

PROTEINURIA IN PREGNANCY – JUST WHAT IS SIGNIFICANT?

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INTRODUCTION

Apart from the measurement of blood pressure, dipstick analysis of urine for protein is the most commonly performed antenatal screening test. The presence of proteinuria is seen as a possible indication of many complications in pregnancy, from urinary tract infection to chronic renal disease and it remains central to the diagnosis of pre-eclampsia in a hypertensive pregnancy. It has both diagnostic and prognostic implications when it is found though the optimal methods for detection and quantification in primary and secondary care remain controversial.¹

Within this review the use of proteinuria in screening programs for preeclampsia will be discussed. The emphasis on this disorder reflects the number of studies completed with regard to this question and to date there are very few studies that have looked at either gestational proteinuria or proteinuria detection in uncomplicated pregnant populations.

Despite intensive investigation into the underlying pathophysiology of pre-eclampsia, the disease remains poorly understood. The definitions of pre-eclampsia, of which there are many, rely on measurements of blood pressure, proteinuria, or a combination of the two.²⁻⁵ There is great inconsistency within the published obstetric literature as to what definition of pre-eclampsia should be employed,⁶ and as such this leads to difficulty in comparing the results of clinical trials and also to difficulties identifying women at risk from this condition. While there is debate amongst obstetricians over what level of blood pressure constitutes significant hypertension in pregnancy, there is universal agreement that the development of proteinuria in a hypertensive pregnancy is a significant finding. The controversy for proteinuria is how to detect it and at what level of proteinuria should action be taken.

THE KIDNEY IN THE NON-PREGNANT WOMAN

In non-pregnant women daily urine protein excretion averages 20–80 mg/day (with an upper limit of 150 mg/day). This is 40% albumin, 15–20% immunoglobulin (IgG 5–10%, IgA 3% and light chains 5–10%) and the remainder is Tamm-Horsfall glycoprotein derived from the tubules and the lower urinary tract.⁷ The movement of protein across capillary walls in most vascular beds is limited by mechanical, electrochemical and haemodynamic restrictions. However, movement in the glomerular capillary is influenced by protein size, configuration and charge. The protein filtered by the glomerulus is reabsorbed, through proximal tubular epithelial cells by endocytosis and lysosomal degradation. This reduces protein excretion but is a process that is easily saturated and therefore can be overwhelmed by a high glomerular protein load which may result in swelling and rupture of the lysosomal particles within the epithelial cells. The resultant interstitial swelling and fibrosis this causes are common in chronic proteinuric renal disease.⁸

THE KIDNEY IN UNCOMPLICATED PREGNANCY

There are striking functional alterations of the urinary tract both anatomical and physiological during pregnancy. These combined with the changes in extracellular volume are fundamental to understanding the kidney and for the accurate interpretation of serological measurements in pregnant women.

The gravid kidney enlarges its length on x-ray increasing by approximately 1 cm whilst the calyces, renal pelvices and ureters all dilate markedly.⁹ The aetiology of this dilatation is disputed, but it is likely that both humoral and obstructive causes are involved. Marked alterations in renal haemodynamics also occur with glomerular filtration rate (GFR) and effective renal plasma flow (ERPF) increasing by approximately 50% compared to prepregnancy values. Creatinine clearance has significantly increased by 4 weeks gestation, peaked at 9–11 weeks gestation and then is sustained until the 36th week of gestation. In the last four weeks of pregnancy creatinine clearance reduces by 15–20%¹⁰ These changes have important clinical consequences with mean serum creatinine and urea both being significantly lower than in the non pregnant state.¹¹

Renal handling of protein

In pregnancy the renal haemodynamic changes mean that greater quantities of colloids and solute pass by the glomerular barrier per unit time. In addition there are changes in glomerular permeability and altered tubular reabsorption of filtered proteins that may result in increased excretion of protein. It is normal in pregnant women for total protein excretion (TPE) and urinary albumin excretion (UAE) to be significantly elevated after 20 weeks gestation. Studies of protein excretion are limited but the currently accepted upper limits of normal are 300 mg/24 hours for TPE and

20 mg/24 hours for UAE.¹² Some authors have suggested that a threshold for pregnancy should be lower at 200 mg/24 hours for total protein excretion¹³ but it is the 300 mg threshold that remains in use for clinical definitions of proteinuria.

Altered tubular reabsorption in pregnancy can be demonstrated by measuring urinary excretion of low molecular weight proteins which have identical plasma concentrations in non-pregnant and pregnant women and which are freely filtered by the glomerulus. These proteins including for example Retinol Binding Protein, β_2 -microglobulin, and α_1 -microglobulin have been compared with albumin whose excretion is affected by the size and charge permselectivity of the glomerular barrier. Their increased excretion in uncomplicated pregnancy is due to a reduced reabsorption capacity in the proximal tubule and therefore despite the fact that the predominant protein in pregnant urine is albumin there is a small tubular contribution to the increase in TPE in uncomplicated pregnancy.¹⁴

THE KIDNEY IN PRE-ECLAMPSIA

Extensive changes occur in the renal system in pre-eclampsia. As part of the “end organ pathology” preeclamptic glomeruli undergo structural changes with pronounced endothelial vacuolisation and hypertrophy of the cytoplasmic organelles first defined as glomerular endotheliosis by Spargo et al¹⁵ Such alterations were thought to be pathognomonic of preeclampsia but it is now accepted that no one feature of glomerular endotheliosis is specific to preeclamptic nephropathy. Under light microscopy the glomeruli appear swollen and bloodless and in contrast to the nephropathy seen in glomerulonephritis and diabetic nephropathy, cellular hyperplasia is absent.¹⁶ There is however, mesangial and endothelial cell hypertrophy with encroachment on the capillary lumen. In the largest postmortem study to date, 50% of affected glomeruli demonstrated extreme swelling with herniation of capillary loops into the proximal tubule.¹⁷

The characteristic nature of the preeclamptic lesion is best revealed by ultrastructural changes which are localised to the endothelial cells. There is extensive enlargement of the cytoplasmic organelles and the lysosomes undergo vacuolisation due to the accumulation of free neutral lipids. The endothelial fenestrae are difficult to discern and there are subendothelial granular deposits which are thought to contain either immune complexes or fibrin related products.¹⁸ The exact content of the deposits remains controversial and it may be that this varies. Other electron dense membrane basement membrane proteins such as laminin, type IV Collagen, fibronectin and proteoglycan may be represented.¹⁹ The epithelial cells (or podocytes) undergo non-specific changes that are seen in nephritic syndrome of any cause and are probably due to an excess of protein movement across the cell secondary to disordered glomerular wall permeability.

