Diagnostic Accuracy of Spot and Timed Measurements of Urinary Albumin Concentration to Determine Microalbuminuria in Sickle Cell Disease

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ABSTRACT

Objective: *Whereas measurement of albumin:creatinine ratio (ACR) in spot urine samples is indicated for determining microalbuminuria, its performance or that of urinary albumin excretion rate (UAER) in predicting microalbuminuria in sickle cell disease (SCD) is unclear. We therefore tested the diagnostic performance of these measures in spot and timed urine samples in predicting a UAER in 24-hour samples.*

Methods: *Thirty participants with SCD had spot, two-hour and four-hour, followed by 24-hour urine collections for ACR, urinary albumin concentration (UAC) and UAER determinations. Receiver operating characteristic (ROC) curve analyses were performed.*

Results: *The areas under the ROC curves for microalbuminuria were 0.99 (CI: 0.97, 1.00) for ACR and 0.97 (CI: 0.92, 1.00) for UAC in spot urine samples. For ACR, at the cut-point of 4.13 mg/mmol, there was 100% sensitivity and 82.6% specificity, allowing an 86.2% correct classification. At the cut-point of UAC = 20.9 mg/L, there was 100% sensitivity and 73.9% specificity, allowing a 79.3% correct classification. Corresponding areas for microalbuminuria in two-hour timed samples were 0.99 (CI: 0.95, 1.00) for ACR and 0.96 (CI: 0.89, 1.00) for UAER. For ACR, the cut-point was 4.64 mg/mmol with 83.3% sensitivity and 91.3% specificity, allowing an 89.7% correct classification. Similarly for UAER, at the cut-point of 21.8 µg/min, there was 83.3% sensitivity and 91.3% specificity, allowing 89.7% correct classification.*

Conclusions: *The diagnostic performance of ACR and UAC in a spot as well as ACR and UAER in two-hour timed urine samples in patients with SCD is excellent. Healthcare professionals can confidently utilize these measures in this patient population.*

Keywords: Albumin:creatinine ratio, diagnostic accuracy, microalbumin measurement, renal disease, sickle cell disease, urinary albumin concentrations

Precisión Diagnóstica de las Mediciones Puntuales y Cronometradas de la Concentración de Albúmina en Orina para Determinar la Microalbuminuria en la Enfermedad de Células Falciformes

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RESUMEN

Objetivo: *Si bien la medición del índice urinario albúmina:creatinina (IAC) en muestras de orina puntuales se indica para la determinación de la microalbuminuria, no está clara su eficacia, ni la de la tasa de excreción de albúmina urinaria (TEAU), en la predicción de microalbuminuria en la enfermedad de células falciformes (ECF). Por lo tanto, sometimos a prueba la eficacia diagnóstica de estas mediciones puntuales y cronometradas de las muestras de orina a la hora de predecir una TEAU en muestras de 24 horas.*

Métodos: *A treinta participantes con ECF se les tomaron muestras puntuales de orina, a las dos horas y a las cuatro horas, seguidas por muestras de orina de 24 horas para el IAC, la concentración de*

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albúmina urinaria (CAU), y las determinaciones de TEAU. Se realizaron análisis de la curva de las características operativas del receptor (ROC).

Resultados: *Las áreas bajo las curvas ROC para la microalbuminuria fueron 0.99 (CI: 0.97, 1.00) para IAC y 0.97 (CI: 0.92, 1.00) para CAU en muestras puntuales de orina. Para IAC, en el punto de corte de 4,13 mg/mmol, hubo 100% sensibilidad y 82.6% de especificidad, lo que permite una clasificación 86.2% correcta. En el punto de corte de CAU = 20,9 mg/L, hubo un 100% de sensibilidad y 73.9% de especificidad, lo que permitió una clasificación 79.3% correcta. Las áreas correspondientes para la microalbuminuria en muestras de tiempo de dos horas fueron 0.99 (CI: 0.95, 1.00) para IAC y 0,96 (CI: 0.89, 1.00) para TEAU. Para IAC, el punto de corte fue 4.64 mg/mmol con 83.3% de sensibilidad y 91.3% de especificidad, lo que permitió una clasificación 89.7% correcta. Del mismo modo para TEAU, en el punto de corte de 21.8 µg/min, hubo una sensibilidad de 83.3% y una especificidad de 91.3%, lo que permitió una clasificación 89.7% correcta.*

Conclusiones: *La eficacia diagnóstica de IAC y UAC en un punto así como el IAC y la TEAU en muestras de orina de tiempo de dos horas en pacientes con ECF es excelente. Los profesionales de la salud pueden utilizar estas mediciones con confianza en esta población de pacientes.*

Palabras claves: Índice albúmina:creatinina, exactitud diagnóstica, medición de microalbúmina, enfermedad renal, enfermedad de células falciformes, concentraciones de albúmina urinaria.

