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Urine congophilia associated with preeclampsia does not persist 6-months postpartum

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ABSTRACT

Introduction: Preeclampsia is a common hypertensive disorder of pregnancy. Several studies have demonstrated that protein aggregates, detected through urine congophilia, is associated with preeclampsia; however, it has yet to be investigated whether urine congophilia remains postpartum in these women. In this study, we aimed to augment prior studies and determine whether urine congophilia is present postpartum.

Methods: Women were recruited from Lyell McEwin Hospital, South Australia. Urine samples were collected during pregnancy and 6-months postpartum from women with non-preeclampsia pregnancies (n = 48) and women with pregnancies complicated by preeclampsia (n = 42). A Congo Red Dot blot test, total protein and creatinine levels from urine, as well as serum Soluble fms-like tyrosine kinase 1 to placental growth factor ratio (sFlt-1:PlGF), were assessed and correlated.

Results: Preeclamptic women exhibited increased urine congophilia ($P < 0.01$), sFlt-1:PlGF ratio ($P < 0.0001$) and total protein ($P < 0.01$) during pregnancy; with a positive correlation between urine congophilia and total protein across the entire cohort ($P < 0.0001$). Although urine congophilia was no longer detected 6-months postpartum in preeclamptic women, total protein remained elevated ($P < 0.05$). sFlt-1:PlGF ratio during pregnancy was positively correlated with congophilia across the cohort ($P = 0.0007$). Serum creatinine was also higher in preeclamptic women during pregnancy ($P < 0.001$).

Discussion: These results support that urine congophilia is significantly elevated in pregnancies complicated with preeclampsia and show that it does not continue postpartum, although larger cohort studies are needed to determine its feasibility as a diagnostic marker.

1. Introduction

Preeclampsia is a common hypertensive disorder of pregnancy, affecting approximately 3.3 % of pregnancies in Australia and 4.6 % (95 % CI 2.7–8.2) worldwide, with incidence varying greatly depending on a number of factors including age and ethnicity [1,2]. If untreated, preeclampsia can progress to eclampsia, a leading cause of maternal mortality worldwide [2,3]. Currently, early pregnancy prediction of preeclampsia and treatment of preeclampsia remains rudimentary. The ratio of serum soluble fms-like tyrosine kinase 1 to placental growth

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factor ratio (sFlt-1:PlGF) is currently one of the most predictive biomarkers available, with an elevated ratio predictive of clinical symptom presentation up to four weeks prior to disease onset [4]. Management of preeclampsia involves low-dose aspirin prophylaxis in women at risk and close monitoring, with prompt delivery of the placenta being the only finite treatment [5]. With diagnosis using sFlt:PlGF ratio measures not currently adopted worldwide, most easily accessible and non-invasive diagnostic biomarkers are only found concurrently with clinically diagnosable disease, indicating the need for early detection prior to atypical pregnancy progression and clinical manifestation.

Recently, protein misfolding and aggregation has been identified as a contributor to the pathogenesis of preeclampsia and may be a possible early biomarker for early-onset preeclampsia [6]. Increases in sFLT-1:PlGF ratios have been associated with disorders relating to abnormalities in angiogenesis. Moreover, changes in sFLT, a marker of endoplasmic reticulum stress, are not limited to preeclampsia but are also indicative of other disorders of placental insufficiency [4].

During placental development and decidual invasion, an accumulation of genetic and environmental factors that alter proteome homeostasis (proteostasis) leads to protein misfolding within the placenta. Analysis of placental transcriptional phenotypes has identified that proteins prone to clustering and aggregation are increased in expression in the placenta of multiple forms of preeclampsia [7,8]. This has led to a proposal that protein misfolding and aggregation is associated with the pathogenesis of preeclampsia, with aggregates of high-ordered fibrous proteins – known as amyloids – a potential biomarker [9]. Amyloidogenic diseases are associated with formation of non-covalent, highly organised, cross-β protein polymers known as amyloid fibrils that are highly resistant to proteolytic agents [10]. There is now evidence that aggregated forms of proteins are present in the placenta, urine and serum of preeclamptic women [9].

Congo red dot (CRD) staining is a simple method that quantifies proteins enriched in β-sheets, which demonstrates the ability to discern women with preeclampsia – from healthy controls – due to the presence of protein aggregates in urine. This proteinopathy manifestation, known as urine congophilia, has the potential to develop up to 10-weeks before clinical manifestation of preeclampsia, when pregnant women remain asymptomatic [9], with a recent prospective study finding positive urine congophilia developing 5-weeks prior to clinical presentation [11].

