HYPERTENSION IN PREGNANCY
2020, VOL. 39, NO. 3, 326–335
https://doi.org/10.1080/10641955.2020.1769643

Taylor & Francis
Taylor & Francis Group

Predisposition to superimposed preeclampsia in women with chronic hypertension: endothelial, renal, cardiac, and placental factors in a prospective longitudinal cohort

Kate Bramham[a*], Pia M. Villa[b*], Jennifer R. Joslin[c], Hannele Laivuori[b,d,e], Esa Hämäläinen[f], Eero Kajantie[g,h], Katri Räikkönen[i], Anukatriina Pesonen[i], Paul Seed [a], R Neil Dalton[j], Charles Turner[j], Max Wong[j], Peter Von Dadelszen[a], James M Roberts[k], Lucilla Poston[a], and Lucy C Chappell[a]

[a]Department of Women and Children's Health, School of Life Course Sciences, King's College London, London, UK; [b]Obstetrics and Gynecology, University of Helsinki and Helsinki University Hospital, Helsinki, Finland; [c]Department of Renal Sciences, School of Immunology and Microbial Sciences, King's College London, London, UK; [d]Medical and Clinical Genetics, University of Helsinki and Helsinki University Hospital, Helsinki, Finland; [e]Institute for Molecular Medicine Finland, University of Helsinki, Helsinki, Finland; [f]Clinical Chemistry, University of Helsinki and Helsinki University Hospital, Helsinki, Finland; [g]Department of Health, National Institute for Health and Welfare, Helsinki, Finland; [h]Children's Hospital, University of Helsinki and Helsinki University Hospital, Helsinki, Finland; [i]Institute of Behavioral Sciences, University Of Helsinki, Helsinki, Finland; [j]WellChild Laboratory, King's College London and King's Health Partners, London, UK; [k]Magee-Womens Research Institute, University of Pittsburgh, Philadelphia, PA, USA

ABSTRACT

Objective: To assess the contribution of maternal and placental factors to the development of superimposed preeclampsia in women with chronic hypertension.

Methods: Endothelial and renal function markers were serially assessed in 90 pregnant women with chronic hypertension and controls.

Results: Syndecan-1 concentrations were lower at 26–27^{+6} weeks in women with chronic hypertension who subsequently developed superimposed preeclampsia compared with those who did not. Decreased PlGF and raised urine albumin:creatinine ratio were also associated with development of superimposed preeclampsia.

Conclusion: Decreased syndecan-1 and PlGF concentrations implicate endothelial glycocalyx disturbance and reduced placental angiogenic capacity, respectively, in the pathophysiology of superimposed preeclampsia.

ARTICLE HISTORY
Received 17 January 2020
Accepted 11 May 2020

KEYWORDS
Chronic hypertension; superimposed preeclampsia; endothelium; kidney; placenta

Introduction

Superimposed preeclampsia occurs in approximately 26% of women with chronic hypertension (CHT) (1), and is associated with adverse outcomes for both mother and fetus. Maternal adverse outcomes include acute renal failure, cardiovascular or respiratory dysfunction, coagulation dysfunction, stroke, eclamptic seizure, or even maternal death. Fetal adverse outcomes include stillbirth, low birth weight, low Apgar score, and neonatal complications due to premature delivery (2). Recognized risk factors for superimposed preeclampsia in women with CHT include severity of preexisting blood pressure, abnormal uterine artery flow velocity waveforms, and angiogenic factor imbalance (3).Endothelial dysfunction and preexisting abnormalities in cardiac and renal function are proposed to contribute to the development of superimposed preeclampsia but there is limited evidence to support this supposition.

The relationship between microalbuminuria and reduced nitric oxide (NO) bioavailability in association with impaired endothelial flow-mediated dilatation in hypertensive non-pregnant individuals is well described. Some studies (4,5) have reported that the albumin:creatinine ratio (ACR) and plasma asymmetric dimethylarginine (ADMA), a specific inhibitor of NO synthase, are elevated prior to the onset of preeclampsia compared to normal pregnancies. However, there are converse results (6,7) and studies focusing exclusively in pregnant women with CHT are few.

The glycocalyx, or endothelial surface layer, plays an important role in the regulation of fluid transfer in the microcirculation and in the mechanotransduction of shear stress-induced NO synthesis. It has also been implicated in hemostasis and in complement and leukocyte activation. Disruption of the glycocalyxoutside

CONTACT Kate Bramham kate.bramham@kcl.ac.uk Department of Women and Children's Health, School of Life Course Sciences, King's College London, 10th Floor North Wing, St Thomas' Hospital, Westminster Bridge Road, London SE1 7EH

*These authors contributed equally to this work.

Supplemental data for this article can be accessed here.

pregnancy is associated with proteinuric disease (8), salt overload (9), and high concentrations of atrial natriuretic peptide (10), all of which are features of CHTand potentially relevant to the development of superimposed preeclampsia. Syndecan-1 (a transmembrane heparin sulfate peptidoglycan) and sialic acid are major constituents of the endothelial glycocalyx which are shed in response to sheddases (e.g., heparanase, matrix metalloproteinase) and neuraminidase enzymatic activity, respectively (11), and plasma syndecan-1 concentrations have recently been reported to be reduced prior to the onset of preeclampsia in uncomplicated pregnancy (12).

Cardiac dysfunction is described in women prior to the onset of preeclampsia (13) but the contribution of preexisting cardiac disease in women with CHTto superimposed preeclampsia is poorly understood.CHT is also associated with abnormalities of renal function and patients with CHToften have undiagnosed chronic kidney disease (CKD) (14) but the contribution of subclinical glomerular and renal tubular injury to superimposed preeclampsia development is unknown.

