First trimester angiogenic and inflammatory factors in women with chronic hypertension and impact of blood pressure control: a case–control study

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Accepted 3 April 2021. Published Online 5 August 2021.

Objectives To assess first trimester serum placental growth factor (PLGF), soluble fms-like tyrosine kinase-1 (sFLT-1), interleukin-6 (IL-6), tumour necrosis factor-α (TNF-α), endothelin and vascular cell adhesion molecule (VCAM) in women with chronic hypertension (CH) stratified according to blood pressure (BP) control.

Design Case–control.

Setting Tertiary referral centre.

Population 650 women with CH, 142 normotensive controls.

Methods In the first trimester, patients with CH were subdivided into four groups. Group 1 included women without pre-pregnancy CH presenting with BP ≥ 140/90 mmHg. Groups 2–4 had pre-pregnancy CH; in group 2 the BP was <140/90 mmHg without antihypertensive medication, in group 3 the BP was <140/90 mmHg with antihypertensive medication, and in group 4 the BP was ≥140/90 mmHg despite antihypertensive medication. PLGF, sFLT-1, IL-6, TNF-α, endothelin and VCAM were measured at 11±1–13±1 weeks’ gestation and converted into multiples of the expected median (MoM) using multivariate regression analysis in the controls.

Main outcome measure Comparisons of MoM values of PLGF, sFLT-1, endothelin, IL-6, TNF-α and VCAM between the entire cohort of women with CH and the control group were made using Student’s t-test or Mann–Whitney U-test. Comparisons between the four CH groups were made using analysis of variance or Kruskal–Wallis tests.

Results Compared with the control group, women with CH had significantly lower MoM of PLGF, sFLT-1 and IL-6 and a significantly higher MoM of endothelin. Between the four groups of women with CH, there were no significant differences in the MoM of sFLT-1, PLGF, sFLT-1/PLGF ratio, endothelin, IL-6 or VCAM, or in the levels of TNF-α.

Conclusion In women with CH, differences exist in first trimester angiogenic and inflammatory profiles when compared with normotensive pregnancies. However, these differences do not assist in the stratification of women with CH to identify those with more severe underlying disease and worse pregnancy outcomes.

Keywords Angiogenic factors, blood pressure, chronic hypertension, endothelin, first trimester, inflammatory mediators, interleukin-6, placental growth factor, pre-eclampsia, pregnancy, soluble fms-like tyrosine kinase-1, tumour necrosis factor-α, vascular cell adhesion molecule.

Tweetable abstract First trimester blood pressure control impacts on serum PLGF, sFLT-1, endothelin and IL-6 in women with chronic hypertension.

Introduction Between 1% and 2% of pregnancies are complicated by chronic hypertension (CH), which represents one of the most significant risk factors for the development of pre-eclampsia (PE). We have proposed that women with CH fall into four categories in relation to their first trimester blood pressure (BP) control. Group 1 includes women...
without a preceding history of CH presenting with BP of \( \geq 140/90 \) mmHg. Groups 2–4 have pre-pregnancy CH; in group 2 the BP is \(< 140/90\) mmHg without antihypertensive medication, in group 3 the BP is \(< 140/90\) mmHg with antihypertensive medication and in group 4 the BP is \(\geq 140/90\) mmHg despite antihypertensive medication.\(^2,^3\) We have previously demonstrated that, across these four groups, the prevalence of superimposed PE is not uniformly distributed with group 4 having the highest rate of 27% compared with only 13% for group 1.\(^2,^3\) We hypothesised that this stratification reflects the severity of endothelial disease in women with CH.

A tendency towards an anti-angiogenic state is thought to contribute to the development of PE.\(^4\) Utero-placental hypoxia in pregnancies destined to develop PE plays an important role in shifting the production in favour of the anti-angiogenic soluble fms like tyrosine kinase-1 (sFLT-1) at the expense of a reduction in the pro-angiogenic placental growth factor (PLGF).\(^5\) This has lead to considerable interest in their ability to predict PE, with PLGF already being incorporated into screening models.\(^6–^8\) However, it has been demonstrated both outside and in pregnancy that differences in the levels of angiogenic factors exist in patients with CH.\(^9,^{10}\) This could impact upon the predictive capacity of PLGF and sFLT-1 and requires further clarification prior to their incorporation into these screening models.

