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Can Staining of Damaged Proteins in Urine Effectively Predict Preeclampsia?

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Key Words
Congo red · Syncytiotrophoblast microparticles · Misfolded proteins · Biomarkers · Proteinuria · Preeclampsia · First trimester

Abstract
Objectives: To assess Congo red urine test in the first trimester for preeclampsia (PE) prediction. ***Sample:*** A Congo red test was developed with a cohort of 81 pregnant women in Bnai Zion hospital, Israel, at 26–41 weeks of gestation (12 PE cases). The test was then applied to a first-trimester cohort of 642 women at King's College Hospital, UK (105 subsequently developed PE, 21 early, i.e., <34 weeks; 537 controls). ***Methods:*** Urine samples were spotted onto nitrocellulose membranes, stained with Congo red, de-stained, dried and quantified with imager and densitometry. ***Results:*** At PE signs and symptoms, the detection rate (DR) was 93% and the false-positive rate (FPR) 4%. However, with first-trimester urine samples, the DR was 33.3%, 16.1% and 20% for early, late and all PE cases, respectively, at 12.8% FPR. The odds ratio (OR) for PE by Congo red alone (including adjusted OR) was superior to body mass index and mean arterial blood pressure (MAP) but inferior to previous PE and black ethnicity. Combining all five parameters generated an adjusted OR of 13.92 for PE ($p < 0.001$). ***Conclusion:*** Congo red urine test at PE verifies the disorder. In the first trimester, it adds accuracy for PE prediction in obese, black women, who had previous PE and over-average MAP.

Introduction

Preeclampsia (PE) is a leading cause of morbidity and mortality during pregnancy, affecting 2.5–7% singleton and 7–21% twin pregnancies [1, 2]. The etiology and the developmental pathways of PE are still a myth, but impaired placentation is widely accepted as a major contributing cause [3, 4]. Additional theories attribute PE to endothelial dysfunction [5] and to increased burden on the maternal heart [6, 7].

A diversity of biomarkers were suggested to predict the risk to develop PE during the first [8] or second trimester [9] together with biophysical markers such as mean arterial pressure (MAP) and uterine artery Doppler sonography [10, 11] and maternal risk factors. The Fetal Medicine Foundation algorithm to predict PE in singleton combines: first-trimester maternal serum pregnancy-associated placental protein (PAPP-A) and placental growth factor (PlGF), maternal uterine artery Doppler pulsatility index (UTPI), MAP and prior risk factors. Statistical

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modelling with this algorithm predicts that for a false-positive rate (FPR) of 10%, the detection rate (DR) will be 96% for early PE and 77% for preterm PE, including early and intermediate cases [12, 13]. However, the use of this marker set, mainly related to impaired placentation, is not possible for many clinics in the developing countries.

Buhimschi et al. [14] have developed a novel test for PE diagnosis. This test is based on the use of Congo red staining of misfolded and damaged proteins in the urine of women with PE. These proteins may be derived from the syncytiotrophoblast microparticles (STBMs), which are membrane-bound vesicles containing misfolded, aggregated and damaged proteins that are shaded by the villous syncytiotrophoblast during the pathological processes leading to the development of PE. During the development of the PE disorder, when the kidney glomeruli are disrupted and kidney function impaired [3, 4], the STBMs and their damaged proteins reach the urine. STBMs appear only in a negligible amount in normal pregnancies or in cases of pure fetal growth restriction [15–17]. The Congo red test of Buhimschi et al. [14] involves pre-amplification of misfolded proteins with antibodies to peptides like serpin A1 (alpha-1 antitrypsin) or a fraction of this peptide that are subsequently stained with Congo red to serve as a simple and rapid test for the diagnosis of PE.

Congo red (disodium 4-amino-3-[4-[4-(1-amino-4-sulfonato-naphthalen-2-yl)diazenylphenyl]phenyl]diazenyl-naphthalene-1-sulfonate) is a pH indicator widely used for staining deposition of aggregates or plaques and inflammation of blood vessels, urine or the cytoplasm [18, 19]. Certain similarities exist between Congo red-positively stained molecules and the nature of the STBMs that are shaded by the placenta [9, 16, 17].

