OBSTETRICS

Urinary congophilia in women with hypertensive disorders of pregnancy and preexisting proteinuria or hypertension

Fergus P. McCarthy, PhD; Adedamola Adetoba, BSc; Carolyn Gill, PhD; Kate Bramham, PhD; Maria Bertolaccini, PhD; Graham J. Burton, PhD; Guillermina Girardi, PhD; Paul T. Seed, ••••; Lucilla Poston, PhD; Lucy C. Chappell, PhD

BACKGROUND: Congophilia indicates the presence of amyloid protein, which is an aggregate of misfolded proteins, that is implicated in the pathophysiologic condition of preeclampsia. Recently, urinary congophilia has been proposed as a test for the diagnosis and prediction of preeclampsia.

OBJECTIVES: The purpose of this study was to determine whether urine congophilia is present in a cohort of women with preeclampsia and in pregnant and nonpregnant women with renal disease.

STUDY DESIGN: With the use of a preeclampsia, chronic hypertension, renal disease, and systemic lupus erythematosus cohort, we analyzed urine samples from healthy pregnant control subjects (n = 31) and pregnant women with preeclampsia (n = 23), gestational hypertension (n = 10), chronic hypertension (n = 14), chronic kidney disease; n = 28), chronic kidney disease with superimposed preeclampsia (n = 5), and chronic hypertension and superimposed preeclampsia (n = 12). Samples from nonpregnant control subjects (n = 10) and nonpregnant women with either systemic lupus erythematosus with (n = 25) and without (n = 14) lupus nephritis were analyzed. For each sample, protein concentration was standardized before it was mixed with Congo Red, spotted to nitrocellulose membrane, and rinsed with methanol. The optical density of the residual Congo Red stain was determined; Congo red stain retention was calculated, and groups were compared with the use of the Mann-Whitney test or Kruskal-Wallis analysis of Variance test, as appropriate.

RESULTS: Congophilia was increased in urine from women with preeclampsia (median Congo red stain retention, 47%; interquartile range, 22—68%) compared with healthy pregnant control subjects (Congo red stain retention: 16%; interquartile range, 13—21%; $P = .002$), women with gestational hypertension (Congo red stain retention, 20%; interquartile range, 13—27%; $P = .008$), or women with chronic hypertension (Congo red stain retention, 17%; interquartile range, 12—28%; $P = .01$). There were no differences in Congo red retention between pregnant women with chronic hypertension and normal pregnant control subjects (Congo red stain retention, 17% [interquartile range, 12-28%] vs 16% [interquartile range, 13—21%], respectively; $P = .72$). Congophilia was present in pregnant women with chronic kidney disease (Congo red stain retention, 32%; interquartile range, 14—57%), being similar to values found in women with preeclampsia ($P = .22$) and for women with chronic kidney disease and superimposed preeclampsia (Congo red stain retention, 57%; [interquartile range, 29—71%; $P = .18$). Nonpregnant women with lupus nephritis had higher congophilia levels compared with nonpregnant female control subjects (Congo red stain retention, 38% [interquartile range, 17—73%] vs 9% [7—11%], respectively; $P < .001$) and nonpregnant women with systemic lupus erythematosus without nephritis (Congo red stain retention, 38% [interquartile range, 17—73%] vs 13% [interquartile range, 11—17%], respectively; $P = .001$). A significant positive correlation was observed between congophilia and protein:creatinine ratio (Spearman rank correlations, 0.702; 95% confidence interval, 0.618—0.770; $P < .001$).

CONCLUSION: This study confirms that women with preeclampsia and chronic kidney disease without preeclampsia have elevated urine congophilia levels compared with healthy pregnant women. Nonpregnant women with lupus nephritis also have elevated urine congophilia levels compared with healthy control subjects. An elevated Congo Red stain retention may not be able to differentiate between these conditions; further research is required to explore the use of congophilia in clinical practice.

Key words: amyloid, chronic kidney disease, Congo red, preeclampsia, renal disease, unfolded protein response, urine congophilia

Preeclampsia, a disease in pregnancy that is characterized by the development of hypertension and multiorgan manifestations that include proteinuria, is a leading cause of maternal death; it accounts for 17—24% of all maternal deaths in low income settings.[1] Current theories suggest that preeclampsia arises from impaired placentation (trophoblast invasion of the maternal uterine spiral arteries), which in turn leads to placental hypoxia and ischemia, and from stimulation of sustained endoplasmic reticulum and oxidative stress.[2-5] It has been proposed that this pathophysiologic cascade generates the characteristic systemic symptoms of the maternal disease.[6] Endoplasmic reticulum stress in the placenta, as in other cell types, leads to up-regulation of the unfolded protein response pathway.[7-9] The unfolded protein response is a common cellular defense mechanism that promotes removal of unfolded or misfolded proteins to prevent potentially toxic accumulation. Activation of placental unfolded protein response has been shown to occur in early onset preeclampsia, but not in late onset preeclampsia, or normotensive control subjects.[5]