West Indian Med J 2013; 62 (9): 809

INTRODUCTION

In recent years, the management of patients with sickle cell disease (SCD) has improved and their life expectancy has been prolonged. However, renal insufficiency has emerged as one of the most prevalent forms of end organ failure occurring in these patients. For example, at the Sickle Cell Unit in Jamaica, the prevalence of renal impairment among clinic attendees is ~ 2%. Additionally, chronic renal failure is responsible for about 18% of deaths among SCD patients (1). Once diagnosis of chronic renal failure is made, life expectancy thereafter is about four years (2).

Glomerular hyperfiltration and enhanced renal blood flow are characteristic findings in young adult patients with SCD. It has been proposed that this hyperfiltration leads to gradual sclerosis of the glomerular capillaries and predisposes to renal insufficiency in these patients (3). Early renal impairment is usually clinically silent but microalbuminuria (MA) and changes in glomerular filtration rate (GFR) are early functional markers (4). In SCD, even though the prognostic implications of MA are not very clear, reduction has been shown in short-term studies using angiotensin converting enzyme (ACE) inhibitor therapy. Hence, screening and early detection of MA may be important in these persons who are especially at high risk for end stage renal disease.

Historically, a 24-hour urine measurement of protein has been considered the gold standard means of quantitating microalbuminuria. However, it is prone to incomplete collections, as it is the most complex method of collection (5). It is also an impractical method for population screening of albuminuria. An untimed spot urine collection is more convenient and it has been further proposed that the measurement of the urinary albumin:creatinine ratio (ACR) on the spot urine (as compared to measurement of urinary albumin concentration alone) may improve the agreement with the measurement of proteinuria on the 24-hour urine collection (5, 6); this has been shown in both non-diabetic (6–8) and diabetic populations (9, 10).

Whereas random ACR has not correlated too well with 12-hour urinary excretion of albumin in SCD (11), how well urinary albumin concentrations (UAC) in spot urine samples or UAC or urinary albumin excretion rates (UAER) in shorter timed collections in persons with SCD correlate with the UAER over a 24-hour period is unknown. In this study, therefore, we compare the diagnostic accuracy of the spot ACR and UAC as well as ACR and UAER in timed urine collections to the gold standard measure of microalbuminuria, *ie* 24-hour urine measurement of UAER in persons with SCD.

SUBJECTS AND METHODS

This study was conducted in accordance with the 'Standards for the reporting of diagnostic accuracy' (STARD) recommendations. Thirty participants with SCD presenting for routine health maintenance care consecutively, and who met the inclusion criteria of being in steady state (*ie* no acute sickle related illness in the last four weeks), having 1+ or more proteinuria on routine dipstick testing, and having a negative midstream urine culture report (*ie* no signs of a urinary tract infection), were recruited to this prospective study at the Sickle Cell Unit, The University of the West Indies, Mona campus in Jamaica. None of the participants was known to have previous renal impairment, and none was on ACE inhibitors/angiotensin receptor blockers (ARB) or hydroxyurea therapy. Written informed consent was obtained prior to inclusion in the study.

The participants were provided with several labelled containers into which they were asked to void urine over a

24-hour period: the samples collected were morning spot urines, followed by a timed collection at two hours, another after another two hours (*ie* four-hour timed collection) and the remaining 20-hour collection. For each timed collection, the volume of urine passed was measured and then a known aliquot of urine was removed for analysis of albumin and creatinine concentrations. To obtain the total 24-hour urinary albumin and creatinine excretion, the measured albumin excretion and urinary creatinine on the pooled sample were adjusted by the values of albumin in the aliquots that were removed during the collection period. Urinary albumin was measured using a competitive chemiluminescent enzyme immunoassay (IMMULITE® 2000 albumin assay, Siemens Medical Diagnostics, LA, USA) with a calibration range of 2.5–60 µg/mL and analytical sensitivity of 1 µg/mL. Urine creatinine was measured using the VITROS CREA Slide method, which is a multi-layered, analytical element coated in a polyester support. The reference interval on this test is 8840–17 680 µmol/day for males and 7072–15 912 µmol/day for females. The tests were performed at the Tropical Medicine Research Institute laboratories located at the Mona campus of The University of the West Indies. Experienced laboratory technicians conducted the tests, and were blinded to the samples being tested.

A sample of venous blood (5 ml) to measure serum creatinine, and complete blood count was taken. Height, weight and blood pressure were also measured with a stadiometer, beam balance and Dinamap™, respectively. The study was a low risk study and no adverse effects were noted in any of the participants during the study. The study was conducted during June to August 2007 and approval was granted by the University Hospital of the West Indies/University of the West Indies/Faculty of Medical Sciences Ethics Committee.