The morphological changes of preeclamptic nephropathy begin to resolve 48 hours following delivery and complete resolution is common by 4–6 weeks postpartum²⁰ though studies have described resolution taking as long as six months.²¹

Fisher et al²² described the postpartum renal biopsy appearances from 176 women with a clinical diagnosis of preeclampsia. They found that only 55% of their group had the renal lesion of preeclampsia alone. In this group of 96 women, 79 were primiparous. Thus if glomerular endotheliosis is pathognomonic of preeclampsia, then the clinical diagnosis could be judged to be incorrect in 24% of primiparous women and 76% of multiparous women.²² This highlights the importance of correctly phenotyping study groups when looking at the hypertensive disorders of pregnancy and many studies have in the past relied upon non-standard or sub-standard definitions.

Haemodynamic changes

There have been more than 20 studies looking at glomerular filtration rate and effective renal plasma flow in pre-eclampsia and uncomplicated pregnancy. Ten of these studies have also had postpartum data. All studies showed a reduction in GFR in preeclampsia (mean reduction 32%) compared to late pregnancy controls and all but one of the studies demonstrated a reduction in ERPF (mean reduction 24%). In the postpartum studies nine from ten studies also demonstrated a reduction in GFR of 22% and ERPF of 22% compared to non-pregnant controls.²³ From clearance studies of neutral dextrans (which are neither secreted or reabsorbed) one can begin to study GFR as this will be directly related to the dextrans' clearance. The GFR is ultimately dependent upon ERPF and a number of other properties of the glomerular barrier and the pressures which act across it. These can be summarised as

- 1 Starling forces (acting across the glomerular wall)
- 2 The net oncotic pressure
- 3 The transglomerular hydrostatic pressure difference (ΔP)
- 4 The ultrafiltration coefficient (K_f), the product of the available surface area for filtration and the porosity of the surface.

As ΔP is not thought to change (at least in uncomplicated pregnancy)²⁴ and hypoalbuminaemia will tend to reduce intraglomerular oncotic pressure favoring ultrafiltration it suggests that the reduction in GFR seen in preeclampsia must be due to either a reduction in K_f and/or ERPF.

In considering ERPF, any drop in systemic arterial pressure is regulated by the afferent glomerular arteriole. Its high sphincter tone, leading to high renal vascular resistance is responsible for the reduction in ERPF consistently seen in preeclampsia.²³ This high afferent arteriolar resistance may serve a protective role in preeclampsia as it will effectively autoregulate intraglomerular pressure (ΔP) which is a critical determinant of glomerular ultrafiltration. This will therefore be maintained within narrow limits despite the presence of systemic hypertension.

Although renal haemodynamics are compromised by preeclampsia, serum creatinine and 24-hour creatinine clearance are relatively insensitive markers of disease severity and significant changes will not be seen until greater than 50% of the nephron population is lost.

Renal handling of protein

Most classification systems for the hypertensive disorders of pregnancy have repeatedly placed emphasis on the appearance and progression of proteinuria above a threshold of ≥ 300 mg/24 hours to separate gestational hypertension from preeclampsia. This threshold therefore defines “significant proteinuria.” Although it is now accepted that proteinuria is not inevitable in preeclampsia it still remains a cardinal sign of the syndrome and one of the two features, along with hypertension that clinicians use to screen the pregnant population for early detection of the disease.

In preeclampsia the glomerular barrier is certainly altered and there is an increased excretion of protein including albumin. When TPE exceeds 1 g/24 hours, tubular protein reabsorption will be saturated and individual proteins excretion rates will be related to their molecular weights. The term selective proteinuria is used when large protein molecules are retained and non-selective proteinuria is used when the glomerular barrier loses this ability. The proteinuria of preeclampsia is considered to be non-selective.²⁵

PROTEINURIA AND CLINICAL OUTCOME

The incidence of proteinuria (and/or hypertension) arising in pregnancy varies according to the definition²⁶ and to parity, age and underlying medical disease,^{27,28} but in most populations it will occur in more than 10% of pregnant women. Proteinuria can be caused by the pregnancy itself, or may exist from before conception (being unrelated to the pregnancy). However as pregnancy may be the first point of medical contact for many women, pre-existing proteinuria may be first diagnosed at this time. Hypertension with proteinuria is clearly associated with increased fetal and maternal morbidity, especially if occurring remote from term.^{29–32} In contrast women with mild chronic hypertension without proteinuria have similar pregnancy outcomes to non-hypertensive women but, if chronic hypertension is complicated by the development of proteinuria, there is a 10% incidence of placental abruption, a 33% incidence of fetal growth restriction and a perinatal mortality rate of up to 24%.³³

The current threshold that defines “significant proteinuria” is based on reference data from the “normal” pregnant population. The determination of an upper centile (95th to 99th) to define an “abnormal population” has previously been utilised.^{10–13} Such studies have varied in their methodology, but the consensus reached is that up to 300 mg protein/24 hours is physiological.^{31,32} Only the study of Kuo et al has suggested that the proteinuria of pregnancy is no more than in non-pregnant subjects in the absence of hypertension. They found the 99th centile at 17–20 weeks gestation to be 300 mg/24 hours and at 33–36 weeks gestation to be 200 mg/24 hours.¹³ There are no studies that derive thresholds related to the prediction of clinical outcome.

The evidence surrounding levels of proteinuria and clinical outcome is conflicting. Some studies suggest a proportional link between the level of proteinuria and adverse clinical outcome. Page et al³⁴ in a prospective study of almost 13,000 pregnant women

found that "significant proteinuria," (defined as 2+ or more on dipstick analysis) was associated with an increase in stillbirth rates, fetal growth restriction and neonatal morbidity, when associated with hypertension. No information regarding the false positive or false negative rate for proteinuria testing was given. Brown et al report this threshold (2+ dipstick) to be associated with up to 50% false positive rates.³⁵

Ferrazzani et al³³ studying a group of 444 hypertensive pregnancies (superimposed and de-novo pre-eclampsia) where a definition of proteinuria was taken as $\geq 1+$ on dipstick or ≥ 0.3 g/L, noted higher serum uric acid levels, lower birthweights and birth percentiles, and more deliveries before 37 weeks gestation if hypertension was associated with proteinuria. Their regression analysis of laboratory protein assay (unspecified) and relative birthweight showed a negative correlation ($R = -0.21$, $p < 0.05$). Despite this correlation they fail to report their tests performance at this threshold (0.3 g/L) as a predictor of the clinical outcomes measured. Furthermore, Chua and Redman have reported that when the level of proteinuria exceeds 5 g/24 hours, delivery is usually required within 2–3 weeks.³⁶

Other studies suggest that it is the presence of proteinuria rather than the severity, which is associated with poorer outcomes. There is evidence that even the finding of trace proteinuria in pregnant women with hypertension is associated with an increase in adverse outcome.³⁷ When screening for proteinuria, it has been suggested that a test should be associated with a low false negative rate (high sensitivity) to avoid missing significant proteinuria and hence pre-eclampsia.³⁸ There has always been controversy over the difficulty of interpreting 'trace proteinuria.' Lowering the threshold for dipstick proteinuria to 'trace' or 15 mg/dl may improve detection and reduce false negative rates. As such, the allocation of this finding as test positive or test negative has a significant effect on the sensitivities and specificities for the detection of significant proteinuria.

