Optical coherence tomography angiography for the characterisation of retinal microvasculature alterations in pregnant patients with anaemia: a nested case–control study

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ABSTRACT
Aims To characterise retinal microvascular alterations in the eyes of pregnant patients with anaemia (PA) and to compare the alterations with those in healthy controls (HC) using optical coherence tomography angiography (OCTA).
Methods This nested case–control study included singleton PA and HC from the Eye Health in Pregnancy Study. Fovea avascular zone (FAZ) metrics, perfusion density (PD) in the superficial capillary plexus, deep capillary plexus and flow deficit (FD) density in the choriocapillaris (CC) were quantified using FIJI software. Linear regressions were conducted to evaluate the differences in OCTA metrics between PA and HC. Subgroup analyses were performed based on comparisons between PA diagnosed in the early or late trimester and HC.
Results In total, 99 eyes of 99 PA and 184 eyes of 184 HC were analysed. PA had a significantly reduced FAZ perimeter (β coefficient=−0.310, p<0.001), area (β coefficient=−0.121, p=0.001) and increased circularity (β coefficient=0.037, p<0.001) compared with HC. Furthermore, higher PD in the central (β coefficient=0.327, p=0.001) and outer (β coefficient=0.349, p=0.007) regions were observed in PA. PA diagnosed in the first trimester had more extensive central FD (β coefficient=4.199, p=0.003) in the CC, indicating impaired perfusion in the CC.
Conclusion It was found that anaemia during pregnancy was associated with macular microvascular abnormalities, which differed in PA as pregnancy progressed. The results suggest that quantitative OCTA metrics may be useful for risk evaluation before clinical diagnosis.

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INTRODUCTION
Anaemia during pregnancy is a common maternal complication that affects 18% of pregnant women in China, which increases the risk of placental abruption, preterm birth, postpartum haemorrhage and fetal malformation.1-3 Most anaemia in pregnancy is nutritional anaemia, which results from a deficiency of iron, folic acid or vitamin B12.4 Among them, iron deficiency anaemia (IDA) contributes to 95% of cases of anaemia during pregnancy.5 Even though diagnosing anaemia through blood count tests is universal and affordable, clinical practice of the method does not suffice to frequently monitor the degree and treatment effect. We are interested in whether anaemia during pregnancy can be exhibited by other organs (such as the eyes) before the patient becomes symptomatic. A novel, rapid and non-invasive tool for screening and real-time monitoring may have the potential to help prevent adverse maternal and fetal outcomes.

Mounting evidence has shown that the eye can serve as a ‘window’, the sentinel sensory organ, to observe microvascular insults referred to as systemic vascular conditions including anaemia.6-9 Mitani et al have trained an artificial intelligence model to

WHAT IS ALREADY KNOWN ON THIS TOPIC
⇒ Abnormalities of retinal microvasculature are found in patients with anaemia. However, little is known about retinal perfusion characteristics in pregnant patients with anaemia (PA) detected by optical coherence tomography angiography (OCTA).

WHAT THIS STUDY ADDS
⇒ The shrinkage and deformation of fovea avascular zone and compensatory alterations of retinal perfusion were observed in PA, different from the ocular change patterns described in previous literature on chronic anaemia.

HOW THIS STUDY MIGHT AFFECT RESEARCH, PRACTICE OR POLICY
⇒ The retinal microvasculature changes in PA detected by OCTA provide initial evidence of ocular characterisations in the early stage of anaemia and facilitate the development of a potentially novel, non-invasive and rapid biomarker to screen and monitor PA.
screen anaemia using retinal fundus images. Optical coherence tomography angiography (OCTA) is one of the newest tools in the armamentarium of ocular imaging, enabling depth-selective visualisation of blood flow in retinal and choroidal capillary vessels without dye injection. The literature has investigated that retinal and choriocapillaris (CC) blood perfusion decrease on OCTA in patients with IDA and sickle cell anaemia. However, most previous studies focused only on patients with chronic anaemia. Studying anaemia during pregnancy allows us to advance our understanding of ocular alterations earlier in the disease course.

In this study, we used OCTA to detect retinal microvascular alterations in pregnant patients with anaemia (PA) and compared those diagnosed in the early and late trimesters with healthy controls (HC). Furthermore, we extracted metadata and quantitative OCTA metrics to construct a model for distinguishing PA, hoping to find a novel non-invasive method for screening and real-time detection of anaemia during pregnancy.

