Predicting 24-hour Urinary Protein Excretion in Afro-Caribbean Barbadians by Comparing Urine Protein Excretion over Different Durations *versus* Spot Collection

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ABSTRACT

Aim: *The gold standard for the determination of proteinuria, an independent risk factor for cardiovascular and renal disease, is the measurement of protein in a 24-hour urine collection. However, this method has been shown to be unreliable mainly due to poor compliance of sampling by patients. This study investigates other appropriate means of predicting 24-hour urinary protein excretion in a sample of Afro-Caribbeans in Barbados by assessing the correlation of actual and estimated urinary protein excretion between a 24-hour urine collection sample, 12-hour (AM and PM) and spot (AM and PM) urine collections.*

Subjects and Method: *A convenient sample of 30 healthy participants of Afro-Caribbean origin between the ages of 21 and 55 years was recruited for the study. The 24-hour urine samples and anthropometric data were collected as documented in the study's standard clinical procedure. A 24-hour urine sample was collected as two separate 12-hour AM and PM samples. In addition, two spot samples (AM and PM) were taken during each 12-hour sample collection period. Analysis of the urinary protein and creatinine was done with a Roche/Hitachi Modular System (Roche Diagnostics, IN, USA). SPSS version 19 was used to analyse the data to make inferences.*

Results: *Thirty Afro-Caribbean persons participated in the study: 16 females and 14 males. The average age and body mass index (BMI) were 38 ± 17 years and 25.32 ± 5.98 kg/m^2, respectively. The Spearman Rho's correlation was used to interpret associations of the urinary parameters in 24-hour collected sample and the other samples. The strongest correlation of the protein:creatinine ratio in the 24-hour collected sample to the other samples was observed with the 12-hour AM sample ($r = + 0.743$, $p < 0.01$) followed by the 12-hour PM sample ($r = +0.672$, $p < 0.01$). On analysing gender, the more significant correlations found were among the males for the 12-hour timed samples with $r = +0.945$, $p < 0.01$ and $r = +0.736$, $p < 0.01$ for the AM and PM samples, respectively. There were very strong correlations between the 24-hour urinary protein excretion and the estimated 24-hour protein excretion from the 12-hour AM and PM samples ($r = +0.846$, $p < 0.01$ and $r = +0.637$, $p < 0.01$, respectively). Both males and females had the strongest correlation for the estimation of 24-hour protein excretion in the 12-hour AM sample ($r = +0.795$, $p < 0.01$ and $r = +0.965$, $p < 0.01$, respectively).*

Conclusion: *The use of a 12-hour timed sample, specifically the morning sample, may be a more convenient way to assess proteinuria in the Afro-Caribbean population. This method allows for a quicker assessment of proteinuria which not only allows earlier diagnosis of renal disease but may also reduce the clinical cost of the disease's management.*

Keywords: Afro-Caribbean, creatinine, excretion, protein, urinary

Predicción de la Excreción de Proteína en Orina de 24 Horas en Barbadenses Afrocaribeños Mediante la Comparación de la Excreción de Proteínas en la Orina en Diferentes Períodos de Tiempo frente a la Recogida al Azar

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RESUMEN

Objetivo: *La regla de oro para la determinación de la proteinuria – un factor de riesgo independiente para las enfermedades cardiovasculares y renales – es la medición de la proteína en una recogida de la orina de 24 horas. Ha quedado demostrado que este método es poco confiable debido principalmente al pobre cumplimiento del muestreo por parte de los pacientes. Este estudio investiga otros medios adecuados para predecir la excreción urinaria de 24 horas de proteínas en los afro-caribeños de Barbados, evaluando la correlación real y estimada de la excreción urinaria de proteínas entre una muestra de recogida de orina de 24 horas, 12 horas (AM y PM) y las recogidas de orina al azar (AM y PM).*