The participants were categorized as being "normoalbuminuric" (defined as UAER < 20 µg/min) or "microalbuminuric" (defined as UAER = 20–200 µg/min), based on testing of the 24-hour urine sample, this being the gold standard reference method. Those who fell out of this range would be classified as having "macroalbuminuria" and were excluded from further analysis. However, the more widely used ACR in 24-hour urine collection was also used to compare the categorization and values of male: 2.5–25 mg and female: 3.5–35 mg of urinary albumin/mmol of creatinine who were defined as "microalbuminuric" and those with ACR male: < 2.5 mg and female: < 3.5 mg of urinary albumin/mmol of creatinine were considered "normoalbuminuric".

Statistical analysis

Albuminuria categories were determined using both 24-hour urine collection UAER and ACR levels defined above and these were cross-tabulated. The participants in the albuminuria categories were further categorized by genotype.

The relationships between spot ACR and UAC, as well as two-hour timed and four-hour timed UAER and ACR with 24-hour UAER and ACR were calculated by pairwise correlation coefficients, and p-values < 0.05 were considered to be significant. As the four-hour timed samples had very weak correlations, no further analyses were done with those measures. Patient flowcharts were created for spot ACR and UAC as well as two-hour UAER and ACR measurements, and from these, various diagnostic parameters (sensitivity, specificity, positive predictive value and negative predictive value) were calculated for each test.

The receiver operating characteristic (ROC) curve approach was used to analyse the performance of the screening tests for microalbuminuria, considering the UAER in the 24-hour sample as the reference standard. The true-positive rate (sensitivity) was plotted against the false positive rate (100-specificity) for each measurement. The areas under the ROC curves were described as mean ± standard error. The estimated area under the fitted smooth curve ranges from 0.5 (no apparent accuracy) to 1.0 (perfect accuracy) as the ROC curve moves toward the left and top boundaries of the ROC graph (12). The first cut-off point was determined where 100% sensitivity intersected with the highest specificity for each measurement. The sensitivity and specificity at the cut-off point recommended in the literature for each test were also determined. The percentages of participants being correctly classified, as well as the positive and negative likelihood ratios (LRs) at each of these levels were reported from the analytic outputs.

Clinical and laboratory parameters were compared for the two groups. Results were expressed as means with standard deviations (SD) or counts/frequencies, as appropriate. Associations between categorical variables were determined by the Chi-squared statistics. For continuous variables, differences between means were determined using t-test. P-values of < 0.05 were considered to be significant. All analyses were conducted using Stata Software version 10.1 for Windows™ (StataCorp, College Station, Texas, USA).

RESULTS

Of the 30 participants, 17 were men and 13 women. The mean age was 35.6 ± 10.5 years, with a range of 18.8–52.4 years. Twenty-four had the homozygous sickle (SS) disease, four had heterozygous haemoglobin S-C (SC) disease and two had Sβ^0-Thallassemia.

Participants were categorized using UAER or ACR cut-offs into albuminuria categories. Using the former UAER criteria, 23 persons were normoalbuminuric (UAER: 6.03 ± 4.52 (4.07, 7.98) µg/min), six had microalbuminuria (UAER: 63.28 ± 34.43 (27.2, 99.4) µg/min) and one person had macroalbuminuria (UAER: 361.1 µg/min). Using the ACR criteria, 22 persons were normal (ACR: 1.12 ± 0.89 (0.72, 1.51) mg/mmol), six persons had microalbuminuria (ACR: 8.75 ± 3.30 (5.28, 12.21) mg/mmol), and two persons were macroalbuminuric (ACR: 61.9 ± 3.90 (59.1, 64.6) mg/mmol) [Table 1]. Of note, the two persons who were mismatched in the UAER and ACR categories had very low

Table 1: Albuminuria categories by urinary albumin excretion rate (UAER) and albumin:creatinine ratio (ACR)

	Normoalbuminuric by albumin excretion rate (UAER)	Microalbuminuric by albumin excretion rate (UAER)	Macroalbuminuric by albumin excretion rate (UAER)	Total
Normoalbuminuric by albumin:creatinine ratio (ACR)	22	0	0	22
Microalbuminuric by albumin:creatinine ratio (ACR)	1	5	0	6
Macroalbuminuric by albumin:creatinine ratio (ACR)	0	1	1	2
Total	23	6	1	30

ACR: albumin:creatinine ratio; UAC: urinary albumin concentration; UAER: urinary albumin excretion rate

urine creatinine levels in one of their timed urine samples each. Hereafter, the categories developed using the UAER criteria were used in further analyses.

Table 2 reports microalbuminuria status by genotype and all those determined as having "microalbuminuria" had the homozygous SS disease. The person with "macroalbuminuria" also had SS genotype (not shown).