Our aim was to evaluate a simpler Congo red-based urine spot staining test for PE diagnosis and the application of this test for the prediction of the risk to develop PE during the first trimester. Accordingly, the test was developed for a cohort of pregnant women at the time of PE signs and symptoms. Then, the test was applied to a different cohort of first-trimester subjects to assess its performance, with the hope to develop a very affordable and simple procedure for PE prediction for developing countries. This paper showed that urine spot staining in the first trimester on its own is not a good marker for the development of PE but it provides fair prediction when combined with history of PE in black and obese women with a MAP of 10 mm Hg above the mean but in the normal range.

Methods

The Definition of PE

PE was diagnosed according to the criteria of the International Society for the Study of Hypertension in Pregnancy as updated by Lindheimer et al. [20–22] and those issued by Sibai [23] on behalf of the American Society of Maternal and Fetal Medicine. The criteria were: systolic blood pressure ≥140 mm Hg and/or diastolic blood pressure ≥90 mm Hg on at least two occasions 4 h apart developed after 20 weeks of gestation in previously normotensive women, combined with proteinuria of ≥300 mg in 24 h, or two readings of at least 2+ on dipstick analysis of midstream or catheter urine specimens if no 24-hour collection was available.

Study Cohorts

Cohort at the Time of PE Signs and Symptoms

Pregnant patients admitted to the Bnai Zion Medical Center, Haifa, Israel, to verify various late pregnancy situations at gestational age (GA) 26–41 weeks were recruited between June 2006 and March 2009 after they signed their written informed consent [24]. Ethical committees at Bnai Zion University Medical Center, Haifa, Israel, approved the study. All patients delivered by C-section, either prescheduled or due to emergency conditions. Women were interviewed to collect medical and pregnancy history and epidemiology data, and delivery records were extracted from the hospital medical records.

PE and normal outcome were identified as described above. In addition to the PE and the normal outcome groups, the cohort also included patients who delivered preterm (PTD) without hypertension disorders to serve as another control group (preterm control) since most PE cases were admitted before term.

First-Trimester Cohort

Patients were prospectively enrolled between March 2006 and September 2009 while they attended King's College Hospital, London, UK for their first pregnancy evaluation visit after they signed their written informed consent approved by the hospital clinical committee. The patients were interviewed to collect medical and pregnancy history and epidemiology data, and screening for adverse obstetric outcomes at 11^{+0} until 13^{+6} weeks of gestation was performed. Gestational week was determined by the fetal crown-rump length [25]. Their MAP was recorded by automated devices [24] along with the collection of blood and urine specimens. Transabdominal color Doppler ultrasound was used to visualize the left and right uterine artery, measure the UTPI in each vessel and calculate the mean UTPI [26, 27]. Samples of urine serum and plasma were stored at –80°C for subsequent biochemical analysis. The inclusion criteria were singleton pregnancy delivering a phenotypically normal stillbirth or live birth at or after 24 weeks of gestation. Pregnancies with major fetal abnormalities and those ending in termination, miscarriage or fetal death before 24 weeks were excluded.

Data on pregnancy outcomes were collected from the hospital maternity records or from the women's general medical practitioners. The obstetric records of all women with preexisting or pregnancy-associated hypertension were examined to determine if the condition was chronic hypertension, PE or gestational hypertension without proteinuria. The PE group was divided according to gestation at delivery into early PE (<34 weeks), intermediate PE (34–36^{+6} weeks) and late PE (≥37 weeks).

Urine Samples

Cohort at the Time of PE Signs and Symptoms. Urine samples were collected prospectively at admission and during the hospital stay. Urine samples were obtained from all patients, centrifuged to clearance and stored at –70°C until assayed.

First-Trimester Cohort. Samples of all cases which developed PE were extracted from the database and their urine samples were retrieved from the storage upon availability. These were matched with normal outcome according to time of enrolment (+2 weeks) and gestational week at enrolment (+1 week) at a 1:5 ratio.

Urine Spot Staining with Congo Red

The test was developed with 5 and 10 μl of urine samples at the time of signs and symptoms of PE using nitrocellulose strips of 10 × 3 cm. After drying, the nitrocellulose membranes were stained with 0.5 mg Congo red dye/ml (40%, Sigma, Rehovot, Israel) freshly prepared in 1 ml double distilled water and incubated for 1 h. Access dye was removed by sequential membrane destaining with methanol (2 min in each of 50%, 70%, 80%, 90% and 94% methanol in double distilled water). The entire assay was carried out at room temperature.