Thus, use of amyloidophilic CRD staining to identify global protein misfolding load in pregnancy – specifically amyloid aggregates based on urine congophilia – may provide a means for early prediction and diagnosis of preeclampsia [9,12]. Albeit, little is known about the postpartum prevalence and accumulation of protein aggregates, and their subsequent impact on kidney function, which may be pertinent to the development of postpartum kidney disease [13,14].

The assessment of urine congophilia with CRD staining may provide a simple, rapid, cost-effective, and non-invasive strategy to diagnose and screen for preeclampsia. However, no studies to date have investigated the postpartum levels of aggregated protein within the urine of previously preeclamptic women or correlated this to renal function and urine creatinine levels. This project aimed to determine whether protein aggregates are present in the urine of preeclamptic women and whether urine congophilia persists in urine postpartum.

2. Methods

To investigate if preeclampsia is associated with increased protein aggregates in urine, samples were collected as part of a larger study from Lyell McEwin Hospital, Adelaide, South Australia. All work was approved by both University of Adelaide and the University of Wollongong Human Research Ethics Committees (HREC/13/TQEHLMH/263). The study cohort consisted of non-preeclampsia pregnancies ($n = 48$) and pregnancies complicated by preeclampsia ($n = 42$). Here, preeclampsia is defined as the new onset of hypertension and proteinuria, or the new onset of hypertension plus significant end-organ dysfunction with or without proteinuria in a previously normotensive patient, with all diagnoses of preeclampsia in the current study made by clinician. Non-preeclampsia pregnancies refers to women without a formal diagnosis of preeclampsia by a clinician, and does not rule out other ailments of pregnancy, or other disease. Urine samples were collected during pregnancy (average gestation 35.26 ± 2.29 weeks) and 6-months postpartum. Of the women with a diagnosis of preeclampsia, urine samples were taken after diagnosis. Urine samples were collected, aliquoted, and subsequently stored at −80 °C for later analyses. To measure the effectiveness of amyloid markers to distinguish preeclampsia from other pregnancy complications that may also result in preterm birth, pregnancies were matched for maternal age and gestation. Samples were unable to be collected from all individuals pre- and postpartum.

To determine the amount of protein in samples, a volume (20 μL) of urine was mixed with an equal volume of HCl-acidified milliQ water (pH 2.0), vortexed for 3 min and placed in a water-bath sonicator to solubilise any protein aggregates that may have been present. Samples were subsequently centrifuged at 20 000 g for 10 min and the supernatant was collected. Samples were further diluted 10-fold by addition of phosphate-buffered saline (PBS) and the final total protein concentration measured using a bicinchoninic acid (BCA) assay kit (Pierce BCA Protein Assay Kit, ThermoScientific, Rockford, IL, USA; catalogue no. 23 227) as per the manufacturer's instructions for the plate-based assay.

A CRD test was performed as previously described by Buhimschi et al. [9]. Briefly, an aliquot (40 μL) of urine was diluted 10-fold by addition of PBS and then mixed with 1 μL Congo Red solution (5 mg/mL Congo Red powder dissolved in milliQ water; Sigma-C6767). All samples were loaded in duplicate into a 96-well plate, with blank samples containing 40 μL of PBS alone. Samples were incubated on an orbital plate shaker for 1 h at room temperature, and then 5 μL of each sample pipetted onto a nitrocellulose membrane. After air drying samples for 15 min, the membrane was rinsed in distilled water for approximately 3 min and washed in increasing concentrations of methanol/ethanol (50 % methanol: 3 min; 70 % ethanol: 1 min; 90 % ethanol: ~10 min). Finally, membranes were imaged using an Amersham gel imager and quantified utilising Quantity1 (BioRad) at an adjusted volume/mm^2 [(density/area)-local background].

Total urine creatinine was determined utilising a DRI Creatinine-Detect kit (ThermoScientific, Rockford, IL, USA; catalogue no. EIA-CUN) as per the manufacturer's instructions. Serum creatinine, carboxyhaemoglobin (COHb), estimated glomerular filtration rate and plasma sFlt-1 and PlGF levels were measured at Lyell McEwin Hospital pathology, South Australia. The sFlt-1/PIGF ratio was calculated from these values.