Table 2: Microalbuminuric categories by genotype

Genotype	Normoalbuminuric	Microalbuminuric	Total
SB^0	2	0	2
SC	4	0	4
SS	17	6	23
Total	23	6	29

Utility of index tests

Simple correlations were done to compare spot urine UAC and ACR as well as UAER and ACR in two- and four-hour timed urine collections with the 24-hour measures of UAER and ACR. Table 3 shows that for these tests, the two- and four-hour timed collections performed better than the measurements in spot urine. The correlations with two- and four-hour timed collections were almost similar. Also, all correlations were better with UAER measurements than with ACR.

Figure 1 describes the patient flowchart for spot urine ACR and UAC, whereas Fig. 2 describes the patient flowcharts for two-hour timed UAER and ACR. These flow charts allow the various diagnostic parameters to be measured for each of the four tests being studied (Table 4). Both the spot and two-hour timed urine samples have low sensitivities but 100% specificities, as well as 100% positive predictive values, when ACR is measured. Their ability to rule out disease, *ie* their negative predictive values is moderately high (82.4% for spot urine and 79.3% for two-hour timed urine).

The spot UAC and two-hour UAER have much higher sensitivities (100% and 85.7%, respectively) but their speci-

Table 3: Pairwise correlations among urinary albumin excretion rate (UAER)/urinary albumin concentration (UAC) and albumin:creatinine ratios (ACR) in timed urine samples

	24-hour urine UAER	Spot urine UAC	2-hour urine UAER	4-hour urine UAER
24-hour urine UAER	1.00			
Spot urine UAC	0.73*	1.00		
2-hour urine UAER	0.97*	0.66*	1.00	
4-hour urine UAER	0.95*	0.87*	0.93*	1.00
	24-hour urine UAER	**Spot urine UAC**	**2-hour urine UAER**	**4-hour urine UAER**
24-hour urine ACR	1.00			
Spot urine ACR	0.41*	1.00		
2-hour urine ACR	0.85*	0.30	1.00	
4-hour urine ACR	0.81*	0.57*	0.95*	1.00

*p-value < 0.05

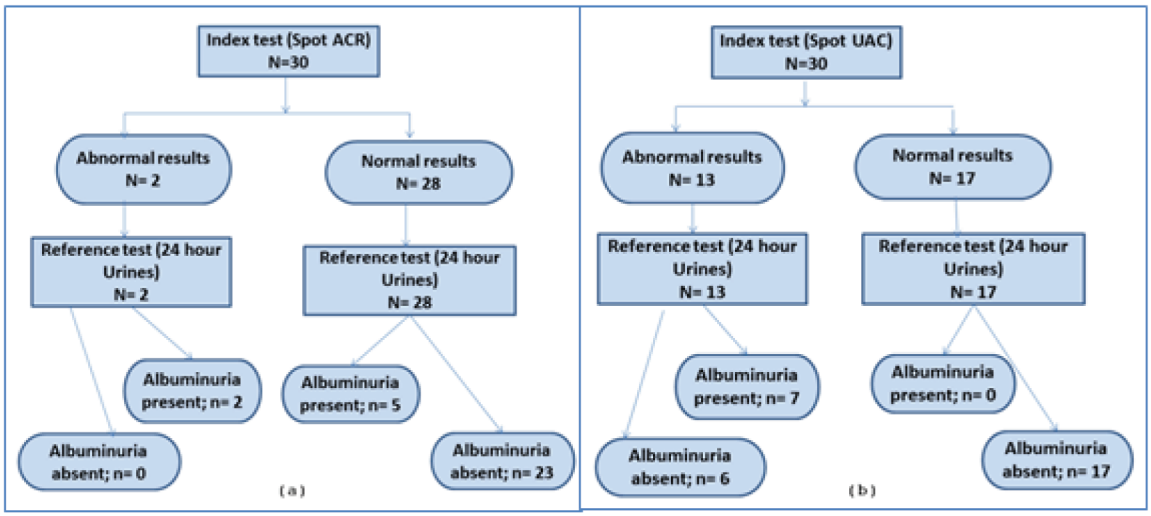

Fig. 1: Patient flowchart for spot urine (a) albumin:creatinine ratio (ACR) and (b) urinary albumin concentration (UAC).