Sujetos y métodos: *Una muestra conveniente de 30 participantes sanos de origen afrocaribeño de edades entre 21 y 55 años fue reclutada para el estudio. Se obtuvieron muestras de orina de 24 horas y datos antropométricos como se indica en el procedimiento clínico estándar del estudio. Se recogió una muestra de orina de 24 horas, separadas en dos muestras de 12 horas AM y 12 horas PM. Además, se tomaron dos muestras al azar (AM y PM) durante cada periodo de recogida de muestras de 12 horas. El análisis del proteína urinaria y la creatinina urinaria se realizó con un sistema analítico modular Roche/Hitachi. La versión 19 de SPSS se utilizó para analizar los datos con el fin de hacer inferencias*

Resultados: *Treinta personas afrocaribeñas participaron en el estudio: 16 mujeres y 14 hombres. La edad promedio y el índice de masa corporal (IMC) fueron 38 ± 17 años y 25.32 ± 5.98 kg/m^2, respectivamente. La correlación Spearman Rho fue utilizada para interpretar las asociaciones de los parámetros urinarios en la muestra recogida de 24 horas y las otras muestras. La correlación más fuerte de la relación proteína: creatinina en la muestra recogida de 24 horas con respecto a las otras muestras, se observó en la muestra de 12 horas AM ($r = +0.743$, $p < 0.01$), seguida por la muestra de la 12 horas PM ($r = +0.672$, $p < 0.01$). En el análisis de género, las correlaciones más significativas fueron aquellas encontradas entre los varones para las muestras cronometradas de 12 horas con $r = +0.945$, $p < 0.01$ y $r = +0.736$, $p < 0.01$ para las muestras de AM y PM, respectivamente. Hubo correlaciones muy fuertes entre la excreción de proteína urinaria de 24 horas y la excreción de proteína de 24 horas estimada de las muestras de 12 horas AM y PM ($r = +0.846$, $p < 0.01$ y $r = +0.637$, $p < 0.01$, respectivamente). Tanto los varones como las hembras mostraron una fuerte correlación con respecto al estimado de la excreción proteica de 24 horas en la muestra de 12 horas ($r = +0.795$, $p < 0.01$ and $r = +0.965$, $p < 0.01$, respectivamente).*

Conclusión: *El uso de muestras cronometradas de 12 horas – específicamente la muestra de la mañana – puede ser una manera más conveniente de evaluar la proteinuria en la población afrocaribeña. Este método permite una evaluación más rápida de la proteinuria, la cual no solamente permite un diagnóstico más temprano de la enfermedad renal, sino que también hace posible reducir el costo clínico del tratamiento de la enfermedad.*

Palabras claves: Afrocaribeño, creatinina, excreción, proteína, urinaria

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INTRODUCTION

Compared to persons of European descent, Afro-Caribbean and other people of African descent have a higher incidence of stroke and end-stage renal disease (1). It is estimated that chronic kidney disease affects over 26 million Americans with the incidence in the South Asian and the Afro-Caribbean population being three- to fourfold higher than the general population (2). The most common method of determining the function of the kidneys is by measuring the protein in the urine with the use of a 24-hour urine collection. Normal levels of protein in the urine are between 150 and 200 mg/day while above 300 mg/day is usually considered significant and is associated with decrease functioning of the kidneys (3). Proteinuria is recognized as an independent risk factor for cardiovascular and renal disease and also a predictor of progression to end organ kidney damage. The gold standard for the determination of this condition is the 24-hour urinary protein estimation, however, it has been shown to be unreliable due to poor compliance during the sampling process by patients (2).

Another possible method for determining urinary protein is the use of a spot sample with a protein:creatinine ratio. This method is more practical as it is less costly and more convenient. The kidneys excrete creatinine which is a breakdown product of creatine, an important part of the muscles (4). The excretion of protein and creatinine is constant

throughout the day when the glomerular filtration rate is stable, therefore the use of a spot sample can be a reliable method for measuring protein rather than the 24-hour urine sample (2). Several studies on kidney disease demonstrate a strong correlation with the protein:creatinine ratio from a spot sample with a 24-hour urine collection (3, 5–8). First morning specimens are usually preferable (6). However, the use of the spot sample is not always ideal as the correlation was absent in studies with pregnant women and those suffering with pre-eclampsia (9, 10). Also, most of the studies were conducted on the South Asian population with very little information on the Afro-Caribbean population (6, 8). Therefore, the aim of this study is to determine if any such correlation of protein:creatinine ratio in the spot and other timed samples exists in a convenient sample of healthy Afro-Caribbean participants.