Waugh et al³⁹ have presented data showing this effect. When using either visual or automated urinalysis, if 'trace proteinuria' is considered test positive then the sensitivity is improved (71% versus 51%) but the specificity significantly reduced (46% versus 78%), resulting in a lower positive predictive value (52% versus 64% for visual testing and 63% versus 78% for automated testing). Thus 'trace proteinuria' cannot be recommended as a positive screen for proteinuria for either visual or automated testing, as it significantly increases the number of false positive results and makes the test ineffective in clinical practice.

Some older studies which have related increasing dipstick proteinuria to worse clinical outcome, are both retrospective and fail to appreciate the potential for very high false positive and false negative rates which may bias the results. As 4+ protein is much less likely to be a false positive result than 1+ protein the results of these studies could be interpreted as saying the more likely the proteinuria is to be genuine the greater the chance of adverse outcome rather than the assumption that the adverse outcome is increased by higher dipstick values. There is further evidence to support this. In a prospective study Schiff et al⁴⁰ found that, in patients with severe disease who are managed conservatively, there is an increase in protein excretion (of >2 g/24 hours) and at least one-third of women will cross the 5 g/24 hour threshold.

There is no evidence to suggest an increase in adverse maternal or fetal outcomes in relation to the degree of proteinuria and therefore, the degree of proteinuria alone does not equate to clinical outcome and cannot be suggested as a clinical indication for delivery.

As such these studies suggest that it is the presence of proteinuria that confers increased maternal and perinatal morbidity, not necessarily the severity and therefore an accurate method of screening for proteinuria is vital if we are to improve pregnancy outcomes in affected women.

If we are therefore to conclude that it is the presence of proteinuria that confers increased perinatal risk rather than the absolute amount or relative increase in protein excretion we should question the threshold that is employed to define significant proteinuria.

The studies of Higby et al¹² and Kuo et al¹³ have attempted to define what is abnormal in relation to a centile definition in an uncomplicated population. As such this definition is likely to include some individuals who despite being above the 95th centile for protein excretion are otherwise uncomplicated. The presence of gestational proteinuria may no doubt contribute to false positive referrals to obstetric day units and potentially lead to iatrogenic intervention.

Studies by Waugh et al⁴⁰ have attempted to define thresholds of proteinuria in hypertensive women that are likely to be useful in predicting adverse outcome. Their data support the findings of Schiff et al⁴¹. In their prospective study of 197 women who collected 24 hour urine specimens they found that the threshold of 300 mg/24 hours was not predictive of adverse outcome. They looked at 4 adverse outcomes common to preeclampsia (severe hypertension, birthweight <10th centile, preterm delivery and a composite biochemical outcome of elevated liver enzymes and/or serum creatinine and thrombocytopaenia). If positive likelihood ratios (LR+ve) are calculated at the 300 mg/L threshold for each clinical outcome then none achieve statistical significance. At the 500 mg/L threshold the positive likelihood ratios did achieve statistical significance for severe hypertension (LR+:1.51 95%CI 0.99–2.28) and for birthweight <10th centile (LR+: 1.72 95%CI 1.11–2.66). They also found that at the 500 mg/24 hour threshold the positive likelihood ratio was significant for severe hypertension (LR+: 2.15 95%CI 1.07–4.34), birthweight <10th centile (LR+: 2.79 95%CI 1.4–5.54) and biochemical disease (LR+: 2.47 95%CI 1.22–5.01) when different proteinuria assays are tested which is discussed further below.

This study suggests that it is the presence of proteinuria that confers an increase in perinatal risk on the pregnancy. Furthermore, whilst 300 mg/24 hours may be above the 95th centile for an obstetric population it is a threshold of 500 mg/24 hours that is more predictive of adverse outcome.

DIPSTICK URINALYSIS AND THE ASSESSMENT OF PROTEINURIA

Despite the prognostic role of proteinuria, it remains a poorly assessed clinical sign in pregnancy. Although accepted by common consensus amongst obstetricians that

proteinuria is most reliably measured by biochemical assay of a 24 hour urine sample, this is an impractical screening test and the most commonly performed front line investigation for proteinuria is semi-quantitative dipstick urinalysis. There are several different dipsticks available from several manufacturers and unlike sphygmomanometers for the measurement of blood pressure, the dipsticks remain unvalidated with as yet no recommendations for the assessment and validation of new dipsticks bought into the market. Clinically urinalysis is performed in a largely unsupervised fashion by untrained doctors, midwives, nurses, auxiliaries and medical students.

The primary reason for this dependence upon dipstick urinalysis performed upon randomly voided urine is the relative low cost and ease with which it can be performed. In addition it is widely believed that the results of dipstick urinalysis correlate with the results of total protein measurements performed on 24-hour urine collections and this has led to an inevitable dependence on the dipstick for both clinical decision making and research definitions of preeclampsia. Thus women may be classified as having significant proteinuria on the basis of a variety of tests.

Dipstick Urinalysis and 24-hour urinary protein excretion

It is the widely held belief of many clinicians that 1+ proteinuria corresponds to 300 mg/24 hours total protein excretion. This assumption is flawed in that 1+ corresponds to a protein concentration of 30 mg/dl. Thus unless urine output is 1000 mls there will be an inevitable false positive and false negative rate. There have now been several studies investigating the relationship between semiquantitative dipstick urinalysis on random voided urine samples and a subsequently collected 24-hour urine sample. Kuo et al in 1992¹³ found a poor correlation with 1+ dipstick proteinuria and subsequent 24-hour protein estimation. They report a false positive rate of 18% and a false negative rate of 40%. In 1994 Meyer et al⁴² in a further retrospective study found that in 300 samples of urine from hypertensive pregnant women, 66% of the women had false negative dipstick urinalysis if significant proteinuria is defined as ≥ 300 mg/24 hours. In the same series they report a false positive rate of 26% at the 1+ level. The series of Brown et al in 1995³⁵ produced false negative results of 8–18% and a very high false positive rate of 67% with 1+ scores. To explain the persistent false positive rate of 1 in 4 they suggest that the dipstick is too sensitive at the 1+ threshold and that as such it is useful for the management of pre-eclampsia as it will minimise the false negative results (missed proteinuria) but the test will be incorrect at least half of the time. Waugh et al's data⁴³ on 197 urines from hypertensive women however, found a high false negative rate where up to 65% of women with <1+ proteinuria on dipstick analysis had significant proteinuria.

All of this data suggests that the correlation between dipstick urinalysis and 24-hour protein estimation is at best imprecise. False positive results may result in over investigation and intervention whereas the potentially more serious issue of a false negative result may place a woman and her pregnancy at risk. There are several

reasons why such a poor correlation may exist. These include observer error, the characteristics of the dipstick tests, the units of protein estimation, the differing nature of the urine specimens involved, as well as possible variation in the “gold standard” assay employed in the laboratory setting.