We hypothesized that preexisting endothelial dysfunction indicated by circulating markers of glycocalyx and subclinical cardiac and renal impairment are contributory to the development of superimposed preeclampsia in women with CHT and therefore the aim of this prospective study of pregnant women with CHT was to use biomarkers to assessthe relative contribution of maternal (endothelium, cardiac, renal) and placental factors.

Materials and methods

Samples were collected in the ´Prediction and Prevention of Preeclampsia'cohort study (PREDO); women with risk factors for developing preeclampsia and healthy controls were prospectively recruited when attending their first ultrasound screening in one of the ten participating hospitals in Finland between September 2005 and December 2009, with maternal blood and urine sample collection at 12^{+0}-13^{+6}, 18^{+0}-19^{+6} and 26^{+0}-27^{+6} weeks' gestation. Overnight urine samples were collected before each visit, and timing and volume of collection recorded. Samples were stored at −80°C. The PREDO Project was approved by the Ethics Committee of Obstetrics and Gynecology Hospital District of Helsinki and Uusimaa and all participants gave written informed consent. The recruitment strategy has been described in detail previously (15).

Standard international definitions were used for clinical inclusion criteria and endpoints (16): *Chronic Hypertension* – blood pressure ≥140/90 mmHg or antihypertension medication before 20^{+0} weeks' gestation without preexisting proteinuria;*Superimposed preeclampsia* – new development of proteinuria >300 mg/24 hours at or after 20^{+0} weeks' gestation. The diagnosis of preeclampsia was adjudicated independently by two physicians and a study nurse who reviewed the maternity and clinical records of each participant.

All women with CHT were included in this study and those healthy controls with greatest sample availability. Samples from the entire cohort (522 samples from 90 women with CHT in the cohort, and 90 healthy controls) were analyzed for plasma markers of placental function (placental growth factor; PlGF), maternal cardiac function (B-type natriuretic peptide; BNP) and renal tubular injury (neutrophil gelatinase-associated lipocalin; NGAL). Additional markers were measured in a nested case–control study using samples that were available at all visits from 12 women who developed superimposed preeclampsia, 24women with CHT who did not develop superimposed preeclampsia matched for BMI and age and 24 randomly selected healthy controls. There were no demographic differences between control women who were included or excluded from the case–control study.

Concentrations of plasma biomarkers which were significantly different between women with and without superimposed preeclampsia were validated in a "time of disease" cohort of women with chronic hypertension and/or chronic kidney disease (CKD) with and without superimposed preeclampsia (at time of diagnosis), women with preeclampsia without preexisting disease and healthy controls (17). Definitions for diagnosis of superimposed preeclampsia are described in online supplementary Figure S1.

Biochemical analysis

All assays were performed without awareness of clinical outcomes.

Endothelial function

(1) Glycocalyx: Syndecan-1 was quantified using a specific ELISA kit according to the manufacturer's protocol: for discovery Cell Sciences, Inc. Canton, Minneapolis, MN, USA; for validation Abnova, Taipei, Taiwan. Plasma-free sialic acid was measured using a chromatographic stable isotope dilution electrospray MSMS method. Additional methodology is given in online supplementary information.

(2) Functional markers: Plasma ADMA was measured using a chromatographic stable isotope dilution fragmentation-specific electrospray mass spectrometry-mass spectrometry (MSMS) method. Urine albumin was measured using

a laser nephelometric immunoassay (Siemens BN ProSpec, www.siemens.com). For the measurement of urinary albumin: creatinine ratio (ARC), urine and plasma creatinine were measured using a chromatographic stable isotope dilution electrospray MSMS method on an AB SCIEX API5000 (www.absciex.com) and reference stable isotope dilution electrospray MSMS, respectively.

Cardiac function

Plasma BNP was tested using the Triage CardioRenal Test (Alere, San Diego CA).

Renal function

Plasma symmetric dimethylarginine (SDMA) was measured simultaneously with plasma ADMA. Plasma cystatin C was analyzed by particle-enhanced laser nephelometric immunoassay (Siemens BN ProSpec, www.siemens.com). Plasma NGAL was assessed using the Triage CardioRenal Test (Alere, San Diego CA). Urine and plasma N-acetyl-β-D-glucosaminidase (NAG) activity were assessed by LC electrospray MS/MS. Urine retinol-binding protein (RBP) was measured using – particle-enhanced laser nephelometric immunoassay (Binding Site, Birmingham, UK), Product Code: LK117. T, on a Siemens BN ProSpec (www.siemens.com).

Placental function

Plasma PlGF was determined using the Triage PlGF Test (Alere, San Diego CA), a fluorescence immunoassay, according to the manufacturer's instructions.

Statistical analysis

Demographic data are presented as medians (interquartile range) or frequencies (percentages). Normality of distribution was explored using a Q–Q plot, and logarithmic transformations used where appropriate. T-tests or Mann–Whitney were used to compare parametric and non-parametric differences between groups, respectively, and Fisher's exact test for contingency tables. To avoid multiple testing of the same hypothesis, data were corrected for inclusion of women who had provided multiple samples, using interval regression analysis with random-effect modeling for individual clustering (18). Interval regression also allows samples measured as below (above) the lower (upper) limit of detection to be treated as being in an appropriate range, rather than replacing them with a single number. Spearman's correlation was used to examine relationships between biomarkers and maternal age and BMI.

For comparison of potential predictive performance of biomarkers, receiver operator curve (ROC) areas were used, with the primary endpoint as development of superimposed preeclampsia. Formal tests were not conducted.

Sample size calculation

A sample size of 12 cases and 24 controls was calculated as adequate to detect one standard deviation difference in means of the concentration of any biomarker between cases and controls to give 81% power at the 5% significance level.