2.1. Data analysis

Maternal, as well as neonatal, clinical outcomes were compared utilising *t* tests (two-tailed, independent). To account for missing data, a two-way analysis of variance (ANOVA) mixed-effects model was used to compare the effects of preeclampsia (Preeclampsia; P_{PE}), during pregnancy or postpartum (Time; P_{Time}) and the interaction (Interaction; P_{Int}) between the two variables. Where a major effect of preeclampsia, time or interaction between preeclampsia and gestation was detected, a Fisher's LSD multiple comparisons *post hoc* test was performed. Correlation analysis was performed using Pearson's rank order correlation. ROC curve analysis was performed on CRD and sFLT-1:PlGF ratio data to determine test specificity and sensitivity. Furthermore, proportions were compared with Fisher's exact tests or Chi-square tests as required. All data were analysed with GraphPad Prism (Software 9.2.1) and are presented as mean ± standard deviation (SD). $P < 0.05$ was considered statistically significant.

3. Results

Urine samples from 90 women were included for investigations and

analyses. There was no significant difference between age or average gestational length between the non-preeclamptic and preeclamptic pregnancy groups (Table 1).

Diastolic and systolic blood pressure, as well as mean arterial pressure, was significantly higher both during pregnancy ($P < 0.0001$) and 6-months postpartum ($P < 0.01$), in women diagnosed with pre-eclampsia. As expected, sFlt-1:PlGF ratio was 10-fold higher in pre-eclamptic pregnancies than non-preeclamptic ($P < 0.0001$). Serum creatinine levels were also higher ($P < 0.001$), although this difference was not apparent postpartum. Preeclampsia had no effect on carboxy haemoglobin levels or estimated glomerular filtration rate within the cohort. Analysis of neonatal outcomes secondary to these pregnancies, showed a significant reduction in birth weight and birth weight centile with subsequent increased rates of intrauterine growth restriction (IUGR) in the preeclamptic pregnancy group (Table 2).

As preeclampsia is partially characterised by proteinuria, evaluation of protein aggregates in urine – evident by CRD detection – and total protein were determined (Fig. 1). Two-way ANOVA of CRD intensity revealed a significant main effect of time (pregnancy vs postpartum) ($P_{Time} = 0.0025$), disease ($P_{PE} = 0.0083$) and interaction between time and disease ($P_{Int} = 0.0243$). Post-hoc analysis showed CRD intensity was 3-fold higher during a preeclamptic pregnancy compared to postpartum levels. Furthermore, average CRD intensity was over 2.7-fold higher in preeclamptic pregnancies compared to non-preeclamptic (Fig. 1A). Total protein levels in urine also showed a significant effect of disease ($P_{PE} = 0.0019$) and time ($P_{Time} = 0.0271$). Urinary total protein levels were 1.3-fold higher in preeclamptic women compared to non-preeclamptic, with levels remaining higher in the preeclamptic group postpartum, at a level similar to that seen during pregnancy (Fig. 1B). There was no effect of time or disease state on urine creatine levels (Fig. 1C).

Correlation analysis was performed to determine the relationship between total protein levels and CRD detection within urine (Fig. 2). It was evident that within the entire cohort, CRD is positively correlated with total protein concentration within urine ($P < 0.0001$, Fig. 2A). This correlation was consistent when analysed within each sub-group; no-preeclampsia pregnant ($P < 0.0001$, Fig. 2B), no-preeclampsia postpartum ($P = 0.0122$, Fig. 2D) and preeclampsia postpartum ($P = 0.0011$, Fig. 2E); however, there was no significant correlation between urine total protein and CRD in preeclampsia during pregnancy (Fig. 2C). Similarly, correlation analysis was performed between total protein levels and creatinine within urine to delineate the relationship between the two in this study (Fig. 3). Creatinine was positively correlated in the entire cohort ($P < 0.0001$). This result was consistent between each sub-group analysed in the cohort.

As sFlt-1:PlGF ratio is the current most sensitive serum biomarker for preeclampsia the relationship between this and urinary CRD, total protein and creatinine levels was also examined, during pregnancy only. The sFlt-1:PlGF ratio was positively correlated with each of these urinary protein measures when examined across the entire cohort (Fig. 4). Within sub-group analysis, only urinary creatinine was positively correlated with sFlt-1:PlGF ratio in non-preeclamptic pregnancies ($P = 0.0198$; data not shown). The sensitivity and specificity of urinary congophilia in the current study was compared to the serum measure sFlt-1:PlGF ratio (Fig. 5). CRD had a 70 % accuracy in diagnosis, but was less sensitive and specific than sFlt-1:PlGF ratio (CRD: AUC = 0.7002 [95 % CI 0.5823 to 0.8181], $P = 0.0017$; sFlt-1:PlGF ratio: AUC = 0.9763 [0.9504 to 1.0, $P < 0.0001$]). At 80 % specificity, CRD had a sensitivity of 57.89 % (42.19–72.15) compared to 100 % (91.24–100) for sFlt-1:PlGF ratio. When examined for 90 % specificity, CRD sensitivity was further decreased at only 34.21 % (21.21–50.11), compared to 95.0 % (83.5–99.11) for the sFlt-1:PlGF ratio.

