Preeclampsia

Changes in Retinal Microvascular Caliber Precede the Clinical Onset of Preeclampsia

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Abstract—Preeclampsia is a leading cause of maternal morbidity and mortality. The degree of maternal cardiovascular dysfunction that precedes the onset of preeclampsia is largely unknown. This prospective cohort study aimed to characterize differences in vivo in retinal microvascular caliber and blood pressure throughout pregnancy in relation to preeclampsia development. Women were recruited from Royal Prince Alfred Hospital, Sydney, Australia, of which 92 women were included in the study. Retinal images and blood pressures were collected at 13, 19, 29, and 38 weeks of gestation. Retinal vessels were analyzed as the central retinal arteriolar equivalent corrected for mean arterial blood pressure and the central retinal venular equivalent corrected for mean arterial blood pressure, using generalized linear models adjusted for age and body mass index. The preeclampsia group were significantly older (P=0.002) and had a significantly higher mean body mass index (P=0.005). The central retinal arteriolar equivalent corrected for mean arterial blood pressure was significantly reduced at 13 (P=0.03), 19 (P=0.007), and 38 (P=0.03) weeks of gestation in the preeclampsia group. The central retinal venular equivalent corrected for mean arterial blood pressure was also significantly lower at 13 (P=0.04) and 19 (P=0.001) weeks of gestation in the women who progressed to preeclampsia. This study directly documents increased peripheral resistance in vivo, observed as the combination of constricted retinal arterioles or venules and elevated blood pressure, in women who later developed preeclampsia. This difference preceded the clinical signs of preeclampsia. (Hypertension. 2013;62:899-904.)

Key Words: imaging, diagnostic ■ microcirculation ■ preeclampsia ■ pregnancy ■ retina

Preeclampsia is a hypertensive disorder of pregnancy, affecting 3% to 5% of all pregnancies, and is one of the leading causes of maternal mortality and morbidity.1 Signs of preeclampsia include de novo hypertension after 20 weeks of gestation, proteinuria, peripheral edema, and liver dysfunction. Preeclampsia can lead to more serious complications including eclampsia, wherein the mother experiences seizures and possibly hemorrhagic stroke;2 and it is associated with a higher likelihood of remote maternal hypertension and stroke.3 Although changes to the placental vasculature have been examined in vitro, the nature and the degree of maternal cardiovascular dysfunction present in preeclampsia have yet to be quantified via direct visualization of the microvascular caliber, which can be measured from retinal images.

An increase in peripheral vascular resistance, partly from systemic endothelial activation, has been suggested to play a key role in the pathogenesis of preeclampsia.4 Activation of the endothelium causes the release of vasoactive factors, resulting in vasoconstriction throughout the microvasculature, increased peripheral resistance, and hypertension.5 Many attempts have been made to quantify the degree of peripheral vascular resistance, endothelial dysfunction, and microvascular constriction indirectly, including measuring the amount of vasoactive molecules present in maternal blood.6 However, a direct method of visualizing and measuring microvascular changes during pregnancy may improve understanding of preeclampsia development and aid clinical risk stratification in pregnancy.

Retinal imaging is a novel method allowing in vivo examination of the microcirculation. The retina is an ideal candidate for directly and objectively assessing microvascular changes in pregnancy that occur because of volume and vasoactive factors because both the placental and the retinal blood vessels lack innervation by the sympathetic nervous system, which is upregulated in preeclampsia.7,8 The aim of this study was to prospectively characterize changes in the retinal microvascular caliber throughout pregnancy between women who subsequently developed preeclampsia and those who had a normotensive pregnancy.