Congo red stain, which initially was developed as a textile dye, has been used most commonly to identify amyloid in

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TABLE 1
Definitions used for classification of women

Definition	Criteria
Healthy control women	No risk factors for preeclampsia
	No history of preeclampsia, hypertension, diabetes mellitus, renal disease, connective tissue disease, or antiphospholipid antibody syndrome
	Systolic blood pressure <140 mm Hg
	Diastolic blood pressure <90 mm Hg
	No protein on dipstick analysis of midstream urine
	Not in labor
Gestational hypertension	Previously normotensive
	Two recordings of systolic blood pressure ≥140 mm Hg or diastolic blood pressure ≥ 90 mm Hg at >4 hours apart
	After 20 weeks of gestation
	Not in labor
Preeclampsia	Gestational hypertension AND proteinuria of >300 mg protein over 24 hours (or protein:creatinine ratio of >30 mg/mmol)
Superimposed preeclampsia on chronic hypertension: hypertension already present	New onset of proteinuria >300 mg protein over 24 hours (or protein:creatinine ratio of >30mg/mmol) OR additional features: severe persistent right upper quadrant pain, epigastric pain that is unresponsive to mediation or alanine transaminase <71U/L or platelet count <100,000/μL, pulmonary edema, new onset cerebral, or visual disturbance
Superimposed preeclampsia: proteinuria already present	Two recordings of systolic blood pressure ≥140 mm Hg or diastolic blood pressure ≥ 90 mm Hg at >4 hours apart OR additional features as listed earlier
Superimposed preeclampsia: hypertension and proteinuria already present	Development of severe hypertension (systolic blood pressure ≥160 mm Hg or diastolic blood pressure ≥110 mm Hg) AND >2-fold increase in proteinuria above 300 mg protein over 24 hours (or protein:creatinine ratio of >30 mg/mmoL) OR additional features as listed earlier
Chronic hypertension	Primary or secondary causes of hypertension
Chronic kidney disease	According to the Kidney Disease Outcomes Quality Initiative guidelines before pregnancy OR persistent proteinuria (>30 mg/mmoL (protein:creatinine ratio) at <20 weeks of gestation OR any recorded serum creatinine >70 μmol before 20 weeks' gestation without risk factors for acute kidney injury.

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tissue sections by demonstration of green birefringence under crossed polarizers,[10] which includes the identification of amyloid beta deposits after death in brain tissue from patients with Alzheimer's disease.[11] As a result of these associations, the presence of Congo red staining itself is now thought to represent protein misfolding because of its propensity to detect proteins with amyloid-like characteristics.[12-14]

Previous work demonstrated the presence of urine congophilia with the use of the Congo red "dot" test, and the authors proposed that it carries diagnostic and prognostic potential for preeclampsia.[15] This Congo red assay is now being investigated as an innovative mobile health solution in countries with limited resources as a diagnostic and prognostic tool for preeclampsia.[16]

The aim of this study was to determine whether urine congophilia is present in a cohort of women with preeclampsia and in pregnant and nonpregnant women with renal disease.

Materials and Methods

We conducted a retrospective analysis of samples that were collected as part of a prospective study. Samples were obtained from participants who were recruited to a multicenter preeclampsia, chronic hypertension, renal disease, and systemic lupus erythematosus (SLE) cohort.[20] A pragmatic approach was adopted; all samples that were available for analysis within the cohort were selected and analyzed, and all data were presented. The groups that were examined consisted of healthy pregnant control subjects (n = 31) and pregnant women with preeclampsia (n = 23), gestational hypertension (n = 10), chronic hypertension (n = 14), chronic kidney disease (CKD; n = 28), CKD with superimposed preeclampsia (n = 5), and chronic hypertension and superimposed preeclampsia (n = 12; Table 1). Exclusion criteria were <18 or [T1] >50 years old, an inability or unwillingness to give informed consent, known HIV, Hepatitis B or C positive, or a multifetal pregnancy.

Three additional groups of nonpregnant women were assessed for urinary congophilia, which included healthy control subjects (n = 10), women with SLE (n = 25), and women with lupus nephritis (n = 14). The patients were identified through the Registry of Connective tissue diseases (10/H0405/35) at St. Thomas' Hospital. The National Health Service National Research Ethics Service approved the collection and use of samples for research purposes. SLE was defined by the American College of Rheumatology criteria for the classification of SLE with and without kidney involvement (category III, IV, and V according to the International Society of Nephrology/Renal Pathology Society glomerulonephritis classification).[21,22]

Midstream urine samples were collected into sterile containers, centrifuged at 1400*g* (10 minutes), and stored in multiple aliquots at −80°C within 3 hours of collection. Total protein con- Q3 centration was quantified with the Pierce Bicinchonic Acid Assay Kit (Life Technologies, Rockville, MD) according to Q4 manufacturer's instructions; each sample was tested in triplicate. Standards

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