Chi-square analysis on the rates of pregnancy complications within both the non-preeclamptic and preeclampsia groups was also performed (Table 3). Overall, there was a significant increase in the rates of babies born with intra-uterine growth restriction, low birth weight or small for gestational age (IUGR/LBW/SGA), preterm birth and gestational diabetes mellitus (GDM) within the preeclamptic group.

Table 1
Maternal demographic data between non-preeclamptic and preeclamptic groups.

Demographic Data		Non-preeclamptic Pregnancy $n = 48$	Preeclamptic Pregnancy $n = 42$	P
Age		29.2 ± 5.1	30 ± 5	NS
Gestation (weeks)		35.2 ± 2.1	35 ± 3	NS
Parity, *n* (%)	Primip	31 ± 65	24 ± 57	NS
	1	15 ± 31	9 ± 21	
	2	1 ± 2	4 ± 10	
	≥3	1 ± 2	5 ± 12	
During Pregnancy BP (mmHg)	DBP	67.1 ± 6	84.9 ± 8	**<0.0001**
	SBP	115 ± 9	138.1 ± 12	**<0.0001**
	MAP	83.1 ± 6	102.7 ± 9	**<0.0001**
Postpartum BP (mmHg)	DBP	67.3 ± 5	74.3 ± 11	**<0.01**
	SBP	114 ± 8	124.1 ± 10	**<0.001**
	MAP	82.9 ± 6	90.8 ± 10	**<0.001**
COHb (%)	Pregnant	2.4 (1.1)	2.4 (1.1)	NS
	Postpartum	2.4 (1.1)	3.1 (1.8)	NS
SrCreat (μmol/L)	Pregnant	44.5 ± 8.5	52.6 ± 13.6	**<0.001**
	Postpartum	58.1 ± 9.1	61.5 ± 10.8	NS
sFLT-1:PlGF ratio (pg/ml)	Pregnant	9.37 ± 8.90	95.07 ± 79.95	**<0.0001**
eGFR (mL/min/1.73m)	Pregnant	>90	>90	NS
	Postpartum	>90	>90	NS

Data presented as mean ± SD and analysed by unpaired *t*-test. Bold numbering indicates significance ($P < 0.05$). SD, standard deviation; NS, nonsignificant; DBP, diastolic blood pressure; SBP, systolic blood pressure; MAP, mean arterial pressure; COHb, carboxyhaemoglobin; SrCreat, serum creatinine; sFLT-1:PlGF ratio, soluble fms-like tyrosine 1 to placental growth factor ratio; eGFR, estimated glomerular filtration rate.

Table 2
Neonatal outcomes data between non-preeclamptic and preeclamptic groups.

Neonatal outcomes	Non-preeclamptic Pregnancy $n = 48$		Preeclamptic Pregnancy $n = 42$		P
	Male	Female	Male	Female	
***n* (%)**	24 (50)	21 (41.8)	19 (45.2)	21 (50)	
Birth weight median (SD)	3390 (521.4)	3347.5 (410.2)	2820 (690.6)	2784 (622.2)	**<0.001**
Minimum	2600	2400	1600	1680	
Maximum	4332	4006	4560	4400	
Birth weight centile (SD)	53.8 ± 32.4	46 ± 22.9	32.4 ± 31.1	29.5 ± 31.5	**<0.01**
IUGR < 5th n, (%)	4 (16.6)	2 (9.5)	4 (21.1)	9 (42.9)	**<0.05**
Fetal APGAR (1 min)	8.1 ± 0.8	8.8 ± 0.6	7.6 ± 1.7	8.1 ± 1.2	NS
Fetal APGAR (5 min)	8.9 ± 0.5	9.1 ± 0.2	8.8 ± 0.7	8.8 ± 0.8	NS

Data presented as mean ± SD. Data analysed by unpaired *t*-test. Bold numbering indicates significance ($P < 0.05$). SD, standard deviation; NS, nonsignificant; IUGR/LBW/SGA, intra-uterine growth restriction/low birth weight/small for gestational age.

