

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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Abstract

Objectives: 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 4 groups. Group 1 included women without pre-pregnancy CH presenting with BP [?]140/90mmHg. Groups 2-4 had pre-pregnancy CH; in group 2 the BP was <140/90mmHg without antihypertensive medication, in group 3 the BP was <140/90mmHg 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+0-13+6 weeks' 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, IL-6, TNF- α , endothelin and VCAM between the 4 CH groups and the controls were made using analysis of variance or Kruskal-Wallis tests. **Results:** In the CH groups, compared to controls, PLGF was reduced in groups 2-4, sFLT-1 was reduced in groups 2 and 3, endothelin was increased in groups 1 and 4 but IL-6 was reduced in group 4. **Conclusion:** In women with CH, differences exist in first trimester angiogenic and inflammatory profiles according to BP control. Further evaluation is needed to determine if these differences are useful in the stratification of care.

INTRODUCTION

Chronic hypertension (CH) complicates 1-2% of pregnancies and represents one of the most significant risk factors for the development of preeclampsia (PE).¹ 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 [?]140/90mmHg. Groups 2-4 have pre-pregnancy CH; in group 2 the BP is <140/90mmHg without antihypertensive medication, in group 3 the BP is <140/90mmHg with antihypertensive medication and in group 4 the BP is [?]140/90mmHg despite antihypertensive medication.² 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 to only 13% for group 1.² We hypothesised that this stratification reflects the severity of endothelial disease in women with CH.

The pathophysiology of PE is thought to involve a tendency towards an anti-angiogenic state.³ 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).⁴ This has led to considerable interest in their ability to predict PE, with PLGF already being incorporated into screening algorithms.⁵⁻⁷ However, it has been

demonstrated both outside and in pregnancy that differences in the levels of angiogenic factors exist in patients with CH.^{8, 9} This could impact upon the predictive performance of PLGF and sFLT-1 and requires further clarification prior to their incorporation into 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- α).^{10, 11} TNF- α promotes both the endothelial expression of vascular cell adhesion molecule (VCAM)¹⁰, an early event in the development of the atherosclerotic plaque, and the production of endothelin, a potent vasoconstrictor.¹² TNF- α , VCAM and endothelin all induce the production of IL-6^{10, 13}, which through its effects on vascular remodelling, contributes to the chronic elevation of BP.¹¹ 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⁺⁰-13⁺⁶ weeks according to BP control.

METHODS

This was a case-control study involving analysis of serum PLGF, sFLT-1, IL-6, TNF- α , endothelin and VCAM levels in stored samples (-80°C) obtained at 11-13 weeks' gestation from 650 singleton pregnancies complicated by CH and 142 normotensive control subjects who attended King's College Hospital (London, UK) for pregnancy care between January 2011 and September 2018. Gestational age was determined from measurement of fetal crown-rump length.¹⁴

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

The inclusion criteria for this study were singleton pregnancies resulting in the live birth or stillbirth of phenotypically normal babies at > 24 weeks' gestation. Pregnancies with fetal aneuploidies or major defects diagnosed in the antenatal or neonatal period and pregnancies ending in miscarriage at <24 week's gestation were excluded.

For every 5 cases with CH, we selected approximately 1 control from uncomplicated pregnancies that resulted in the live birth of phenotypically normal neonates that were matched to the cases for storage time of maternal serum and racial origin, because the incidence of chronic hypertension is 3 times higher in Black than white women.

Main outcome measures

Women were classified as CH if they had pre-pregnancy hypertension or had BP [?]140/90 mmHg on two consecutive clinical visits prior to 20 weeks' gestation in the absence of renal or liver disease.¹⁵ Preeclampsia was defined according to the International Society for the Study of Hypertension in Pregnancy (ISSHP)¹⁵ as the presence of hypertension along with at least one of the following: renal involvement (proteinuria [?]300 mg/24h and/or creatinine [?]90 μ mol/L or 1 mg/dL), liver impairment (transaminases >70 IU/L), neurological complications (e.g. eclampsia), thrombocytopenia (platelet count <150,000/ μ L).

Assay analysis

Angiogenic factors

Maternal serum concentrations of PLGF and sFLT-1 in pg/mL were measured from thawed samples using an automated biochemical analyser (BRAHMS KRYPTOR compact PLUS; Thermo Fisher Scientific, Hennigsdorf, Germany). In this analyser, the inter-assay coefficients of variation for the low and high concentrations

were 22% and 5% for PlGF and 5% and 5% for sFLT-1, respectively. Assays cover a measurement range from 3.6-7000 pg/mL for PlGF and from 22-90,000pg/mL for sFLT-1.