METHODS

Participants

The overall workflow chart is displayed in online supplemental eFigure 1. This nested case–control study involved 321 participants in total, and 283 of them were included in the final analysis. We evaluated 99 eyes from 99 PA subjects and 184 eyes from 184 HC from the Eye Health in Pregnancy Study by the Zhongshan Ophthalmic Centre, Sun Yat-sen University, and the department of obstetrics and gynecology, the First Affiliated Hospital, Sun Yat-sen University. The inclusion criteria for the study were as follows: age ≥18 years old, singleton pregnancy and live fetus without major fetal abnormality at the time of recruitment. The exclusion criteria for the study were hypertension during pregnancy, pregestational diabetes, anaemia caused by chronic diseases (such as cancers and tuberculosis), a history of ocular surgery, and ocular diseases including cataracts, glaucoma and fundus changes due to pathological myopia. Only data from participants’ oculi dexter were included. This study was registered in the Clinical Research Internal Management System of Zhongshan Ophthalmic Centre and prospectively registered at the Chinese Clinical Trial Registry.

Collections of clinical data

A pregnancy profile was created at the first prenatal visit during 11–13+6 weeks of gestation. Regular blood count tests, including haemoglobin (Hb) measurements, were performed in the first, second and third trimesters separately. Based on the guidelines of WHO, an Hb concentration of less than 110 g/L is diagnosed as anaemia during pregnancy. In our study, the definition of PA was that the patient was anaemic according to her recent blood count test results as the OCTA image was captured. The PA cases were further divided into the pregnant patients with anaemia diagnosed in the first trimester (PA-ST) group and the pregnant patients with anaemia diagnosed in the second or third trimester (PA-ST) group. To be more specific, PA-ST referred to those whose Hb was normal in the first trimester while getting anaemic in the following trimesters. Those who failed to acquire a blood test in the first trimester would not be assigned to the PA-ST group, even if they were anaemic in the second or third trimester.

Gestational age was determined by measuring fetal crown-rump length at 11–13+6 weeks. At the same time, clinical measurements of each participant were collected, including height, prepregnatal weight, gestational body mass index (BMI), diastolic and systolic pressures. The first, second and third trimesters were defined as ≤13+6 weeks, 14–27+6 weeks and ≥28 weeks of gestation, respectively.

OCTA image acquisition and processing

All participants underwent OCTA imaging with a Cirrus high-definition OCT 5000 (Carl Zeiss Meditec, Dublin, California, USA) equipped with an optical microangiography algorithm for analysis and had a scan rate of 68 000 A-scans per second. Besides, all OCTA images were acquired within 2 weeks of the blood count test. We assessed the parameters using 6×6 mm OCTA images centred on the fovea. OCTA images with poor scan quality (signal strength index less than 7/10), motion artefacts, segmentation artefacts or poor centration were excluded. Moreover, retinal fundus images were taken with the Retinal Fundus Camera RetiCam3100 (SYSEYE, Chongqing, China) at the time of OCTA image acquisition. Subjects were excluded if their fundus images demonstrated category 2 or 3 pathological myopia according to the international photographic classification and grading system for myopic maculopathy.

The Cirrus HD-OCT 5000 Review Station software V.10.0 (Carl Zeiss Meditec) automatically segments macular scans into the superficial capillary plexus (SCP), spanning from the internal limiting membrane to the lower border of the inner plexiform layer (IPL); and the deep capillary plexus (DCP), extending from the IPL to the outer plexiform layer; and the CC, defined as a thin layer spanning from 29 μm to 49 μm below the retinal pigment epithelium (RPE). The accuracy of automatic segmentation was checked, and the manual correction was performed if needed. The built-in functions of the software removed projection artefacts from large superficial vessels on the DCP and CC automatically.

All raw SCP, DCP and CC raw images were analysed in Fiji software V.2.0.0.rc-69/1.52 p (National Institutes of Health) to extract the following measures: FAZ area, FAZ perimeter, FAZ circularity, FAZ aspect ratio (AR), perfusion density (PD) and flow deficits (FDs). The microvascular measures were extracted in accordance with the previously reported methods with modifications. Additionally, PD was defined as a percentage of the area of perfused vasculature over the area in a measurement region. FD was calculated as the percentage of the area without a flow signal over the entire scanned region in the CC. FAZ metrics were traced for each image using Fiji software. PD and FD were measured over the 6×6 mm ETDRS centre region (d=1 mm), the inner ring (d=1–3 mm) and the outer ring (d=3–6 mm). In addition, both the inner and outer rings are divided into four quadrants separately (temporal, superior, nasal and inferior). The overall subregions are shown in online supplemental eFigure 2.