Outside of pregnancy, the pathophysiology of CH involves a coordinated interaction between the endothelium and circulating inflammatory mediators that include interleukin-6 (IL-6) and tumour necrosis factor alpha (TNF-\(\alpha\)).\(^{11,12}\) TNF-\(\alpha\) promotes both the endothelial expression of vascular cell adhesion molecule (VCAM),\(^11\) an early event in the development of the atherosclerotic plaque, and the production of endothelin, a potent vasoconstrictor.\(^{13}\) TNF-\(\alpha\), VCAM and endothelin all induce the production of IL-6,\(^{11,14}\) which through its effects on vascular remodelling, contributes to the chronic elevation of BP.\(^{12}\) Thus, these inflammatory mediators, as markers of endothelial dysfunction outside of pregnancy, have been identified as possible biomarkers, in addition to angiogenic factors, for the prediction of PE.

Our objective was to examine differences in angiogenic and inflammatory factors in women with CH at 11–13\(^{\text{th}}\) weeks according to BP control.

**Methods**

The design of our study was case–control and involved the analysis of serum PLGF, sFLT-1, IL-6, TNF-\(\alpha\), endothelin and VCAM levels in samples stored at \(-80^\circ\)C. The samples were obtained at 11–13\(^{\text{th}}\) weeks’ gestation from 650 singleton pregnancies complicated by CH and 142 normotensive control subjects who attended King’s College Hospital (London, UK) for antenatal care between January 2011 and September 2018. Measurement of fetal crown-rump length was used to determine gestational age.\(^{15}\)

**Patient involvement**

Participants were invited to provide feedback on their pregnancy care at King’s College Hospital and to be contacted with the study findings. Otherwise, they were not involved in obtaining funding, study design or in the data analyses.

**Inclusion and exclusion criteria**

Inclusion criteria were singleton pregnancies that resulted in the live birth or stillbirth of phenotypically normal babies at \(\geq 24\) gestational weeks. Pregnancies ending in miscarriage at \(< 24\) gestational weeks as well as those where fetal aneuploidies or major defects diagnosed in the antenatal or neonatal period were excluded.

We selected approximately 1 control from uncomplicated pregnancies that resulted in the live birth of phenotypically normal neonates for every five cases with CH. The controls were matched to the cases for storage time of maternal serum and racial origin, because the incidence of CH is 3-fold higher in Black than white women.

**Main outcome measures**

Chronic hypertension was diagnosed in women if they had pre-pregnancy hypertension or had BP \(\geq 140/90\) mmHg on two consecutive occasions prior to 20 gestational weeks\(^6\) in the absence of renal or liver disease.\(^16\) Pre-eclampsia was defined according to the International Society for the Study of Hypertension in Pregnancy (ISSHP).\(^16\) as the presence of hypertension along with at least one of the following: liver involvement (transaminases \(> 70\) IU/l), renal impairment (proteinuria \(\geq 300\) mg/24 hours and/or creatinine \(\geq 90\) \(\mu\)mol/l or 1 mg/dl), thrombocytopenia (platelet count \(< 150,000/\mu l\)) and/or neurological sequelae (e.g. eclampsia).

**Assay analysis**

**Angiogenic factors**

Samples were thawed and an automated biochemical analyser (BRAHMS KRYPTOR compact PLUS; Thermo Fisher Scientific, Hennigsdorf, Germany) was used to quantify serum concentrations of PLGF and sFLT-1 in pg/ml. The inter-assay coefficients of variation in this analyser for the low and high concentrations were 22 and 5% for PLGF and 5 and 5% for sFLT-1, respectively. The measurement ranges were 3.6–7000 pg/ml for PLGF and from 22 to 90 000 pg/ml for sFLT-1.

**Inflammatory mediators**

TNF-\(\alpha\) and IL-6. The Meso Scale Proinflammatory 2 Plex (Mesoscale Discovery, Rockville, MD, USA) was used to
determine serum concentrations of TNF-α and IL-6. For this analyser, the intra-assay coefficients of variation were 10.1 and 4.5% for low concentrations of TNF-α and IL-6, respectively, and 6.1 and 3.6% for high concentrations for TNF-α and IL-6, respectively. The inter-assay coefficients of variation were 6.2 and 7.3% for the low concentrations of TNF-α and IL-6, respectively, and 7.2 and 5.2% for the high concentrations of TNF-α and IL-6, respectively. The detection limits were 0.04 ng/l for TNF-α and 0.06 ng/l for IL-6 with a measurement range from 0.10 to 1.75 ng/l for TNF-α and 0.16 to 27.2 ng/l for IL-6.