Inflammatory mediators

TNF- α and IL-6

Serum concentrations of TNF- α and IL-6 were determined using the Meso Scale Proinflammatory 2 Plex (Mesoscale Discovery, MD, USA). The detection limits were 0.04 and 0.06 ng/L for TNF- α and IL-6, respectively, with a measurement range from 0.10 to 1.75 ng/L and 0.16 to 27.2 ng/L for TNF- α and IL-6, respectively. The intra-assay coefficients of variation for the low and high concentrations were 10.1% and 6.1% for TNF- α and 4.5% and 3.6% for IL-6. The inter-assay coefficients of variation for the low and high concentrations were 6.2% and 7.2% for TNF- α and 7.3% and 5.2% for IL-6.

Endothelin

Serum concentrations of endothelin were determined using the Quantikine Endothelin-1 ELISA kit (Biotechne, R&D Systems, Oxon, UK). The detection limit was 0.09 ng/L with a measurement range of 0.45 to 2.00 ng/L. The intra-assay coefficients of variation for the low and high concentrations were 4.0% and 1.9%. The inter-assay coefficients of variation for the low and high concentrations were 7.6% and 5.3%.

VCAM

Serum concentrations of VCAM were determined using the Quantikine VCAM ELISA kit (Biotechne, R&D Systems, Oxon, UK). The detection limit was 0.60 μ g/L with a measurement range 349 to 991 μ g/L. The intra-assay coefficients of variation for the low and high concentrations were 2.3% and 3.6%. The inter-assay coefficients of variation for the low and high concentrations were 7.8% and 5.5%.

Statistical analysis

Normality of the distribution of continuous variables was assessed by the Kolmogorov-Smirnov test. 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, IL-6, TNF- α , endothelin and VCAM were logarithmically transformed to approximate Gaussian distribution. All with the exception of log TNF- α were converted into multiples of the expected median (MoM) after adjustment for maternal characteristics. Log TNF- α was not converted to MoMs as there were no significant independent maternal contributions identified in the multiple regression model. Between group comparisons were made by the ANOVA or the Kruskal-Wallis test, with 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).

RESULTS

Population characteristics

The inclusion criteria for women with CH were met by 650 pregnancies 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 11-13⁺⁶ weeks gestation and the incidence of previous and/or family history of preeclampsia 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 to 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 to group 1, systolic and diastolic pressure were lower. Women of group 2 were more likely to be younger and nulliparous when compared to group 3 and with a lower diastolic blood

pressure when compared to group 4. Those of group 1 were also more likely to weigh more and be nulliparous when compared to groups 2 and 3, respectively, and with a lower systolic blood pressure compared to group 4. Groups 1 and 2 had a significantly lower rate of preterm PE and higher gestation at delivery when compared to groups 3 and 4 and a significantly lower incidence of SGA neonate with higher birth weight centile when compared to group 3, alone.

Serum PLGF, sFLT-1 and sFLT-1 / PLGF ratio

Creation of multiples of the median using data from controls

In the multiple regression model for log sFLT-1, significant independent contributions were provided by age, weight, Black racial origin, parity and history of previous preeclampsia ($p = <0.001$, $R^2 = 0.251$): Log sFLT-1 expected = $3.142305 + 0.007276 \times \text{age (in years)} - 0.003325 \times \text{weight (in kg)} + 0.102288 \times \text{Black racial origin} - 0.172146 \times \text{multiparity} + 0.158197 \times \text{previous preeclampsia}$.

In the multiple regression model for log PLGF, significant independent contributions were provided by age, weight and Black racial origin ($p = <0.001$, $R^2 = 0.207$): Log PLGF expected = $1.524666 + 0.008217 \times \text{age (in years)} - 0.002721 \times \text{weight (in kg)} + 0.170813 \times \text{Black racial origin}$.

In the multiple regression model for log sFLT-1/PLGF ratio, significant independent contributions were provided by parity alone ($p = 0.001$, $R^2 = 0.082$): Log sFLT-1/PLGF ratio expected = $1.486950 - 0.151459 \times \text{if parous}$.

