Retinal microvascular and microstructural alterations in the diagnosis of meibomian gland dysfunction in severely obese population: a new approach

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Abstract

AIM: To study retinal microvascular and microstructural alterations in meibomian gland dysfunction (MGD) in severely obese population using optical coherence tomography angiography (OCTA).

METHODS: Twelve MGD patients with severely obese population (PAT group; 24 eyes) and 12 healthy controls (HC group; 24 eyes) were recruited. OCTA images were segmented into five [superior (S), nasal (N), inferior (I), temporal (T), and central foveal (C)] or nine [inner superior (IS), outer superior (OS), inner nasal (IN), outer nasal (ON), inner inferior (II), outer inferior (OI), inner temporal (IT), outer temporal (OT), and C] subregions. The superficial vessel density (SVD), retinal thickness (RT), foveal avascular zone (FAZ) parameters, and retinal volume were measured.

RESULTS: Visual acuity was significantly different between two groups (0.8±0.17 in PAT group vs 0.2±0.06 in HC group). SVD was significantly lower in PATs in N, T, OS, IN, OT, and ON. The area under the receiver operating characteristic curve (AUC) for T was 0.961 [95% confidence interval (CI): 0.908 to 1.000], for OS was 0.962 (95% CI: 0.915 to 1.000). RT was significantly lower in PATs in IS, OS, OI, OT, ON, IT, IN, and II. AUC for OT was 0.935 (95% CI: 0.870 to 0.999), for IS was 0.915 (95% CI: 0.838 to 0.992). Angiography results showed significantly lower area and perimeter of FAZ, SVD of the inner retina and both retinal volume and the average volume thickness in the PAT group.

CONCLUSION: Vision may be affected in patients with MGD due to changes in retinal microvessels and microstructures. These changes detected by OCTA may be a potential marker for diagnosing MGD in severe obesity.

KEYWORDS: superficial vessel density; retinal thickness; severely obesity; meibomian gland dysfunction; optical coherence tomography angiography

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INTRODUCTION

Obesity is a serious health risk[1]. Body mass index (BMI) is an indicator used to evaluate obesity. BMI is calculated by dividing weight (kg) by height squared (m²), with values <25 indicating low weight or regular weight,
25-30 overweight, 30-35 moderate obesity and ≥35 severe obesity[2].

There is growing evidence that obesity is strongly related to metabolic diseases such as diabetes, dyslipidemia and hyperuricemia[3]. Dyslipidemia is also thought to initiate the development of meibomian gland dysfunction (MGD), leading to dry eye and loss of ocular surface homeostasis[4-5]. The meibomian gland is a modified sebaceous gland in cutaneous eyelid that generates meibum, the lipid component of tears[6]. On blinking, meibum is distributed evenly across the ocular surface, protecting the water layer from evaporating and stabilizing the tear film[7]. Any meibomian gland lesions will affect the formation of meibum, thus destabilizing the tear film, and ultimately affecting the corneal steady state, inducing epithelial disruption. As a result, most MGD patients have ocular irritation, such as eye pain and discomfort, and they may also experience symptoms of inflammation[8].

Optical coherence tomography angiography (OCTA) is a significant advance in ocular imaging, producing blood flow images of all retinal vascular layers with unprecedented resolution. OCTA is quick and non-invasive and can offer volumetric data. It allows localization and identification of pathology while providing information about structure and blood flow. OCTA can also be repeated in a single imaging process to obtain comprehensive, extensive microvascular information, or to assess the microvascular response to functional stimuli. In comparison with fluorescein angiography or indocyanine green angiography, OCTA images are not masked by high fluorescence due to dye leakage, so OCTA produces high-contrast, clear microvascular images. Since OCTA does not require an exogenous contrast agent, it can be performed in any patient, especially if fluorescein angiography or indocyanine green angiography are not possible. Finally, OCTA can be executed faster than these other methods, simplifying clinical workflow[9]. It is currently limited, however, by a comparatively small visual field, inability to identify leaks, and image artifacts due to patient movement or blinks[10].

OCTA has provided many important clinical findings, including areas of macular telangiectasia[11], microvascular impairment[2], microaneurysms[13], capillary remodeling[14], and neovascularization[15]. More importantly, OCTA provides unprecedented depth imaging information. It has been applied in assessing many kinds of diseases, including diabetic retinopathy[16], retinal vein obstruction[7], uveitis[18], and glaucoma[19]. It has also been used in neurological research[20] and retinal diagnostics[21]. Therefore, in the present research, we used this technology to evaluate retinal microvasculature and microstructure in MGD patients in severely obese population.