Results

Patient characteristics and clinical outcomes

Cohort study: Demographics and clinical details at study entry and maternal and neonatal outcomes of 90 women with CHT, and 90 healthy controls are shown in Table 1, and in greater detail in Tables S1–4. Women with CHT had a higher BMI, were less likely to be nulliparous than healthy controls and a higher proportion had bilateral notching of uterine artery Dopplers at 12–13 weeks' gestation. Women with CHT were more likely to deliver at an earlier gestation than healthy controls. Women with superimposed preeclampsia were more likely to deliver at an earlier gestation, and to have delivered infants of lower birth weight than women with CHT without superimposed preeclampsia. Women with chronic hypertension did not have evidence of cardiac or renal clinical disease. However, 34.8% were subsequently diagnosed with gestational diabetes vs. 1.1% of healthy controls (treated with diet only) (Table S1).

Nested case–control group: Demographics and outcomes for the nested case–control group are shown in Tables S5 and S6. Significant differences exist between women with CHT who did and did not develop superimposed preeclampsia included maternal birth weight, gestational age at delivery, and neonatal birth weight. Eighteen women with CHT also participated in the PREDO trial, including six women who developed superimposed preeclampsia (2 aspirin; 4 placebo) and 12 who did not (4 aspirin; 8 placebo).

Biomarker data and maternal demographics

Biomarker concentrations and ratios are reported in Table S7. Some markers of renal function were correlated with BMI ((NGAL ($R = 0.320$; $P < 0.001$), SDMA ($R = 0.238$; $P = 0.010$), cystatin ($R = 0.49$); $P < 0.001$))

Table 1. Baseline demographics and pregnancy outcomes in healthy controls and women with CHT, with and without superimposed preeclampsia.

	Healthy Controls n = 90	CHT n = 90	CHT without superimposed preeclampsia n = 76	CHT with superimposed preeclampsia n = 14
Maternal age (years)	30.2 ± 4.2*	32.2 ± 4.7*	32.4 ± 4.9	31.0 ± 4.5
BMI (kg/m^2)	22.5 ± 2.7†	30.0 ± 6.9†	29.9 ± 6.7	30.2 ± 8.0
Multiparity	40 (44.4%)‡	58 (64.4%)‡	48 (63.2%)	10 (71.4%)
Mother's own birth weight (grams)	3500 (3280–3750)§	3255 (3001–3600)§	3260 (3025–3655)	3050 (2890–3340)
Aspirin	0	17 (18.9%)	15 (19,7%)	2 (14.2%)
Early preeclampsia (diagnosis <34 weeks' gestation)	0	4 (4.4%)	0	4 (28.6%)
First antenatal systolic BP (mmHg)	115.7 ± 9.7†	139.8 ± 12.4†	138.3 ± 11.4$^{\square}$	147.7 ± 14.9$^{\square}$
First antenatal diastolic BP (mmHg)	70 ± 7.5†	90.9 ± 10.6†	89.8 ± 9.9	96.4 ± 12.7
Mode of Delivery	n = 89	n = 88	n = 74	n = 14
Spontaneous VD	66 (74.2%)	58 (62.5%)	51 (68.9%)	7 (50.0%)
Elective CS	5 (5.6%)	5 (5.7%)	4 (5.4%)	1 (7.1%)
Emergency CS	9 (10.1%)$^{\square}$	20 (22.7%)$^{\square}$	15 (20.2%)	5 (35.7%)
Instrumental	9 (10.1%)	5 (5.7%)	4 (5.4%)	1 (7.1%)
Gestation at Delivery (weeks)	40.4 (39.3–41.1)*	39.6 (38.3–40.7)*	39.9 (38.6–40.8)$^{\#}$	38.3 (36.7–39.6)$^{\#}$
Preterm delivery <34 weeks' gestation	0$^{\square}$	5 (5.6%)$^{\square}$	1 (1.3%)**	4 (28.6%)**
Preterm delivery <37 weeks' gestation	3 (3.4%)	9 (10.0%)	4 (5.3%)††	5 (35.7%)††
Birth weight (grams)	3435 (3240–3705)	3440 (2973–3757)	3510 (3021–3835)$^{\#}$	2889 (2470–3532)$^{\#}$

CHT: chronic hypertension; BMI: body mass index; BP: blood pressure; SGA: small for gestational age; GDM: gestational diabetes.
*P = 0.001; †P < 0.0001; ‡ P = 0.01; §P = 0.046; $^{\square}$P = 0.03; P = xx; $^{\#}$P = 0.007; **P = 0.002; ††P = 0.004.
Data shown are given as mean ± standard deviation, median (interquartile range) or n (%).

and with maternal age ((NGAL (R = 0.271; P = 0.003), SDMA (R = 0.335; P < 0.001), cystatin (R = 0.321); P < 0.001)). There was no association between any other biomarkers and maternal demographics.

Biomarker data in women with CHT with and without superimposed preeclampsia

The plasma syndecan-1 concentration was lower at 26^{+0}-27^{+6} weeks (p = 0.03) in women who subsequently developed superimposed preeclampsia compared to women with CHT without superimposed preeclampsia, but not at earlier gestations, nor was there any difference over the gestational age range assessed in this marker of the endothelial glycocalyx between women with CHT and healthy controls (Figure 1(a); Table S7). The plasmaPlGF concentration (across gestation) was lower in women with CHT who developed superimposed preeclampsia compared to those who did not (p = 0.002) and was also discriminatory for superimposed preeclampsia at 26^{+0}-27^{+6} weeks (p < 0.0001) (Figure 1(b) and Table S7).

The ACR was elevated (across gestation) in women with CHT who subsequently developed superimposed preeclampsia compared with women with CHT (p = 0.007) or healthy controls (P = 0.002), and discriminated between women with CHT who did and did not develop superimposed preeclampsia at 12^{+0}-13^{+6} weeks' and 26^{+0}-27^{+6} (p = 0.009 and p = 0.006, respectively) (Figure 1(c) and Table S7).

There were no differences in any other marker between women with CHT who did and did not develop superimposed preeclampsia.