Endothelin. The Quantikine Endothelin-1 ELISA kit (R&D Systems, Oxon, UK) was used to determine the serum concentrations of endothelin. For this analyser, the intra-assay and inter-assay coefficients of variation for low concentrations were 4.0 and 7.6% and, for high concentrations, 1.9 and 5.3%. The detection limit was 0.09 ng/l with a measurement range of 0.45–2.00 ng/l.

VCAM. The Quantikine VCAM ELISA kit (R&D Systems) was used to determine the serum concentrations of VCAM. The intra-assay and inter-assay coefficients of variation for the low concentrations were 2.3 and 7.8% and, for high concentrations, 3.6 and 5.5%. The detection limit was 0.60 μg/l with a measurement range 349–991 μg/l.

Statistical analysis
The Kolmogorov–Smirnov test was used to assess normality of the distribution of continuous variables. Numerical data were expressed as mean and standard deviation (SD) or median and interquartile range (IQR) for normally and non-normally distributed data, respectively.

The distribution of sFLT-1, PLGF, sFLT-1/PLGF ratio, endothelin, IL-6, TNF-α and VCAM were logarithmically transformed to approximate Gaussian distribution. Linear multivariate regression models were used only to determine the maternal characteristics that impacted upon the angiogenic and inflammatory factors and, thus, to enable the creation of MoMs. To ensure the validity of the results of the model, we employed the use of bootstrapping with 1000 replications. Only log TNF-α was not converted to MoMs as there were no significant independent maternal contributions identified in the multivariate regression model.

Comparisons between the entire cohort of women with CH and the controls were made using the student t-test or Mann–Whitney U test for normally and non-normally distributed data, respectively. Comparisons between groups 1–4 for the women with CH were made by the ANOVA or the Kruskal–Wallis test, with Dunn–Bonferroni correction for post-hoc analysis, for normally and non-normally distributed data, respectively. The Chi-square test was used to compare categorical variables.

Statistical analysis was performed using SPSS (Version 24; SPSS Inc, Chicago, IL, USA).

Results
Population characteristics
A total of 650 pregnancies complicated with CH met the inclusion criteria and were classified as group 1 (n = 81), group 2 (n = 199), group 3 (n = 221) and group 4 (n = 149). The control group consisted of 142 normotensive women. Maternal and pregnancy characteristics of the four groups of women with CH and the control group are compared in Table 1. In the total cohort of women with CH, compared with controls, maternal age, weight and systolic and diastolic blood pressure at 11–13th weeks gestation and the incidence of previous and/or family history of pre-eclampsia were higher. Approximately 93% of women in groups 3 and 4 were taking one anti-hypertensive medication with 7% taking 2 or more. In groups 3 and 4, compared with group 2, there was a significantly higher weight, systolic blood pressure, incidence of black racial origin and previous PE. In groups 2 and 3, compared with group 1, systolic and diastolic pressure were lower. Women of group 2 were more likely to be younger and nulliparous when compared with group 3 and to have a lower diastolic blood pressure when compared with group 4. Those of group 1 were also more likely to weigh more and be nulliparous when compared with groups 2 and 3, respectively, and to have a lower systolic blood pressure compared with group 4. Groups 1 and 2 had a significantly lower rate of preterm PE and higher gestation at delivery when compared with groups 3 and 4 and a significantly lower incidence of SGA neonate with higher birthweight centile when compared with group 3, alone.

Serum PLGF, sFLT-1 and sFLT-1/PLGF ratio
Creation of multiples of the expected median using data from controls
Significant independent contributions were provided by age, weight, black racial origin, parity and history of previous pre-eclampsia in the multiple regression model for log sFLT-1 (P < 0.001, R² = 0.251): Log sFLT-1 expected = 3.142305 + 0.007276 × age (in years) − 0.003325 × weight (in kg) + 0.102288 × black racial origin − 0.172146 × multiparity + 0.158197 × previous pre-eclampsia.