Subjects and Methods

Ethical Approval The study methods and protocols were approved by the Medical Ethics Committee of the First Affiliated Hospital of Nanchang University (Nanchang, China; No.2021039) and followed the principles of the Declaration of Helsinki. All subjects were notified of the objectives and content of the study and latent risks, and then provided written informed consent to participate.

Participants Twelve MGD patients in severely obese population (PAT group; 4 females and 8 males) were selected from the Department of Ophthalmology, the First Affiliated Hospital of Nanchang University. Recruitment criteria: 1) BMI≥30; 2) waist circumference ≥85 cm in female, waist circumference ≥90 cm in male; 3) diagnosed as MGD. There are symptoms of dry eye: dryness, foreign body sensation, signs of dry eye; narrowing or interruption of the tear river, and evidence of meibomian gland abnormalities: such as absent meibomian glands, abnormal meibomian gland openings, or abnormal meibomian gland secretions[22]; 4) able to perform OCTA.

This study recruited 12 healthy controls (HC group; 6 females and 6 males), all matched for age and gender in the PAT group. Recruitment criteria: 1) 20≤BMI≤24; 2) able to perform OCTA.

Exclusion criteria: patients in both groups were required to have no 1) mental illness, 2) drug or alcohol abuse history, 3) current pregnancy, 4) history of diseases that lead to OCTA changes: macular hole, cystic macular edema, diabetic retinopathy, age-related macular degeneration, glaucoma, hypertension, diabetes, cerebrovascular disease, Alzheimer’s disease, and some intracranial pathologies.

Methods TVue Avanti XR OCTA system (Optovue, Fremont, CA, USA) was used to image both microvascular and retinal cross sections. Scan parameters were scanning rate 70 000 A-scans per second, horizontal definition 22 μm, axial resolution 5 mm, center fold wavelength 840 nm, and bandwidth 45 nm. B-scans were conducted in 3×3 and 6×6 mm² scanning modes, five repeated images each at 216 grating positions, were focused on the central fovea, and took 3.9 seconds to complete. A 1080 B-scan (216×five positions) was acquired at 270 frames per second[23]. We used two horizontal and two vertical gratings to obtain 3×3 and 6×6 mm² OCTA images through a series of four individual scans. OCTA images of 3-dimensional 3×3 and 6×6 mm² en-face were recorded from both eyes.

After scanning, each retinal image was divided into five [superior (S), nasal (N), inferior (I), temporal (T), and central foveal (C)] or nine [inner superior (IS), outer superior (OS), inner nasal (IN), outer nasal (ON), inner inferior (II), outer inferior (OI), inner temporal (IT), outer temporal (OT), and...
C] subregions[24], consisting of two or three concentric circles, and their microvessels and microstructure were investigated. Each retinal layer covered (I) the inner retina (from the inner boundary membrane to the inner plexiform layer), and (II) the whole retina (from the inner boundary membrane to the retinal pigment epithelium). A two-dimensional panoramic image of the superficial retina (between vitreoretinal interface and anterior boundaries of ganglion cell layer) was formed using a threshold method to determine vascular density. According to the pixel size, we scaled the skeleton plate average of the region of interest to calculate the superficial vessel density (SVD) from the fovea to the 3×3 and 6×6 mm$^2$ brightness gradient image edges. SVD and retinal thickness (RT) were measured and compared. SVD images of each participant were divided into five and nine subregions, and RT images were divided into nine subregions (Figures 1 and 2).

Statistical Analysis SPSS (version 26.0) and GraphPad Prism (version 8.0) were used to analyze data, which were recorded in the form of mean±standard deviation (SD). Independent samples t-tests were utilized to analyze data between PAT and HC groups. The relationship between visual acuity and related factors was studied by univariate and multivariate regression analyses. To examine the difference between HC and PAT groups, receiver operating characteristic curves (ROC) were plotted for SVD, RT, FAZ, and retinal volume. $P<0.05$ was considered statistically significant.

RESULTS Each group included 12 participants (24 eyes). The groups were statistically matched in age and gender. Visual acuity in PATs was significantly poorer than in HCs ($P<0.0001$; Table 1).

Analysis of the Retinal Superficial Vessel Density SVD of each group is shown in Table 2, Figure 3A, 3B. SVD of PATs...
in N (P<0.0001) and T (P<0.0001) was significantly lower than that of HCs. S, I and C showed no statistically significant difference between the groups. SVD was significantly decreased in PATs in OS, IN, OT (all P<0.0001), and ON (P=0.0068), while no difference was found in IS, II, OI, IT, or C.