More recently the poor performance of dipstick urinalysis was highlighted in a systematic review. The review focused on prospective observational studies or comparative cross-sectional studies in which the results of the diagnostic test of interest were compared with the results of a ‘reference standard’. The population of interest was pregnant women. This included uncomplicated pregnancies, women with hypertension and pregnancies complicated by pre-existing renal disease. The diagnostic intervention was a point of care test for urine protein. The diagnostic reference standard was a laboratory assay for urine protein preferably from a 24 hour urine sample. The advantage of this approach is that studies are assessed and compared for methodological quality as well as their reported results. From over 1500 papers identified in the initial searches the number suitable for inclusion in the review was only seven. The review of the literature demonstrated that the accuracy of dipstick urinalysis using a 1+ threshold in the prediction of significant proteinuria is poor. Neither a positive or negative dipstick result (1+ threshold) substantially raises or lowers the probability of having clinically significant proteinuria thereby limiting its usefulness in informing clinical decision making. Accuracy may be improved at higher thresholds (greater than 1+ proteinuria), but available data is sparse and of poor methodological quality. It is not therefore possible to make meaningful inferences about accuracy at higher urine dipstick thresholds.⁴⁴

It is, however, not possible for dipstick urinalysis to be removed from antenatal care without a viable alternative being sought to replace it. As such without improvements we continue to rely on this imprecise tool and interpret the results with caution. Some women with proteinuria will certainly be missed and in some there will be the anxieties of a positive result which subsequently proves to be false. There have been several studies reported that have explored the sources of error with proteinuria detection with a variety of methods and as such these studies have led to modifications of dipstick techniques that offer real improvements in accuracy. These studies and the benefits of automation are discussed below.

Dipstick Urinalysis and Observer error – the role of automation

There have been many reports of the contribution made through observer errors to the measurement of blood pressure in pregnancy. Great emphasis is now placed on correct measurement technique. Given the wide variety of individuals who dipstick urine with little or no training it is of little surprise that Bell et al⁴⁵ have shown that nursing auxiliaries record a higher number of false positives than do midwives (47% versus 17%). This study was performed on a series of predetermined solutions of human serum albumin at ‘non-proteinuric’ and ‘proteinuric’ concentrations. To explore whether this observation might be due to a lack of training, a further study

was performed using untrained laboratory staff who initially achieved a false positive rate of 35%. Following a period of minimal training and guidance in the interpretation of the dipstick, this was reduced to a false positive rate of 5%.⁴⁵

A similar improvement was reported by Saudan et al⁴⁶ with the introduction of automated technology to read the dipstick (52% false positive with visual testing versus 26% with automated testing). Automated technology has been evaluated outside pregnancy with encouraging results (only 2–3% incidence of false positive results).⁴⁷ When evaluated for accuracy in pregnancy using both test solutions and clinical specimens automated dipstick readers demonstrate very good intra- and inter-observer variability.^{46,48}

This may well imply that when specimens are tested from hypertensive women the nature of the proteinuria itself can affect the sensitivity of the dipstick. This argument is not without some precedence as we know from electrophoretic analysis of urine in pregnancy, that there are marked alterations in the constituent proteins within the urine.⁴⁹ As such, the ability of a dipstick to detect all types of proteinuria may vary with the condition being tested (see below).

Waugh et al have performed a study comparing visual dipstick testing with automated methods.⁵⁰ They performed receiver operating characteristic curves and found significant improvements in the area under the curve for automated dipstick urinalysis compared with visual testing. In practical terms automated dipstick urinalysis has a significantly better positive and negative predictive value for detecting 300 mg/24 hours protein excretion. (PPV 78% versus 64%, NPV 84% versus 65%).

The use of automated readers has the potential to improve the accuracy and reproducibility of antenatal screening. The more accurate definition of pathology will improve diagnosis and should reduce hospital admissions.

The value of automated readers was also assessed in the systematic review of Waugh et al⁴⁴ Two studies looked at the effect of automation. However these two studies used different laboratory reference standards so that data could not be pooled. The study of highest quality, evaluated both automated and visual techniques in a blinded comparison. Estimates of accuracy were better for both positive and negative test results for automated compared to visual urinalysis (LR+ of 4.27 (95% confidence interval [CI] 2.78–6.56) and an LR– of 0.22 (95% [CI] 0.14–0.36) for automated urinalysis compared to an LR+ of 2.27 (95% confidence interval [CI] 1.47–3.51) and an LR– of 0.64 (95% [CI] 0.49–0.82) for visual urinalysis). This would suggest that studies of automated dipstick readers and automated technologies should be pursued as they offer the potential for the improvements in dipstick urinalysis that clinicians desire.

Urine Specimen (Concentration versus Total protein content)

Having considered the practical aspects of dipstick urinalysis, it is apparent that inter- and intra-observer variability and observer error are not the only factors that contribute to the variable correlation between dipsticks and total protein excretion.

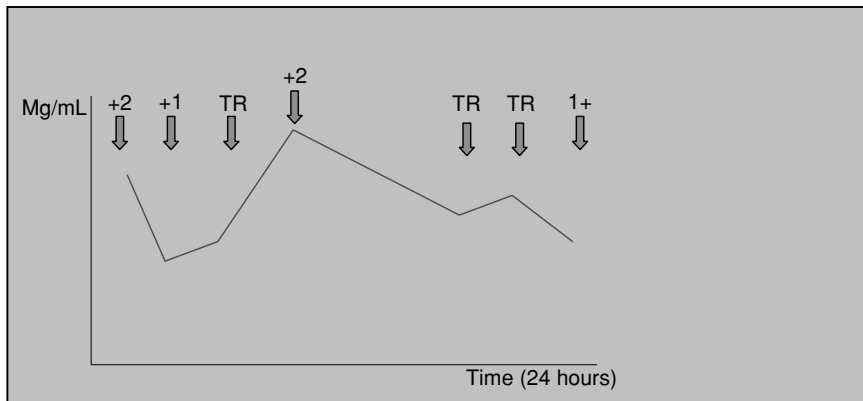


Figure 1 Plot of urine concentration vs time for a sequentially collected 24 hr urine.

When dipstick urinalysis is performed on a random sample of urine, it gives a measure of the protein concentration in that specimen and as such it is affected by a number of variables such as contamination (false positive), exercise (increased excretion), posture, osmolality and urinary pH. It is unusual to find data on urine specific gravity or pH when reporting on dipstick accuracy. It is also widely accepted that protein excretion has its own circadian variation and that this can change dipstick values from negative to as much as 3+ (i.e. non-proteinuric to proteinuric) over a 24-hour period. This has been reported in pregnancy in a study of 17 women with hypertension and proteinuria where considerable variation in protein excretion was observed throughout a 24 hour period.⁵¹ A typical sequential urine collection from this study (Figure 1) shows the variation in protein concentration throughout the day as well as the number of voided samples that dipstick negative for protein despite the 24 hour excretion of >600 mg/24 hours. Clearly such variation is controlled for by the 24-hour specimen as the entire collection is combined before a protein concentration is measured.