Material and Methods

Study Design
We performed a prospective cohort study. Ethical approval for this study was obtained from the Sydney South West Area Health Service...
Human Research Ethics Committee (NSW, Australia) and was ratified by the University of Western Sydney Human Ethics Committee. All participants gave informed consent, and the procedures followed were in accordance with Sydney South West Area Health Service guidelines. Recruitment of 129 women at 13±2 weeks of gestation was undertaken between July 2010 and April 2011 at Royal Prince Alfred Hospital, a major tertiary referral hospital with a specific interest in the treatment of hypertension in pregnancy in Sydney, Australia. All women presenting for a nuchal translucency ultrasound (performed between 11 and 13 weeks of gestation) were approached in the Fetal Medicine Unit waiting room to participate in this study; ≈15% of women approached agreed to participate. Each participant provided a clinical history, including the number of previous pregnancies, episodes of hypertensive disorders of pregnancy and gestational diabetes mellitus, smoking history, alcohol intake, and family history of cardiovascular disease. A physical examination was performed at a participant’s first antenatal visit, which included the measurement of height, weight (and calculation of body mass index [BMI]), and blood pressure.

Postpartum data including primary clinical diagnosis were collected from medical records before the analysis of retinal images. Women were excluded if they miscarried; delivered elsewhere; were diagnosed with gestational diabetes mellitus or gestational hypertension according to the Royal Australian and New Zealand College of Obstetricians and Gynaecologists (RANZCOG)9 or the Society of Obstetric Medicine of Australia and New Zealand (SOMANZ)10 guidelines, respectively; had a pregnancy of multiple gestation; or were diagnosed with gestational diabetes mellitus, smoking history, alcohol intake, and family history of cardiovascular disease. A physical examination was performed at a participant’s first antenatal visit, which included the measurement of height, weight (and calculation of body mass index [BMI]), and blood pressure.

Blood pressure was measured throughout pregnancy as part of the participant’s routine antenatal visits. Retinal images were collected at the same time points. Blood pressure was measured by a study author or a midwife using an automated Intellisense Digital Blood Pressure Monitor HEM-907 (Omron, Canada). A cuff size appropriate for patient BMI was chosen,11 and readings were collected from the upper right arm with the patient sitting. Three readings were collected during a period of 5 minutes, and the average of these readings was calculated and used in analyses. Where blood pressure data were unavailable on the day of the retinal image, a blood pressure reading taken within 1 week of the retinal image was included in the data. The mean arterial blood pressure (MAP) was calculated using the formula MAP=DP+1/3 (SP–DP), where DP and SP represent diastolic and systolic blood pressure levels, respectively.

**Retinal Image Analysis**

Retinal imaging was performed using a 45° nonmydriatic retinal fundus camera (Canon CR-1 with a 10D SLR digital camera back, Canon, Tokyo, Japan). Photographs were taken at 13±2, 19±2, 29±2, and 38±2 weeks of gestation. Women rested for 5 minutes in a darkened room before retinal photography to achieve pupil dilatation without pharmacological mydriasis. Because retinal vessel characteristics are comparable between the right and left eyes,12 1 eye was chosen for analyses. One image of the left eye, centered on the optic disc, was analyzed for each participant at each time point. The left eye was chosen because 1 patient had suffered fetal monocular toxoplasmosis, resulting in fundus destruction in the right eye. Images were graded using a semiautomated retinal vascular caliber measurement software program,13 which identifies and measures all retinal vessels that pass through an area between 0.5 and 1.0 disc diameter from the optic disc margin (zone B). The calibers of the 6 largest arterioles and the 6 largest venules were summarized to form the central retinal arteriolar equivalent (CRAE) and the central retinal venular equivalent (CRVE), respectively. This is according to a standardized protocol, based on the revised Knudtson–Parr–Hubbard formula, previously described elsewhere.14,15 Because blood pressure is known to affect retinal vascular caliber,16 retinal vascular measures were corrected for MAP by dividing the CRAE and CRVE by MAP to produce the cCRAE and cCRVE, respectively.17

Retinal image graders were blinded to the pregnancy outcome. To determine intergrader reliability, a subset of 40 retinal images were randomly selected and independently measured by 2 graders. One grader repeated these measurements after 2 months to determine intrgrader reliability. Overall, interrater reliability (measured by Cronbach’s Alpha) was 0.96 for CRAE and 0.95 for CRVE, and intrarater reliability was 0.96 for CRAE and 0.94 for CRVE.

