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ORIGINAL ARTICLE

Screening for late preeclampsia at 35–37 weeks by the urinary Congo-red dot paper test

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ABSTRACT

Background: Several cross-sectional studies have investigated the incidence of urinary Congo-red dye positivity in women with preeclampsia (PE), compared to unaffected pregnancies, and reported very high sensitivity and low false positive rate in the diagnosis of PE.

Objective: To determine the performance of the urinary Congo-red dot paper test at 35–37 weeks' gestation in the prediction of delivery with PE at ≤2 and >2 weeks after assessment.

Methods: This was a prospective observational study in women attending for a routine hospital visit at 35^{+0} to 36^{+6} weeks' gestation in a maternity hospital in England. Urine samples were collected and the Congo-red dot paper test was used to assess the degree of Congo-red dye positivity. The test uses a scoring system from 1 to 8 and the higher the score the greater the degree of Congo-red dye positivity. We examined and compared the degree of Congo-red dye positivity in the groups that delivered with PE at ≤2 and >2 weeks with those that remained normotensive. Reproducibility was assessed by examining the inter- and intra-observer reliability of scoring on stored images with the researchers blinded to previous results.

Results: The study population of 2140 women included 46 (2.1%) that subsequently developed PE (2.1%). The urinary Congo-red dot test was positive in 8.3% (1/12) and 2.9% (1/34) that delivered with PE at ≤2 and >2 weeks from assessment and in 0.2% (4/2094) of the unaffected pregnancies when the cutoff for Congo-red dye positivity was ≥5. The respective values when the cutoff used was ≥3 were 66.7%, 23.5%, and 16.5%, respectively. The intraclass correlation coefficient for the inter-observer reliability was 0.926 (95% CI 0.890–0.953, $p<.0001$) and Cohen's kappa coefficient for the intra-observer reliability was 0.904, $p<.0001$.

Conclusions: The performance of the urinary Congo-red dot paper test at 35–37 weeks' gestation in the prediction of PE is very poor.

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Introduction

Effective screening for preterm preeclampsia (PE) with delivery at <37 weeks' gestation can be provided by a combination of maternal demographic characteristics and medical history with measurements of uterine artery pulsatility index (UtA-PI), mean arterial pressure (MAP), and serum placental growth factor (PlGF) [1–5]. Administration of aspirin (150 mg/day from 11 to 14 weeks' gestation to 36 weeks) in the high-risk group reduces the rate of preterm-PE by 60–70%, but has no significant effect on term-PE [6,7]. Therefore, term PE is neither predictable nor is it preventable by first trimester screening and prophylactic pharmacological intervention. Although the adverse consequences of PE, in terms of maternal and fetal/neonatal mortality and morbidity, are more severe in preterm than in term PE, the overall contribution to adverse outcome may be the same because term PE is three times as common as preterm PE [4,5,8]. Effective screening for term PE is provided by a combination of maternal factors with measurements of MAP, PlGF, and serum soluble fms-like tyrosine kinase-1 (sFLT-1) at 35–37 weeks' gestation, with detection rate of about 75%, at screen positive rate of 10% [9–12].

There is some evidence that a potentially useful biomarker for the diagnosis and prediction of PE may be urinary Congo-red dye positivity, which indicates the presence of amyloid protein, an aggregate of misfolded proteins [13–18]. Several cross-sectional studies have investigated the incidence of urinary Congo-red dye positivity in women with PE, compared to

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unaffected pregnancies, and reported very high sensitivity and low false positive rate in the diagnosis of PE [13–16]. One study has also investigated the potential value of urinary Congo-red dye positivity in the prediction of subsequent development of PE. Sammar et al., examined the urine of 642 women at 11–13 weeks' gestation, which included 21 cases that subsequently developed early PE at <34 weeks' gestation and 84 that developed late PE at ≥34 weeks [15]. Congo-red dye positivity was observed in 33%, 16%, and 20% of cases of early PE, late PE, and unaffected pregnancies, respectively. Initial studies for assessment of Congo-red dye positivity necessitated a laboratory based analysis, but more recently a simple point-of-care test that provides results within a few minutes of testing was introduced [17,18].

