Assessing the usability and clinical utility of the Congo Red Dot Test: A case control study

Gynuity Health Projects

NCT 02610972

Document date: January 20, 2018

Clinical.trials.gov: August 20, 2019

Protocol	
2.1 Objectives & Hypotheses	2.1 List the objectives. The aim of this study is: 1. To evaluate progression of urine congophilia postpartum by following patients diagnosed with preeclampsia and clinically healthy women 2.1.1 List the clinical hypotheses. • Preeclampsia is characterized by urine congophilia which can be followed in the postpartum period using the Congo Red test GV-005
2.2 Background	Preeclampsia is a pregnancy-specific hypertensive disorder and a leading cause of maternal and perinatal morbidity and death worldwide. WHO estimates that 16% of global maternal mortality (~ 63,000 maternal deaths annually) is due to preeclampsia alone [1]. The risk of maternal death is much higher in resource-limited settings. Often the factor responsible for major maternal and fetal morbidity is the failure to recognize preeclampsia in a timely manner to allow transfer to a health care facility for magnesium sulfate, steroid therapy and/or emergent delivery prior to eclampsia, maternal hypertensive stroke or fetal death [2]. Although preeclampsia is frequently diagnosed before delivery and may be the indication for an induced delivery or cesarean section, 75% to 89% of maternal deaths occur postpartum [3-4]. The purpose of this research is to provide a solution for screening and risk assessment for preeclampsia that could be used by women themselves throughout their pregnancy and the postpartum period. Traditionally, the diagnosis of preeclampsia relies on hypertension and proteinuria [5]. Recently, researchers discovered that women with preeclampsia excrete high amounts of misfolded proteins in urine [6]. This feature was found to increase in severity and precede by up to 10 weeks the onset of clinical manifestations of the disease. In principle, this phenomenon categorizes preeclampsia as a protein conformational disorder similar to Alzheimer's and prion disease [6]. Buhimschi et al hypothesized that the presence of misfolded proteins would cause urine from preeclamptic women to exhibit congophilia (affinity for the azo-dye Congo Red). Congo Red is a self-assembling dye which binds to misfolded proteins and amyloids. Based on these premises, the researchers designed, developed and validated a simple colorimetric urine diagnostic test for preeclampsia - the Congo Red Dot (CRD) [7-9]. In a large population of unselected pregnant women enrolled at a tertiary medical site in the U.S., the Congo Red test alone (cut-off ≥15%) had a sensitivity: 87.7% [95%CI: 83.5-91.2]; specificity: 84.9% [80.2-88.8]; positive likelihood ratio (LR): 5.8 [5.4-6.2]; negative LR: 0.14 [0.01-0.2] in predicting preeclampsia requiring medically indicated delivery (MIDPE). This was significantly better compared to the currently recognized clinical screening criteria for diagnosis of preeclampsia (Congo Red vs. blood pressure P=0.024; Congo Red vs. protein dipstick P=0.009). The combination of the Congo Red test and blood pressure was significantly better than the combination of blood pressure and dipstick proteinuria in predicting MIDPE (P=0.007). The CRD test may offer significant advantages over a urine protein dipstick. Urine dipsticks are associated with significant false positives and negatives which make them unreliable to detect or exclude proteinuria in pregnant women [10-12]. Applied in a low-risk population and in the absence of hypertension, urine protein dipstick is a