**Statistical Analysis**

All statistical analyses were performed using SPSS statistics version 20.0 (IBM Corp, New York, NY). χ² test and unpaired t tests were used to determine differences in demographic data between

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**Figure 1.** Study cohort, detailing inclusion and exclusion criteria. ART indicates assisted reproductive technology.
the normotensive and preeclampsia groups. Generalized linear models adjusted for age and BMI were used to determine differences in corrected retinal vascular caliber between the normotensive group and the preeclampsia group at each time point. Ratios were log-transformed before statistical analysis. Differences between the groups were considered significant for \( P < 0.05 \). Data are presented as means±standard error. Women with missing data at a time point were excluded from the analysis at the relevant time point.

**Results**

A total of 92 women were included in the analysis, of which 83 had a normotensive pregnancy, and 9 were subsequently diagnosed with preeclampsia. The mean age was significantly different between the normotensive (32.3±0.5 years) and the preeclampsia (37.1±1.5 years) groups (\( P = 0.002 \)). The mean BMI was also significantly different between the normotensive (24.2±0.5 kg/m\(^2\)) and the preeclampsia (29.0±1.8 kg/m\(^2\)) groups (\( P = 0.005 \)). Because age and BMI were significantly different between the 2 groups, further analysis adjusted for these variables. The proportion of women who experienced hypertension in a previous pregnancy was significantly higher in the preeclampsia group compared with the normotensive group (\( P = 0.02 \)). A higher proportion of infants were born by lower segment cesarean delivery in the preeclampsia group (55.6%) than in the normotensive group (22.9%; \( P = 0.05 \)). There were no significant differences between the 2 groups in the gestational age at delivery (\( P = 0.34 \)) or in the birth weight (\( P = 1.00 \)) of the infants. Further characteristics of the study population are described in the Table.

The mean cCRAE was significantly lower in the preeclampsia group at 13 weeks (\( P = 0.03 \)), 19 weeks (\( P = 0.007 \)), and 38 weeks (\( P = 0.03 \)) of gestation. The mean cCRAE was also lower at 29 weeks of gestation in the preeclampsia group but did not reach statistical significance (\( P = 0.50 \); Figure 2).

The mean cCRVE was reduced in the preeclampsia group at every time point, reaching significance at 13 (\( P = 0.04 \)) and 19 weeks (\( P = 0.001 \)) of gestation. The differences between the 2 groups did not reach significance at the 29th week (\( P = 0.70 \)) or the 38th week (\( P = 0.10 \)) time point (Figure 3).

**Discussion**

This prospective cohort study measured retinal vessel caliber and blood pressure at 4 time points during pregnancy. Of the 92 women included, 9 developed clinical preeclampsia. The women in the preeclampsia group displayed known risk factors for hypertensive disorders of pregnancy, including an increased age, a higher BMI, and a previous history of a hypertensive disorder of pregnancy. After adjusting for age and BMI in the statistical analysis, we found a significant difference in the cCRAE at 13, 19, and 38 weeks of gestation, and in the cCRVE at 13 and 19 weeks of gestation, between those who were normotensive and those who developed preeclampsia. It has previously been documented by some of these authors and others that, in nonpregnant populations, retinal arteriolar narrowing precedes the development of hypertension, reflecting peripheral vascular resistance as a key pathogenic factor in hypertension. This is the first study using retinal imaging to document that this mechanism may reflect the same phenomenon in preeclampsia.
preeclampsia (PE; open squares), adjusted for age and body mass index. *P<0.01; **P<0.05; ***P<0.001.

Figure 2. Comparison of central retinal arteriolar equivalent (CRAE) corrected for mean arterial blood pressure (cCRAE) of normotensive women (NT; solid squares) and women with preeclampsia (PE; open squares), adjusted for age and body mass index. *P<0.05; **P<0.01.