Significant independent contributions were provided by age, weight and Black racial origin in the multiple regression model for log PLGF (P < 0.001, R² = 0.207): Log PLGF expected = 1.524666 + 0.008217 × age (in years) − 0.002721 × weight (in kg) + 0.170813 × black racial origin.
| Clinical feature                  | Controls (n = 142) | All CH (n = 650) | P-value<sup>1</sup> | Group 1 (n = 81) | Group 2 (n = 199) | Group 3 (n = 221) | Group 4 (n = 149) | P-value<sup>2</sup> |
|----------------------------------|--------------------|------------------|---------------------|------------------|------------------|------------------|------------------|------------------|
| Antihypertensive medications     |                    |                  |                     |                  |                  |                  |                  |                  |
| One drug, n (%)                  | –                  | 345.0 (53.1)     | –                   | –                | –                | 206.0 (93.2)     | 139.0 (93.3)     | 0.97             |
| Two or more drugs, n (%)         | –                  | 25.0 (3.8)       | –                   | –                | –                | 15.0 (6.8)       | 10.0 (6.7)       | 0.97             |
| Age (years), median (IQR)        | 31.5 (28.2–34.5)   | 34.0 <0.001      | 34.0 (30.0–37.0)    | 34.0 (30.0–37.0) | 34.0 (30.0–37.0) | 34.0 (30.0–37.0) | 34.0 (30.0–37.0) | 0.02             |
| Weight in kg, median (IQR)       | 81.9 (71.7–90.0)   | 84.0 (71.0–97.0) | 0.04 (<72.3–97.5)**| 89.0 (72.3–97.5)**| 82.0 (72.0–96.8) | 84.0 (72.0–97.1) | 86.8 (74.3–101.8) | 0.25             |
| Height (m), median (IQR)         | 165.0 (160.0–169.6)| 165.0 (160.0–169.6)| 0.45 (<162.2–169)| 166.0 (162.2–169)| 165.0 (161.0–170)| 165.0 (160.0–170)| 165.0 (160.0–170)| 0.01             |
| Family history of PE, n (%)      | 7.0 (4.9)          | 86.0 (13.2)      | 0.005 (<16.0 (19.8)| 16.0 (19.8)     | 24.0 (12.1)     | 26.0 (11.8)     | 20.0 (7.4)       | 0.34             |
| Multiparous, n (%)               | 102 (71.8)         | 446.0 (68.6)     | 0.45 (<52.0 (64.2)|| 52.0 (64.2)     | 120.0 (60.3)     | 171.0 (77.4)     | 103.0 (69.1)     | 0.03             |
| Previous pre-eclampsia, n (%)    | 5.0 (3.5)          | 255.0 (39.2)     | <0.001 (<28.0 (34.6)| 28.0 (34.6)     | 55.0 (27.6)     | 111.0 (50.2)    | 61.0 (40.9)       | 0.01             |
| Racial origin                    |                    |                  |                     |                  |                  |                  |                  |                  |
| Black, n (%)                     | 87 (61.3)          | 389.0 (59.8)     | 0.75 (<43.0 (53.1)| 43.0 (53.1)     | 99.0 (49.7)     | 150.0 (67.9)    | 97.0 (65.1)      | <0.001           |
| White, n (%)                     | 52 (36.6)          | 209.0 (32.2)     | 0.30 (<33.0 (40.7)| 33.0 (40.7)     | 83.0 (41.7)     | 51.0 (23.1)     | 42.0 (27.2)*      | <0.001           |
| Other, n (%)                     | 3 (2.2)            | 52 (8.0)         | 0.01 (<5.0 (6.2)| 5.0 (6.2)       | 17.0 (8.5)      | 20.0 (9.0)      | 10.0 (6.7)       | 0.99             |
| SBP in mmHg, median (IQR)        | 118.1 (112.9–125.0)| 130.0 (<140.0)   | <0.001 (<1140.0–140.0)| 140.0 (140–148)| 120.0 (120–140)| 124.0 (120–140)| 145.0 (140–151)**| <0.001           |
| DBP in mmHg, median (IQR)        | 73.0 (66.0–77.1)   | 80.0 (<75.0–88.0)| <0.001 (<90.0 (82.0–93)| 90.0 (82.0–93)| 78.0 (70.0–80.0)***| 80.0 (70.0–80.0)***| 90.0 (85.0–97.0)***| <0.001           |
| Pregnancy outcome                |                    |                  |                     |                  |                  |                  |                  |                  |
| Preterm PE, n (%)                | –                  | 119.0 (18.3)     | –                   | 10.0 (12.3)     | 19.0 (9.5)      | 53.0 (24.0)     | 37.0 (24.8)      | <0.001           |
| Delivery gestation, median (IQR) | 40.2 (39.4–40.9)   | 38.9 (<37.7–39.8)| <0.001 (<38.4–40.0)| 39.1 (38.4–40.0)| 39.3 (38.0–40.0)| 38.6 (37.4–39.4)| 38.7 (37.5–39.7) | <0.001           |
| Birthweight centile, median (IQR)| 45.6 (30.2–69.0)  | 28.8 (9.8–54.2)  | 0.01 (<16.7–59.8)***| 39.1 (16.7–59.8)**| 32.4 (12.6–58.1)**| 27.8 (7.3–49.2)   | 25.2 (9.1–53.9) | 0.02             |
| Birthweight <10th centile, n (%) | 2.0 (1.4)          | 163.0 (25.1)     | <0.001 (<14.0 (17.3)***| 14.0 (17.3)***| 38.0 (19.1)***| 71.0 (32.1)***| 40.0 (26.8)*** | 0.011            |