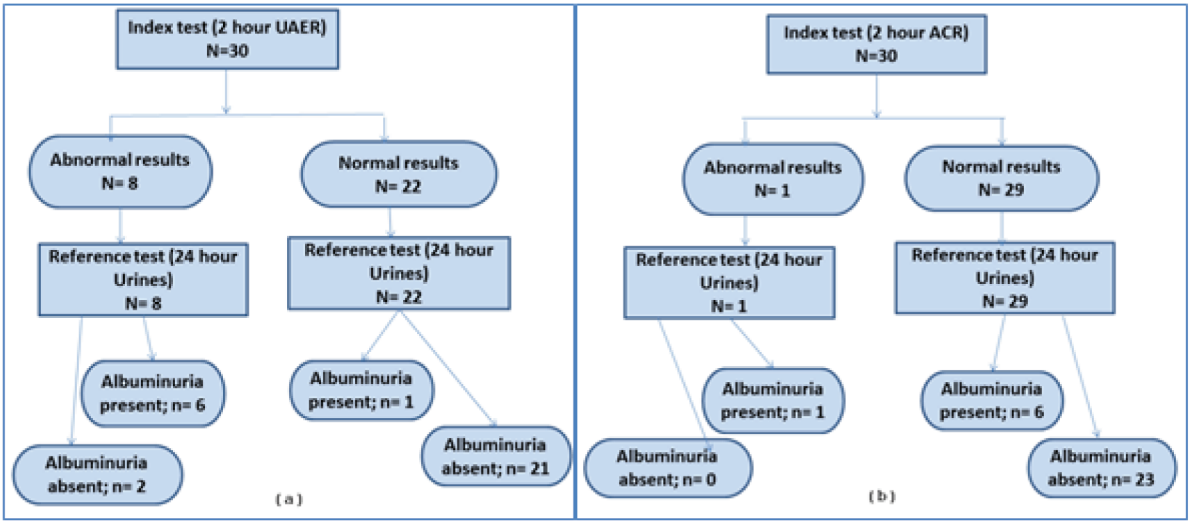

Fig. 2: Patient flowchart for two-hour timed (a) urinary albumin excretion rate (UAER) and (b) albumin:creatinine ratio (ACR).

Table 4: Diagnostic abilities of the four tests being studied

	Sensitivity	Specificity	PPV	NPV
Spot ACR	28.6%	100%	100%	82.4%
Spot UAC	100%	73.9%	53.9%	100%
2-hour ACR	14.3%	100%	100%	79.3%
2-hour UAER	85.7%	91.3%	75%	95.5%

ACR: albumin:creatinine ratio; UAC: urinary albumin concentration; UAER: urinary albumin excretion rate;
PPV: positive predictive value; NPV: negative predictive value

ficities are lower than ACR (73.9% and 91.3%, respectively). Correspondingly, they have much higher negative predictive values (100% and 95.5%) than positive predictive values (53.9% and 75%).

ROC curves and analyses

Receiver operating characteristic curve analyses were then performed for each of these tests.

Spot urine analyses

Table 5 and Fig. 3 describe the ROC curve characteristics for spot urine measurements. The area under the curve for ACR was 0.99 (CI: 0.97, 1.00). The cut-point was 8.71 mg/mmol with 100% sensitivity and 95.7% specificity. This cut-point accurately classified 96.6% of participants. At the level closest to the established cut-point in the literature, the cut-point was 4.13 mg/mmol with 100% sensitivity and 82.6% specificity, allowing an 86.2% correct classification of individuals.

