1.68 years; range: 20–60 years) were significantly younger (P < 0.001) than DR patients. However, the FAZ area and OCTA parameters in normal eyes were unaffected by age (P > 0.05). A recent OCTA study on normal Indian eyes (age: 20–67 years) using a local fractal-based method demonstrated that vessel density and FAZ did not change significantly with age. Based on these observations, the age difference between normal and DR eyes was not considered in the analyses. However, other studies have shown a significant decrease in vessel density with age in Chinese and Caucasian eyes. Therefore, a larger cohort study may be needed to reassess these correlations in Indian eyes. A recent study showed that the vessels in the superficial plexus superimposed on the deep plexus in 68% of the cases. A study on image artifacts in OCTA described this artifact as “projection artifact.” In this study, images of the deep retinal vascular plexus with significant projections of the large vessels from the superficial layer were excluded. However, it was difficult to completely avoid projection artifacts and these might still have some confounding effects on the analyses. Some recent techniques aim to reduce these artifacts, which can only help in improving the analyses with local fractal dimension in the near future. Despite the upcoming techniques used to compensate for axial eye motions, transverse motions from fixation changes remain a major cause of artifacts in OCTA. Additionally, the OCTA software can introduce artifacts such as loss of detail, doubling of vessels, stretching defects, and false flow artifact. In this study, OCTA images with significant motion artifacts or stretching defects or doubling of vessels were excluded from this study. However, other artifacts which were not apparent could have some confounding effect on the results of this study.

Optical coherence tomography angiography is proving to be a very useful modality in evaluating vascular changes in healthy as well as diseased eyes. However, a lot of improvement and understanding is still needed to correctly interpret the data and its clinical significance. Thus, spacing between the large vessels, which correlated positively with HbA1c, FBS, and PPBS, may be a sensitive marker to quantify the progression of DR. This may be used in combination with HbA1c, FBS, and PPBS for identifying patients who are at high risk of developing DR early and for grading of DR. Further studies with long-term follow-up are needed to confirm these findings.

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