The objective of this screening study is to determine the performance of the urinary Congo-red dot paper test at 35–37 weeks' gestation in the prediction of subsequent delivery with PE at ≤2 and >2 weeks after assessment.

Methods

Study design and participants

This was a prospective observational study in women attending for a routine hospital visit at 35^{+0} to 36^{+6} weeks' gestation at King's College Hospital, London, UK between June and November 2019. This visit included recording of maternal demographic characteristics and medical history, ultrasound examination for fetal anatomy and growth and collection of a midstream urine sample in a sterile bottle that was stored at −70 °C until analysis. Gestational age was determined by the measurement of fetal crown-rump length at 11–13 weeks or the fetal head circumference at 19–24 weeks [19,20]. The women gave written informed consent to participate in the study, which was approved by the NHS Research Ethics Committee.

The inclusion criteria for this study were singleton pregnancies that did not have PE at the time of their visit at 35^{+0} to 36^{+6} weeks' gestation and delivering a non-malformed live birth or stillbirth. We excluded pregnancies in women <18 years of age, those with serious mental health illness or learning difficulties, and those with fetal aneuploidies and or major abnormalities.

Outcome measure

The outcome measures were delivery with PE at ≤2 and >2 weeks after assessment. Data on pregnancy outcome were collected from the hospital maternity records or the general medical practitioners of the women. The obstetric records of all women with pre-existing or pregnancy associated hypertension were examined to determine the diagnosis of PE. This was based on the finding of hypertension (systolic blood pressure of ≥140 mmHg or diastolic blood pressure of ≥90 mmHg on at least two occasions four hours apart developing after 20 weeks' gestation in previously normotensive women) and at least one of the following: proteinuria (≥300 mg/24 h or protein to creatinine ratio ≥30 mg/mmol or ≥2 + on dipstick testing), renal insufficiency (serum creatinine >1.1 mg/dL or twofold increase in serum creatinine in the absence of underlying renal disease), liver involvement (blood concentration of transaminases >70 IU/L or twofold increase in the normal level), neurological complications (e.g. cerebral or visual symptoms), thrombocytopenia (platelet count <100,000/μL), or pulmonary edema [21].

Diagnosis of urinary Congo-red dye positivity

The basis of the test is that Congo-red bound to misfolded proteins in an aqueous solution migrates differentially on cellulose membrane, forming different dyeing patterns compared with a free Congo-red solution (Shuwen Biotech Co., Ltd., Deqing, China). A drop of urine is placed in a well containing a Congo-red solution and the mixture is then transferred to a cellulose membrane. The test produces a result within 3 min and the dyeing pattern is classified into one of eight stains in a reference panel (Figure 1). Based on the specifications by the company, the numbers 1–4 are classified as a negative result and the numbers 5–8 as positive. We also explored different cutoffs for the detection of PE. The operators had received training prior to undertaking the study and they were blinded to pregnancy outcome.

Reproducibility

The inter-observer agreement was evaluated by asking 10 different researchers to classify 50 randomly selected pictures of Congo-red dot paper tests using the reference panel described above. Similarly, the intra-observer agreement was evaluated by the same researcher who assessed 50 consecutive pictures of Congo-red dot paper tests at two different time points. All assessments for both inter- and intra-observer reliability were performed on stored images, with the researchers blinded to previous results.