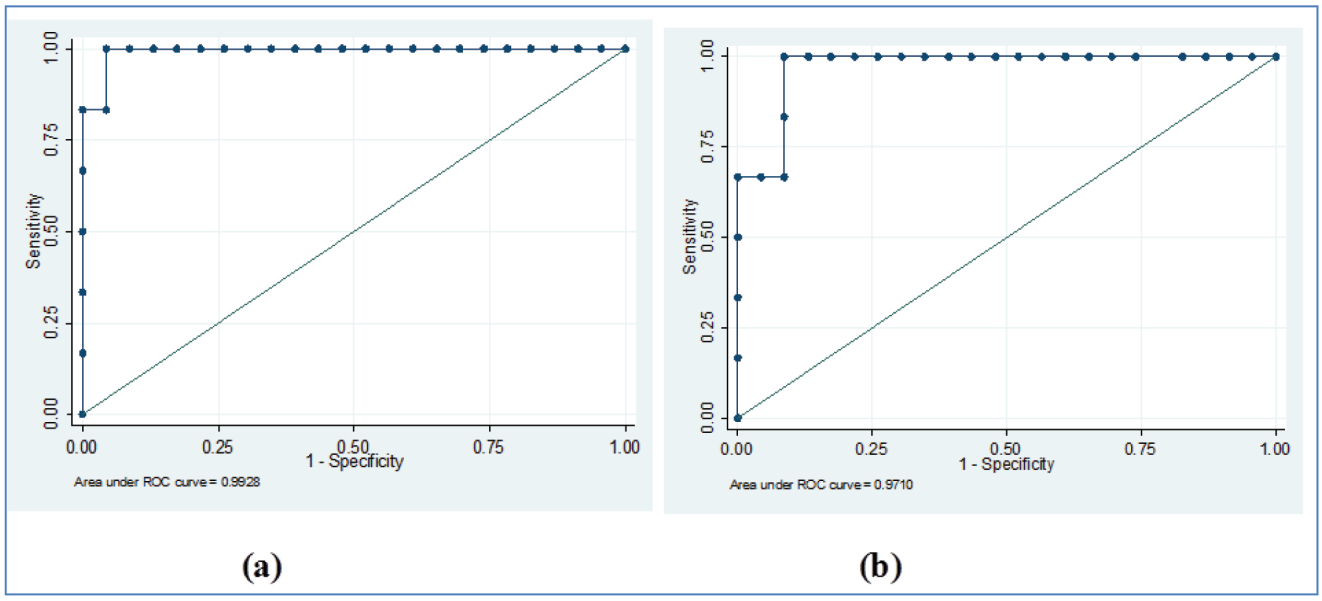

Fig. 3: Receiver operating characteristic (ROC) curve for albumin:creatinine ratio (ACR) with area under the curve (AUC) = 0.9928 (a) and urinary albumin concentration (UAC) with AUC = 0.9710 (b) in spot urine to determine microalbuminuria.

The area under the curve for UAC was 0.97 (CI: 0.92, 1.00). The cut-point was 35.7 mg/L with 100% sensitivity and 91% specificity. This cut-point accurately classified 93.1% of participants. At the level closest to the established cut-point in the literature, the cut-point was 20.9 mg/L with 100% sensitivity and 73.9% specificity, allowing a 79.3% correct classification of individuals. The positive likelihood ratios are correspondingly high for both cut-points in each test.

Two-hour timed urine analyses

Table 5 and Fig. 4 describe the ROC curve characteristics for two-hour timed urine measurements.

The area under the curve for ACR was 0.99 (CI: 0.95, 1.00). The cut-point was 2.60 mg/mmol with 100% sensitivity and 91.3% specificity. This cut-point accurately classified 93.1% of participants. At the level closest to the established cut-point in the literature, the cut-point was 4.64 mg/mmol with 83.3% sensitivity and 91.3% specificity, allowing an 89.7% correct classification of individuals.

The area under the curve for UAER was 0.96 (CI: 0.89, 1.00). The cut-point was 9.9 µg/min with 100% sensitivity and 78.3% specificity. This cut-point accurately classified 82.8% of participants. At the level closest to the established cut-point in the literature, the cut-point was 21.8 µg/min with 83.3% sensitivity and 91.3% specificity, allowing an 89.7%

Table 5: Receiver operating characteristic (ROC) curve for spot and two-hour urine measures

Variable	ROC area	Cut-point	Sensitivity	Specificity	Correctly classified	LR+	LR-
Spot urine measures							
Albumin/creatinine ratio (ACR) mg/mmol	0.99 ± 0.01	8.71	100	95.7	96.6%	23.0	0.0
		4.13	100	82.6	86.2%	5.75	0.0
Albumin concentration mg/L	0.97 ± 0.02	35.7	100	91	93.1%	11.5	0.0
		20.9	100	73.9	79.3%	3.83	0.0
2-hour urine measures							
Albumin/creatinine ratio (ACR) mg/mmol	0.99 ± 0.02	2.603	100	91.3	93.1%	11.5	0.0
		4.64	83.3	91.3	89.7%	9.58	0.18
Albumin excretion rate (UAER) µg/min	0.96 ± 0.04	9.9	100	78.3	82.8%	4.6	0.0
		21.8	83.3	91.3	89.7%	9.58	0.18