SUBJECTS AND METHODS

The study was approved by the Institutional Review Board of The University of the West Indies, Cave Hill campus/ Ministry of Health, Barbados. A convenient sample of 30 healthy participants of Afro-Caribbean origin was recruited for the study from The University of the West Indies' Health Services Clinic and the staff clinic at the Hilton Barbados. The exclusion criteria included alcohol intake greater than 14 units per week for women and 21 units per week for males, recreational drug use, smokers (greater than one year), diabetics (diagnosed or on fasting blood glucose), hypertensive patients on medication (Joint National Committee (JNC) pre-hypertension was acceptable), clinically evident vascular disease and participants with a body mass index (BMI) less than 18 kg/m^2 or greater than 36 kg/m^2.

The 24-hour urine sample was collected as documented in the study's standard clinical procedure. Hence, a 24-hour urine sample was collected as two separate 12-hour AM and PM samples. In addition, two spot samples (AM and PM) were taken during each 12-hour sample collection period. The AM spot sample was defined as the second urine voided for the day and the PM spot sample was defined as the first urine voided after a 12-hour period (collected in the evening). Urine samples were analysed in the Chemical Pathology Laboratory at the Queen Elizabeth Hospital, Bridgetown, Barbados. Analysis of the urinary parameters of protein and creatinine was done with a Roche/Hitachi Modular System (Roche Diagnostics, IN, USA). SPSS version 19 was used to analyse the data to make inferences.

The main purpose of this study was to determine if there were any correlations between the estimates of the 24-hour urinary protein in the timed and spot samples and the gold standard of the 24-hour urinary protein measurement. The following formula was used for the estimation in the timed and spot samples:

$$\text{Estimated protein excretion (mg/L)} = \frac{\text{AM spot protein measure (mg/L)}}{\text{AM spot creatinine measure (mmol/L)}} \times \text{24-hour creatinine (ummol/L)}$$

RESULTS

Thirty persons participated in the study: 16 females and 14 males. All participants were of Afro-Caribbean descent with an average age and BMI of 38 ± 17 years and 25.32 ± 5.98 kg/m^2, respectively. The Spearman Rho's correlation was used to interpret all the data. Statistical significance was noted at the 95% confidence level.

Correlations of the protein:creatinine ratios between the 24-hour collected sample and other timed and spot samples were investigated. As seen in Table 1, no significant correlation was found with the protein:creatinine ratio in the 24-hour collected sample and the AM spot sample. However, a strong correlation was found with the timed 12-hour AM sample ($r = +0.743$, $p < 0.01$). On analysing gender, the more significant correlations found were among the males for the 12-hour timed samples with $r = +0.945$, $p < 0.01$ and $r = +0.736$, $p < 0.01$ for the AM and PM samples, respectively.

Table 1: Correlation of urinary protein:creatinine ratio in a 24-hour collection sample with 12-hour timed and spot urine samples

Results	Total	Males	Females
AM spot protein-creatinine ratio	r = -0.207 $p = 0.311$	r = -0.261 $p = 0.467$	r = -0.180 $p = 0.505$
PM spot protein-creatinine ratio	r = +0.296 $p = 0.150$	r = +0.200 $p = 0.55$	r = +0.394 $p = 0.164$
Timed sample 12-hour AM protein-creatinine ratio	**r = +0.743 $p < 0.01$	**r = +0.945 $p < 0.01$	**r = -0.508 $p = 0.045$
Timed sample 12-hour PM protein-creatinine ratio	**r = +0.672 $p < 0.01$	**r = +0.736 $p = 0.04$	**r = 0.561 $p = 0.024$