Comparison of multiples of the median between groups

Groups 2-4, compared to the control group, had significantly lower mean levels of PLGF and groups 2 and 3 had significantly lower mean levels of sFLT-1 (Table 2, Figure 1). Women with CH, compared to the control group had a higher sFLT-1/PLGF ratio, but the differences did not reach statistical significance (Table 2, Figure 1). Between the four groups of women with CH, there were no significant differences in the levels of sFLT-1, PLGF and sFLT-1/PLGF ratio (Table 2, Figure 1).

Σερωμ ΙΑ-6, TNF-α, ενδοθηλιν ανδ VCAM

Creation of multiples of the median using data from controls

In the multiple regression model for log VCAM, significant independent contributions were provided by Black racial origin ($p = <0.01$, $R^2 = 0.067$): Log VCAM expected = $2.754330 - 0.051222 \times \text{Black racial origin}$.

In the multiple regression model for log IL-6, significant independent contributions were provided by weight and 'Other' racial origin ($p = <0.001$, $R^2 = 0.125$): Log IL-6 expected = $5.496581 + 0.004763 \times \text{weight (in kg)} + 0.309845 \times \text{'Other' racial origin}$.

In the multiple regression model for log endothelin, significant independent contributions were provided by Black racial origin and parity ($p = <0.001$, $R^2 = 0.138$): Log endothelin expected = $2.297770 + 0.076133 \times \text{Black racial origin} + 0.073548 \times \text{parity}$.

In the multiple regression model for log TNF-α, there were no significant independent contributions provided by maternal characteristics.

Comparison of multiples of the median between groups

Groups 1 and 4, compared to the control group, had significantly higher mean levels of endothelin and, only group 4 had significantly lower mean levels 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 levels of IL-6, TNF-α, endothelin or VCAM (Table 2, Figure 2).

DISCUSSION

Main findings

The findings of this study demonstrate that at 11-13⁺⁶ weeks' gestation in the CH groups, compared to controls, serum PLGF was reduced in those with pre-pregnancy CH (groups 2-4) and serum sFLT-1 was reduced in those with normal BP (groups 2-3). In addition, serum endothelin was increased in women with CH who are hypertensive at presentation (groups 1 and 4) but IL-6 was reduced only in those that remained hypertensive despite antihypertensive medications (group 4).

Strengths and limitations

The strengths of this study are examination of a large population of women with CH recruited in the first trimester of pregnancy. Exclusion of women with underlying renal and/or liver disease has reduced the potential for bias in the diagnosis of PE. A limitation of this study is the absence of longitudinal measurements of angiogenic and inflammatory factors throughout and outside of pregnancy to correlate with possible changes that can occur during pregnancy and the postnatal period.

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 in the progression from normal blood pressure to mild and then severe hypertension there is increasing endothelial dysfunction. Groups 1 and 2 are likely to represent the early phase of CH with mild endothelial dysfunction but sufficient ability to achieve physiological adaptation to pregnancy with normalisation of BP in the latter group. Groups 3 and 4 may represent moderate to severe endothelial dysfunction with less capacity to achieve early vasodilation 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 is 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 five weeks prior to the clinical onset of the disease.³ However, little is known as to 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 with a consequent 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.¹⁶ 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 birth weight adjusted for gestational age at delivery is skewed to the left of the distribution for uncomplicated pregnancies and that there is an approximate 2-fold increase in the risk of having a small for gestational age infant even in the absence of PE.¹ 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 who had well-controlled BP only was difficult to interpret. We postulated that an impaired placenta, as expected in women with CH, would have less capacity to produce sFLT-1. However, should this be the case, one would expect low sFLT-1 in all four groups. It is likely that, although the risk of PE is not homogenous across all women with CH, alteration in first trimester serum sFLT-1 does not have a significant role in the later development of PE and / or, alternatively, our study was underpowered to detect subtle differences between the four groups. Although there are studies examining first trimester serum sFLT-1 in women with CH, none of them included normotensive controls for comparison.^{9, 17}

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 to those in the lower quartile.¹⁸ 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 to those without.^{19, 20} TNF- α has been shown to induce structural as well as functional alterations in endothelial cells, enhances the production of endothelin and other inflammatory mediators, including IL-6 and VCAM, that ultimately lead to atherosclerotic plaque formation and progression.^{12, 21} In addition, the increases in serum IL-6, TNF- α , endothelin and VCAM have been correlated to the degree of cardiovascular risk in patients with CH and, thus, have emerged as predictors of cardiovascular morbidity.²²