Subsequently, the test was applied to the first-trimester samples using nitrocellulose membranes of 8 × 12 cm matrixes that were placed in a dot-blotter apparatus (Cleaver Scientific, UK) operated with a mild vacuum pump to enable high-throughput testing. All other details were the same as above. Each matrix included 8 cases of positive control and 8 cases of negative control.

Images of the stained membrane were captured by LAS300 Imager (Fuji Photo Film Co., Ltd., Japan) and a quantitative and semi-quantitative densitometry assessment of the intensity of each spot was performed by the Image Gauge software (Fuji Photo Film Co., Ltd.).

Spot Intensity Semi-Quantitative Calculation

Staining intensity of the urine spots for the cohort of signs and symptoms was obvious to the naked eye and a yes/no answer could be achieved without the densitometer. However, to reach standard approach, the spot intensity was determined by the densitometer for all. Background subtraction from each sample was conducted using the mean of eight spots with no urine, which were run on each matrix.

The spot relative intensity after subtracting the background was then calculated by dividing the spot intensity (I_X) value by the mean of the eight positive samples (I_{PC}). The relative intensity ($I_{X\%}$) was between 0% to 159%, and the standard deviation was 19.8%. Accordingly, each spot which has $I_{X\%} \geq 20\%$ was scored positive (1) and those below were scored negative (0).

Statistics

For categorical variables, summary tables are provided giving absolute frequencies. For continuous variables, summary tables are provided giving arithmetic mean and standard deviation. Pearson's χ^2 or Fisher's exact tests were applied for testing the differences between the study groups for the categorical parameters.

Comparisons between cases and controls were analyzed by independent Student's t test. Comparisons between controls and the two groups of PE were analyzed using Kruskal-Wallis non-parametric test. A Pearson correlation was used to evaluate the distribution of the relative intensity (I_X) of Congo red spot distribution and Fisher's r-to-z transformation to assess the significant differences between the correlation coefficients of spot distribution between cases and control.

Univariate logistic regression model was used to calculate the odds ratio (OR) for Congo red and each of the major maternal risk factors associated with prediction of PE as individual markers, which were previous PE, black ethnicity, body mass Index (BMI) and MAP.

Adjusted OR was obtained with logistic regression using correlation coefficients of the interrelations between the markers and χ^2 analysis for the 4 risk factors and Congo red combined. The OR for all five markers was calculated by logistic regression of all the adjusted OR of all 5 markers combined. All p values were two-tailed, $p < 0.05$ was considered statistically significant. Data were analyzed using SPSS.

Results

Cohort at the Time of Signs and Symptoms of PE

The cohort features were previously described by Sammar et al. [24]. There were 63 normal-outcome patients delivered at term and 12 patients who developed PE. All PE cases delivered preterm, i.e., before 37 weeks, of which 10 delivered before 34 weeks (early cases). There were 6 cases who delivered early (<34 weeks) not due to hypertensive disorders (PTD).

Maternal age was similar among all tested groups; the proportion of nulliparity was similar between the term control and the PTD group, but significantly higher in PE. GA at delivery was earlier in all disorder groups compared with the control group. Overall, only 11% of the patients enrolled were of Arabian minorities compared to 19% in the Israeli population. Among the PE cases, the blood pressure was lower in term and preterm controls compared with the PE patients.

Newborns' birth weight was significantly lower for PE and PTD groups compared with term control due to earlier delivery, but it was not different between the PE group or the PTD group. All babies were above the 10th centile for GA indicating that in this cohort, there were no small-for-gestational-age infants. As anticipated, the mean highest blood pressures, either systolic or diastolic, were higher in the PE group compared to control or PTD.

First-Trimester Cohort Characteristics

The first-trimester cohort included 105 PE cases, 21 of them developed early PE (delivered <34 weeks), and 537 unaffected controls. All samples were retrieved from 11–13 gestational weeks' urine depository.

