Article
Quantitative Analysis of Different Foveal Avascular Zone Metrics in Healthy and Diabetic Subjects
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Abstract: The primary aim of this study was to assess the size and shape of the Foveal Avascular Zone (FAZ) in patients with type 2 diabetes mellitus compared to healthy subjects. The study used 80 OCTA images from the FAZID dataset. The FAZ size was measured by its area, perimeter, and maximum/minimum Feret diameters. The shape was assessed using the axial ratio, circularity, roundness, and solidity. These metrics were calculated automatically using Matlab® R2020b. Statistical analysis was performed using SPSS statistical software version 28.0, with a p-value of less than 0.01 considered significant. The results showed that the FAZ area was significantly larger in diabetic eyes (mean = 0.50 mm²) compared to control eyes (mean = 0.37 mm²), with a p-value of less than 0.01. Both the maximum and minimum diameters of the FAZ were also significantly larger in diabetic groups compared to the control group. Parameters associated with FAZ’s shape were significantly smaller in the diabetic groups than in the control group, except for the axial ratio. The main finding of this study is that diabetic eyes without clinically detectable diabetic retinopathy exhibit morphological changes and irregularities at the FAZ border.

Keywords: type 2 diabetes mellitus; diabetic retinopathy; FAZ

1. Introduction

Diabetic retinopathy (DR) is the most well-known ocular complication associated with diabetes mellitus and the fourth leading cause of blindness worldwide. According to Flaxman et al. [1], the incidence of blindness due to DR increased from 0.2 million to 0.4 million between 1990 and 2015, and the number of people with vision impairment due to DR rose from 1.4 million to 2.6 million. This disease occurs when changes in blood glucose levels associated with diabetes damage the blood vessels that nourish the retina [2]. The principal risk factors related to the onset and progression of DR are as follows [3]: (i) The duration of diabetes, as retinal complications increase as the disease progresses. (ii) High blood glucose levels (hyperglycemia), which is a critical risk factor for DR as it is closely related to developing cardiovascular disease [4]. (iii) Dyslipidemia, which is characterized by elevated levels of low-density lipoprotein (LDL) cholesterol, triglycerides, and low HDL cholesterol, is also linked with the progression of DR pathology. It has been demonstrated in patients with type 2 diabetes mellitus that there is a statistically significant correlation between dyslipidemia and the development and severity of DR [5]. Dyslipidemia is also a risk factor for the development of diabetic macular edema (DME) [6], the leading cause of central vision loss in patients with DR. (iv) Arterial hypertension, in addition to maintaining a healthy level of blood sugar, hypertension is another risk factor for heart disease and the vascular system, which should be controlled to delay or reduce the progression of DR. Numerous studies have demonstrated a significant correlation between DR and both systolic and diastolic hypertension in patients with type 2 diabetes [7–9].

Diabetic retinopathy diagnosis is based on clinical manifestations associated with retinal vascular abnormalities. However, in the initial stages, patients do not experience any symptoms. Diabetic retinopathy may, therefore, be divided into two stages: an early
stage, where symptoms are mild or nonexistent, known as non-proliferative diabetic retinopathy (NPDR), and a later stage called proliferative diabetic retinopathy (PDR), which is characterized by the growth of abnormal new blood vessels on the retina. This neovascularization can lead to serious effects on vision, and even blindness [10].

Nowadays, technological advances have improved the diagnostic screening methods for this disease. Optical Coherence Tomography Angiography (OCTA) has emerged as a non-invasive technique for automated DR detection. It enables precise imaging of retinal capillary beds in vivo, provides detailed morphological microvascular information, and quantifies retinal capillary network dimension [11,12]. Several studies using OCTA have evaluated the macular complications of retinal vascular disease, such as Diabetic Retinopathy (DR), DME, and age-related macular degeneration, among others [11].