Brown et al³⁵ explored this issue by performing dipstick urinalysis before, during and after a 24 hour urine sample was collected. They found that the positive and negative predictive values for dipstick urinalysis did vary and the best correlation was between an aliquot of the 24-hour sample that was tested with dipstick urinalysis rather than a voided sample either before or after the 24-hour urine was collected: negative predictive value 87% vs. 38% and positive predictive value 60% vs. 46%.

A further problem with the assessment of proteinuria is that the threshold used to define an abnormal protein excretion is based on an absolute protein excretion over 24 hours and the dipstick measures protein concentration. The correlation at the 1+ level is confounded by urine volume (as discussed above). When we collected 197 24-hour urine samples from the maternity unit in our hospital the volume varied from 256 mls to 3761 mls. We have further explored this issue and found that when dipstick urinalysis is performed on an aliquot of the 24-hour specimen the correlation between dipstick urinalysis and the laboratory gold standard is better if a threshold

Table 1 Comparison of dipstick urinalysis with Benzethonium chloride and Bradford laboratory protein assays. (Threshold for proteinuria: 1+ for dipstick urinalysis; 300 mg/24 hours for laboratory assay.)

		Dipstick Urinalysis		
		-ve	+ve	
Benzethonium Chloride Assay	-ve	58	1	Sensitivity = 22.5% (15.8%, 30.3%)
	+ve	107	31	Specificity = 98.3% (90.9%, 99.9%)
				NPV = 35.2% (27.9%, 43.0%)
				PPV = 96.9% (83.8%, 99.9%)
Bradford Assay	-ve	144	4	Sensitivity = 57.1% (42.2%, 71.2%)
	+ve	21	28	Specificity = 97.3% (93.2%, 99.3%)
				NPV = 87.3% (81.2%, 91.9%)
				PPV = 87.5% (71.0%, 96.5%)
				Difference in NPV's = 52.1% (43.7%, 60.5%), P < 0.001
				Difference in PPV's = -9.4% (-22.6%, 3.8%), P = 0.25

NPV = Negative Predictive Value

PPV = Positive Predictive Value

Table 2 Comparison of dipstick urinalysis with Benzethonium chloride and Bradford laboratory protein assays. (Threshold for proteinuria : 1+ for dipstick urinalysis; 300 mg/ml for laboratory assay.)

		Dipstick Urinalysis		
		-ve	+ve	
Benzethonium Chloride Assay	-ve	89	1	Sensitivity = 29.0% (20.6%, 38.5%)
	+ve	76	31	Specificity = 98.9% (94.0%, 99.9%)
				NPV = 53.9% (46.0%, 61.7%)
				PPV = 96.9% (83.8%, 99.9%)
Bradford Assay	-ve	152	2	Sensitivity = 69.8% (53.9%, 82.8%)
	+ve	13	30	Specificity = 98.7% (95.4%, 99.8%)
				NPV = 92.1% (86.9%, 95.7%)
				PPV = 93.8% (79.2%, 99.2%)
				Difference in NPV's = 38.2% (30.0%, 46.4%), P < 0.001
				Difference in PPV's = -3.1% (-12.3%, 6.0%), P = 0.31

NPV = Negative Predictive Value

PPV = Positive Predictive Value

for significant proteinuria is based on concentration and not the absolute amount of protein excreted (i.e. dipsticks perform better when we compare tests with equivalent units of measurement). (Table 1 and 2). Thus the effect of urine volume has been controlled for and as such it is important to realise this confounding effect when assessing the severity of any individual case.

SPECIFIC "GOLD STANDARD" PROTEIN ASSAY AND THE URINARY PROTEIN COMPOSITION

Another common misconception is that the laboratory measurement of proteinuria is standard across different studies and in different hospital laboratories. However, there

are more than 10 different assays for urinary protein and no single proteinuria assay has gained wide acceptance as the 'gold standard' method of assessing urinary protein excretion and different methods have different reference intervals for the normal excretion of proteins in urine. This can be ascribed to several factors including the ability of alternative methods to detect different quantities of low molecular weight proteins as well as albumin and globulins, the ability of these methods to respond differently to the diverse protein groups and the fact that high concentrations of urinary pigments can interfere with some assays. Any reliable urinary protein assay must be able to cope with a wide variety of protein types and concentrations and ideally it should be unaffected by the type of protein molecule. So far no method has been described that is free from this variation. Apart from the lack of universal acceptance of any one assay, there are also no data relating performance of the different assays to important clinical outcomes for the various proteinuric conditions in which they are employed, including pregnancy and pre-eclampsia.

There have been few studies comparing the different methods of analysis. McElderry et al⁵² compared six common laboratory assays for protein outside pregnancy. They compared linearity, ease of standardisation, precision, comparability of assay values, technical ease of the assay and cost. All were available in kit form and all were in clinical use in various laboratories. The study found that no single assay could obviously be recommended above any other. Some observations are important, however. The turbidometric method (also referred to as the benzethonium chloride method)⁵³ is the assay that has been adapted for use in most large automated urinalysers in laboratories throughout the world. It is also the technique that has been used as a gold standard for most urine dipstick accuracy studies, although this is not universal. McElderry et al⁵² go on to suggest that the Coomassie brilliant blue dye-binding techniques offer the most advantages as a routine assay for protein.

Waugh et al⁵⁴ have studied the effect of using different protein assays in pregnancy. They measured proteinuria in an aliquot of a 24-hour urine sample by 3 methods; firstly dipstick urinalysis; secondly the benzethonium chloride assay; and thirdly the Bradford assay, another widely accepted assay for total protein that relies on the ability of proteins to bind the dye Coomassie blue. The results of this study are shown in Table 2. It can be clearly seen that the correlation between the dipstick and the 24-hour protein estimation is highly dependent upon the assay employed. The incidence of proteinuria varied greatly in this study depending upon which method of testing was used and what threshold is used to define 'significant proteinuria'.

They report a prevalence that ranges from a minimum of 16.2% with dipstick urinalysis to a maximum of 70% when the Benzethonium chloride assay is employed to measure total protein excretion in 24 hours. The choice of assay is also important when it comes to testing urine dipsticks. The predictive value of dipstick urinalysis is highly dependent upon which biochemical assay is employed as a gold standard. In Waugh et al's study, when dipsticks were compared with the benzethonium chloride assay they had a sensitivity of 22.5% (95% CI 15.8–30.3%) and a specificity of 98.3% (95% CI 90.9–99.9%). The false positive rate was only 1/32 (3%) but the false negative rate was 107/165 (65%). When compared with the Bradford assay, dipstick urinalysis

