Proteinuria Testing

Technology Opportunity Assessment

Prepared for the Merck for Mothers Program
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Summary

Preeclampsia and eclampsia (PE/E) are life-threatening disorders that can occur during a woman’s pregnancy, childbirth, and postpartum period. They are characterized by high blood pressure and protein in the urine (proteinuria). Existing proteinuria tests and technologies vary in accuracy, cost, simplicity, and feasibility, particularly when used in low-resource settings. Several new technologies show promise but are not yet available for widespread use. Combining two existing methods, dipstick protein and protein-to-creatinine ratio testing, may be the best available option to improve detection and save lives.

Statement of Need

Preeclampsia (PE) is a life-threatening disorder that only occurs during pregnancy, childbirth, and the postpartum period. It is characterized by high blood pressure (hypertension) and protein in the urine (proteinuria). Convulsions (fits) with signs of PE indicate eclampsia, although occasionally convulsions occur in the absence of hypertension and/or proteinuria. Preeclampsia and eclampsia (PE/E) are among the leading causes of maternal death and disability worldwide. The World Health Organization (WHO) estimates that PE/E account for at least 16% of maternal deaths in settings with low resources that lack the skilled providers and facilities required for prevention, identification, and management of the condition.¹ In most countries, PE/E ranks second only to hemorrhage as a specific, direct cause of maternal death. The risk of PE/E varies greatly depending on where a woman lives; the risk that a woman in a low-resource country will die of PE/E is approximately 300 times greater than that for a woman in a high-resource country.²

Positive maternal and perinatal outcomes for women with PE/E depend on how soon the condition is identified and how quickly the woman can access the recommended treatment package.¹ Secondary prevention for PE/E has focused on antenatal screening for high blood pressure and proteinuria as part of focused antenatal care (ANC).³ The presence of proteinuria changes the diagnosis from gestational hypertension to PE, and detection of proteinuria is key for making a diagnosis of PE.

There are currently several ways to measure protein in the urine, but these vary in accuracy, cost, simplicity, and feasibility. Measurement of protein in a 24-hour urine collection is considered the gold standard and more recently 12-hour collections (and even 2-hour collections) have been validated.³

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¹ The treatment package includes inpatient monitoring, anticonvulsant and antihypertensive therapy, optimal timing of childbirth, and skilled attendance at birth.
² Focused ANC is evidence-based, goal-directed care that is tailored to the gestational age of the pregnancy and individualized to each woman. It emphasizes quality of visits over quantity of visits and is conducted by a skilled health care provider. Goals of focused ANC include early detection and treatment of complications, prevention of problems, birth preparedness/complication readiness, and the promotion of healthy practices to help ensure a positive health outcome for the woman and her baby. Focused ANC is provided through a women-centered approach that values the dignity and value of each woman and her family.
However, such collections are not always feasible and may be impractical in low-resource settings. There is a need for a reliable, high-performing screening method that meets or approaches the WHO ASSURED criteria (affordable, sensitive, specific, user-friendly, rapid and robust, equipment-free, and deliverable to end-users). To impact morbidity and mortality from PE/E, the proteinuria test should not only improve accuracy of proteinuria measurements but should also potentially increase access to proteinuria measurement at the community level by simplifying how proteinuria is measured. Increased access to tests would greatly improve the identification of women with PE and increase their opportunity to access treatment packages; therefore, these interventions could ultimately increase the likelihood of survival for mothers with PE and their infants.

In this short analysis we discuss the benefits and shortcomings of tests and technologies currently used for testing for proteinuria and outlines several technologies in development but not yet available for widespread use. We also recommend investment in one methodology, a combination of dipstick protein and protein-to-creatinine ratio testing, which may improve detection and save lives, particularly in low-resource settings.

Technology Solutions Landscape

Although the measure of proteinuria is a poor predictor of either maternal or fetal complications in women with PE, it remains one of the essential criteria for the clinical diagnosis of PE. The diagnostic criterion for protein levels in PE is proteinuria of at least 300 mg of protein in a 24-hour urine collection (or 2+ on urine dipstick, or urine protein-to-creatinine ratio [PCR] of at least 0.3). A 24-hour urine collection is the standard test by which proteinuria is assessed and remains the gold standard.

Currently, protein is measured in the urine with a 2-, 12-, or 24-hour urine collection, and spot checks of random urine samples using dipsticks that measure protein, creatinine, and protein ratios, or creatinine and albumin ratios. There are proteinuria tests that are promising, such as a cartridge-based automated analyzer. Dipstick microalbumin:creatinine ratio testing has been suggested, but a review found that their use did not improve overall detection rates compared with automated or visual testing.