We have previously described retinal microvascular and blood pressure changes throughout healthy pregnancy in the same cohort.17 In healthy pregnancy, dilatation of the retinal arterioles and venules was observed between weeks 13 and 19, and this occurred concurrently with a decrease in MAP. Combining these measurements as the cCRAE and the cCRVE, respectively, allowed quantification of the decrease in peripheral resistance experienced within the maternal circulation during gestation. The peak in dilatation of the microvasculature, and trough of MAP, in these women was seen at 19 weeks of gestation. In the present study, there was a significant difference in the cCRAE and cCRVE at the same time points between the preeclampsia group and those who had a normotensive pregnancy, and additionally in the cCRAE at pregnancy term. A combination of reduced dilatation and increased MAP among women who were later diagnosed with preeclampsia reflects an increase in peripheral vascular resistance many weeks before the onset of overt signs of preeclampsia.

To our knowledge, this is the first study to use retinal imaging to show that an increase in peripheral vascular resistance occurs during pregnancy before a clinical diagnosis of preeclampsia. The mean cCRAE and cCRVE were lower in the preeclampsia group at the 13- and 19-week time points when compared with normotensive women. The maximum difference in these measurements was reached at 19 weeks, observed in the cCRAE as a 16.5% reduction and in the cCRVE as an 18.1% reduction in the preeclampsia group when compared with the normotensive group. This demonstrates a relative constriction of the retinal arterioles and venules concurrently with higher MAP at these time points, indicating an increase in peripheral resistance experienced by the preeclampsia group. In healthy pregnancy, a reduction in maternal peripheral resistance, mediated by vasodilatation, commences at ≈5 weeks of gestation.23 This becomes disturbed in preeclampsia, resulting in the observed increase in maternal peripheral resistance.24 The failure to maximally dilate may be because of several mechanisms, including endothelial dysfunction,25,26 a decrease in nitric oxide release,27 increased responsiveness to thepressor effect of angiotensin II,28 or vasospasm.29 Endothelial dysfunction leads to an alteration in the production of vasoactive molecules, such as sFlt-1, endoglin, and placental growth factor,30 derangements of which have previously been associated with preeclampsia. Further studies are required to determine the mechanisms involved in the reduced dilatation observed here, and the possible association with levels of angiogenic and vasoactive molecules.

The reduction of the cCRVE before the appearance of the clinical signs of preeclampsia reported in this study adds to the growing body of knowledge that venules are more than passive conduits of deoxygenated blood, but form a dynamic part of the microvasculature,31 and are actively involved in disease processes. Previous investigation of the behavior of retinal venular caliber in disease has revealed venular widening in the context of endocrine dysfunction, including impaired glucose function32 and diabetes mellitus type II,33 and in systemic inflammatory states.34 The pathogenesis of preeclampsia has a recognized inflammatory component, observed as a derangement of inflammatory cytokines including tumor necrosis factor-α and interleukins 6, 8, and 10.35 Altered concentrations of these cytokines may mediate the changes in the venular caliber observed here. Future research investigating inflammatory mediators and retinal venular caliber is required to further elucidate venular behavior in preeclampsia.

Our study is supported by and is consistent with data in nonpregnant populations in which retinal imaging has previously documented that retinal arteriolar narrowing precede and predict the development of hypertension,21,36 suggesting that peripheral vascular resistance plays a key role in the pathogenesis of essential hypertension. Our results now indicate that peripheral vasoconstriction also occurs before the appearance of hypertension in women with preeclampsia, supporting observations by Easterling et al17 that an increase in cardiac output, and therefore of peripheral resistance, occurs before an elevation of blood pressure.
Previous methods of analyzing microcirculatory changes in pregnancy have included measuring erythrocyte velocity by periangual microscopy,38 ex vivo analysis of maternal small arteries,5 peripheral deep body temperature responsiveness,39 and ophthalmic artery Doppler ultrasonography.40.41 These studies describe a disturbance of the mechanism of vasodilatation in subjects with preeclampsia. Our study agrees with these findings and is the first study to use retinal imaging as a direct, objective, and in vivo method to obtain measurements of the microcirculation in assessing the microvascular response to pregnancy dysfunction, at multiple time points. In contrast to the above studies, our study examines women as early as 13 weeks of gestation, allowing data collection both before and after the appearance of elevated blood pressure or proteinuria. These results enable further understanding of the maternal microvascular caliber and blood pressure during pregnancy. The use of retinal imaging in this study emphasizes the usefulness of retinal imaging in elucidating the pathogenesis of disorders involving the microvasculature.42