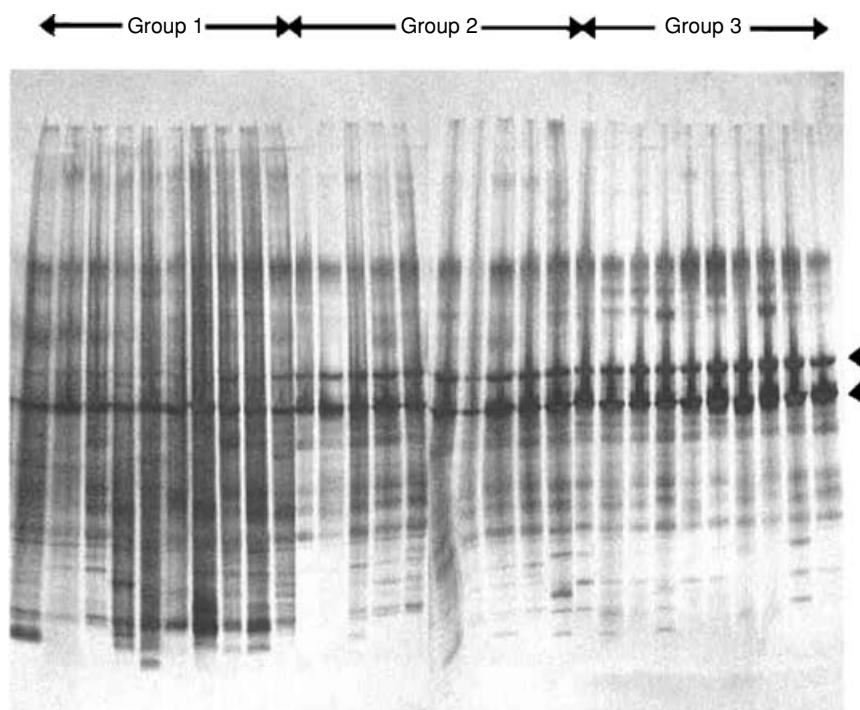


Figure 2 2D electrophoresis on selected samples from three groups of women. Group 1 are samples from women who were proteinuric by Benzethonium chloride but not by Bradford assay or dipstick. Group 2 were non-proteinuric by all 3 methods of analysis. Group 3 was proteinuric by all 3 methods of analysis.

had a sensitivity of 57.1% (95% CI 42.2–71.2%) but a specificity of 97.3% (95% CI 93.2–99.3%). The false positive rate was 4/32 (12.5%) and the false negative rate was 21/165 (13%). This was reflected in similar positive predictive values (PPV): 96.9% (95% CI 83.8–99.9%) for the benzethonium chloride assay compared with 87.5% (95% CI 71.0–96.5%) for the Bradford assay. The difference in PPVs was non significant – 9.4% (95% CI – 22.6% to 3.8%; $P=0.25$). However the negative predictive values (NPV) were very different: 35.2% (95% CI 27.9–43.0%) for the benzethonium chloride assay compared with 87.3% (95% CI 81.2–91.9%) for the Bradford assay. The difference in the NPVs was 52.1% (95% CI, 43.7–60.5%; $P < 0.001$).

The absence of agreed standards makes the enforcement of rigid thresholds suggested in guidelines difficult. Variability in standardisation makes it very likely that different studies are not comparing like with like, due to a lack of harmonisation in quantification.⁵⁵

In an attempt to explain this difference Waugh et al have performed 2D electrophoresis on selected samples from three groups of women. The 2D electrophoretic gel shown in Figure 2, where all wells were loaded with an equivalent amount of total protein, shows the spread of proteins from 3 different groups: Group 1 are samples from women who were proteinuric by Benzethonium chloride but not

by Bradford assay or dipstick. Group 2 were *non-proteinuric* by all 3 methods of analysis, and group 3 was *proteinuric* by all 3 methods of analysis. It is quite clear that the proteinuric specimens that are only detected by benzethonium chloride assay are far more heterogenous in their protein composition. Those samples where there is a better correlation between all three types of assay (whether proteinuric or non-proteinuric) are composed mainly of mid molecular weight proteins such as albumin or transferrin. These observations have been reported elsewhere in relation to the risk of pre-eclampsia.⁵⁶ Studies of the urinary protein patterns in confirmed pre-eclamptic patients have shown significant reductions in Tamm-Horsfall glycoprotein as well as low molecular weight protein bands.⁴⁹ These changes have been correlated to disease severity.⁵⁷ There is as yet no specific electrophoretic pattern of proteinuria that correlates with the severity of preeclampsia, or with glomerular endotheliosis. These findings do however demonstrate the importance of the type of assay employed as the “gold standard” for both the measurement of urinary protein and therefore the definition of pre-eclampsia. Currently, in clinical practice there is a lack of universal acceptance of any one assay although, there is already some evidence that once present, the amount of protein excreted is unrelated to clinical outcome.^{41,58} It may therefore be the type of proteinuria and not the absolute amount of protein excreted that is important in assessing disease severity in pre-eclampsia, and it may be true that the assays to date are detecting changes in urinary protein composition rather than changes in absolute protein excretion and this is why urinary markers have been absent in so many patients presenting with eclampsia.⁵⁹

We can thus see that whilst urine volume, and hence concentration, can account for a proportion of false negative results there is clearly a selective ability for methods of urinalysis to detect proteinuria.

URINALYSIS IN PREGNANCY . . . WHERE NEXT?

Protein: creatinine ratios in spot urine samples

Measurements on random urine samples of the protein:creatinine ratio have been reported to show a good correlation with subsequent 24 hour urine protein estimation in non-pregnant populations. (renal impairment,⁶⁰ kidney transplants⁶¹ and diabetics⁶²). There has also been good evidence of a strong correlation between random sample protein:creatinine estimation and subsequent 24 hour protein excretion in the hypertensive pregnant population.^{63,64} There have been two studies assessing the best threshold for the prediction of subsequent significant proteinuria and they both conclude that a threshold of 30 mg protein/mmol creatinine gives the best predictive values.^{46,65} Only one study to date in pregnancy has not found the protein creatinine ratio to be predictive of total protein excretion. Lindow and Davey⁶⁶ found in a small cohort (n = 22) that the protein creatinine ratio had a coefficient of variation of 27% amongst 8 hourly urine collections in women with heavy proteinuria ($\geq 2+$).

Four from five studies in pregnancy suggest that the correlation from random sample to 24 hour estimation is excellent at protein excretions < 500 mg/24 hours. The authors have also found that use of a laboratory protein/creatinine ratio of 30 mg/mmol has a positive predictive value for proteinuria levels of 300 mg/24 hour of 79% and a negative predictive value of 98% determining 500 mg/24 hours. All studies agree that as the degree of proteinuria increases there is a greater variation in the protein/creatinine ratio over time. However, if the important question is, "Is the threshold for proteinuria exceeded?" rather than "Precisely how much protein is present?" protein creatinine ratio measurement can be recommended as a confirmatory test. This method although removing the need for a 24 hour sample to be collected still requires that samples are sent for laboratory protein estimation and therefore it is still not applicable at point of care. If it were possible to obtain results quickly it would represent an ideal "rule in/rule out" test.