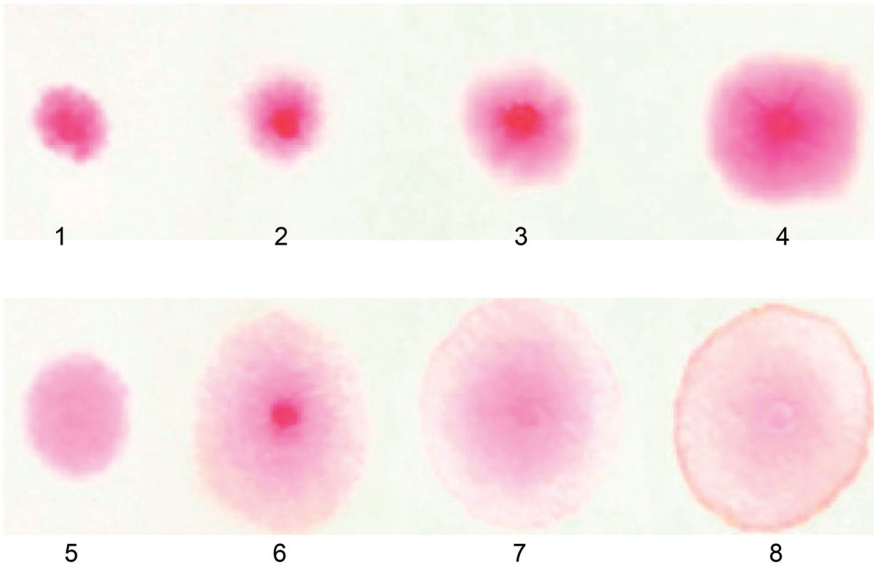

Figure 1. Staining patterns of urine Congo-red staining. According to the recommendations of the manufacturers numbers 1–4 are classified as a negative result and the numbers 5–8 as positive result.

Statistical analysis

Descriptive data were presented as medians and interquartile ranges for continuous variables and as numbers and percentages for categorical variables. Comparison between the outcome groups was done by χ^2 or Fisher's exact test for categorical variables and by the Mann–Whitney *U* test for continuous variables. The inter-observer reliability was assessed using intraclass correlation coefficients (ICCs) with 95% confidence intervals (CIs) and the intra-observer reliability was assessed by Cohen's kappa coefficient.

The statistical software package SPSS 27.0 (SPSS Inc., Chicago, IL) was used for all data analyses.

Results

Characteristics of the study population

The urinary Congo-red dot test was performed in 2140 women out of which 46 women (2.1%) developed subsequent PE. The characteristics of the study population are summarized in Table 1. In the PE group, compared to unaffected pregnancies the median maternal weight and body mass index were higher and there were more nulliparous women and less parous women without a history of PE in their previous pregnancy.

Performance of screening for PE

Table 2 shows the distribution of Congo-red dying patterns in PE and unaffected pregnancies. The urinary Congo-red dot test was positive in 8.3% (1/12) and 2.9% (1/34) of the cases that delivered with PE at $\leq$2 and $>$2 weeks from assessment and in 0.2% (4/2094) of the unaffected pregnancies. However, a cutoff score of $\geq$3 would detect 66.7% (8/12) and 23.5% (8/34) of the cases that delivered with PE at $\leq$2 and $>$2 weeks from assessment at a false positive rate of 16.5% (346/2094) (Table 2).

Reproducibility

The ICC for the inter-observer reliability was 0.926 (95% CI 0.890–0.953, $p<.0001$) and Cohen's kappa coefficient for the intra-observer reliability was 0.904, $p<.0001$.

Discussion

Main findings of the study

In this screening study at 35–37 weeks' gestation examination of the maternal urine for Congo-red dye positivity in asymptomatic women provided very poor prediction of subsequent development of PE when using the recommended score of $\geq$5. However, the prediction of PE was improved when the scoring cutoff was set $\geq$3, with detection rates for PE at $\leq$2 and $>$2 weeks of 67% and 24%, respectively, at false positive rate of 17%. The urinary Congo-red dot paper test was simple to perform and it provided highly reproducible results within 3 min of testing.

Table 1. Characteristics of the study population.