During pregnancy, there is extensive literature to support a 2-3 fold increase in the production of IL-6, TNF- α , endothelin and VCAM in pregnancies already complicated with PE.^{23, 24} It has been proposed that, similar to outside of pregnancy, these inflammatory mediators lead to the development of atherosclerotic-like lesions.²⁵ Within the fetoplacental circulation, these lesions result in placental hypoxia and, within the systemic circulation, result in endothelial dysfunction and the maternal syndrome of PE.²⁶ However, it remains to be clarified as to 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 who are hypertensive have elevated first trimester serum endothelin when compared to normotensive controls. Women in group 4 who are taking antihypertensive medications are likely to have had a longer duration of disease than those in group 1 who are previously undiagnosed. 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.^{23, 27}

Endothelin is also known to increase the expression of other inflammatory cytokines such as IL-6, another biomarker of endothelial disease.¹³ The finding of lower first trimester serum IL-6 for group 4 only when compared to normotensive controls was unexpected. In normal pregnancy, current literature has demonstrated a doubling in the maternal serum concentration of IL-6 when compared to non-pregnant controls due to trophoblastic production in the early first trimester.²⁸ It has been suggested that IL-6 may have a major role in the normal paracrine regulation of placental development and hormone production.²⁸ We propose that in women with CH, particularly those with more severe disease as in group 4, there is less capacity of the impaired placenta to up-regulate its production of IL-6, as with sFLT-1. In contrast, first trimester maternal serum concentration of endothelin is approximately halved when compared to non-pregnant controls and is considered to be a protective mechanism against its vasoconstrictive properties.²⁹ 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, when compared to normotensive controls, there are differences in the first trimester angiogenic and inflammatory profiles according to BP control and use of antihypertensive medications. Further evaluation is needed as to whether these differences are clinically useful in the stratification of care.

Disclosures of interest

The authors report no conflict of interest

Contribution of authorship

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

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 17th February 2017.

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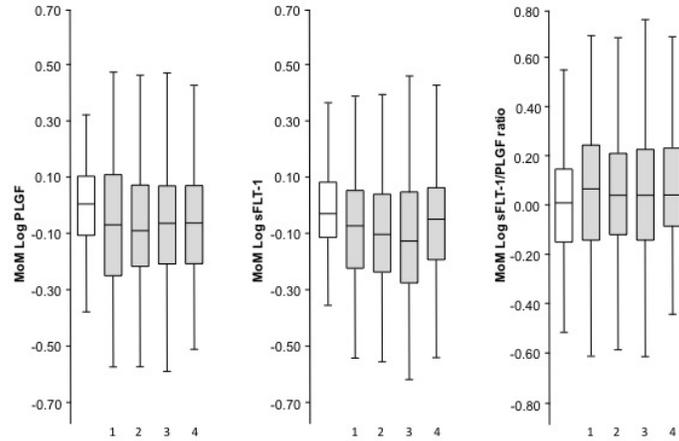


Figure 1: Serum levels of MoM Log PLGF, MoM Log sFLT-1 and MoM Log sFLT-1/PLGF ratio in controls (white) and groups 1-4 (grey) of women with CH.

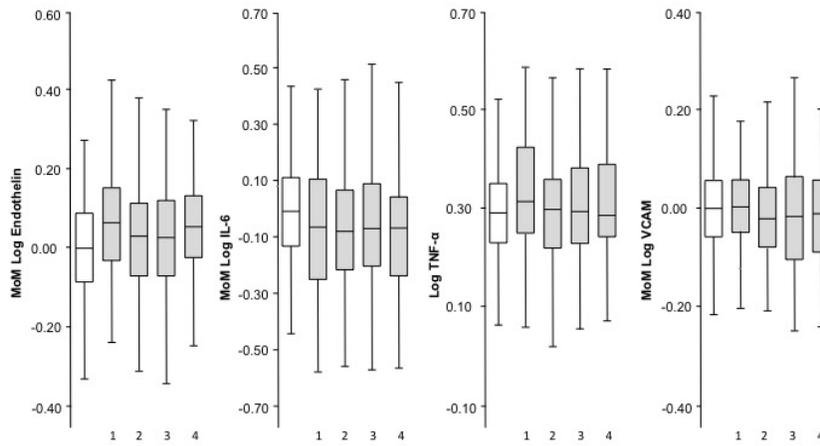


Figure 2: Serum levels of MoM Log endothelin, MoM Log IL-6, Log TNF-α and MoM Log VCAM in controls (white) and groups 1-4 (grey) of women with CH.