More recently dipstick manufacturers have combined semiquantitative creatinine pads onto multipad urine dipsticks, offering the potential for a point of care dipstick test for protein creatinine ratios. Preliminary studies of these dipsticks in pregnancy have been performed and results suggest that the dipsticks offer real improvements in diagnostic accuracy. In attempting to predict 300 mg protein/24 hr in a 24 hour urine the PCR dipstick had significantly improved sensitivity (94.5% vs 82%) and specificity (95.7% vs 81%) compared to automated dipstick urinalysis [authors personal observations].

It is likely that these improvements are due to a reduction in concentration related errors as well as a reduction in observer error. The significantly reduced false negative rate should result in fewer women with preeclampsia being misdiagnosed as non-proteinuric and the lower false positive rate should reduce inappropriate admissions.

Microalbumin measurements in preeclampsia

General considerations

Microalbumin is defined as urinary excretion of albumin that is persistently above normal, although below the sensitivity of conventional semiquantitative test strips. In the non-pregnant population it reflects glomerular or, less commonly tubulointerstitial dysfunction, and is considered only after structural abnormalities and infection of the renal tract have been excluded. As point of care technologies improve and assay sensitivities increase it is becoming increasingly possible to screen for microalbuminuria with greater accuracy. It is possible that there is a microalbuminuric phase that precedes overt proteinuria in preeclampsia and that we might devise strategies to detect this.

The classification of microalbuminuria has been hampered by a lack of consensus as to the preferred method of urine collection, the quantitative expression of albuminuria,⁶⁷ considerable postural and diurnal variation in subjects⁶⁸ and possible

ethnic differences.⁶⁹ As such, using sensitive immunoassays several groups have defined the normal reference range for the healthy adult population.^{70,71} This is either expressed as 20–200 µg/min or 30–300 mg/24 hr.

For a diabetic population it is accepted that for an overnight urine collection 20 µg/min is diagnostic for microalbuminuria. An early morning urine specimen with an albumin creatinine ratio of >3 reliably predicts an overnight excretion of 300 µg/min. If such a ratio is greater than 1 then it is mandatory to collect a 24 hour sample of urine to quantify the albuminuria.⁷²

Microalbuminuria in pregnancy

Proteinuria in pregnancy is the result of selective glomerular filtration and non-selective (proximal tubule) reabsorption. Although the glomerulus is relatively impermeable to albumin it is known that in non-pregnant women there is an albumin filtration of 500–600 mg/day (i.e. a filtrate concentration of 0.3 mg/dl).⁷³ Proteinuria gradually increases through pregnancy. Levels of 5 mg/100 mL in the first and second trimesters and 10 mg/100 mL in the third trimester are normal and levels may reach 300 mg/mL day in a normal pregnancy in the third trimester.⁷⁴ There is now agreement on this increase in protein but not on the underlying cause or the relative contributions made by different protein categories. Values in excess of 15 mg/100 mL or 300 mg/24 hr are usually associated with either pre-eclampsia or underlying renal disease. Douma et al⁷⁵ describe the circadian variation in albumin excretion concluding that the majority of additional albumin excretion in pregnancy is from nocturnal excretion.

It is apparent from these figures that urinary albumin excretion can be 10–20 times higher than normal without being detected by conventional dipstick or laboratory tests.

Waugh et al⁷⁶ have described the gestation-specific reference range for microalbuminuria in pregnancy (Figure 3) and explored the predictive powers of microalbuminuria measurement techniques for detecting clinical proteinuria. Other studies to describe the excretion of albumin in normal and hypertensive pregnancies are confounded by differing methods of sample collection, though all use sensitive radioimmunoassays allowing the detection of microalbumin levels. Lopez-Espinoza et al⁷⁷ found increased levels in the third trimester compared to the first and second trimester with persistence until 1 week postnatally. Wright et al⁷⁸ using 2-hour collections found no change in the albumin excretion rate over non-pregnant women. They did find that the albumin:creatinine ratio was higher than the non-pregnant state at all stages in pregnancy and that this was also raised at 36 weeks compared to 14 weeks. This rise has been confirmed by others^{79,80} though only using random urine samples.

It has been hypothesized that the increasing increments in albumin:creatinine ratio correspond to increasing glomerular permeability to albumin through normal pregnancy.⁷⁸ Alternatively the findings of Beetham et al⁸¹ of increasing low molecular

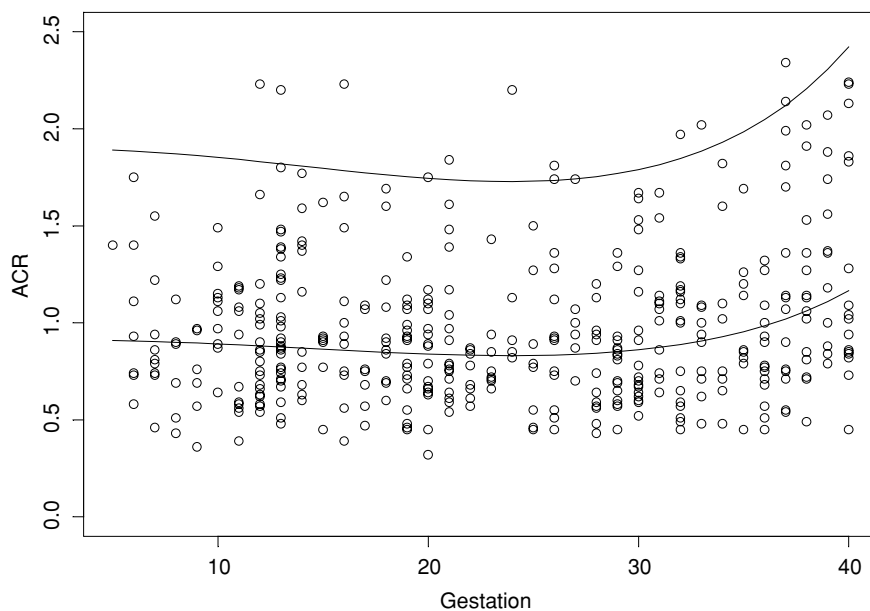


Figure 3 Gestation specific 95% reference range for urinary microalbumin/creatinine ratio (DCA[®] 2000).

weight protein excretion through pregnancy might suggest that tubular reabsorption is progressively decreased.

Microalbuminuria and hypertensive disorders of pregnancy

Proteinuria (≥ 0.3 g/24 hr) is considered a late sign of pre-eclampsia,⁸² and carries a poor perinatal prognosis.^{31–33,36,83} Lopez-Espinosa⁷⁷ could find no evidence that gross proteinuria was preceded by a phase of microalbuminuria, and Konstantin-Hansen et al⁸⁰ testing first morning urine samples also found no significant differences in albumin concentration or albumin:creatinine ratio in women who developed pre-eclampsia from a low risk population compared to those who remained normal.