The macula is an elliptical specialized region of the retina. The central zone of the macula contains an important and specialized region for high-acuity vision, the fovea. Within the fovea is a capillary-free area known as the Foveal Avascular Zone (FAZ). The function of the FAZ in vision has been investigated extensively. Studies have shown that the size of the FAZ correlates with central visual function [13,14]. The leading cause of vision loss in DR is DME [15]. A statistically significant enlargement of the FAZ in patients with clinically significant macular edema compared with non-diabetic eyes has been found [16,17]. Since interconnected networks surround the FAZ, it is affected by microvascular changes in the macula [18]. Therefore, variations in the FAZ can play a crucial role in the diagnosis and prognosis of retinal diseases, such as DR [19].

The purpose of the present work was to quantify the size and shape of the FAZ, including the area, perimeter, circularity, roundness, solidity, axial ratio, and maximum and minimum Feret diameters in healthy and diabetic eyes.

2. Materials and Methods

A public dataset named FAZID was made available by Agarwal et al. [20]. The FAZID dataset was approved by the Institutional Review Board (IRB) of the Vision Research Foundation, Chennai, India, and this study was conducted following the tenets of the Declaration of Helsinki. This database is licensed under a Creative Commons Attribution 4.0 International (CC BY 4.0) License and is available for researchers on the ICPSR website (https://doi.org/10.3886/E117543V2) (accessed on 20 June 2024). The dataset consists of clear, manually marked FAZ images, including patients with type 2 Diabetes Mellitus (DM) both with and without diabetic retinopathy (DR), as well as clinically normal images. In this study, a total of 80 eyes were analyzed: 20 eyes from healthy subjects and 60 eyes from diabetic patients. The patients were divided into the following subgroups: (1) no diabetic retinopathy (NoDR, n = 20), (2) moderate non-proliferative diabetic retinopathy (MDR, n = 20), and (3) severe non-proliferative diabetic retinopathy (SDR, n = 20). OCTA imaging was performed using the Zeiss Cirrus 5000 with AngioPlex™ OCTA software (Carl Zeiss Meditec Inc., Dublin, CA, USA). Each image size is approximately 420 × 420 pixels, corresponding to 6 mm × 6 mm.

Quantitative Measurements of FAZ Using Matlab®

For all the OCTA fundus images, a trained clinical expert performed manual marking of the FAZ area [20]. In these images, the FAZ region marked manually was extracted from the original image using Matlab (Figure 1A,B). In this study, the following parameters were calculated for each region of interest: the area, perimeter, circularity, roundness, solidity, axial ratio, and maximum/minimum Feret diameters. All the quantitative measurements of the FAZ were carried out in MATLAB® software (Matlab R2020b, MathWorks, Inc., Natick, MA, USA).
The area, perimeter, and maximum/minimum Feret diameters are parameters associated with the size of the FAZ. The area is defined as the size of the segmented FAZ region. It was calculated from the mask of the FAZ in square pixels and then converted to square millimeters (mm$^2$) according to the formula:

$$\text{FAZ}_{\text{area}} (\text{mm}^2) = \frac{\text{FAZ}_{\text{area}} (\text{pixel}^2)}{\text{TS}_{\text{area}} (\text{pixel}^2)} \cdot \text{TS}_{\text{area}} (\text{mm}^2)$$

(1)

where $\text{TS}_{\text{area}} (\text{pixel}^2)$ is the total surface area in square pixels (i.e., 420 pixels $\times$ 420 pixels) and $\text{TS}_{\text{area}} (\text{mm}^2)$ is the total surface area in square millimeters (i.e., 6 mm $\times$ 6 mm).

The perimeter and Feret’s diameter of the FAZ in pixels were also computed from the mask and converted to millimeters. Since images for the 6 mm $\times$ 6 mm protocol exported from the Cirrus 5000 Angioplex were 420 $\times$ 420 pixels, the unit of pixel was converted to millimeters at a ratio of 420 to 6. The perimeter was determined by the length of the FAZ’s contour. However, the maximum (MaxFeret) Feret diameter was determined by the maximum distance between any two points along the selection boundary, also called the maximum caliper. Finally, the minimum Feret diameter (MinFeret) was the minimum caliper diameter.