Comparison between women with CHT and healthy controls

Plasma NGAL and serum cystatin C concentrations were significantly higher across gestation in women with CHT without superimposed preeclampsia than healthy controls (p < 0.0001 and p = 0.008, respectively) (Table S7). Creatinine clearance was higher across gestation in women with CHT without superimposed preeclampsia than healthy controls (p = 0.03) (Figure 1(d) and Table S7). There were no differences in PlGF concentrations between healthy controls and women with CHT without superimposed preeclampsia, nor any other markers of cardiac, renal, or endothelial function.

Gestational profile

The PlGF concentration increased with gestation in healthy controls and women with CHT with and without superimposed preeclampsia (p < 0.0001), except between 18^{+0}-19^{+6} and 26^{+0}-27^{+6} weeks in women who subsequently developed superimposed preeclampsia (p = 0.70). Similarly, the syndecan-1 concentration also increased with gestation in healthy controls and women with CHT without superimposed preeclampsia (p < 0.01). Plasma sialic acid and NAG concentrations

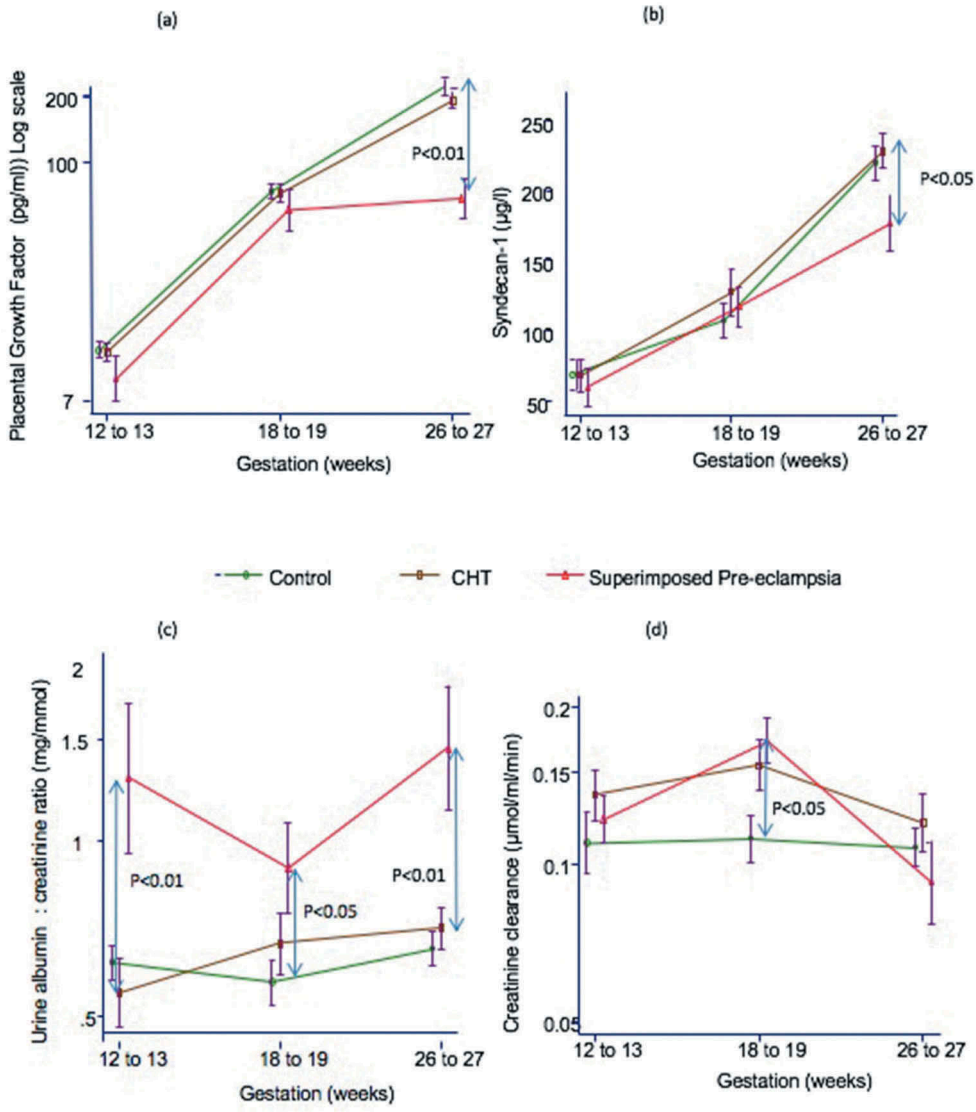

Figure 1. (a–d) Placental growth factor concentrations, overnight urine albumin:creatinine ratio, plasma syndecan-1 concentration, overnight creatinine clearance, and according to gestation in women with chronic hypertension with and without superimposed preeclampsia and healthy controls. Bars represent standard errors. In Figure 1(a–c), comparisons are shown between women with CHT with and without superimposed preeclampsia. Figure 1(d) comparison is shown between women with CHT and healthy controls. Plasma placental growth factor concentrations are shown for prospective cohort study (CHT with superimposed pre-eclampsia N = 14; CHT without superimposed preeclampsia N = 76; healthy controls N = 90). Other biomarkers are shown for nested case–control study (CHT with superimposed preeclampsia N = 12; CHT without superimposed preeclampsia N = 24; healthy controls N = 24).

also increased with gestation ($p < 0.0001$) but there were no differences between healthy controls and those with CHT with and without superimposed pre-eclampsia (Table S7).

There were no changes with gestation in the ACR in healthy controls or women with CHT who developed superimposed preeclampsia. BNP concentrations fell with gestation in women with CHT without superimposed pre-eclampsia ($p = 0.011$) and healthy controls ($p = 0.005$) but did not decrease with gestation in women with subsequent superimposed preeclampsia (Table S7).