LR+: positive likelihood ratio; LR-: negative likelihood ratio

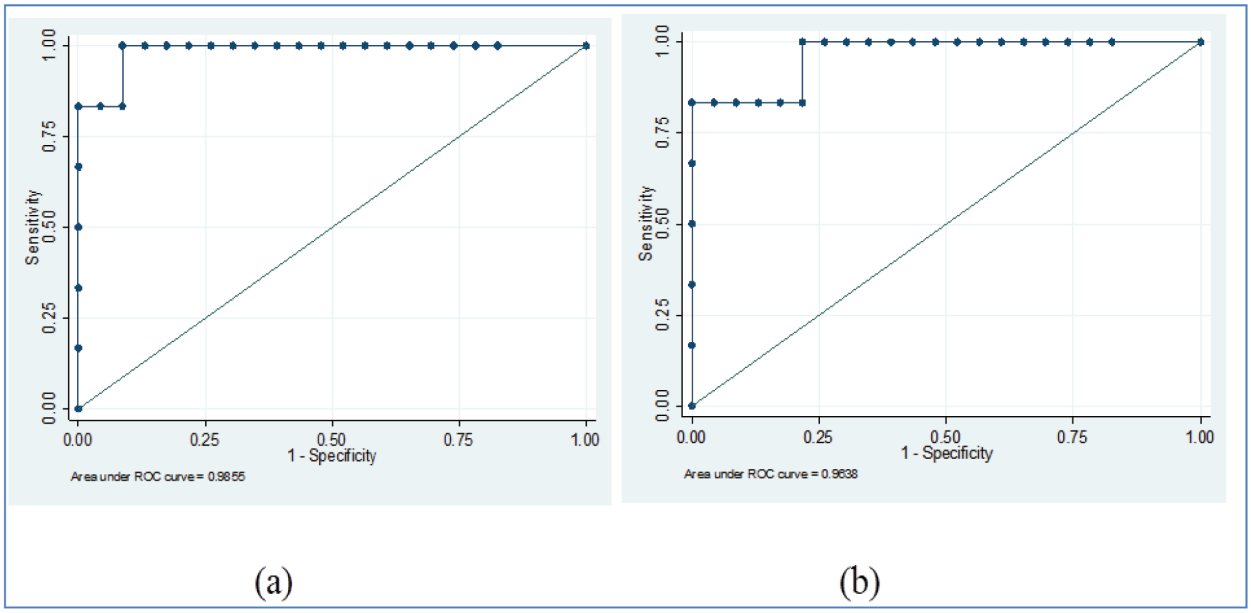

Fig. 4: Receiver operating characteristic (ROC) curve for albumin:creatinine ratio (ACR) with area under the curve (AUC) = 0.9855 (a) and urinary albumin excretion rate (UAER) with AUC = 0.9638 (b) in two-hour timed urine to determine microalbuminuria.

correct classification of individuals. The positive likelihood ratios for cut-points closest to the accepted cut-offs in the literature are very good for each test (9.58 for ACR and UAER each).

Correlates of microalbuminuria

Table 6 describes the participant characteristics by albuminuria categories. Patients classified as having "microalbuminuria" had significantly lower haemoglobin (6.9 ± 1.4 *vs* 9.1 ± 1.7 gm/dL; *p*-value: 0.007), but significantly higher serum creatinine (95.2 ± 27.5 *vs* 71 ± 18.7 µmol/L; *p*-value: 0.016). Even though these differences were not significant, the participants with microalbuminuria also tended to be older, had higher systolic and diastolic blood pressures, higher reticulocytes and lower haemoglobin F levels.

DISCUSSION

It is important to have validated methods and laboratory tests to screen for early nephropathy in patients with SCD as end stage renal disease is a major cause of morbidity and mortality in these patients. There are also unique features of the renal pathophysiology in persons with SCD which could render the usual screening methods useless. For example, the hyposthenuria (13) and the increased tubular secretion of creatinine (14, 15) that occur in them would impact on the total urinary creatinine excretion. There is also an increased

Table 6: Participants' characteristics by albuminuria categories

	Normoalbuminuric (n = 23)	Microalbuminuric (n = 6)	*P*-value
Age (years)	33.4 ± 10.8	42.3 ± 4.9	0.0630
Weight (kg)	58.6 ± 11.7	60.4 ± 2.8	0.7083
Height (cm)	168.4 ± 9.2	175.8 ± 10.1	0.0927
BMI (kg/m^2)	20.6 ± 3.1	19.7 ± 2	0.5083
Hb (gm/dL)	9.1 ± 1.7	6.9 ± 1.4	**0.0073**
WBC	9.8 ± 2.1	10.2 ± 3.4	0.7567
Retics (%)	8.9 ± 3.7	11.3 ± 4.4	0.180
HbF	8.9 ± 5	6.1 ± 4.5	0.2163
Serum creatinine (µmol/L)	71 ± 18.7	95.2 ± 27.5	**0.0164**
Average SBP (mmHg)	106.6 ± 12	111.4 ± 8.4	0.3680
Average DBP (mmHg)	62.9 ± 7.2	66.1 ± 8.6	0.3550

BMI: body mass index; Hb: haemoglobin; WBC: white cell count; HbF: foetal haemoglobin; SBP: systolic blood pressure; DBP: diastolic blood pressure

prevalence of bacteriuria (16) and haematuria (15) in persons with SCD, and these could impact measurements of urine proteins, as both contribute to the proteins being excreted in the urine.