As shown in table 1, there was no difference in GA at the time of enrolment between cases and control. As previously shown by Akolekar et al. [11], maternal age was

Table 1. Demographic and characteristics of the study population

Characteristics	Control (n = 537)	All PE (n = 105)	p_1	Early PE (n = 21)	Late PE (n = 84)	p_2
At enrolment						
Maternal age, years	31.8±5.6 (16.6–44.3)	31.4±6.4 (17.9-47.6)	0.556	31.6±6.7 (20.3-44.6)	31.4±6.4 (17.9–47.6)	0.803
BMI	25.2±4.2[b] (16.4–47.7)	28.6±6.2 (18.0–49.4)	<0.001	27.6±5.0[a] (18.7–38.1)	28.8±6.5[a] (18.0–49.4)	<0.001
Ethnicity, black	15.6	43.8	<0.001	66.7	38.1	<0.001
Current smoker	8.8	7.6	0.704	0	9.5	0.372
Mean PI 1st	1.7±0.5[b] (0.6–3.2)	2.0±0.5 (0.6–3.4)	<0.001	2.3±0.6[a] (1.3-3.1)	1.9±0.5[b] (0.6–3.4)	<0.001
PAPP-A	3.3±2.1 (0.5–17.8)	3.1±2.3 (0.1–11.0)	0.634	3.0±2.1 (0.1–8.1)	3.2±2.4 (0.3–11.0)	0.847
Previous PE	1.3	15.2	<0.001	14.3	15.5	<0.001
Vascular function, mm Hg						
Central systolic pressure	116±10[c] (87–150)	125±12 (96–157)	<0.001	129±15[a] (106–157)	123±11[b] (96–143)	<0.001
Central diastolic pressure	70±7[b] (49–95)	77±8 (60–99)	<0.001	79±10[a] (60–99)	77±8[a] (61–99)	<0.001
MAP	85.2±7.4[b] (63.0–111.0)	92.8±9.0 (73.5–118.0)	<0.001	95.3±11.0[a] (80.5–118.0)	92.2±8.4[a] (73.5–112.5)	<0.001
At delivery						
GA, weeks	40.1±1.2[a] (37.1–42.7)	36.7±3.8 (25.0–41.7)	<0.001	30.3±2.2[c] (25.0–33.0)	38.3±1.9[b] (33.5–41.7)	<0.001
Baby weight, g	3,473±398[a] (2,469–4,425)	2,615±935 (458–4,180)	<0.001	1,183±341[c] (458–1,650)	2,973±648[b] (1,617–4,180)	<0.001

Values are means ± standard deviations (range) or percentages. p_1 is the p value for the comparison of control to all PE, where [a] stands for significantly higher and [b] for significantly lower; p_2 stands for the comparison of early and late PE to controls, where [a] represents significantly higher and [b] and [c], respectively, represent significantly lower.

Table 2. Congo red spot staining intensity in the first trimester

Group	5 µl			10 µl		
	n	DR, %	OR	n	DR, %	OR
Normal (n = 537)	73	12.8*		85	15.8*	
Early PE (n = 21)	7	33.3	2.60	8	38.1	2.43
Late PE (n = 84)	14	16.1	1.26	23	27.4	1.75
All PE (n = 105)	22	20.9	1.63	26	24.7	1.57

Using the relative spot intensity $I_{X\%}$, samples were categorized as positive (1) or negative and the DR (detected/all cases in the group) was determined. * FPR.

similar between cases and control. BMI, MAP and the frequency of black ethnicity and nulliparity were higher ($p < 0.001$) in the PE group compared to unaffected control. The frequencies of previous PE and chronic hypertension were higher among the PE groups. Newborn weight and GA at delivery were lower in the PE group compared to unaffected control.

Congo Red Test at the Time of PE Signs and Symptoms

As shown with the first phase of the study (fig. 1a), urine spots from pregnant women who have PE signs and symptoms stained strongly compared with controls. Staining intensity was reduced by sequential dilution of the PE urine samples but remained positive to the naked eye even at 1:16 dilution (e.g. 0.2 g urine protein/dl) without the need for any imaging analyzer or densitometry. Accordingly, the DR was 94.1% for PE cases of the urine spots staining by Congo red and the FPR was 15.3%, whereas staining of spots from PTD cases was not different from control. This test was then applied to analyze first-trimester samples.

Urine Spots Staining with Congo Red in the First Trimester

Urine spot staining intensity was way lower in the first trimester (fig. 1b). Using the $I_{X\%}$ relative intensity scoring conducted on all matrixes, the DR for the 5-µl urine spots was 33.3%, 16.1% and 20% for early, late and all PE cases, respectively, and the FPR was 12.8%. For the 10-µl spots, the DR was 38.1%, 27.4% and 24.7% for early, late and all PE cases, respectively, and the FPR was 15.8% (table 2). The Pearson correlation coefficient for scattered spot intensity distribution according to the $I_{X\%}$ measure was $r = 0.338$ ($p < 0.001$) for the control and $r = 0.632$ ($p < 0.001$) for all PE cases. Using Fisher's r-to-z transformation to assess the significance of the differences between the two correlation coefficients yielded a significant difference ($p < 0.001$).