There were no changes with gestation in creatinine clearance in women with CHT without superimposed preeclampsia or healthy controls, but women who developed superimposed preeclampsia had a significant increase in creatinine clearance at 18^{+0}-19^{+6} than 12^{+0}-13^{+6} weeks ($P = 0.007$) and then lower values at 26^{+0}-27^{+6} weeks ($P < 0.0001$).Women with CHT with and without superimposed preeclampsia demonstrated a decrease in SDMA clearance at 26^{+0}-27^{+6} compared to18^{+0}-19^{+6} weeks' ($P = 0.045$ and $P = 0.001$, respectively), which was not evidenced in healthy controls (Table S7).

Cystatin C concentration increased with gestation in all groups (P < 0.0001) with no differences between groups.

There were significant increases in the urine NAG: creatinine ratio with gestation in healthy control women (P < 0.0001) and women with CHT without superimposed preeclampsia (P < 0.0001); the change with gestation was less marked in women with CHT who developed superimposed preeclampsia (26^{+0}-27^{+6} v 12^{+0}-13^{+6} P = 0.034) but did not discriminate between groups (Table S7). Similarly, the urinary RBP:creatinine ratio increased with gestation in all groups (P < 0.001) but no differences between subgroups were seen (Table S7).

There were no significant differences between groups or with gestation in plasma ADMA concentrations or SDMA:ADMA ratio (Table S7).

Prediction of superimposed preeclampsia

The highest observed ROC areas for prediction of superimposed preeclampsia were for ACR 12^{+0}-13^{+6} weeks 0.87 (0.73 to 1.0), ACR 26^{+0}-27^{+6}weeks 0.79 (0.57 to 1.00) and PlGF at 26^{+0}-27^{+6} weeks 0.78 (0.55 to 1.00) (Table 2 and Figure 2).

Validation cohort

Demographic, clinical details at study entry and maternal and neonatal outcomes of women with CHT or CKD with (N = 9) and without (N = 9) superimposed preeclampsia, and women with preeclampsia (N = 12) without preexisting disease and healthy controls (N = 9) are shown in Table S8. For women with chronic hypertension with superimposed preeclampsia, samples were taken at 37.1 weeks' gestation (IQR 32.2, 38.3), and without superimposed preeclampsia at 34.0 weeks (28.3, 37.3), at 36.2 weeks (34.2, 38.1) for women with preeclampsia without preexisting disease and at 37.9 weeks (31.2, 39.2) from healthy controls.

PlGFconcentration was lower in women with superimposed preeclampsia than those with CHT and/or CKD (P = 0.003), and in women with preeclampsia without preexisting disease compared with healthy controls (P < 0.001) (Table S8). Syndecan-1 was lower with superimposed preeclampsia than those with CHT and/or CKD (P = 0.001) and after adjustment for gestation remained significantly lower (P = 0.005) (Figure 3). Syndecan-1 was also lower in healthy controls than women with CHT and/or CKD without superimposed preeclampsia (P = 0.045) and after adjustment for gestation (P = 0.045). There were no difference in syndecan-1 concentrations (P = 0.615) between

Table 2. Receiver operator curves values (95% confidence intervals) for prediction of superimposed preeclampsia.

	12^{+0}–13^{+6} weeks' gestation	18^{+0}–19^{+6} weeks' gestation	26^{+0}–27^{+6} weeks' gestation
[Placental Growth Factor]	0.48 (0.22 to 0.74)	0.52 (0.31 to 0.72)	0.78 (0.55 to 1.00)*
Albumin: Creatinine Ratio	0.87 (0.73 to 1.0)*	0.73 (0.56 to 0.90)	0.79 (0.57 to 1.00)*
[Syndecan-1]	0.44 (0.16 to 0.71)	0.40 (0.23 to 0.57)	0.66 (0.45 to 0.87)

[] indicates low concentration of biomarker/ratio correlated to disease.
*Indicates significant result.

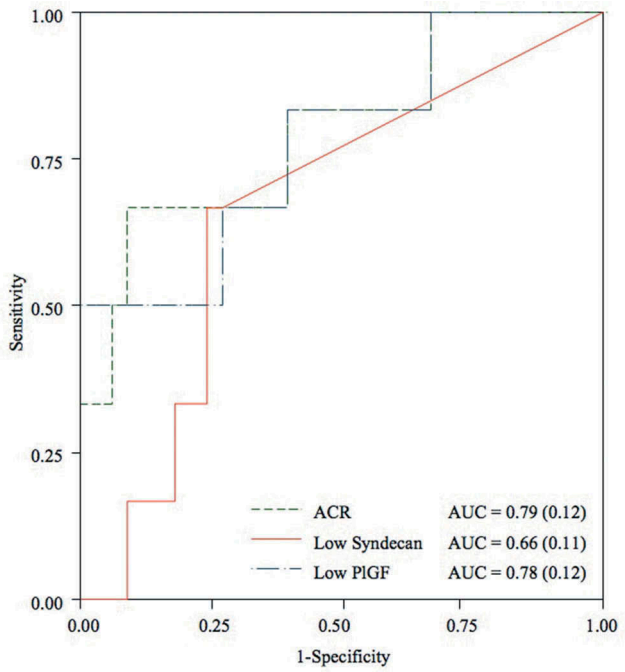

Figure 2. Receiver operator curves (standard errors) for low placental growth factor, albumin:creatinine ratio, and low syndecan-1 at 26^{+0}-27^{+6} weeks' gestation.