**Analysis of the Retinal Thickness**  
RT of each group is shown in Table 3, Figure 3C. Compared with HCs, RT was significantly decreased in PATs in IS, OS, OI, OT, ON (P<0.0001), IT (P=0.0002), IN (P=0.0014), and II (P=0.0108).

C showed no statistically significant difference between the groups.

Univariate regression analysis showed that visual acuity was negatively correlated with RT (β=-0.634, P<0.001), SVD (β=-0.486, P<0.001), retinal volume (β=-0.651, P<0.001), average volume thickness (β=-0.597, P<0.001), FAZ perimeter (β=-0.507, P<0.001), and FAZ area (β=-0.0326, P=0.024), but not for FAZ substantiation, blood pressure, age, or gender. Multivariate regression found a significant positive correlation between visual acuity and FAZ perimeters (β=-1.406, P<0.001; Table 4).

**ROC Analysis of Superficial Vessel Density and Retinal Thickness**  
Sensitivity and specificity of SVD and RT as diagnostic indicators were investigated by using ROC (Figure 4). Significant between-group differences were found in SVD in all four quadrants and in the central fovea. The
area under the ROC curve (AUC) in the temporal quadrant was 0.961 [95% confidence interval (CI): 0.908 to 1.000], and in the nasal quadrant 0.890 (95%CI: 0.782 to 0.998; Figure 4A), indicating that SVD has a medium to high diagnostic sensitivity in PAT\textsuperscript{[25]}. Significant differences were found between groups in all nine subregions. The AUC in OT was 0.935 (95%CI: 0.870 to 0.999), and 0.915 in IS (95%CI: 0.838 to 0.992), indicating that SVD has a high diagnostic sensitivity for retinal anomalies in PAT (Figure 4B).

Analysis of RT results showed significant differences between groups in all nine subregions. The AUC for OS was 0.962 (95%CI: 0.915 to 1.000), and 0.885 for OT (95%CI: 0.766 to 1.000), indicating medium to high diagnostic sensitivity in PAT (Figure 4C).

Analysis of the Foveal Avascular Zone FAZ analysis in PAT and HC is shown in Table 5 and Figure 5A. In the 3×3 and 6×6 mm\textsuperscript{2} angiography results, the area (P=0.0015 and P=0.0039) and perimeter (P=0.0068 and P<0.0001) were significantly decreased in PATs. The AUC was 0.729 for area (95%CI: 0.572 to 0.887), and 0.838 for perimeter (95%CI: 0.704 to 0.972), indicating medium diagnostic sensitivity in PAT.

Analysis of Superficial Vessel Density at Different Retinal Layers The SVDs at different retinal layers in PATs and HCs are shown in Table 5 and Figure 5B. In the 3×3 mm\textsuperscript{2} angiography result, the SVD of the retinal inner layer (P<0.0001) was significantly lower in PATs than in HCs, while the central foveal and the full layer showed no difference between groups. In the 6×6 mm\textsuperscript{2} angiography result, the SVDs at the retinal inner layer (P<0.0001) and the full layer (P=0.0194) were significantly lower in PATs than HCs. The AUC for 3×3 and 6×6 mm\textsuperscript{2} angiography in the retinal inner layer were 0.858 (95%CI: 0.739 to 0.977) and 0.828 (95%CI: 0.712 to 0.944) respectively, indicating medium diagnostic sensitivity in PAT.

Analysis of Retinal Volume and Average Volume Thickness The retinal volume and average volume thickness of the PAT and HC groups are shown in Table 6 and Figure 5C. Compared with HCs, the retinal volume (P=0.0001) and average

Table 4 Univariate and multivariate regression analyses of association between visual acuity and related factors

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Univariate regression analysis</th>
<th>Multivariate regression analysis</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>β</td>
<td>SE</td>
</tr>
<tr>
<td>Age</td>
<td>0.090</td>
<td>0.007</td>
</tr>
<tr>
<td>Gender</td>
<td>-0.156</td>
<td>0.099</td>
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<tr>
<td>Systolic blood pressure</td>
<td>-0.077</td>
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<tr>
<td>Diastolic blood pressure</td>
<td>0.237</td>
<td>0.005</td>
</tr>
<tr>
<td>RT</td>
<td>-0.634</td>
<td>0.003</td>
</tr>
<tr>
<td>SVD</td>
<td>-0.486</td>
<td>3.757</td>
</tr>
<tr>
<td>Volume</td>
<td>-0.651</td>
<td>0.074</td>
</tr>
<tr>
<td>Average volume thickness</td>
<td>-0.597</td>
<td>0.003</td>
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<tr>
<td>FAZ area</td>
<td>-0.326</td>
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<tr>
<td>FAZ perimeter</td>
<td>-0.507</td>
<td>0.125</td>
</tr>
<tr>
<td>FAZ substantiation</td>
<td>-0.065</td>
<td>1.188</td>
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SE: Standard error; RT: Retinal thickness; SVD: Superficial vessel density; FAZ: Foveal avascular zone.
volume thickness (P=0.001) in PATs were both significantly decreased. The AUC for the retinal volume was 0.897 (95%CI: 0.803 to 0.990) and for average volume thickness 0.872 (95%CI: 0.765 to 0.980), indicating moderate to high diagnostic sensitivity in PATs.