Data analyses

All statistical analyses were conducted using SPSS software V26.0.0.0. The clinical and blood count test measurements were described as the mean (SD) or ratio. Through the Shapiro-Wilk test, we assessed the normality of the clinical features and blood count test results. All the data are presented in the mean (SD) and ratio. Moreover, independent t-tests were used to compare the clinical data between the outcome groups. Linear regressions were performed to assess the associations between OCTA measurements of PA and those of HC, with maternal age and gestational age included as covariates. Subgroup analyses were performed in the PA-ST group and the PA-ST group. Receiver operating characteristic (ROC) curve analyses were
conducted to assess the ability of the OCTA measurements to discriminate PA from HC. The significance level was set to 0.05 for a two-sided alternative hypothesis test.

**RESULTS**

**Descriptive statistics**

A total of 283 participants were included in the final analysis, including 99 eyes from PA and 184 from HC. The demographic and clinical characteristics of the enrolled participants are described in Table 1. There were no differences in age, gestational age, height, pregestational weight, pregestational BMI, parity, systolic pressure and diastolic pressure. PA had significantly decreased Hb, MCV and HCT than HC. BMI was calculated as a person’s weight (kg) divided by the square of the height (m). Haematocrit (%).

**Microvascular group differences**

The results of linear regression analysis of the associations between OCTA parameters of PA and HC are displayed in Table 2. Compared with HC, PA demonstrated decreased FAZ perimeter (β coefficient = −0.310, 95% CI −0.441 to −0.180; p < 0.001), decreased FAZ area (β coefficient = −0.121, 95% CI −0.189 to −0.035; p < 0.001) and increased FAZ circularity (β coefficient = 0.037, 95% CI 0.020 to 0.054; p < 0.001) in the DCP. In the SCP of PA, the FAZ perimeter decreased sharply (β coefficient = 0.015, 95% CI 0.006 to 0.023; p = 0.028) and the circularity increased significantly (β coefficient = −0.310, 95% CI −0.441 to −0.180; p = 0.001). Additionally, in examining PA, we observed a higher PD in the centre regions of both the DCP (β coefficient = 3.237, 95% CI 1.333 to 5.141; p = 0.001) and the SCP (β coefficient = 2.858, 95% CI 0.883 to 4.833;
The blood count tests indicated that the group suffered from a more chronic anaemia that possibly existed before pregnancy. The shrinkage and irregularity of the FAZ were more evident in the PA-group than HC did ($\beta=4.199$, $p=0.003$), indicating impaired CC perfusion. No differences in FD were found between the PA-ST and HC groups (figure 2B,C and online supplemental eTable 1).

### Diagnostic accuracy of OCTA parameters

We aimed to validate the hypothesis that OCTA measurements could have an added-on value for distinguishing PA from HC. To achieve that, we built a clinical data-only model, an OCTA data-only model, and a combined model using logistic regression. The metadata model contained seven metrics derived from the previous work of anaemia prediction model using clinical data, including age, gestational age, height, pregestational weight, pregestational BMI, and systolic and diastolic pressures.$^9$ The principles for OCTA metrics selection were to be significantly different between PA and HC, not strongly associated with each other, and representative of different characteristics of FAZ and retinal layers. Thus, FAZ perimeter in the DCP, FAZ circularity in the DCP, FAZ circularity in the SCP and centre PD in the DCP were chosen in the OCTA model construction eventually. Moreover, all of the 11 variates mentioned previously were included in the combined model. As was shown in the results, the area under the curve (AUC) for the metadata model was 0.788 (95% CI 0.735 to 0.841); that for the OCTA data model was 0.728 (95% CI 0.68 to 0.78); and that for the combined model reached 0.874 (95% CI 0.835 to 0.914). We found that the AUC of the combined model was better than that of the clinical data-only model using the permutation test ($p<0.001$) (figure 2).