	poor predictor of preeclampsia [13]. Therefore, the test cannot be used to diagnose the need for transfer where transfer is difficult or unreliable. Urine congophilia could be used as a diagnostic and predictive marker for preeclampsia in asymptomatic women. Dipstick's colorimetric reagent (tetrabromphenol blue) detects only a fraction of the proteins in preeclampsia urine and does not detect proteins aggregated due to misfolding, a process closer to the pathophysiology of preeclampsia than total proteinuria [14]. The CRD test may substantially help in making appropriate decisions including expectant vs. active management or transfer at a point in gestation when preeclampsia cannot be diagnosed or predicted based on the current clinical criteria.
2.3 Study Design	***Component 1: Assessment of clinical utility of CRD in the postpartum period*** A case-control study (n=150) will evaluate the clinical utility of the Congo red test (GV-005) in following women with a clinical diagnosis of preeclampsia and clinically healthy women in the postpartum period. Women with preeclampsia (n=100) (cases) and clinically healthy women (n=50)(controls) who are hospitalized postpartum will be recruited for the trial. All women will be asked to provide a urine sample at delivery and daily during a woman's hospitalization, up to 5 days post-partum. The CRD test and a standard urine dipstick proteinuria test will be performed by a lab technician within 72 hours of sample collection. *Study 1: Eligibility Criteria* • Pregnant women admitted for delivery • Clinical diagnosis of preeclampsia (severe, mild or superimposed) including eclampsia (n=100) OR Clinically healthy (n=50) • Eligible to consent for research • Agree to comply with study procedures • Able to give informed consent
2.4 Study Procedures	**Study sites** The assessment of the diagnostic value of the Congo red test (GV-005) in the postpartum period will be conducted at Dhaka Medical College. **Screening** All women who present at the study site and meet the eligibility criteria (described above) will be given the option of participating in the study. Women who meet the eligibility criteria will be provided with a description of the study, detailing the study rationale, protocol, risks and benefits. Women who agree to participate will be administered the informed consent form by study staff. **Informed consent** Women who are eligible to participate based on clinician's assessment, will be given a consent form to review and sign. If the woman is unable to read, the form will be read and explained to her, and her consent will be indicated by a mark, such as the woman's thumbprint. Those who agree to participate will be asked a few background and demographic questions after signing the consent form. Information provided will be collected on the **Screening and Enrollment Form (Form 1).** **Study procedures** After signing the consent form, women will be asked to provide a sample of urine (10ml). Urine samples will be collected serially on each day that a woman is

	hospitalized for treatment up to 5 days postpartum. For the purpose of the study, the urine sample will be collected in a sterile 15ml conical container which will be transported to the research laboratory for processing of the urine samples, testing and storage. Urine samples will be refrigerated up to 72 hours or immediately processed. All samples will be labeled with a unique study ID number. Within 72 hours from the collection of the urine samples, study staff will perform the Congo red test (GV-005). The Congo red test (GV-005) is a simple dye test. Urine will be inserted into the sample well of the Congo Red Dot test cassette. After three minutes, study staff will record the results of the test, label the test with the patient's study ID number and take a photo of the completed test. In addition to the Congo red test (GV-005), the collected urine will be used to perform a urine proteinuria dipstick test.Only the results of the tests and a photo of the completed Congo red test will be retained in the study files. **Data Collection** Form 1: Screening Form. This form will determine a woman's eligibility for enrollment in the study. The form will also document basic demographic characteristics and obstetrical history. Form 2: Urine Sample Collection. This form will document each daily urine sample collection, clinical indications of preeclampsia, and results of laboratory analyses performed on that day. Form 3: Results of Congo red test GV-005 and proteinuria dipstick. The results of the Congo Red test and urine dipstick test laboratory analyses performed will be recorded here. The technician will also take a photo of each test and include this with the patient CRF. Form 4: Case Summary. Clinicians will complete a case summary form for each woman after she is discharged from the study. The form will summarize data recorded on other forms and confirm the number of samples obtained.
2.6 Study Duration	6 months
2.7 Statistical Analysis and Sample Size Justification	**Data Policy** At the end of the study, study investigators will have exclusive access to the data until the publication of the results in a journal. The consent form will include a clause for the woman to give permission for her anonymous data to be used for future research studies. <u>Variables/Time Points of Interest</u> The variables of interest are the outcomes of the Congo red test (GV-005) and the standard urine dipstick test. The two tests will also be correlated with a composite clinical severity score. The score will be composed of the following variables: blood pressure (systolic blood pressure >160mmHg or diastolic blood pressure >110mmHg), visual or cerebral symptoms, epigastric or right upper quadrant pain; pulmonary edema; oliguria, worsening lab parameters such as evidence of impaired liver function, increased maternal serum creatinine levels, and HELLP syndrome. <u>Statistical Methods</u> Statistical analysis will be performed with the aid of SPSS. The data will be

	presented as mean and standard error of the mean (SEM) for normally distributed data or as median followed by the range for skewed data. Statistical comparisons between groups will be performed using Student t or Mann-Whitney test or One way- or Two-way repeated analysis of variance (ANOVA) followed by post hoc Holm-Sidak tests as appropriate. Pearson product moment correlation will be used to estimate association between variables. A p value of <0.05 will be considered to indicate statistical significance. The two tests will be correlated with changes in the woman's condition. Comparative accuracy analysis will be performed using ROC analysis (MedCalc software). Additional statistical modeling techniques will be employed if needed. <u>Power/Sample Size:</u> As indicated above, there is no prior experience with the CRD in this clinical situation and little data on the results of the Congo red test (GV-005) postpartum. For the first component, we propose a sample size of 150 women hospitalized postpartum. The sample will include 100 women diagnosed with preeclampsia and 50 clinically health women. All women will be followed daily during their hospital stay until discharge.
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