On the other hand, the axial ratio, circularity, roundness, and solidity are parameters related to the shape of the FAZ. The best-fit ellipse of the FAZ estimates the axial ratio, and it was calculated with the following formula:

$$\text{Axial Ratio} = \frac{\text{Length of major axis}}{\text{Length of minor axis}}$$

(2)

FAZ circularity reveals the degree of similarity to a perfect circle. Its value is limited in the range of 0–1. A circularity of 1 indicates a perfect circle, while values closer to 0 indicate an increasingly elongated shape. This metric can be defined by Equation (3):

$$\text{Circularity} = 4\pi \cdot \frac{\text{area}}{(\text{perimeter})^2}$$

(3)

Roundness of objects considers the major axis of the best-fit ellipse. Circularity represents how close to circular an object is, while roundness measures the extent to which its edges and corners have been smoothed or rounded. It is determined by the following formula:

$$\text{Roundness} = 4 \cdot \frac{\text{Area}}{\pi \cdot (\text{Length of major axis})^2}$$

(4)
Solidity represents the extent to which a structure is convex or concave. The solidity of a completely convex shape is 1.0. The farther the solidity value deviates from 1, the greater the extent of concavity in the object. This parameter is described by the following formula:

$$\text{Solidity} = \frac{\text{Area}}{\text{Convex area}} \quad (5)$$

For comparing two samples, a statistical analysis was conducted using the independent samples t-test. A p-value less than 0.01 was considered statistically significant.

Statistical analysis was performed with commercially available software SPSS 28.0 (Chicago, IL, USA). In this work, the mean ± standard deviation (SD), skewness, and kurtosis of all the parameters were analyzed.

3. Results

In this study, 20 eyes (10 men and 10 women) were analyzed in the control group. Their mean age was around 37.60 ± 18.50 years. A total of 20 eyes (13 men and 7 women) were included in the NoDR group. Their mean age was around 59.30 ± 8.70 years. A total of 20 eyes (8 men and 12 women) were contained in the MDR group. Their mean age was around 55.90 ± 7.90 years. Finally, a total of 20 eyes (15 men and 5 women) were analyzed in the SDR group. Their mean age was 54.70 ± 9.90 years. The demographics of the subjects are presented in Table 1.

<table>
<thead>
<tr>
<th>Table 1. Demographics of control, NoDR, MDR, and SDR subjects.</th>
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</thead>
<tbody>
<tr>
<td>Age (in Years)</td>
</tr>
<tr>
<td>----------------</td>
</tr>
<tr>
<td>Control</td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td>NoDR</td>
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<tr>
<td></td>
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<tr>
<td>MDR</td>
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<td></td>
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<tr>
<td>SDR</td>
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</tbody>
</table>

A summary of the descriptive statistics (mean (µ), SD (σ), skewness, and kurtosis) characterizing the shape and size of the FAZ for all groups are presented in Table 2. From this table, we can observe that the parameters associated with the size of the FAZ were significantly higher in the NoDR, MDR, and SDR groups than in the control group (p < 0.01). All average values of the area, perimeter, and Feret diameters of patients with and without DR were higher than normal. Furthermore, the values of the circularity, roundness, and solidity of the FAZ in the eyes with DM were also significantly different from those in healthy ones. These parameters had significantly lower values in the NoDR, MDR, and SDR groups, except for the axial ratio value, which was higher in the NoDR, MDR, and SDR groups than in the control group.

In statistics, skewness is a measure of the asymmetry of the distribution. The skewness for a normal distribution is zero. A positive skewness value indicates that the bulk of the data values lie to the left of the mean. In contrast, a negative skewness value indicates that the bulk of the data values lies to the right of the mean. On the other hand, kurtosis is a measure of the relative tailedness of the probability distribution. It is a shape parameter that characterizes the degree of peakedness [21]. In the current work, skewness and kurtosis were different between healthy and pathological subjects. Skewness was higher in parameters related to the size of the FAZ and lower in parameters related to the shape of the FAZ (except for the axial ratio). On the other hand, kurtosis values were higher in almost all FAZ parameters compared to the control group.