### DISCUSSION

This study, based on the application of OCTA, has found that PA participants had a smaller FAZ area and perimeter, higher FAZ circularity, and higher PD in the centre and outer regions of the retina than HC. In addition, the PA-ST and PA-FT subjects exhibited different patterns of ocular changes. The shrinkage and irregularity of the FAZ were more evident in the PA-ST group than in the PA-FT group when compared with HC. The PA-FT group showed more impaired microvascular perfusion (a
The reason for the discrepancy may be the duration of anaemia. Compared with anaemia that has plagued some participants for years, anaemia during pregnancy is a relatively short-term state caused by a temporary increase in blood volume, hormone fluctuations, a lack of iron, etc. Since the foveal region is packed with cone photoreceptors and thus consumes the most oxygen, it is especially sensitive to energy alterations. Therefore, the FAZ readily shrunk to adapt to low oxygen transport capacity, so it may serve as a sensitive biomarker to discriminate subjects with early-course anaemia from HC. Meanwhile, the central macular PD increased as the FAZ was reduced. However, the increase in the outer region of the PD suggested a regulatory mechanism to compensate for the oxygen reduction by increasing the blood flow to maintain the function of photoreceptors, which was in line with the former study of sickle cell anaemia. In addition, these ischaemic abnormalities are more predominant in the DCP than in the SCP. Minvielle et al. and Jung et al. confirmed that the DCP was clearly more affected than the SCP was. The middle retinal layers perfused by the DCP are thought to be a watershed-like region that requires a high amount of oxygen; therefore, it is more susceptible to ishaemia.

We subclassified the PA group into the PA-ST group and the PA-FT group to further explore whether the duration influenced retinal and CC blood perfusion. We speculated that the PA-ST group represented the early-course anaemia with a relatively short duration, while the PA-FT group was related chronically anaemic presumably from before pregnancy. We found that both PA-ST and PA-FT showed decreased FAZ and increased PD. However, this trend was more evident in PA-ST than in PA-FT, indicating a compensatory process in early-course anaemia. In addition, the two groups demonstrated different alterations in the CC layer, the main vascular bed in the choroid that feeds the highly active RPE and photoreceptors. The essential functions of the CC are to transport nutrients to photoreceptors and take metabolic waste to the general blood circulation. In this study, we found that the FAZ was higher in the central region in the PA-FT group than in the HC group (the increase in FAZ indicates that more areas are in a lack of blood perfusion). At the same time, there was no difference between the PA-ST and HC groups. In the PA-FT group, CC perfusion became impaired as anaemia progressed and the fovea region was most affected since the foveal region had the highest oxygen demand.

The results of this study provided evidence that the retinal vessel adapted as the anaemia progressed. We speculated that there was a ‘trilogy of retina microvascular and CC alterations’ in anaemia, reflecting a pathological pathway initiated and moulded by an ischaemic environment. At first, in early-course anaemia (PA-ST), the size of the FAZ readily reduces, while the perfusion of the periphery region of the retina increases in compensation. The pattern is more evident in DCP on account of its high oxygen demand. As the disease progresses (PA-FT), the FAZ in the CC starts to increase, indicating lower perfusion in the central region of the CC, which forms the hallmark of stage two. Finally, whole retina vessel perfusion decreases in chronic anaemia, and the FAZ enlarges due to devascularisation resulting from the long-term lack of oxygen and nutrition.

The difference in the distributions of OCTA metrics between PA and HC potentially provides a clinically useful monitoring tool. We collected seven basic clinical variables and four metrics from a single non-invasive OCTA examination of each subject and, to discriminate PA, built a metadata model, an OCTA data model and a combined model. The AUC of the combined model reached 0.87 (95% CI 0.84 to 0.91), better than that of...
the metadata model (0.79, 95% CI 0.74 to 0.84). These results confirmed the added-on value of OCTA metrics for PA detection.

In summary, our research suggests that OCTA could potentially serve as a novel non-invasive real-time detection tool for anaemia during pregnancy. The strengths of our study include a standardised clinical data and image acquisition protocol. All eligible pregnant individuals underwent OCTA within 2 weeks of the blood count test to ensure that OCTA acquisitions were matched with the current Hb levels. Furthermore, each OCTA image was acquired and analysed in a standardised approach and was carefully screened to ensure its quality.

Nonetheless, limitations of this study should be noted. First, our study lacked ocular axial length data. To minimise the influence of axial length and optical magnification on OCTA measurements, we ruled out pathological myopia fundus based on their retinal fundus images. Furthermore, we used spectral domain OCTA, which is not as good as swept-source OCTA in visualising blood flow in the choroidal capillary layer. Another limitation of the nested case–control study is that we could not tell whether the ocular changes happened before or after the anaemia. In addition, due to the standard care setting, the blood count could not be tested regularly, which may further increase the difficulty in sequencing anaemia and ocular changes during pregnancy. However, our results indicate a novel method for monitoring the disease status and the prevention effects of PA.

In conclusion, our study suggests that OCTA may serve as a rapid, non-invasive tool for PA screening and potentially real-time monitoring. In addition, we noticed that different ocular alterations occur as anaemia progressed. Longitudinal studies will be necessary to determine whether the findings have substantial value in the early diagnosis of PA, which means timely care to pregnant individuals and help avert related adverse maternal and neonatal outcomes.

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REFERENCES