Recent efforts in the development of preeclampsia screening and risk stratification algorithms have focused on the first trimester of pregnancy. Studies evaluating the use of algorithms taking into account maternal factors and biophysical and biochemical markers at 11 to 14 weeks of gestation have shown promise.43 The data provided by retinal imaging may strengthen these algorithms because differences between women who had normotensive pregnancies and women who developed preeclampsia were observable at 13 weeks of gestation. Further research is needed in a larger sample size to allow the assessment of this method as an addition to current screening algorithms.

The limitations of this study include that retinal photographs could not be collected from every woman at every time point. We also had a small number of women in the preeclampsia group. Of the 92 women included, 9 (9.8%) women developed preeclampsia, >3% to 5% incidence rate described in the literature. This discrepancy may be because of the Fetal Medicine Department of Royal Prince Alfred Hospital being a tertiary hospital which has a specialist unit for women who have previously suffered a high-risk pregnancy. In addition, prospective studies examining the incidence of preeclampsia have found it to be higher than that described in observational studies.44 We also would have expected to observe a difference in the gestational age at delivery between the normotensive and the preeclampsia groups. This was not observed. We hypothesize that this is attributable to the mildness of disease experienced by the majority of women in the preeclampsia group because late-onset preeclampsia is hypothesized to arise from an underlying maternal susceptibility.45 Studies investigating the prepregnancy maternal cardiovascular system would identify whether the differences in retinal vessel caliber observed here are a cause or consequence of preeclampsia.

In conclusion, narrowing of retinal arterioles and venules, combined with an absence in physiological blood pressure dipping, occurs before the appearance of the clinical syndrome of preeclampsia. The constriction observed in the retinal vasculature at 13, 19, and 38 weeks of gestation indicates that maternal peripheral resistance in preeclampsia is increased above that found in normotensive pregnancies. Future studies are needed to determine the clinical use of retinal imaging in pregnancy as a predictor of preeclampsia.

**Perspectives**

This is the first study to show differences in peripheral resistance, by direct observation and measurement of the retinal microvasculature, in healthy compared with diseased pregnancies. Women who developed preeclampsia had significantly reduced peripheral resistance in both arterioles and venules as early as 13 weeks of gestation, when compared with women who had a healthy pregnancy. Measurement of retinal microvasculature is a unique tool that has the potential to be incorporated into risk calculation for the development of preeclampsia in early pregnancy. This study provides evidence that the 13- and 19-week gestational time points should be the focus of a larger trial examining the use of retinal imaging as a screening tool in pregnancy.

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**Disclosures**

None.

**References**

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**What Is New?**

- This is the first study to show differences in retinal microvasculature, corrected for blood pressure, between women with a healthy pregnancy and those that develop preeclampsia, measured using retinal imaging.
- A reduction in the microvascular caliber, corrected for blood pressure, occurs before the appearance of the clinical syndrome of preeclampsia.

**What Is Relevant?**

- Preeclampsia is the leading cause of maternal morbidity and mortality, and is associated with future maternal hypertension and stroke.

**Summary**

Increased maternal peripheral resistance is detectable in women who develop preeclampsia as early as 13 weeks of gestation by measuring retinal microvascular caliber corrected for blood pressure.
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