Correlation between Urine Spot Staining with Congo Red and Prior Risk Factors

In linear regression analysis, there was no correlation between staining intensity in control cases and maternal

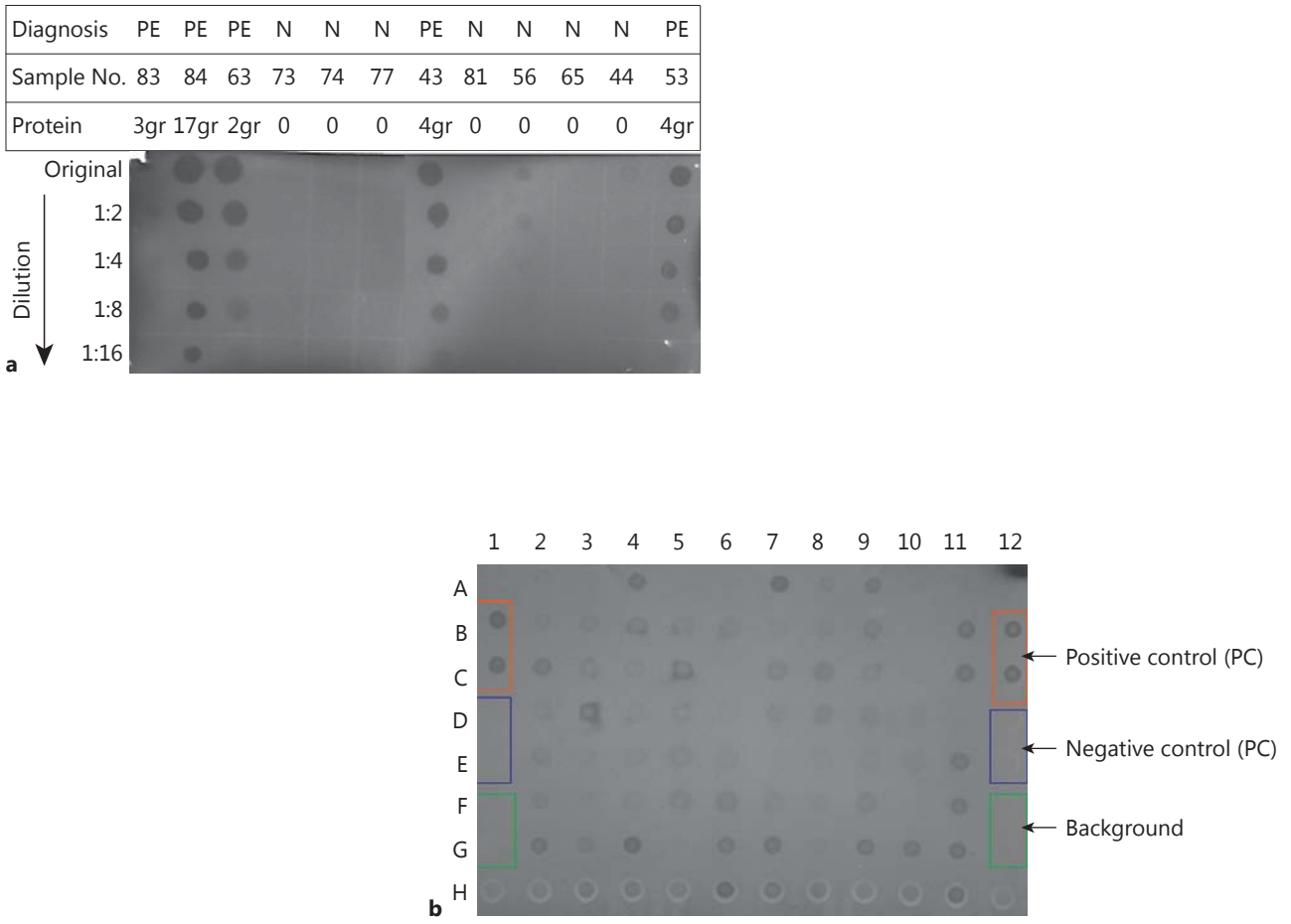
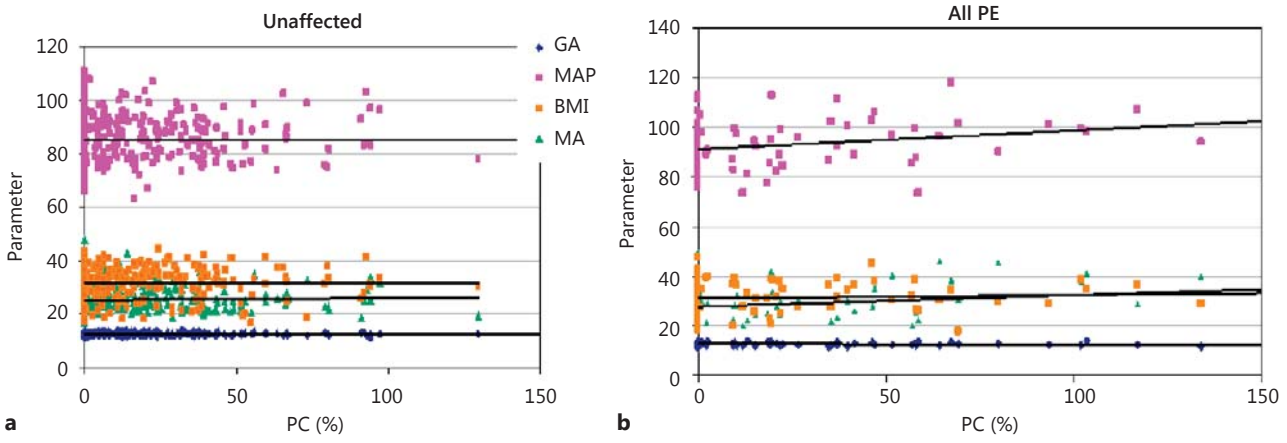