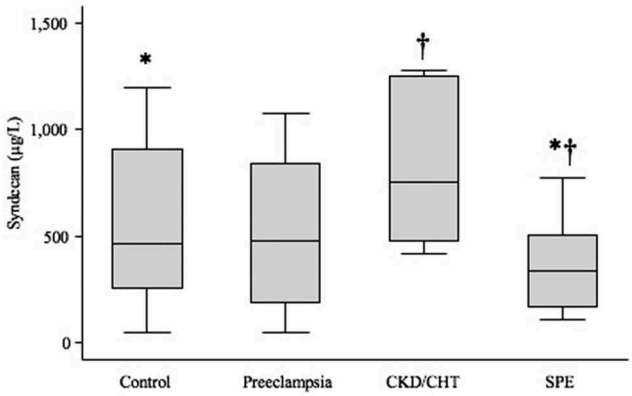

Figure 3. Figure 2: Relative concentrations of plasma syndecan-1 in controls, preeclampsia, women with chronic kidney disease (CKD) or chronic hypertension (CHT), and superimposed preeclampsia (SPE).

women with preeclampsia compared with healthy controls even after adjustment for gestation.

Discussion

In this comprehensive study of markers of maternal endothelial, cardiac, renal, and placental function in women with chronic hypertension, those who developed superimposed preeclampsia were characterized by failure to sustain a normal gestational increase in the plasmasyndecan-1 concentration (a marker of endothelial glycocalyx function), in some cases several weeks before clinical features of disease, in parallel with a reduction of plasma PlGF. Furthermore, women with CHT or CKD had lower plasma syndecan-1 concentrations after diagnosis of superimposed preeclampsia compared to those without. The blunted increase in syndecan-1 may reflect a decline in placental or systemic synthesis of glycocalyx constituents and may occur as a precursor to the onset and contribute to the clinical manifestations of preeclampsia. Overnight urinary ACR was significantly higher at all gestations in women with CHT who developed superimposed preeclampsia compared to those who did not, supporting the implication that systemic endothelial injury may play a role in development of preeclampsia. However neither ADMA nor any of the markers of cardiac, renal glomerular, or tubular injury were significantly different between women with CHT who did and did not develop superimposed preeclampsia, providing no evidence for a discriminatory role.

Superimposed preeclampsia contributes to poor pregnancy outcomes in women with CHT, and some authors propose that preexisting maternal disease may be contributory (19), but few studies have explored underlying mechanisms. Modulators of the glycocalyx, including shear stress, tumor necrosis factor-α, reactive oxygen species, and matrix metalloproteinases increase in pregnancy, particularly in women with preeclampsia compared with healthy controls (20). In healthy controls (and in women with CHT without superimposed preeclampsia), plasma syndecan-1 and sialic acid increased with gestation. The plasma concentration of syndecan-1 at later gestations was comparable to that reported in patients on hemodialysis (21). Syndecan-1 protein expression has previously been demonstrated in the placenta, localized to the syncytiotrophoblast interface (22), and raised plasma concentrations could reflect placental release; however increased endothelial permeability, evident even in healthy pregnancy, is suggestive of systemic glycocalyx changes. A recent study demonstrated greater glycocalyx degradation and impaired sublingual microvascular perfusion in women with early-onset preeclampsia without preexisting CHT compared to normotensive pregnant controls (23).

Total sialic acid has been demonstrated to be elevated in healthy pregnancy (24) but free sialic acid concentrations have not previously been reported. In all groups there was a previously unreported gestational increase in the plasma concentration of N-acetyl-B-D-glucosaminidase, a surrogate biomarker of the degree of lysosomal enzyme release, including neuraminidase, into the circulation: these enzymes are capable of substantial endothelial glycocalyx disruption, including release of free sialic acid. This leads to the novel suggestion that normal pregnancy is associated with an increase in glycocalyx turnover.

The estimation of glycocalyx function in women with CHT has not previously been attempted. In the present study women with CHT who subsequently developed superimposed preeclampsia had reduced syndecan-1 concentrations compared to women with CHT, and also those with normal pregnancy outcomes. Similarly, women with CHT or CKD (preexisting vascular disease) had lower syndecan-1 concentrations after diagnosis of superimposed preeclampsia. Syndecan-1 is a natural competitive inhibitor of neuraminidase; hence, a reduction in concentration in those with superimposed preeclampsia will lead to further destruction of the glycocalyx.

The "insult" of preeclampsia is indolent and the ability to maintain the protective glycocalyx barrier in those with preexisting endothelial injury could be compromised, become "exhausted" and thereby contribute to the endothelial manifestations of preeclampsia. There is also evidence for reduced trophoblast expression of syndecan-1 in women with preeclampsia, which could reflect reduced placental synthesis (22,25).

Some authors have reported no difference in Syndecan-1 concentrations between preeclampsia cases and controls (23). In other hypertensive complications, such as hemolysis, elevated liver enzymes, low platelets (HELLP) syndrome, increased plasma syndecan-1 concentrations have been observed, potentially due to augmented trophoblast or systemic release (26). It is possible that Syndecan-1 could be a marker of disease severity, or could differentiate between different underlying etiologies of the heterogenous syndrome of preeclampsia.

Overnight higher urinary ACR as quantified by immunonephelometry was predictive of superimposed preeclampsia in women with CHT, particularly at the earlier gestations studied. Microalbuminuria is strongly associated with several factors (e.g., obesity) that are prevalent in women with chronic hypertension, and endothelial dysfunction is proposed to be a common

mediator. The association between ACR in early pregnancy and subsequent superimposed preeclampsia provides potential evidence that preexisting endothelial dysfunction in women with CHT may contribute to its development.

ADMA concentrations and SDMA:ADMA ratios were unaffected by the presence of CHT or superimposed preeclampsia, in keeping with other studies comparing women with and without preeclampsia reporting no differences in plasma ADMA concentrations (27) or SDMA: ADMA ratio (6). Hence, inhibition of NOS by ADMA in women with CHT and depletion of NO, as a contributory pathway to superimposed preeclampsia is unlikely.

Women with CHT without superimposed preeclampsia had higher glomerular filtration estimated by creatinine clearance than healthy controls which may reflect impaired autoregulation. Hyperfiltration is a recognized feature of early hypertension, which occurs prior to the development of CKD and is considered to be the consequence of fewer functional nephrons (28). It was interesting to observe that women with CHT had lower maternal birth weights than healthy controls, and may have contributed to the development of CHT in these women.