*Statistically significant compared with group 1.
**Statistically significant compared with group 2.
***Statistically significant compared with group 3.
****Statistically significant compared with group 4.
Table 2. Comparison of maternal serum PLGF, sFLT-1, sFLT-1/PLGF ratio, endothelin, IL-6 and VCAM and levels of TNF-α between normotensive controls, the total cohort of women with CH and groups 1–4. P-values are presented separately for the comparisons between the normotensive controls1 and the total cohort of women with CH and (2) groups 1 and 4 only2.

| Clinical feature                  | Controls (n = 142) | All CH (n = 650) | P-value1 | Group 1 (n = 81) | Group 2 (n = 199) | Group 3 (n = 221) | Group 4 (n = 149) | P-value2 |
|----------------------------------|-------------------|-----------------|----------|-----------------|-----------------|-----------------|-----------------|----------|
| Angiogenic factors               |                   |                 |          |                 |                 |                 |                 |          |
| PLGF MoM, median (IQR)           | 1.0 (0.8–1.3)     | 0.9 (0.6–1.2)   | <0.001   | 0.9 (0.6–1.3)   | 0.8 (0.6–1.2)   | 0.9 (0.6–1.2)   | 0.9 (0.6–1.2)   | 0.91     |
| sFLT-1 MoM, median (IQR)         | 1.0 (0.8–1.3)     | 0.8 (0.6–1.2)   | <0.001   | 0.9 (0.6–1.2)   | 0.8 (0.6–1.1)   | 0.8 (0.5–1.2)   | 0.9 (0.7–1.2)   | 0.07     |
| sFLT-1/PLGF ratio MoM, median (IQR) | 1.0 (0.7–1.4) | 1.1 (0.8–1.7)   | 0.056    | 1.2 (1.4–1.8)   | 1.1 (0.8–1.6)   | 1.1 (0.7–1.7)   | 1.1 (0.8–1.7)   | 0.61     |
| Inflammatory mediators           |                   |                 |          |                 |                 |                 |                 |          |
| Endothelin MoM, median (IQR)     | 1.0 (0.8–1.2)     | 1.1 (0.9–1.3)   | 0.004    | 1.2 (0.9–1.4)   | 1.1 (0.9–1.3)   | 1.1 (0.8–1.3)   | 1.1 (0.9–1.4)   | 0.27     |
| IL-6 MoM, median (IQR)           | 1.0 (0.7–1.3)     | 0.9 (0.6–1.2)   | 0.001    | 0.9 (0.6–1.3)   | 0.8 (0.6–1.2)   | 0.9 (0.6–1.2)   | 0.9 (0.6–1.1)   | 0.77     |
| TNF-α, median (IQR)              | 2.0 (1.7–2.2)     | 2.0 (1.7–2.4)   | 0.42     | 2.1 (1.8–2.6)   | 2.0 (1.7–2.3)   | 2.0 (1.7–2.4)   | 1.9 (1.8–2.4)   | 0.38     |
| VCAM MoM, median (IQR)           | 1.0 (0.9–1.1)     | 1.0 (0.8–1.1)   | 0.16     | 1.0 (0.9–1.1)   | 1.0 (0.8–1.1)   | 1.0 (0.8–1.2)   | 1.0 (0.8–1.1)   | 0.37     |

Figure 1. Serum levels of MoM Log PLGF, MoM Log sFLT-1 and MoM Log sFLT-1/PLGF ratio in controls (white) and groups 1–4 (grey) of women with CH.
Significant independent contributions were provided by parity alone in the multiple regression model for log sFLT-1/PLGF ratio ($P = 0.001$, $R^2 = 0.082$): Log sFLT-1/PLGF ratio expected $= 1.486950 - 0.151459$ if parous.