4. Discussion

In this study we have retrospectively investigated urine protein aggregates levels with CRD staining, as well as urine total protein and creatinine levels, in pregnant women with preeclampsia and postpartum. We observed that preeclamptic women had increased urinary congophilia, total protein and creatinine during pregnancy. As first described by Buhimschi et al. [9], these findings are also consistent with several other studies, confirming that CRD staining is an effective tool

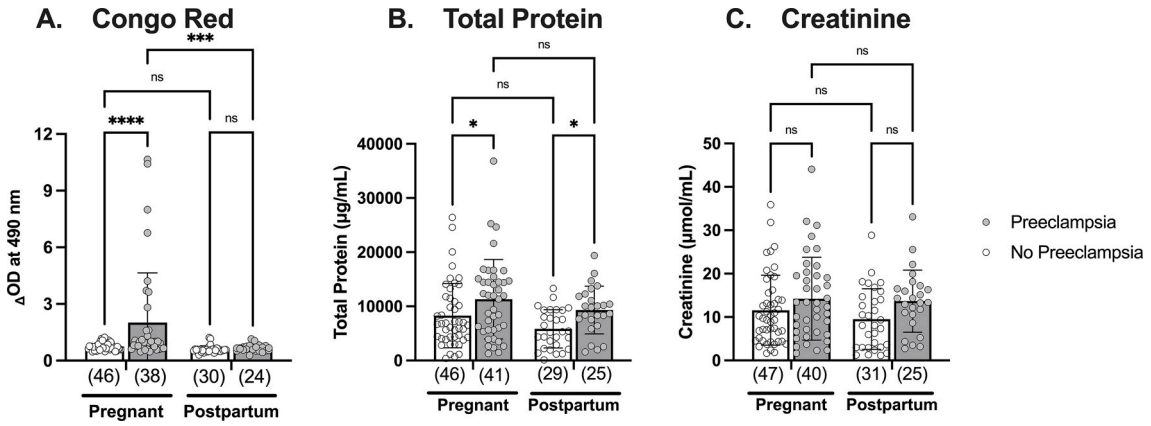

Fig. 1. Urine levels of (A) Congo Red detection, (B) Total Protein (C) Creatinine of no-preeclampsia (open bars) and preeclampsia (grey bars) women both during pregnancy and postpartum. Data are mean ± SD. Urine levels were analysed by two-way ANOVA with preeclampsia and time stage (pregnancy vs postpartum) as major factors. A Fisher's post hoc test was performed for multiple comparisons. $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$, $^{****}P < 0.0001$. Across the series n ranges from 24 to 47 and is presented in brackets under each column.