Others have however reported that microalbumin may be of benefit in screening for pre-eclampsia. Rodriguez et al⁸⁴ reported that when urinary microalbumin concentration was combined with a low calcium:creatinine ratio, the test had a sensitivity of 50% and a specificity of 99% (PPV 83%, NPV 94%) for the detection of subsequent pre-eclampsia in 88 low risk nulliparous women. Holm et al⁸⁵ combined serum uric acid measurement with relative albumin clearance measurements in two cohorts of women at 24–41 weeks gestation (54 normal, 14 pre-eclampsia). They report a positive predictive value of 100% and a negative predictive value of 100%. These studies are obviously limited by small numbers and differing methodologies.

Thong et al⁸⁶ measured the albumin:creatinine ratio in 162 women in the third trimester from a single voided urine, following a diagnosis of either pre-eclampsia

(n = 27), non-proteinuric hypertension (n = 72), or a normal control (n = 63). They report significantly different albumin creatinine ratios for all three groups but could not correlate ACR to outcomes such as birthweight, serum uric acid or liver function results.

Nakamura et al⁸⁷ measured albumin : creatinine ratio in 199 normotensive pregnant women at 20 weeks and 30 weeks gestation. They concluded that the best sensitivity and specificity were for a ratio of 16 mg/g (PPV 50% and NPV 94% at 20 weeks gestation and PPV 43% and NPV 96% at 30 weeks gestation). They concluded therefore that this was a useful screening tool for pregnancy induced hypertension and preeclampsia.

Bar et al⁸⁸ actually described a phase of microalbuminuria that preceded clinical proteinuria and that this test has some predictive value for severe disease. They also suggest that the accepted definition of gestational proteinuria should be reconsidered.

These studies all relied on random urine samples that were sent for laboratory analysis. Higby et al⁸⁹ compared two point of care screening tests for the detection of significant proteinuria (> 300 mg/24 hours) in a normotensive and hypertensive pregnant population. They compared methods of urine screening against a gold standard urine biochemical assay (rather than clinical outcomes). This assay would have defined the pre-eclamptic population. The micro-bumintest (a dipstick for microalbuminuria) had a sensitivity of 87% compared with 36% for the Multistix 10 SG. It also had a higher specificity and positive and negative predictive values. They concluded that this test was a better screening test to identify pre-eclampsia at point of care. Das and colleagues⁹⁰ testing the micral test, a dipstick for microalbuminuria (Boehringer Mannheim, Germany) found it to have a sensitivity of 68% and specificity of 92% (PPV 56% and NPV 94%). They also concluded that microalbumin testing was a good predictor of pregnancy induced hypertension though not all of the women in this study who developed hypertension actually developed proteinuria by conventional testing.

These studies although not conclusive do suggest that microalbuminuria has a potential for improving the detection of pre-eclampsia.

NEW DEVELOPMENTS IN MEASUREMENT AT POINT OF CARE

Waugh et al⁹¹ have also explored other urinalysis devices that can give fully quantitative urine protein measurements at the point of care. The DCA 2000[®] (Bayer Corporation, Elkhart USA) estimates the microalbumin/creatinine ratio utilising a cartridge system and a 40 µl sample of urine. They have validated the DCA[®] 2000 in women with uncomplicated pregnancies, hypertensive pregnancies and for urine from a variety of specimens from early morning samples to 24 hour samples. They found the device to be highly accurate and as such a viable alternative to a laboratory albumin creatinine ratio test.⁹¹ This is the first new urinalysis technique to be rigorously tested/validated in this way.

Having validated the device and constructed a 95th centile reference range (Figure 3) they tested the device for proteinuria prediction in hypertensive pregnancies.

The ACR threshold for normality in the non-pregnant population is 3.4 mg/mmol. This threshold is above that identified for pregnancy from the studies above. Waugh et al⁹¹ constructed receiver operating characteristic curves for the DCA 2000 and found the optimal threshold was 2.0 mg/mmol. Using this threshold we found the device has a sensitivity of 94% (95% CI 85–98%) and a specificity of 94% (95% CI 85–98%). These results now allow a highly predictive assessment of proteinuria for women with hypertension in an obstetric day unit (i.e as an outpatient).

The finding in this study that the false positive results from the DCA[®] 2000 all went on to develop significant pre-eclampsia (proteinuric hypertension with evidence of multisystem disease), supports the argument that there is a phase of microalbuminuria that precedes overt proteinuria and may allow earlier detection of this condition. This study was not sufficiently powered to allow an estimation of the length of the microalbuminuric phase but the maximum time from positive ACR without proteinuria to positive ACR with proteinuria was 13 days. It is of note that of those who were apparent false negatives with the DCA[®] 2000 none developed significant pre-eclampsia. All had gestational hypertension with no evidence of multisystem disease apart from gestational proteinuria. This never exceeded 370 mg/24 hours and in some cases subsequently dropped to below 300 mg/24 hours. As the threshold for “significant proteinuria” is based on 95th centile data from uncomplicated pregnancies these women may have been excreting total protein amounts that were above the 95th centile but they were not significantly albuminuric as they did not have the glomerular damage that accompanies pre-eclampsia. As such proteinuria in the absence of significant microalbuminuria might have a better prognosis and run a more benign course. Microalbuminuria may correlate more closely with other clinical measurements of disease severity as it may more accurately reflect the glomerular dysfunction associated with the glomerular endotheliosis of pre-eclampsia.

CONCLUSIONS

So what is significant proteinuria? It is certain that significance is achieved when a threshold is exceeded though whether this should be 300 mg or 500 mg/24 hours can be debated. Evidence now suggests that increasing proteinuria once present is not a good predictor of perinatal risk and therefore alone should not influence timing of delivery. We have highlighted the known failings of dipsticks as well as the less well appreciated failings of the laboratory 24 hour urine protein measurement and while pre-eclampsia remains a diagnosis based on the recognition of new hypertension and proteinuria in pregnancy the ‘gold standard’ assay for the assessment of proteinuria remains undefined.

Screening for significant proteinuria should now have a different meaning for a clinician. The recently published NICE guidelines¹ make three recommendations with regard ‘to significant proteinuria; 1. The use of automated dipstick readers can

significantly improve false positive and false negative rates for the detection of proteinuria.; 2. A finding of dipstick proteinuria should be confirmed by either a 24 hour urine collection or a protein creatinine ratio; and 3. Future research is required to determine the optimal frequency and timing of blood pressure measurements and on the role of screening for proteinuria in pre-eclampsia management.

The emphasis now should be to improve diagnostic techniques so that we can confirm the presence or absence of proteinuria above a threshold of 300 or 500 mg/24 hours in any clinical setting preferably with a test as simple as a dipstick and worry less about the absolute amount of protein once above this threshold. If this is done reliably then the need for confirmation through 24 hour urine collections and the need to wait 48 hours for a diagnosis of preeclampsia become a thing of the past. Studies are ongoing with new desktop assays that should begin to answer the questions raised by the NICE recommendations. With the development of new dipsticks to measure a protein creatinine ratio and fully quantitative albumin assays at point of care the diagnosis of proteinuria and hence pre-eclampsia moves ever closer to the clinician's desktop.

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