**DISCUSSION**

Our study found significant decrease in visual acuity, retinal SVD, RT, FAZ area and perimeter, retinal volume and average volume thickness in MGD patients in severely obese population than in controls.

MGD is the major causal factor of dry eye and loss of ocular surface homeostasis. More and more research have indicated that dyslipidemia can promote the development of MGD, which is highly associated with severe obesity [26]. Previous studies have shown that moderate to severe MGD is significantly related to high BMI in Chinese adults [27].

The periphery of the retina is a pathological site for several eye diseases. Changes in the retina can provide markers to diagnose and monitor these and to evaluate their therapeutic response. Retinal changes have also been reported in neurodegenerative diseases such as Parkinson’s disease [28] and Alzheimer’s disease [29]. The retina and the cerebrum develop from the same embryonic tissue, so retinal research may offer a new way to understand systematic diseases that are related to the central nervous system. Since retinal vessels with tight junctions between endothelial cells are similar to cerebrovascular vessels, it is assumed that imaging of the former may be helpful in research on the latter. OCTA has been effectively applied in predicting retinal vascular anomalies in neurological diseases [30]. Schönfeld-Lecuona et al [31] have provided further evidence that in schizophrenia spectrum disorder patients structural changes observed in the brain are also observable in the retina. Retinal research may help to further understand a nervous system that changes in highly complex diseases.

**Figure 5 ROC curve analysis of FAZ, inner SVD, and volume**

A: The area under the ROC curve was 0.729 for area (95%CI: 0.572 to 0.887), and 0.838 for perimeter (95%CI: 0.704 to 0.972); B: The area under the ROC curve was 0.858 for 3×3 mm² angiography of inner SVD (95%CI: 0.739 to 0.977), and 0.828 for 6×6 mm² angiography of inner SVD (95%CI: 0.712 to 0.944); C: The area under the ROC curve was 0.897 for retinal volume (95%CI: 0.803 to 0.990), and 0.872 for average volume thickness (95%CI: 0.765 to 0.980). ROC: Receiver operating characteristic; FAZ: Foveal avascular zone; SVD: Superficial vessel density.

**Table 5 Analysis of FAZ results and SVD results in different retinal layers**

<table>
<thead>
<tr>
<th>Parameters</th>
<th>HC group</th>
<th>PAT group</th>
<th>t</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Angiography 3×3 mm²</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>FAZ area (mm²)</td>
<td>0.35±0.13</td>
<td>0.26±0.02</td>
<td>3.386</td>
<td>0.002</td>
</tr>
<tr>
<td>FAZ perimeter (mm)</td>
<td>2.37±0.47</td>
<td>2.08±0.13</td>
<td>2.835</td>
<td>0.007</td>
</tr>
<tr>
<td>FAZ substantiation</td>
<td>0.74±0.05</td>
<td>0.74±0.06</td>
<td>0.1019</td>
<td>0.919</td>
</tr>
<tr>
<td>Central SVD</td>
<td>0.18±0.06</td>
<td>0.19±0.02</td>
<td>0.380</td>
<td>0.706</td>
</tr>
<tr>
<td>Inner SVD</td>
<td>0.41±0.01</td>
<td>0.40±0.01</td>
<td>6.096</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Full SVD</td>
<td>0.38±0.02</td>
<td>0.38±0.01</td>
<td>0.141</td>
<td>0.889</td>
</tr>
<tr>
<td>Angiography 6×6 mm²</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>FAZ area (mm²)</td>
<td>0.33±0.13</td>
<td>0.25±0.03</td>
<td>3.044</td>
<td>0.004</td>
</tr>
<tr>
<td>FAZ perimeter (mm)</td>
<td>2.25±0.39</td>
<td>1.85±0.07</td>
<td>4.842</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>FAZ substantiation</td>
<td>0.80±0.05</td>
<td>0.79±0.04</td>
<td>0.584</td>
<td>0.562</td>
</tr>
<tr>
<td>Central SVD</td>
<td>0.21±0.06</td>
<td>0.21±0.01</td>
<td>0.145</td>
<td>0.885</td>
</tr>
<tr>
<td>Inner SVD</td>
<td>0.45±0.02</td>
<td>0.42±0.02</td>
<td>4.926</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Outer SVD</td>
<td>0.47±0.01</td>
<td>0.47±0.02</td>
<td>0.215</td>
<td>0.831</td>
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<tr>
<td>Full SVD</td>
<td>0.46±0.01</td>
<td>0.45±0.01</td>
<td>2.424</td>
<td>0.019</td>
</tr>
</tbody>
</table>