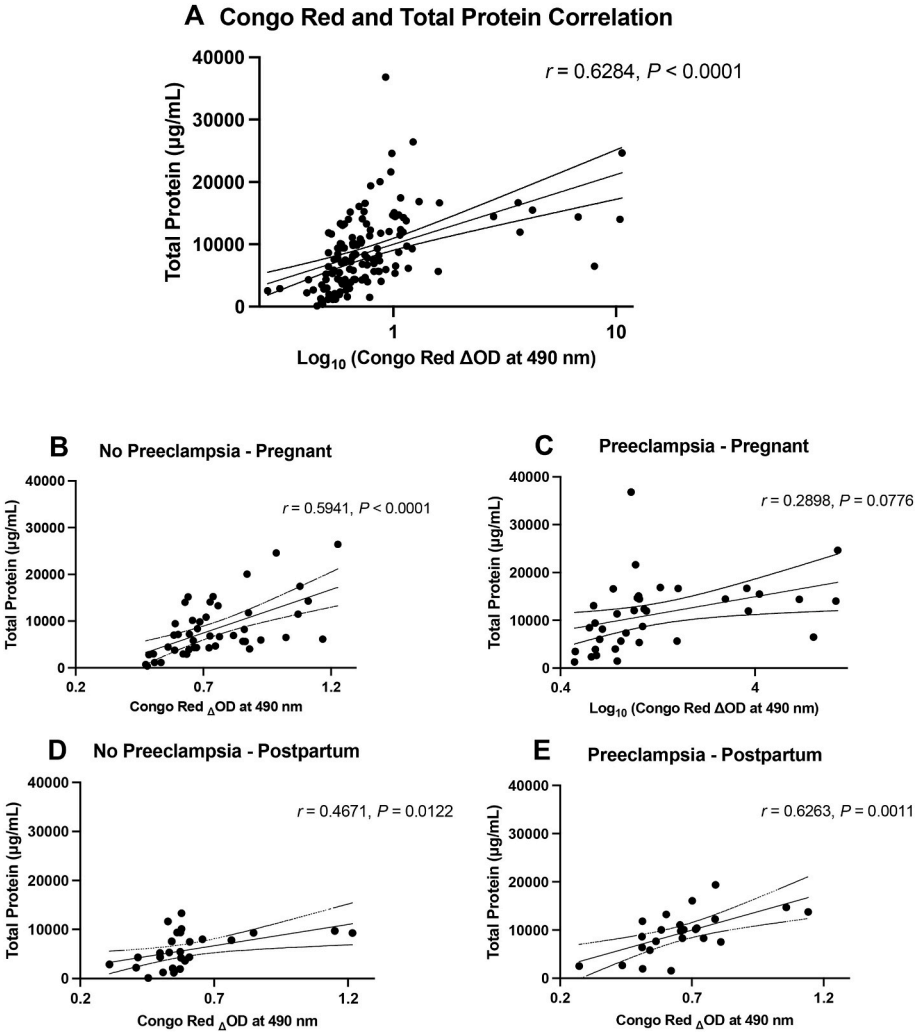

Fig. 2. Correlation of urine Congo Red detection with urine total protein within (A) the total cohort, (B) no-preeclampsia pregnancy, (C) preeclampsia pregnancy, (D) no-preeclampsia pregnancy postpartum and (E) preeclampsia pregnancy postpartum. Correlation between Congo Red staining and total protein were analysed by correlation analysis using Pearson's test. $P < 0.05$ was considered statistically significant. Note that in panels A and C the x-axis is a $\log_{10}$ scale. Data were fit to a linear regression model. Dotted lines in plots show 95 % confidence intervals for the fit to the data.

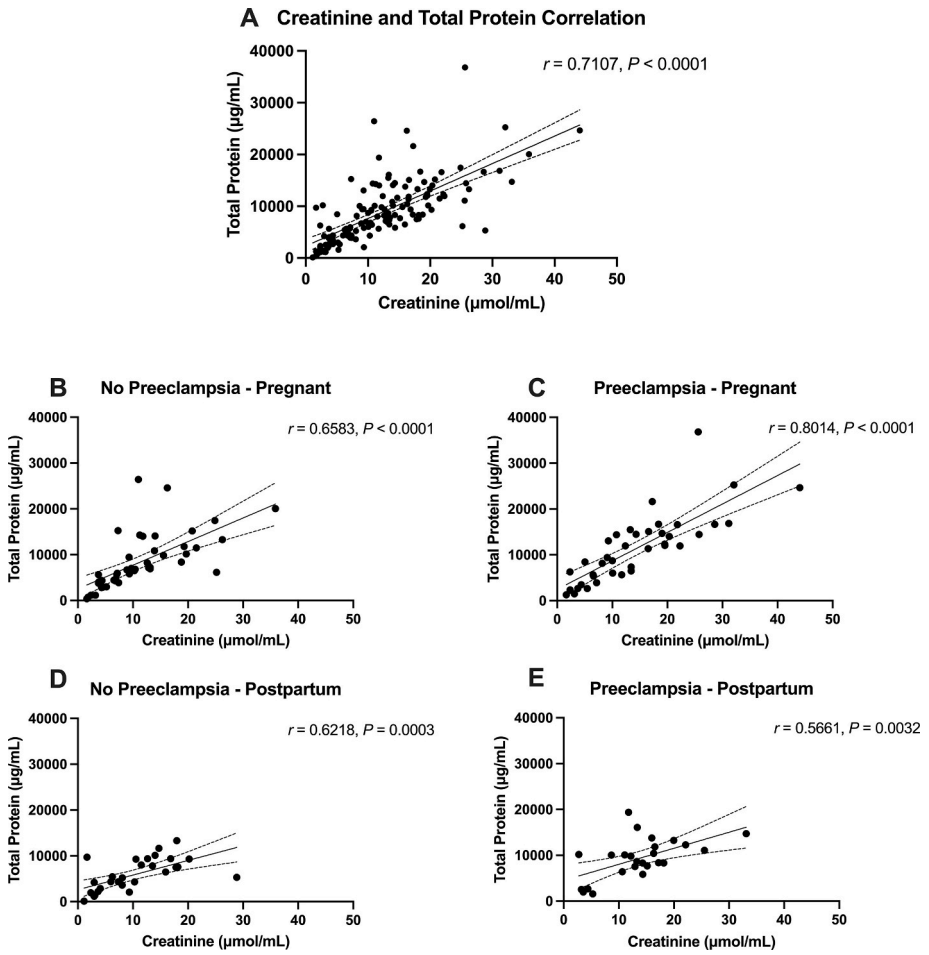

Fig. 3. Correlation of urine creatinine with urine total protein within (A) the cohort, (B) no-preeclampsia pregnancy, (C) preeclampsia pregnancy, (D) no-preeclampsia pregnancy postpartum and (E) preeclampsia pregnancy postpartum. Correlation between creatine levels and total protein were analysed by correlation analysis using Pearson's test. $P < 0.05$ was considered statistically significant. Data were fit to a linear regression model. Dotted lines in plots show 95 % confidence intervals for the fit to the data.