Cystatin C has been proposed as a useful diagnostic (29) and predictive marker of preeclampsia (30) but was not associated with the development of superimposed preeclampsia in this study. Urine RBP: creatinine and NAG: creatinine ratios increased with gestation in healthy controls, as reported by others (31), but unlike other case–control studies of preeclampsia increased urine NAG (31) and urine RBP (32) excretion were not discriminatory for development of superimposed preeclampsia in the present study.

The observation of low plasma PlGF in women who developed superimposed preeclampsia in the present study is consistent with other (33) but not all (34) studies. PlGF concentrations did not differ between women with CHT without superimposed preeclampsia and healthy controls, also in keeping with other reports (35). PlGF-2 has a uniquely potent heparan binding domain (36) which may contribute to glycocalyx maintenance and could also contribute to a reduction in syndecan-1 concentration. Assessment of temporal changes in these markers would facilitate understanding of interplay between angiogenesis and glycocalyx synthesis.

Others have observed that women born at low birth weights have increased risk of developing both preeclampsia and CHT. In this cohort women with CHT and superimposed preeclampsia had lower maternal birth weights than both healthy controls and women with CHT without superimposed preeclampsia. However, there were no differences between those with CHT without superimposed preeclampsia and healthy controls. These novel findings require further validation in a larger cohort.

A major strength of this study is that it is a well-characterized prospective cohort with novel observations contributing to an understanding of the underlying pathophysiology of superimposed preeclampsia women with a high prevalence of adverse pregnancy outcomes, with validation in a "time of disease" cohort of women with CHT or CKD. A limiting factor was the small sample size which may have prohibited identification of relationships between markers of organ dysfunction; however, the sample size was sufficient to detect one standard deviation difference between group means, although in view of the number of biomarkers studied, a type-1 error is possible. Similarly, the high proportion of women with early-onset or severe disease may limit the generalizability of these findings. Lack of data regarding other factors that may influence glycocalyx integrity which has been found by others is a further limitation. The absence of *in vivo* assessment of function using cardiac indices, endothelial function, and formal measurement of glomerular filtration prevents absolute confirmation of the relationship between preexisting disease and the development of superimposed preeclampsia. It would also be interesting in future studies to include exploration of endothelial changes after delivery too.

Our findings support the hypothesis that preexisting endothelial dysfunction, assessed by overnight ACR, in women with CHT contributes to the development of superimposed preeclampsia. Decreased PlGF concentrations and reduced shedding of syndecan-1 also predate disease development, but no other markers of cardiac or renal dysfunction were associated with future superimposed preeclampsia in this study. Women with chronic hypertension had glomerular hyperfiltration compared with healthy controls, which potentially reflects impaired autoregulation. Glycocalyx shedding with gestation in healthy controls, and its potential effect on clinical manifestations in both normal and hypertensive pregnancy is intriguing and warrants further study. Importantly, if glycocalyx depletion is contributory to the development of superimposed preeclampsia, restoration of glycocalyx protection may provide an opportunity for therapeutic intervention, e.g., sulodexide.

Acknowledgments

We acknowledge the support of the Global Pregnancy Collaboration. The Global Pregnancy Collaboration is part of the Pre-eclampsia-Eclampsia Monitoring, Prevention & Treatment (PRE-EMPT) initiative funded by the University of

British Columbia, a grantee of the Bill & Melinda Gates Foundation.

Disclosure statement

The authors report no conflict of interest.