Fig. 1. Congo red urine spot test. **a** Urine samples obtained at the time of PE signs and symptoms were sequentially diluted and spotted onto nitrocellulose membranes and stained with Congo red to examine signal intensity. Top upper line: patient outcome; top middle line: sample code; top lower line: the urine protein level determined by the standard 24-hour urine collection test. Signal intensity is followed by the dilution factor indicated to the left. N = Normal outcome. **b** Representative figure: A 8 × 12 nitrocellulose matrix of first-trimester urine samples stained with Congo red. Positive and negative controls represent pools of 8 PE cases or 8 unaffected cases, spotted on the same matrix, respectively. The background does not contain urine. Samples were spotted by the scientist who was blinded to the clinical outcome. Staining with Congo red was performed as described in the Methods section.

age, BMI, MAP or GA at testing (correlation coefficient <0.006 for all). In comparison, in PE cases, the staining intensity increased with BMI ($r = 0.241$, $p < 0.001$) and MAP ($r = 0.276$, $p < 0.001$) but not with maternal age or gestational week (fig. 2). For the a-parametric values (ethnicity, method of conception, parity), staining intensity was higher for the PE group but only among the cases who had previous PE or black ethnicity ($p < 0.001$, not shown).

In a univariate logistic regression model, similar ORs to develop PE were obtained by staining with 10 µl as estimated using the $I_{X\%}$ scoring system, i.e., 1.67 ($p < 0.038$), 1.73 ($p < 0.042$) and 1.44 ($p < 0.491$) for all, late and early PE, respectively. The 5-µl spots gave similar results but lower confidence values (table 3).

ORs for previous PE were 13.61, 13.86 and 12.62 for all, late and early PE, respectively ($p < 0.001$; table 3). The



Color version available online

	BMI	GA	MAP	MA
Unaffected	0.0002	0.0037	0.0006	0.0001
All PE	0.058	0.0068	0.076	0.0048

c

Fig. 2. First-trimester urine spot intensity versus maternal risk factors. **a**, **b** Congo red spot staining intensity ($I_{X\%}$) depicted against individual maternal risk factors for normal controls (unaffected; **a**) and for all PE cases (**b**). Purple dots and line: MAP [calculated as (2 × diastolic blood pressure + systolic blood pressure)/3]; orange: BMI (weight/height squared); blue: GA at enrolment; green: maternal age (MA, years). PC = Positive control. **c** The linear regression coefficient R^2 calculated by two-way ANOVA for each parameter in control and all PE.