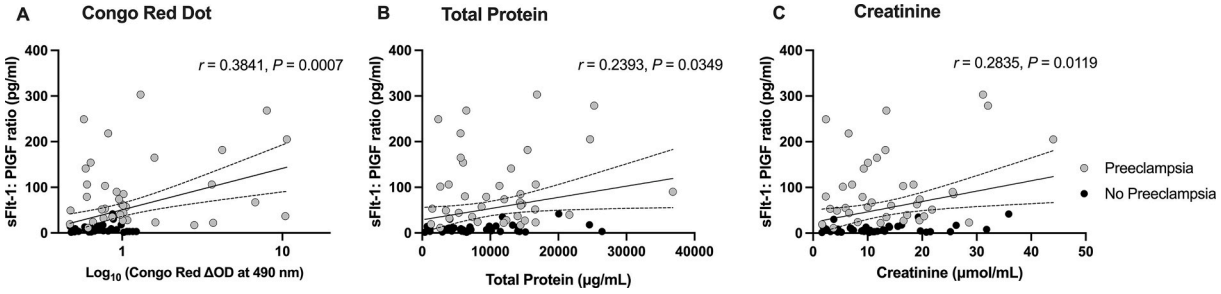

Fig. 4. Correlation of serum sFlt:PlGF ratio with the urinary markers A) Congo Red, B) Total Protein and C) urinary creatinine. Note that in panel A the x-axis is a $\log_{10}$ scale. Correlation between sFlt-1:PlGF ratio and other protein measures were analysed by correlation analysis using Pearson's test. $P < 0.05$ was considered statistically significant. Data were fit to a linear regression model. Dotted lines in plots show 95 % confidence intervals for the fit to the data.

for detection of urine congophilia, and has potential as a useful tool for identifying preeclamptic women [15,16]. This is the first study to show urine congophilia may be isolated to preeclamptic pregnancies independent of total protein levels – total protein in urine remained elevated 6-months postpartum despite a decrease in detected amyloid. Previous studies indicate that postpartum proteinuria in preeclamptic women resolves 6-months postpartum, with persistence indicating undiagnosed renal disease. Roberts et al. [17], showed 31 % of preeclamptic women had persistent proteinuria with 7 % developing chronic kidney disease. There is no evidence of chronic or acute kidney injury in the current study, as eGFR (as a marker of kidney function) remained >90 (mL/min/1.73 m^2) throughout; however, persistent proteinuria may result in renal damage through other more subtle mechanisms, such that manifestation of chronic kidney deterioration had not yet occurred in this cohort.

Correlation analysis indicated that both urine congophilia and creatinine are positively correlated with total protein levels; however, our data indicate there was a weak correlation between urine congophilia and total protein within pregnant preeclamptic women. Thus, our findings suggest that urine congophilia associated with

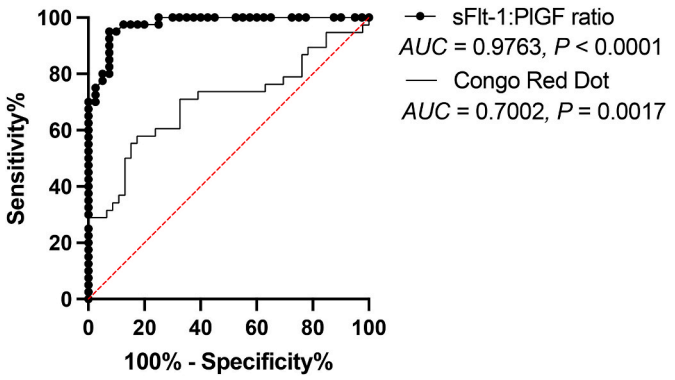

Fig. 5. ROC curves of serum sFlt-1:PlGF ratio and urinary Congo Red Dot with non-preeclamptic population being the control group.

Table 3
Other pregnancy complications associated with the no-preeclampsia and pre-eclampsia groups.