Comparison of multiples of the expected median between groups
Compared with the control group, women with CH had significantly lower MoM of sFLT-1 and PLGF. However, the difference in the MoM of sFLT-1/PLGF ratio between the control group and women with CH did not reach statistical significance ($P = 0.056$) (Table 2, Figure 1). Between the four groups of women with CH, there were no significant differences in the sFLT-1, PLGF or sFLT-1/PLGF ratios (Table 2, Figure 1).

Serum IL-6, TNF-α, endothelin and VCAM
Creation of multiples of the expected median using data from controls
Significant independent contributions were provided by black racial origin in the multiple regression model for log VCAM ($P < 0.01$, $R^2 = 0.067$): Log VCAM expected $= 2.754330 - 0.051222 \times$ black racial origin.

Significant independent contributions were provided by weight and ‘Other’ racial origin in the multiple regression model for log IL-6 ($P < 0.001$, $R^2 = 0.125$): Log IL-6 expected $= 5.496581 + 0.004763 \times$ weight (in kg) + $0.309845 \times$ ‘Other’ racial origin.

Significant independent contributions were provided by black racial origin and parity in the multiple regression model for log endothelin ($P < 0.001$, $R^2 = 0.138$): Log endothelin expected $= 2.297770 + 0.076133 \times$ black racial origin + $0.073548 \times$ parity.

There were no significant independent contributions provided by maternal characteristics in the multiple regression model for log TNF-α.

Comparison of multiples of the expected median between groups
Compared with the control group, women with CH had a significantly higher MoM of endothelin and a significantly lower MoM of IL-6 (Table 2, Figure 2). There were no significant differences between the control group and women with CH in TNF-α or VCAM (Table 2, Figure 2). Between the four groups of women with CH, there were no significant differences in the MoM of IL-6, endothelin or VCAM and no significant difference in the levels of TNF-α (Table 2, Figure 2).

Discussion
Main findings
The findings of this study demonstrate that in women with CH, at 11–13\textsuperscript{th} weeks’ gestation, serum levels of PLGF, sFLT-1 and IL-6 were significantly reduced, whereas serum endothelin was significantly elevated when compared with
normotensive controls. Between the four groups of women with CH, there were no differences in the levels of first trimester angiogenic and inflammatory factors.

**Strengths and limitations**

The strengths of this study are the inclusion of a large population of women with CH recruited between 11 and 13 weeks’ gestation and the exclusion of those with pre-existing renal and/or liver impairment, which has minimised the potential for bias in the diagnosis of PE. Among the limitations of this study, we acknowledge the absence of longitudinal measurements of angiogenic and inflammatory factors throughout and outside of pregnancy in order to correlate these with possible changes that can occur during pregnancy and the postnatal period in women with CH.

**Interpretation of findings**

We hypothesised that the four subgroups of women with CH represent different stages of the disease. Studies in non-pregnant individuals have demonstrated that there is increasing endothelial dysfunction in the progression from normal blood pressure to mild and then severe hypertension. Groups 1 and 2 are likely to represent the early phase of CH with mild endothelial dysfunction, but the latter group has sufficient ability to achieve physiological adaptation to pregnancy with normalisation of BP. Groups 3 and 4 may represent moderate to severe endothelial dysfunction with less capacity to achieve early vasodilatation and normalisation in BP without antihypertensive medications. We further postulated that the severity of underlying endothelial disease, as represented by the four groups, might be reflected in the first trimester angiogenic and inflammatory profiles.

**Angiogenic factors**

During pregnancy, it has been well established that, in the general obstetric population, PLGF is reduced from as early as the first trimester in pregnancies later complicated by PE, and sFLT-1 is increased up to 3 weeks prior to the clinical onset of the disease. However, little is known about the impact of CH on first trimester serum PLGF and sFLT-1.