ORs of black ethnicity were also high, corresponding to 4.21, 3.32 and 10.79 for all, late and early PE, respectively ($p < 0.001$). The OR of Congo red was better compared to the ORs of BMI or MAP, which were in the range of 1.11–1.15 ($p < 0.001$; table 3).

Adjusted Multivariate Regression and Multiple Marker Analysis

There was high correlation between the parameters above and thus, adjusted OR was calculated for each parameter given the other correlated factors. Accordingly, the adjusted ORs were calculated and although lower compared to the unadjusted, they remained significant (table 4). For each adjusted model, the ORs were slightly higher with the 10-μl spot series compared to the 5-μl spot series (table 4).

Based on the above, a multivariate combined analysis was performed with the adjusted ORs yielding the OR = 13.58 (prediction accuracy 85.8%) for all five parameters for the 5-μl series and OR = 13.92 (prediction accuracy 86%) for the 10-μl one (table 4). The results correspond with added value for Congo red spot test over the use of BMI, black ethnicity, previous PE and MAP alone.

Discussion

The main finding of this study is that a very simple Congo red test can diagnose PE at very high accuracy at the time of signs and symptoms. The test performance in the prediction of PE in the first trimester is not very strong.

Table 3. First-trimester single variate analysis of Congo red and maternal risk factors assessed by the OR for PE development

Parameter	All PE		Late PE		Early PE	
	OR (95% CI)	p	OR (95% CI)	p	OR (95% CI)	p
Congo red 5 µl (PC)	1.69 (0.99–2.8)	0.054	1.50 (0.82–2.72)	1.87	2.54 (0.96–6.76)	0.062
Congo red 10 µl (PC)	1.67 (1.03–2.72)	0.038	1.73 (1.02–2.94)	0.042	1.44 (0.51–4.01)	0.491
Previous PE	13.61 (5.45–34.02)	<0.001	13.86 (5.35–35.91)	<0.001	12.62 (3.02–52.82)	<0.001
Black ethnicity	4.21 (2.68–6.6)	<0.001	3.32 (2.02–5.46)	<0.001	10.79 (4.23–27.52)	
BMI	1.14 (1.09–1.19)	<0.001	1.14 (1.09–1.2)	<0.001	1.11 (1.02–1.20)	0.016
MAP	1.12 (1.09–1.15)	<0.001	1.12 (1.08–1.15)	<0.001	1.15 (1.09–1.21)	<0.001

The OR of PE prediction by Congo red according to the results of the relative spot intensity ($I_{X\%}$) is shown for the 5- and 10-µl urine samples as a single predictor along with the major maternal factors including previous PE, black ethnicity, BMI and MAP, each evaluated as a single predictor as well. The results with other factors (maternal age, parity, GA at testing, for the 10–13-week period) were not significant. PC = Positive control.

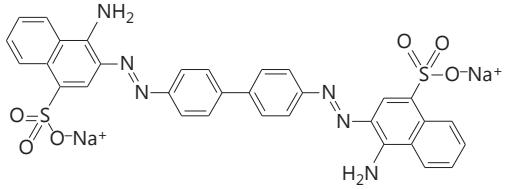
Table 4. Multivariate logistic regression models to predict all PE by adjusted OR

Parameter	Model 1		Model 2	
	OR (95% CI)	p	OR (95% CI)	p
Congo red 5 µl (PC)	1.17 (0.61–2.25)	0.642		
Congo red 10 µl (PC)			1.28 (0.54–1.75)	0.929
Previous PE	6.79 (0.39–19.29)	<0.001	6.87 (2.43–19.43)	<0.001
Black ethnicity	3.51 (2.11–5.82)	<0.001	3.52 (2.12–5.86)	<0.001
BMI	1.06 (1.01–1.12)	<0.011	1.07 (1.01–1.12)	<0.008
MAP	1.09 (1.06–1.13)	<0.001	1.09 (1.06–1.13)	<0.001
All markers	13.58	<0.001	13.92	<0.001

Model 1: $R^2 = 0.30$, $\chi^2_{(5)} = 123.72$, $p < 0.001$. The overall accuracy of classification is 85.8% (523 of the controls and 28 with any PE), adjusted combined multivariate 13.58. Model 2: $R^2 = 0.30$, $\chi^2_{(5)} = 123.72$, $p < 0.001$. The overall accuracy of classification is 86% (522 of the controls and 30 with any PE), adjusted combined multivariate 13.92. The adjusted OR of PE prediction by Congo red according to the results of the relative spot intensity ($I_{X\%}$) is shown for the 5- (model 1) and 10-µl (model 2) urine sample as adjusted predictor given the correlation between all parameters including previous PE, black ethnicity, BMI and MAP. PC = Positive control.