Protein dipstick tests

Point-of-care dipstick urinalysis for detection of proteinuria is the standard of care in most low-resource settings. However, urinary protein dipsticks have been shown to have low sensitivity and low specificity for urinary protein excretion over 24 hours. A prospective study found that the false-positive dipstick tests ranged from 7% at the 3+ level to 71% at the 1+ proteinuria level while false-negative rates were 7% for “nil” and 14% for “trace” proteinuria. Dipstick proteinuria was, however, significantly more likely to be correct (true positive/true negative when compared with 24-hour collection) if diastolic blood pressure was elevated > 90 mmHg (p = 0.032) and in the absence of ketonuria (p = 0.001). Even with automated
urinalysis, the false-positive rate for dipstick levels greater than or equal to 1+ is very high, particularly in the presence of ketonuria. In general, clinicians accept readings of greater than or equal to 2+ dipstick proteinuria to improve overall diagnostic accuracy for PE at the expense of a higher false-negative rate.

Some reasons for the low sensitivity and specificity include incorrect colorimetric interpretation with visual testing (automated dipstick urinalysis is a more accurate screening test than visual testing), inaccuracy due to the hour-to-hour variability of protein excretion, inaccuracy if the urine is dilute or concentrated, and dependency on observer interpretation. Other factors such as diabetes, presence of urinary tract infection, severe anemia, kidney disease, presence of vaginal secretions or pus from sexually transmitted infections, or maternal age may also confound the diagnostic result. For example, a person’s state of hydration and diuresis can influence the end result (other related factors include time of day, stress level, etc). False-negative results may be seen when the urine volume is high and the urine is dilute, whereas false-positive results may be seen when urine volume is low and it is concentrated. Finally, the dipsticks are susceptible to deterioration from humidity and temperature.

The cost of dipsticks—approximately US$.05 to US$.10 per strip—is an additional barrier. Manufacturers abound, both in the United States and internationally. Dipstick assays are available as part of a quantitative automated system such as the Clinitek® assay or Atlas® assay, or as semiquantitative manual reagent strips like the Multistix PRO® 10SG.

There is no standardization, however, between manufacturers. A retrospective analysis of 2,224 dipstick measurements from 296 patients showed a wide range of sensitivity and specificity of the Clinitek, Atlas, and Multistix 10SG dipstick assays; fluctuations depend on the threshold of detection (e.g, 1+, 2+, etc.). Interestingly, the same study showed that the manual dipstick (Multistix 10SG) performed as well as, if not better than, the automated systems (Clinitek and Atlas). Another option would be dipsticks capable of simultaneously measuring protein and creatinine (PCR), but these require additional testing or adaptation to make them more accurate, simple, and affordable.

**Spot urine PCR**

Studies have demonstrated that measurement of PCR in a spot urine sample accurately reflects 24-hour urinary collection for detection and quantification of protein in the urine and is simpler, faster, and a more useful method for the diagnosis of significant proteinuria. A Urine PCR of greater than or equal to 0.19 yields a sensitivity of 90% and a specificity of 70% for detecting proteinuria. The slightly higher values found for sensitivity compared with specificity suggest that the ratio test might be more valuable as a rule-out test. For a PCR of 130 mg/g to 150 mg/g, sensitivity ranged from 90% to 99% and specificity ranged from 33% to 65%; for a PCR of 300 mg/g, sensitivity ranged from 81% to 98% and specificity ranged from 52% to 99%; for a PCR of 600 mg/g to 700 mg/g, sensitivity ranged from 85% to 87%, and specificity ranged from 96% to 97%. This suggests that random PCR determinations are helpful primarily when they are below 130 mg/g to 150 mg/g.

iv. Clinitek, Atlas, and Multistix PRO are registered trademarks of Siemens Aktiengesellschaft.
The Multistix PRO strips—are the only urinalysis products providing PCR on the market. Among the variety of tests strips, three strips are used for protein, albumin, and creatinine ratios: (1) the protein-low pad, specific for albumin (sensitivity, 80 mg/L to 150 mg/L albumin), (2) the protein-high pad (sensitivity, 300mg/L protein), and (3) a pad for establishing urinary creatinine levels. In an evaluation of 881 freshly voided urine samples, the Multistix PRO 10LS demonstrated a higher sensitivity and specificity for albumin:creatinine ratios (ACR) than PCR determinations.13 While ACR sensitivity and specificity were found to be 96% and 92% to 94% respectively, when using either visual or the Clinitek automated system, for PCR the values were lower at around 75% and 96%, respectively. Therefore, ACR performs more robustly than PCR, and the visual evaluation can be as effective as using an automated system. The study, however, lacks correlation to PE, and the results merit a prospective study to determine positive and negative predictive values to PE.

Spot urine ACR

An additional test is the spot ACR. Albumin is the predominant protein in the vast majority of proteinuric kidney diseases, including hypertension. Albumin measurement offers greater sensitivity and improved precision for the detection of lower but clinically significant levels of proteinuria compared to total protein. In addition, ACR holds an advantage of not being time dependent as the “ratio cancels out the time factor and allows for an estimation of 24-hour protein excretion if stable renal function is assumed.”14 The ratio to creatinine also helps adjust for variances in protein concentration.