In women with CH, it could be anticipated that the degree of early placental hypoxia would be greater than in normotensive pregnancies due to pre-existing endothelial disease leading to a higher production of sFLT-1 and lower PLGF. The latter is in keeping with our finding, along with other studies, of significantly lower first trimester serum PLGF in women with CH. However, we were unable to demonstrate any differences in first trimester serum PLGF between the four groups of women with CH despite the differences in the rates of, particularly preterm, PE. It has previously been shown that, in women with CH, the distribution of birthweight adjusted for gestational age at delivery is skewed to the left of the distribution for uncomplicated pregnancies and that there is an approximate two-fold increase in the risk of having a small-for-gestational-age infant even in the absence of PE. Along with our findings, this suggests that CH is associated with impaired placentation irrespective of the development of PE.

Our finding of lower sFLT-1 in women with CH is difficult to interpret. We postulated that an impaired placenta, as expected in women with CH, would have less capacity to produce sFLT-1. It is likely that, although the risk of PE is not homogeneous across all women with CH, alteration in first trimester serum sFLT-1 does not play a significant role in the later development of PE. Although there are studies examining first trimester serum sFLT-1 in women with CH, none of them have included normotensive controls for comparison.

**Inflammatory mediators**

Outside of pregnancy, increased serum IL-6 and TNF-α has been reported in patients with CH. In one study, the prevalence of hypertension was double in those in the upper quartile of IL-6 and TNF-α when compared with those in the lower quartile. Two smaller studies further support a role for TNF-α in the pathophysiology of hypertension by reporting elevated TNF-α in those with CH compared with those without. TNF-α has been shown to induce structural as well as functional alterations in endothelial cells, and enhances the production of endothelin and other inflammatory mediators, including IL-6 and VCAM, that ultimately lead to atherosclerotic plaque formation and progression. In addition, the increases in serum IL-6, TNF-α, endothelin and VCAM have been correlated with the degree of cardiovascular risk in patients with CH and, thus, have emerged as predictors of cardiovascular morbidity.

During pregnancy, there is extensive literature to support a two- to three-fold increase in the production of IL-6, TNF-α, endothelin and VCAM in pregnancies already complicated with PE. It has been proposed that, similar to outside of pregnancy, these inflammatory mediators lead to the development of atherosclerotic-like lesions. Within the feto-placental circulation, these lesions result in placental hypoxia and, within the systemic circulation, result in endothelial dysfunction and the maternal syndrome of PE. However, it remains to be clarified whether this inflammatory process predates the placental impairment or whether it is a consequence of it.

Our work has demonstrated that, in women with CH, first trimester alterations in IL-6 and endothelin exist. We have shown that women with CH have elevated first trimester serum endothelin when compared with normotensive
controls. Along with our findings, this supports the existing literature where a positive correlation between levels of endothelin and mean arterial pressure but not duration of disease has been demonstrated.24,28

Up-regulation of endothelin production is known to stimulate the expression of other inflammatory cytokines such as IL-6, another biomarker of endothelial dysfunction.14 The finding of lower first trimester serum IL-6 in women with CH when compared with normotensive controls was unexpected. In normal pregnancy, current literature has demonstrated a doubling in the maternal serum concentration of IL-6 when compared with non-pregnant controls.30 Thus, the endothelin levels measured are a reflection of maternal systemic production rather than a trophoblastic contribution, as with IL-6.

Conclusion

In women with CH, differences exist in first trimester angiogenic and inflammatory profiles when compared with normotensive pregnancies. However, these differences do not assist in the stratification of women with CH to identify those with more severe underlying disease and worse pregnancy outcomes.

Disclosure of interests

None declared. Completed disclosure of interests forms are available to view online as supporting information.

Contribution to authorship

DN was involved in the collection and organisation of data, statistical analysis and writing the first draft of the manuscript. KHN and NAK were involved in the conception of the study and editing the manuscript. In addition, NAK was involved in the collection and organisation of data and statistical analysis.

Details of ethics approval

Written informed consent was obtained from women who agreed to participate in the study on early prediction of pregnancy complications, approved by the National Health Service Health Research Authority, Dulwich Research Ethics Committee (REC reference 02-03-033) on 17 February 2017.

Funding

The study was funded by the Fetal Medicine Foundation (Registered charity No. 1037116). The reagents and equipment for the measurement of serum placental growth factor and soluble fms-like tyrosine kinase-1 were provided by Thermo Fisher Scientific. These bodies had no involvement in the study design; in the collection, analysis and interpretation of data; in the writing of the report; or in the decision to submit the article for publication.

Data availability

The data that support the findings of this study are available from the corresponding author upon reasonable request.

Supporting Information

Additional supporting information may be found online in the Supporting Information section at the end of the article.

Video S1. Presentation of author insights.

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