ORCID

Paul Seed http://orcid.org/0000-0001-7904-7933

References

[1] Bramham K, Parnell B, Nelson-Piercy C, et al. Chronic hypertension and pregnancy outcomes. Obstet Anesth Dig. 2015;35(1):6–7.
[2] Abalos E, Cuesta C, Carroli G, et al. Pre-eclampsia, eclampsia and adverse maternal and perinatal outcomes: a secondary analysis of the world health organization multicountry survey on maternal and newborn health. BJOG. 2014;121(Suppl):14–24.
[3] Ankumah NAE, Sibai BM. Chronic hypertension in pregnancy: diagnosis, management, and outcomes. Clin Obstet Gynecol. 2017;60(1):206–214.
[4] Bar J, Hod M, Erman A, et al. Microalbuminuria in early pregnancy in normal and high-risk patients. Early Pregnancy. 1996;2(3):197–200.
[5] Savvidou MD, Hingorani AD, Tsikas D, et al. Endothelial dysfunction and raised plasma concentrations of asymmetric dimethylarginine in pregnant women who subsequently develop pre-eclampsia. Lancet. 2003;361(9368):1511–1517.
[6] Poon LCY, Kametas N, Bonino S, et al. Urine albumin concentration and albumin-to-creatinine ratio at 11 +0 to 13+6 weeks in the prediction of pre-eclampsia. BJOG An Int J Obstet Gynaecol. 2008;115(7):866–873.
[7] Ehsanipoor RM, Fortson W, Fitzmaurice LE, et al. Nitric oxide and carbon monoxide production and metabolism in preeclampsia. Reprod Sci. 2013;20(5):542–548.
[8] Chen JW, Gall MA, Yokoyama H, et al. Raised serum sialic acid concentration in NIDDM patients with and without diabetic nephropathy. Diabetes Care. 1996;19(2):130–134.
[9] Oberleithner H, Peters W, Kusche-Vihrog K, et al. Salt overload damages the glycocalyx sodium barrier of vascular endothelium. Pflugers Arch Eur J Physiol. 2011;462(4):519–528.
[10] Bruegger D, Jacob M, Rehm M, et al. Atrial natriuretic peptide induces shedding of endothelial glycocalyx in coronary vascular bed of guinea pig hearts. Am J Physiol - Hear Circ Physiol. 2005;289(5):58.
[11] Tarbell JM, Cancel LM. The glycocalyx and its significance in human medicine. J Intern Med. 2016;280:97–113.
[12] Gandley RE, Althouse A, Jeyabalan A, et al. Low soluble syndecan-1 precedes preeclampsia. PLoS One. 2016;11(6):1–18. .
[13] Melchiorre K, Sutherland G, Sharma R, et al. Mid-gestational maternal cardiovascular profile in preterm and term pre-eclampsia: A prospective study. BJOG An Int J Obstet Gynaecol. 2013;120(4):496–504.
[14] Crews DC, Plantinga LC, Miller ER, et al. Prevalence of chronic kidney disease in persons with undiagnosed or prehypertension in the United States. Hypertension. 2010;55(5):1102–1109.
[15] Villa PM, Kajantie E, Räikkönen K, et al. Aspirin in the prevention of pre-eclampsia in high-risk women: A randomised placebo-controlled PREDO Trial and a meta-analysis of randomised trials. BJOG An Int J Obstet Gynaecol. 2013;120(1):64–74.
[16] Tranquilli AL, Dekker G, Magee L, et al. The classification, diagnosis and management of the hypertensive disorders of pregnancy: A revised statement from the ISSHP. Pregnancy Hypertens. 2014;4:97–104.
[17] Bramham K, Seed PT, Lightstone L, et al. Diagnostic and predictive biomarkers for pre-eclampsia in patients with established hypertension and chronic kidney disease. Kidney Int. 2016;89(4):874–885.
[18] Skrondal A, Rabe-Hesketh S. Generalized latent variable modeling: multilevel, longitudinal, and structural equation models. Generalized Latent Variable Modeling: multilevel, Longitudinal, and Structural Equation Models. Chapman and Hall, London. 2004. p. 1–508.
[19] Redman CWG, Sargent IL. Pre-eclampsia, the placenta and the maternal systemic inflammatory response - A review. Placenta. 2003;24(SUPPL.A):S21-S27.
[20] Chaiworapongsa T, Chaemsaithong P, Yeo L, et al. Pre-eclampsia part 1: current understanding of its pathophysiology. Nat Rev Nephrol. 2014;10:466–480.
[21] Padberg JS, Wiesinger A, Di Marco GS, et al. Damage of the endothelial glycocalyx in chronic kidney disease. Atherosclerosis. 2014;234(2):335–343.
[22] Jokimaa V, Inki P, Kujari H, et al. Expression of syndecan-1 in human placenta and decidua. Placenta. 1998;19(2–3):157–163.
[23] Weissgerber TL, Garcia-Valencia O, Milic NM, et al. Early onset preeclampsia is associated with glycocalyx degradation and reduced microvascular perfusion. J Am Heart Assoc. 2019;8(4). DOI:10.1161/JAHA.118.010647.
[24] Von Versen-Hoeynck FM, Hubel CA, Gallaher MJ, et al. Plasma levels of inflammatory markers neopterin, sialic acid, and C-reactive protein in pregnancy and preeclampsia. Am J Hypertens. 2009;22(6):687–692.
[25] Heyer-Chauhan N, Ovbude IJ, Hills AA, et al. Placental syndecan-1 and sulphated glycosaminoglycans are decreased in preeclampsia. J Perinat Med. 2014;42(3):329–338.
[26] Hofmann-Kiefer KF, Knabl J, Martinoff N, et al. Increased serum concentrations of circulating glycocalyx components in HELLP syndrome compared to healthy pregnancy: an observational study. Reprod Sci. 2013;20(3):318–325.
[27] Maas R, Böger RH, Schwedhelm E, et al. Plasma Concentrations of Asymmetric Dimethylarginine (ADMA) in Colombian women with pre-eclampsia [4]. J Am Med Assoc. 2004;291:823–824.
[28] Helal I, Fick-Brosnahan GM, Reed-Gitomer B, et al. Glomerular hyperfiltration: definitions, mechanisms and clinical implications. Nat Rev Nephrol. 2012;8:293–300.
[29] Strevens H, Wide-Swensson D, Torffvit O, et al. Serum cystatin C for assessment of glomerular filtration rate in pregnant and non-pregnant women. Indications of

altered filtration process in pregnancy. Scand J Clin Lab Invest. 2002;62(2):141–147. .

[30] Thilaganathan B, Wormald B, Zanardini C, et al. Early-pregnancy multiple serum markers and second-trimester uterine artery doppler in predicting preeclampsia. Obstet Gynecol. 2010;115(6):1233–1238.

[31] Hayashi M, Ueda Y, Hoshimoto K, et al. Changes in urinary excretion of six biochemical parameters in normotensive pregnancy and preeclampsia. Am J Kidney Dis. 2002;39(2):392–400.

[32] Xiao J, Niu J, Ye X, et al. Combined biomarkers evaluation for diagnosing kidney injury in preeclampsia. Hypertens Pregnancy. 2013;32(4):439–449.

[33] Perni U, Sison C, Sharma V, et al. Angiogenic factors in superimposed preeclampsia: A longitudinal study of women with chronic hypertension during pregnancy. Hypertension. 2012;59(3):740–746.

[34] Powers RW, Jeyabalan A, Clifton RG, et al. Soluble fms-Like Tyrosine Kinase 1 (sFlt1), endoglin and placental growth factor (PlGF) in preeclampsia among high risk pregnancies. PLoS One. 2010;5:10.

[35] Sunderji S, Gaziano E, Wothe D, et al. Automated assays for sVEGF R1 and PlGF as an aid in the diagnosis of preterm preeclampsia: a prospective clinical study. Am J Obstet Gynecol. 2010;202(1):40.e1-40.e7.

[36] Martino MM, Briquez PS, Güç E, et al. Growth factors engineered for super-affinity to the extracellular matrix enhance tissue healing. Science. 2014;343(6173):885–888.