A common feature in the origins of various diseases is build-up of damaged proteins in the body fluids or tissues [18, 19]. In PE, several reports have demonstrated the accumulation of such proteins in maternal blood and urine during the development of the PE pathology. Placental shedding of the STBMs containing such damaged proteins is considered as the main source to such damaged protein build-up in the development of the PE disorder [3, 8, 15–17].

Secondary diazo dyes (general formulation of $C_{32}H_{22}N_6Na_2O_6S_2$) have



SO^-_3 group that exchange the sodium ions for charged peptides residues. Our results have shown that at the time of PE signs and symptoms, the test could accurately diag-

nose the patients in a very simple and accurate manner. Puchtler et al. [28] pioneered in 1962 the use of Congo red staining to diagnose rare infections and also in degenerating brain diseases such as Alzheimer, Creutzfeldt-Jacob disease and prion disease, among others [18, 19, 28, 29]. Congo red is widely used as a pH indicator to stain the deposition of aggregates or plaques and inflammation of blood vessels, urine or the cytoplasm. Known stained candidates are various hormones and proteins, particularly inflammatory proteins. A typical staining with diazo dye involves proteins who exhibit decreased solubility under physiological conditions, a phenomena coined as 'congophilia'. It reflects the loss of biological function due to aggregation or misfolding and suggests common features in the origins of various diseases associated with increased build-up of damaged proteins in body fluids or tissues [18, 19]. Thus, it is no wonder that Buhimschi et al. [14] suggested to use the most common marker of damaged proteins – the Congo red indicator – as a test for the diagnosis and potentially the prediction of PE.

Taking a simpler test procedure than the one used by Buhimschi et al. [14], we could also show very strong urine spot staining by Congo red, which was visible to the naked eye at severe PE (total urine protein of 300 mg/dl) or even milder PE (30 mg/dl). Thus, the test appears to be ideal for aiding the physicians decide whether the hypertension should be followed by hospitalization, induction of labor, etc. [30].

Buhimschi et al. [14] introduced a test amplification using anti-peptide antibodies. The simpler test described here requires water, Congo red, ethanol and nitrocellulose paper. It takes 30 min and costs ~10–30 cents. In this regards, it can perform the same functions in PE diagnosis compared to Alere's PlGF stick test [31] and Roche's sFlt-1/PlGF ratio tests [32], in a small fraction of their cost. Its simplicity and low price offers improved diagnosis of PE in South and Sub-Saharan Africa that need such simple and cheap methods, especially in light of the higher frequency of this condition in these countries [30, 33] and their poor population.

The test performance in the first trimester is limited. Only a very small fraction of patients who will subsequently develop PE could be identified by a positive staining. Accordingly it appears that on its own, the test cannot be considered as a marker of PE in the first trimester as it is a weaker marker than previous PE or black ethnicity, although it was stronger than BMI and MAP. However, it seems interesting that combining all five parameters – Congo red spot staining intensity, previous PE, black ethnicity, MAP and BMI – yielded a very high OR. As these are simple and common means of evaluation of the patients' risk to develop PE, we could say that there is a positive value to Congo red staining in combination with taking maternal weight and height, measuring her blood pressure, asking for her pregnancy history and recording her ethnicity, especially if more sophisticated methods are not available.

Limitations

The cohort examined at the time of PE signs and symptoms was relatively small and larger studies are warranted, especially in the context of developing country setting. Facing the aim of developing simple means to predict PE, the standards biochemical markers and Doppler UTPI were not added to evaluate Congo red performance under more advanced medical setting.

Conclusions

Urine staining with the Congo red direct and simple method of spot staining could be a useful tool at the time of signs and symptoms of PE for aiding the physicians in taking decisions on patient management and prevention of bad PE outcome. The test is simple and cheap, enabling its implementation in developing countries. For first-trimester PE prediction, the value of the test appears to be useful particularly for obese women with a history of PE, black origin, and a slightly elevated MAP, even if in the normal range. When this combination is found for a given patient, it should be considered for PE prevention with low-dose aspirin according to the World Health Organization recommendations [30] and in accordance with the ASPRE mode of using 150 mg aspirin daily at bedtime [34].

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Disclosure Statement

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