Characteristic	No preeclampsia ($n = 2094$)	Preeclampsia ($n = 46$)	p Value
Maternal age (years)	33.7 (20.5–36.9)	34.0 (30.0–36.7)	.967
Maternal weight (kg)	78.0 (70.9–87.2)	84.8 (74.9–92.0)	.021
Maternal height (cm)	166 (162–171)	166 (162–171)	.905
Body mass index (kg/m^2)	28.2 (25.7–31.5)	30.0 (27.0–33.8)	.024
Gestational age (weeks)	35.9 (35.6–36.1)	35.7 (35.4–36.0)	.345
Racial origin			
White	1509 (72.1)	35 (76.1)	.621
Black	323 (15.4)	9 (19.6)	.413
South Asian	115 (5.5)	1 (2.2)	.513
East Asian	65 (3.1)	1 (2.2)	1.000
Mixed	82 (3.9)	0	.420
Medical history			
Chronic hypertension	29 (1.4)	1 (2.2)	.481
Diabetes mellitus type 1	4 (0.2)	1 (2.2)	.103
Diabetes mellitus type 2	28 (1.3)	1 (2.2)	.470
SLE/APS	7 (0.3)	0	1.000
Smoker	26 (1.2)	2 (4.3)	.120
Family history of preeclampsia	104 (5.0)	4 (8.7)	.290
Method of conception			
Natural	1978 (94.5)	43 (93.5)	.740
In vitro fertilization	116 (5.5)	3 (6.5)	.740
Ovulation drugs	0	0	1.000
Parity			
Nulliparous	1110 (53.0)	38 (82.6)	<.0001
Parous, no previous preeclampsia	941 (44.9)	7 (15.2)	<.0001
Parous, previous preeclampsia	43 (2.1)	1 (2.2)	.619

SLE: systemic erythematosus lupus; APS: antiphospholipid syndrome.

Table 2. Detection rate and false positive rates for preeclampsia according to different scoring cut-offs to define urine Congo-red dye positivity.

Scoring cutoff	Preeclampsia ≤2 weeks ($n = 12$)	Preeclampsia >2 weeks ($n = 34$)	Unaffected ($n = 2094$)
8	0 (0.0)	0 (0.0)	0 (0.0)
≥7	0 (0.0)	0 (0.0)	0 (0.0)
≥6	1 (8.3)	0 (0.0)	1 (0.04)
≥5	1 (8.3)	1 (2.9)	4 (0.2)
≥4	1 (8.3)	1 (2.9)	9 (0.4)
≥3	8 (66.7)	8 (23.5)	346 (16.5)
≥2	11 (91.7)	18 (52.9)	1192 (56.9)

Data presented as n (%).

Comparison with results of previous studies

Most previous studies investigated urinary Congo-red dye positivity as a diagnostic test in pregnancies with established disease and reported a high performance in distinguishing between PE and unaffected pregnancies [13–18]. There is only one previous study that investigated the value of urinary Congo-red dye positivity in the prediction of subsequent development of the disease; screening at 11–13 weeks' gestation provided poor prediction of PE [15].

Two previous studies used a paper-based point-of-care test to diagnose PE within a few minutes of testing [17,18]. Rood et al. evaluated for PE 346 consecutive pregnant women who presented to a triage unit; PE was confirmed in 96 cases and the detection rate and false positive rates of Congo-red dye test were 80% and 11%, respectively [17]. Li et al. evaluated 1532 pregnant women hospitalized at 20–41 weeks' gestation; PE was diagnosed in 140 cases and the detection rate of preterm and term PE and false positive rates of the Congo-red dye test were 86%, 50%, and 3%, respectively [18]. In contrast to these two studies, we investigated pregnancies before the development of PE and found the predictive performance of the test to be very poor.

In a previous study at 35–37 weeks' gestation, we reported that effective screening for imminent PE developing within two weeks of assessment can be provided by a combination of maternal demographic characteristics and medical history with MAP, PlGF, and sFLT-1; the detection rates were 85% and 93%, at respective screen positive rates of 5% and 10% [12].

Strengths and limitations

The strengths of this study are first, examination of a large population of pregnant women attending for routine care in a gestational age range which is

increasingly being used for assessment of fetal growth and wellbeing, and second, use of a simple and reproducible method of assessing urine Congo-red dye positivity. The main limitation is that the study examined women before the clinical onset of PE and therefore the results are not comparable to those of studies in women with established PE.

Conclusions

On the basis of previous studies examination of maternal urine for Congo-red dye positivity in women with suspected PE may be useful, but our findings indicate that the test is not useful in screening for subsequent development of PE.

Disclosure statement

No potential conflict of interest was reported by the author(s).

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