Unfortunately ACR testing, such as the Biuret method, needs to be performed in a clinic and is not available for home testing or field use. Studies on ACR are also conflicting in terms of sensitivity, specificity, and correlation to the 24-hour urinalysis standard.15 Some studies have suggested that ACR is not confounded by maternal age and gestational periods and has been correlated with adverse maternal and fetal outcomes.16 However, several recent studies determined a lower correlation for substituting ACR for timed collections and prompted researchers to discourage use of ACR in place of 24-hour diagnosis of total urine protein.17

Although ACR testing can offer a significant improvement over conventional dipsticks, pregnancy-specific thresholds must be used. Researchers have also found that dipstick assessments of ACR do not improve the detection rate of significant proteinuria.12 Therefore, more work is needed to clarify the utility of ACR in PE detection. As mentioned earlier, a worthwhile example would be a greater examination of the use of Siemens Multistix PRO 10LS for PE. Alternatively, the DCA Vantage™ Analyzer system by Siemens is an automated system specific for quantifying microalbumin:creatinine ratio; the high cost could make it inappropriate for low-resource settings.

Proteinuria pen

Harshad Sanghvi and his team at Jhpiego, a nonprofit global health affiliate of Johns Hopkins University, have been developing a proteinuria pen for low-resource settings.18 The pen dispenses ink which creates a colorimetric (color) change when in contact with the target albumin protein. A special substrate paper or cloth (also to be supplied to the end-user) is used to absorb urine from a patient. The proteinuria pen and
protein dipsticks both use tetrabromophenol blue. Therefore, sensitivity and specificity of the proteinuria pen will likely be low and variable; however, the cost may be comparable, if not better, than protein strips.

**High-tech solutions**

New and more sophisticated (but often costly) methods for detection of urine protein are also being developed. High performance liquid chromatography (HPLC) provides higher sensitivities and specificities than available tests because the technique can measure both immunoreactive and immunounreactive intact albumin, and size exclusion allows quantification of fragmented albumin.

Other screening methods similarly use costly reagents and/or costly instrumentation (i.e., fluorescence immunoassay, enzyme-linked immunosorbent assay, radioimmunoassay, immunoturbidimetric assay, immunonephelometry, and chip electrophoresis). One elegant development is the use of thin-layer liquid crystals as a readout system, potentially providing accurate quantitation of urine protein. Another idea under development is the use of a piezoelectric immunosensor read out of electrical impulses gathered from antibody-antigen complexes (or in this case, albumin-specific antibodies) that form on a physico-chemical substrate/sensor (transducer). Although these developments are not currently suited for use in low-resource settings, they may lead to innovative diagnostic solutions that can be applied more widely.

**Gap Analysis**

In summary, there are numerous methods available to measure proteinuria during pregnancy. Measuring protein in a 2-, 12-, or 24-hour urine collection is the most accurate, but such collections are not always feasible and may be impractical in low-resource settings. Newer, sophisticated developments in protein measurements generally involve cost-prohibitive instrumentation and are not yet well understood. There are promising urinalysis devices, such as a cartridge-based automated analyzer and creatinine/protein dipsticks, but these require additional testing or adaptation to make them more accurate, simple, or affordable. The current WHO diagnostic criteria for PE are based on urinary protein levels as measured by dipsticks.

One way problems with dipstick analysis can be addressed is by measuring the urine’s PCR as a rule-out or confirmatory test. Using the PCR in this way could maximize the use of this relatively more expensive test and minimize problems associated with it, including lack of reliability for quantifying proteinuria during pregnancy.

Getting approval from obstetric and midwifery associations as well as incorporating the PCR spot tests into national and international protocols for the diagnosis of PE will require clinical evidence and justification of cost-effectiveness. Once clinical protocols have been changed, considerable efforts will need to be made to update clinical providers and integrate new products into essential medicines lists and national procurement and logistics systems.
Investment Opportunity

Technology that improves the accuracy of proteinuria assessment has the potential to improve safety and save the lives of women and their infants by reducing incorrect diagnoses and increasing correct diagnoses and treatment. The PCR on a random urine specimen can be used in conjunction with urinary protein dipsticks to confirm proteinuria in hypertensive women in situations where urinary dipsticks are less reliable—proteinuria less than 2+ and cases when the woman has ketonuria (is dehydrated). This would improve accuracy of dipstick testing for urinary protein without substantially changing existing diagnostic criteria and protocols or increasing costs for urine testing.

Dipsticks for checking proteinuria and PCR already exist. However, the investment opportunity lies in seeking a more affordable PCR test and developing clinical protocols and algorithms that include the use of urinary protein dipsticks with spot PCR as a rule-out test. It will be necessary to gather clinical evidence, cost-effectiveness data, and information on programmatic implications to support the use of urinary protein dipsticks with spot PCR testing to improve screening in the following situations:

- Diastolic blood pressure is greater than or equal to 90 mm Hg, the woman appears well hydrated (or does not have ketonuria), and dipstick proteinuria is less than 2+.
- Diastolic blood pressure is greater than or equal to 90 mm Hg and the woman appears dehydrated (has ketonuria), regardless of dipstick proteinuria.
- Diastolic blood pressure is greater than or equal to 90 mm Hg, the urine appears very dilute and dipstick proteinuria is less than 2+. 
References