	Non-preeclamptic Pregnancy $n = 48$	Preeclamptic Pregnancy $n = 42$	χ^2	P
IUGR/LBW/ SGA *n (%)*	6 (12.5)	13 (31)	4.58	**<0.05**
% of Total (*N = 90*)	6.7	14.4		
PTB *n (%)*	7 (14.6)	18 (42.9)	8.93	**<0.01**
% of Total (*N = 90*)	7.8	20.0		
PP *n (%)*	2 (4.2)	0 (0)	1.79	NS
% of Total (*N = 90*)	2.2	0		
GDM *n (%)*	4 (8.3)	15 (35.7)	10.1	**<0.01**
% of Total (*N = 90*)	4.4	16.7		

Chi-square analysis of morbidity incidence within the no-preeclampsia and preeclampsia pregnancy groups during pregnancy with bold text indicating significance ($P < 0.05$). Data is presented as n (%). IUGR/LBW/SGA, intrauterine growth restriction/low birth weight/small for gestational age; PTB, preterm birth; PP, Placental previa; GDM, gestational diabetes mellitus.

preeclampsia may be independent of proteinuria during pregnancy. As proteinuria and hypertension are thought to be late manifestations of preeclampsia, with underlying pathological processes leading to these late signs, this may be a significant finding.

As preeclampsia has been recently identified as a proteinopathy disease, CRD staining may provide a simple, rapid and non-invasive strategy to identify preeclampsia through detection of amyloid aggregates. Our study only examined women post diagnosis of preeclampsia, after manifestation of clinical signs (by definition >20 weeks gestation); however, it is important to delineate if urine congophilia is present early in women who later develop preeclampsia. This may be possible with the GV-005 beta prototype device, a lateral chromographic test used bedside to detect urine congophilia in pregnant women, as described by Bracken et al. [18].

Women with preeclampsia also exhibited increased serum creatinine during pregnancy, which resolved 6-months postpartum. This was concomitant with the increased urine levels of creatinine. Reduced creatinine clearance, indicated by increased serum creatinine levels, may suggest renal dysfunction. Reversion of serum creatinine levels postpartum is consistent with transient disruption to nephron filtration, which may be consistent with glomerular endotheliosis [14], although this was not formally assessed in this work. As expected, blood pressure of preeclamptic women was significantly higher during pregnancy. This may have also contributed to mild renal damage, contributing to the rise in serum creatinine during pregnancy [19].

Interestingly, although blood pressure dropped 6-months postpartum, blood pressure in the preeclamptic group on average remained higher than that of women who had an uncomplicated pregnancy. Although blood pressure of preeclamptic women during pregnancy in this study is not at clinical levels of hypertension, this may be due to medical management and intervention to control blood pressure already ensuing before urine sampling for this study – the goal of keeping blood pressure below a systolic of 140 mmHg and diastolic 90 mmHg [20].

The study would benefit from increasing the sample size and recruitment of patients from multiple hospital sites, with correlation of results to other comorbidities and other complications of pregnancy. Thus, it is important to note that this study should be interpreted as an initial attempt to determine the usefulness of CRD staining postpartum. Furthermore, due to the finding of independence between urine total protein levels and congophilia, there may be potential for CRD testing to be examined as an earlier indicator of urinary congophilia in first-term pregnancies. Additionally, other pregnancy complications were significantly increased within the preeclamptic group as indicated by chi-square analysis, and future studies would benefit from utilising true control and preeclamptic groups or larger cohorts with best subsets regression models. Urinary congophilia was determined to have a similar sensitivity and specificity compared to other studies and, while far less sensitive than the serum marker sFlt-1:PlGF ratio, CRD testing is potentially a more widely accessible and less invasive measure that the sFlt-1:PlGF ratio, which is particularly relevant in regions where the sFlt-1:PlGF ratio cannot be easily measured. In these scenarios, CDR testing may be the best available means to detect preeclampsia before the onset of clinical symptoms. In this study CRD was found to have a ROC-AUC of 70 % for preeclampsia detection – there is potential for other fluorescent stains to be more sensitive than CRD and these may enable easier and earlier detection of preeclampsia [12]. As protein misfolding postpartum has not previously been investigated in women with prior preeclampsia, it may also be important to also consider investigations into urine and/or blood levels of α1-antitrypsin (SERPINA1) and transthyretin (TTR/prealbumin), both of which are associated with amyloid-related chronic disease and have been observed to increase in patients with preeclampsia [21]. This would allow observations into the difference in amyloid profiles between early and late onset preeclampsia and if these alterations continue up to 6-months postpartum.

This study reiterates that urine amyloid levels are increased in preeclampsia during pregnancy, independent of total urine protein, with levels reverting postpartum. This may indicate urine congophilia is an earlier manifestation of preeclampsia than signs currently diagnostic of preeclampsia, including hypertensive pregnancy and proteinuria. If amyloid formation is involved in the causation and manifestation of preeclampsia, it is likely CRD testing may have a significant role in future obstetric screening; however, further research is required to determine its usefulness for detection at early stages of disease. Investigation of CRD testing postpartum is warranted to determine when the level of urine congophilia is re-established as normal and proteostasis is reattained.

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CRediT authorship contribution statement

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Declaration of competing interest

None.

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