In this study of persons with SCD, spot urine ACR and UAC as well as two-hour timed urine collection measurements of ACR and UAER have shown excellent performance as screening tests for microalbuminuria, the spot urine measure being somewhat more accurate than the two-hour collection. Similar studies have been done in nephropathies associated with other chronic diseases such as diabetes mellitus (8, 10, 17–20), even though all have been done with random spot urine samples and none with timed collections. Gansevoort *et al* (6) have recommended pre-screening by measuring UAE in a spot morning urine sample in order to keep the burden and costs involved in widespread population screening for microalbuminuria as low as possible. Even though albumin:creatinine ratios tend to perform better than albumin concentration in spot or timed samples (17, 21, 22), the differences with measurement of albumin excretion rates or concentrations are small, and hence if cost of test is a crucial issue for the patient, measurement of albumin concentrations may suffice. Testing for UAC in spot samples in persons with diabetes mellitus has also been shown to be the most cost-effective laboratory test (12). On the other hand, however, early morning spot urine ACR measurements have been shown to be the most superior method to predict worsening renal outcomes in patients with Type 2 diabetes (23). The ACR measure may actually have dual advantage: not only does it increase with increasing albumin excretion, but lowering urinary excretion of creatinine also causes an elevation in ACR; low urinary creatinine itself has shown association with cardiovascular risk and mortality (24, 25), independent of body composition, kidney function, and other traditional coronary artery disease risk factors.

Even though first-void urine testing tends to be more accurate over measurements from spot urine collections, as it negates the effects of physical activity and hydration status (26), the latter is still preferred as the ease of collection of a spot urine during a routine clinic visit is much greater. Other important confounders to consider when quantifying microalbuminuria are the effects of exercise, fever and other acute illnesses, the possibility of higher albumin excretion with higher blood pressures, urinary tract infections and stressors such as surgery (27). Albumin:creatinine ratio also tends to have different discriminator values that are gender- and age-specific (22), however, our study did not categorize by gender or age as the sample size was relatively small.

Possibly due to the variations inherent in urinary excretion of creatinine that are present in SCD, such as reduced production of creatinine due to lower body muscle mass and increased tubular secretion in the SCD kidneys, the ACR in both spot as well as in two-hour timed urine samples has much lower sensitivities that the sensitivities of measuring urinary albumin excretion alone in these tests. However, specificity is 100% with the ACR measures in both spot and two-hour urine samples, which also equates to 100% positive predictive value of these tests. The ROC analysis shows the strongest measure of diagnostic accuracy, the area under the ROC curve, to also be the highest with spot urine tests, especially with spot urine ACR.

Recent studies (28, 29) indicate that increases in albuminuria, even within the 'normoalbuminuric' range, may be associated with increasing cardiovascular risks. Whether this fact is of importance in SCD remains to be seen, however, it is important to determine utility of tests that are valid to measure albuminuria as a continuous variable. Use of correlations and ROC analysis as methods for comparisons of tests fill that criterion and hence recommendations from these studies could be used with confidence.

The prevalence of microalbuminuria rises with age in SCD and the higher the level of albumin excretion, the greater the progression to further glomerular and other damage to the kidney (27). Increasing serum creatinine, increasing blood pressure (even though not significantly so in our study) and falling haemoglobin levels are also indicators that renal function may be worsening (2), and this also was borne out in our study.

This study does not report on the coefficients of variation for albumin excretion, but as in other diseases, this variation is expected within and between individuals (26). Till other studies are done to confirm this, once a screen for microalbuminuria is positive, the test should be repeated twice more in a short time period to diagnose early sickle glomerulopathy in the individual. The use of ACE inhibitors in persons with SCD and microalbuminuria has been shown to be beneficial in past studies, even though they have been few and of very short intervention periods (30–33). Once again, until more prospective studies define the progression of sickle glomerulopathy and the effects of albumin excretion on this progression, as well as long term beneficial effects of ACE inhibitor therapy on halting progression, clinicians should plan on instituting ACE inhibitor therapy once persistent microalbuminuria is found to be present.

Determination of spot urine albumin levels has been found to be a good predictor of cardiovascular morbidity and all-cause mortality in general populations (21, 34), and whether there is any such link in SCD remains to be determined.

The main limitation of this study is its small sample size; it would be ideal to repeat the study, and hence duplicate the results in a larger sample. Nevertheless, the study has shown strong utility of spot urine and short timed urine assessments to detect microalbuminuria. A larger study may also enable a clearly defined cut-point to be determined for these measurements.

In conclusion, healthcare professionals working with patients with sickle cell disease can utilize a spot random urine measurement of albumin concentrations or albumin:

creatinine ratio as a marker of early nephropathy in this patient population.

ACKNOWLEDGEMENTS

The authors would like to thank Dr Raphael Fraser (Statistician), the late Nurse Norma Lewis (Study Nurse), Mrs Stacy Chin (Laboratory Technician) and Dr Asha Badaloo (Technical Advisor), all of Tropical Medicine Research Institute, for their contributions to this project. The work was supported by a grant from the Caribbean Health Research Council.

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