Figures 2 and 3 represent the FAZ parameters’ probability density functions. A non-parametric Kruskal–Wallis test was also applied. So, in each subfigure, the results of the Kruskal–Wallis test are shown in the form of a Tukey box plot. The results presented in
both figures and those shown in Table 2 confirm statistically significant differences between the control and diabetic groups.

Table 2. FAZ parameters of control, NoDR, MDR, and SDR subjects. Asterisks indicate significant differences using Student’s t-test ($p < 0.01$).

<table>
<thead>
<tr>
<th></th>
<th>Healthy $\mu$</th>
<th>Healthy $\sigma$</th>
<th>NoDR $\mu$</th>
<th>NoDR $\sigma$</th>
<th>Moderate DR $\mu$</th>
<th>Moderate DR $\sigma$</th>
<th>Severe DR $\mu$</th>
<th>Severe DR $\sigma$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Area (mm$^2$)</td>
<td>0.37, 0.09</td>
<td>−0.20, 1.97</td>
<td>0.50 *, 0.20</td>
<td>0.58, 2.54</td>
<td>0.50 *, 0.10</td>
<td>0.47, 2.50</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Max. Feret (mm)</td>
<td>0.78, 0.09</td>
<td>−0.24, 1.91</td>
<td>0.90 *, 0.10</td>
<td>0.15, 1.83</td>
<td>0.96 *, 0.10</td>
<td>0.31, 2.34</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Min. Feret (mm)</td>
<td>0.65, 0.08</td>
<td>−0.63, 2.36</td>
<td>0.80 *, 0.10</td>
<td>0.45, 2.50</td>
<td>0.70 *, 0.10</td>
<td>0.20, 2.80</td>
<td></td>
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<tr>
<td>Perimeter (mm)</td>
<td>2.30, 0.30</td>
<td>−0.46, 1.94</td>
<td>2.80 *, 0.50</td>
<td>0.59, 2.69</td>
<td>2.80 *, 0.70</td>
<td>0.43, 2.79</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Axial ratio</td>
<td>1.13, 0.07</td>
<td>1.18 *, 0.09</td>
<td>1.30 *, 0.20</td>
<td>1.30 *, 0.20</td>
<td>0.77, 2.79</td>
<td></td>
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<tr>
<td>Solidity</td>
<td>0.95, 0.01</td>
<td>−1.84, 6.69</td>
<td>0.91 *, 0.05</td>
<td>0.93 *, 0.03</td>
<td>0.43, 2.79</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Circularity</td>
<td>0.88, 0.06</td>
<td>−0.67, 2.81</td>
<td>0.81 *, 0.08</td>
<td>−1.55, 5.93</td>
<td>0.80 *, 0.10</td>
<td>−0.58, 2.12</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Roundness</td>
<td>0.88, 0.05</td>
<td>−0.14, 2.30</td>
<td>0.83 *, 0.06</td>
<td>−0.85, 4.02</td>
<td>0.80 *, 0.10</td>
<td>−0.37, 2.26</td>
<td></td>
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</tr>
</tbody>
</table>

Figure 2. Results of the probability density function (pdf) and Kruskal–Wallis test for all size FAZ parameters—comparison between healthy and diabetic groups. C, control. NoDR, no diabetic retinopathy. MDR, moderate non-proliferative diabetic retinopathy. SDR, severe non-proliferative diabetic retinopathy. (A) The area, (B) perimeter, (C) maximum Feret diameter, and (D) minimum Feret diameter.
The current study has evaluated the size and shape of the FAZ in healthy and diabetic eyes to explore the morphological changes of the FAZ due to diabetes. All FAZ parameters were evaluated in the superficial vessel plexus. Results indicated that parameters associated with the size of the FAZ were significantly higher in all diabetic groups than in the healthy group. For example, the average area in patients with type 2 diabetes mellitus with NoDR was $0.50 \pm 0.20 \text{ mm}^2$ compared with $0.37 \pm 0.09 \text{ mm}^2$ in those without diabetes ($p < 0.01$). This finding aligns with prior reports investigating the FAZ area in NoDR patients, which also found a statistically significant increase in the moderate and severe NPDR groups [17,22]. Kim et al. [23] have also investigated the FAZ area using OCTA in diabetic eyes with and without DR and found that the FAZ area was greater in NoDR subjects and diabetic patients with various grades of diabetic retinopathy. Enlargement of the FAZ in diabetic subjects results from retinal capillary occlusion and loss of blood vessel integrity [24], confirming that microvascular impairment already exists even before clinical signs of DR are detected [25,26].