FAZ: Foveal avascular zone; SVD: Superficial vessel density; HC: Health control; PAT: Meibomian gland dysfunction patient in severely obese population.

**Table 6 Analysis of retinal volume and average volume thickness results**

<table>
<thead>
<tr>
<th>Parameters</th>
<th>HC group</th>
<th>PAT group</th>
<th>t</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Volume (mm³)</td>
<td>10.49±0.41</td>
<td>9.80±0.34</td>
<td>6.303</td>
<td>0.0001</td>
</tr>
<tr>
<td>Average volume thickness (μm)</td>
<td>289.25±10.43</td>
<td>275.21±7.77</td>
<td>5.288</td>
<td>0.0001</td>
</tr>
</tbody>
</table>

HC: Health control; PAT: Meibomian gland dysfunction patient in severely obese population.
Decreased SVD has been detected in many diseases. OCTA in glaucomatous eyes is characterized by reduced density of superficial blood vessels at the optic disk and in the macular region, and complete loss of choroidal capillaries in localized areas of peripapillary atrophy\[19\]. Previous research revealed that superficial parapapillary microvascular density is reduced in high myopia\[32\]. Our study found decreased SVD in MGD patients in severely obese population, and we speculate that those patients may be at risk of the eye diseases mentioned above. Recent research has reported that decreased RT in patients with Alzheimer’s disease is correlated with disease severity\[33\] and outer parapapillary total retinal ring volumes reportedly differentiate papilledema from pseudopapilledema\[34\]. As these subclinical changes also existed in the PATs group in the present study, we speculate that retinal microvascular and microstructural alterations may facilitate understanding of the pathogenesis of MGD in severely obese population and can be used for early diagnosis and staging of MGD in this population.

Our regression analysis demonstrated that impaired visual acuity was highly correlated with retinal thinning, SVD decrease and FAZ reduction, and a number of previous studies are consistent with these findings. A study on Sjögren’s syndrome patients showed that the thinning of the retina affects visual acuity\[35\] and Islam\[36\] reported that vision was highly related to RT in diabetic macular edema. Nakajima et al\[37\] found that deep vessel density was highly related to loss of visual field in patients with retinitis pigmentosa. Roesel et al\[38\] reported that foveal thickness was related to vision. Our ROC curve analysis of SVD, RT, FAZ, and retinal volume illustrates possible approaches for early discovery of retinal variations in MGD patients in severely obese population. Early diagnosis is key to more effective treatment and better prognosis. OCTA and functional magnetic resonance imaging are revolutionary methods for visualization of blood vessels at different layers of the retina and brain\[19-42\]. Being non-invasive and time-efficient, it has been widely used in retinal diagnostics. Microvascular and microstructural changes in MGD patients in severely obese population may precede clinically distinguishable retinopathy. The SVD, FAZ, RT, and retinal volume detected by OCTA may provide new avenues for the diagnosis of PAT. However, more studies are needed to provide evidence for future application in clinic. Therefore, in view of the important results of our study, more participants need to be enrolled in future research.

Our research has some limitations. The study included a relatively small sample, but despite this, SVD and RT were significantly different between the two groups. These results may contribute to future progress toward more precise methods to test retinal microvascular and microstructural alterations at an early stage. In addition, we found no correlation between SVD and RT in the PATs, which may need to be verified by more large-scale studies in the future.

In conclusion, we used OCTA to detect the retinal microvascular and microstructural alterations in MGD patients in a severely obese population. Our results showed reduced SVD in N, T, OS, IN, ON, OT; thinning of the RT in all subregions except central foveal; reduction in FAZ area and perimeter; significantly reduced SVD in the inner retina; and reduced retinal volume and average volume thickness. In addition, perimeter thinning at the FAZ was found to affect vision. OCTA may provide a new approach for early diagnosis of MGD in severely obese population.

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