FAZ length and width were estimated as the maximum and minimum Feret diameters, respectively. Both parameters were significantly larger in all diabetic groups than in the control group. To the author’s knowledge, this is the first report to determine FAZ deformation in diabetic patients using Feret’s diameter.

On the other hand, results revealed that parameters associated with the shape of the FAZ were significantly smaller in all diabetic groups than in the control group, except for the axial ratio. In humans, the normal FAZ is known to be circular when viewed en face [27]. In this research, FAZ circularity showed a decrease in patients with diabetes compared with the control subjects. This finding was consistent with previous studies [28,29], where a decrease was correlated with a reduction in visual function. Various studies have shown
that FAZ circularity decreases after a period of pathophysiologic processes in several disease states [30,31]. In patients with NoDR or MDR, a decrease in FAZ circularity was also associated with macular ganglion cell/inner plexiform layer thinning [32] and correlated with microcirculation impairment in DR [33]. Choi et al. [34] suggested that decreased FAZ circularity could result from vascular endothelial dysfunction, leading to arteriolar changes and causing an uneven dropout of capillaries at the FAZ border. Several studies have revealed that irregularities in FAZ borders are one of the early hallmarks of diabetic retinal vascular injury [35,36].

The axial ratio was significantly higher in the NoDR, MDR, and SDR groups compared to the control group. An increased axial ratio was associated with worse stages of retinopathy [33]; it appears that the FAZ in DR becomes more elongated with more severe disease.

In summary, the size of the FAZ was larger for diabetic eyes than for healthy eyes, regardless of the presence of DR. Additionally, the FAZ of diabetic eyes had lower values of circularity, roundness, and solidity, and a higher axial ratio, indicating a more irregular shape. It is apparent from the results of this study that, unlike other indicators such as area, perimeter, and length, shape parameters can also quantify FAZ geometry and detect FAZ changes occurring much earlier in the stage of DR. The analysis of the FAZ’s morphology may be a potential biomarker for evaluating the risk of developing DR.

The size and shape of the FAZ play an important role in providing information about FAZ damage in pathological conditions and can serve as a potential indicator of retinal microvascular abnormalities. Evaluating the shape and size of the Foveal Avascular Zone could permit the early detection of diabetic retinopathy in clinical settings and help clinicians understand the impairment of microcirculation in DR patients. Further studies are needed to confirm these findings.

5. Limitations

This research study had some limitations. First, some studies indicate that axial length and retinal thickness significantly affect the FAZ area [37,38]. However, there are several limitations regarding open-source medical imaging datasets and their usage; in the present study, a lot of data that could help prevent errors in research are missing, such as the information about axial length, refractive error of subjects, and retinal thickness. A second limitation worth noting comprises the image artefacts, which are considerably high in OCTA as they are extremely motion-sensitive. In this work, many images with artefacts were excluded.

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Institutional Review Board Statement: The images used in this work are from a public image dataset known as the Foveal Avascular Zone Image Database (FAZID). Moreover, this study was approved by the Institutional Review Board (IRB) of the Vision Research Foundation, Chennai, India. This study was conducted following the tenets of the Declaration of Helsinki. The database used in this study is available for researchers on the ICPSR website (https://doi.org/10.3886/E117543V2) (accessed on 20 June 2024).

Informed Consent Statement: This study conformed to the tenets of the Declaration of Helsinki, and signed informed consent was obtained from all subjects.

Data Availability Statement: The raw data supporting the conclusions of this article will be made available by the authors on request.

Conflicts of Interest: The author declares no conflicts of interest.


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