**Significant $p < 0.05$

Table 2 shows that there are very strong correlations between the 24-hour urinary protein excretion and the

Table 2: Correlation of urinary protein excretion in a 24-hour collection sample with 12-hour timed and spot urine samples

Results	Total	Males	Females
AM spot sample	r = +0.198 $p = 0.333$	r = +0.164 $p = 0.650$	r = +0.174 $p = 0.520$
PM spot sample	r = +0.337 $p = 0.099$	r = -0.128 $p = 0.709$	**r = +0.748 $p < 0.01$
Timed 12-hour AM sample	**r = +0.846 $p < 0.01$	**r = +0.795 $p < 0.01$	**r = +0.965 $p < 0.01$
Timed 12-hour PM sample	**r = +0.637 $p < 0.01$	r = +0.366 $p < 0.219$	**r = +0.752 $p < 0.01$

**Significant $p < 0.05$

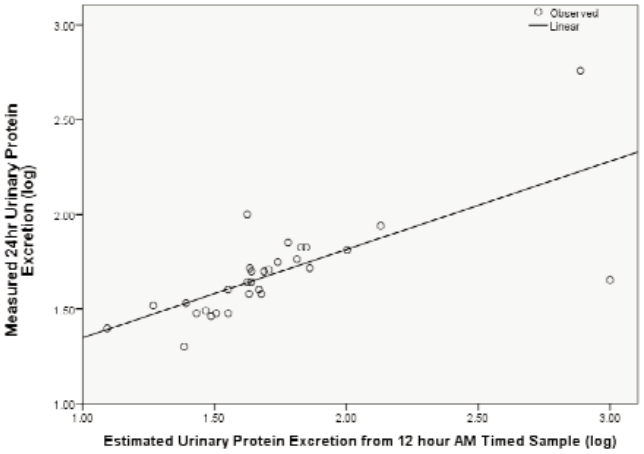

Figure: Correlation with 24-hour urinary protein excretion and estimated 24-hour protein excretion using the timed 12-hour AM samples (log).

estimated 24-hour protein excretion from the 12-hour AM and PM samples (r = +0.846, $p < 0.01$ and r = +0.637, $p < 0.01$, respectively). No correlations were found with the AM spot sample. The correlations were consistent in both males and females with the strongest correlation for the estimation of 24-hour protein excretion in the 12-hour AM sample. The Figure demonstrates the strong correlation between the 24-hour urinary protein excretion and the estimated 24-hour protein excretion using the timed 12-hour AM sample.

DISCUSSION

The 24-hour urinary collection is considered the gold standard for obtaining urinary parameters including urinary protein levels which are necessary for determining kidney function (2). Due to poor patient compliance when collecting the 24-hour urine sample, other methods are being investigated for their adequacy in determining protein excretion (2). An alternative approach is the use of random urine collections and assessing the protein:creatinine ratio (3). Several studies have demonstrated a smaller variation in the protein:creatinine ratio in participants compared with the 24-hour protein concentration in urine samples (3). In a study from Ginsberg *et al*, they reported a strong correlation of r = 0.972 between 24-hour protein excretion and protein-creatinine ratio (11). Based on the findings of this study, correlations of the protein:creatinine ratio in the 24-hour collections and spot and timed samples were found amongst the timed 12-hour samples only.

Significant correlations were reported in this study between the 24-hour collected sample and the 12-hour timed samples. In a systematic review by Price *et al*, it was concluded that protein:creatinine ratio can be used as a tool to measure proteinuria as defined by a 24-hour urinary excretion (3). Similarly, a study from Xin *et al* reported that spot samples of protein:creatinine ratio can be used as an alternative to 24-hour urine samples. This study demonstrates no significant correlation with the morning or afternoon spot samples which is inconsistent with the literature (6). Several studies have similar conclusions with strong correlations between the 24-hour urine samples and the morning spot samples; however, not all of those studies were conclusive on the morning spot samples but rather looked at random samples throughout the day (7, 8, 12). Other studies focussed on the use of timed samples instead of the 24-hour urine sample.

In a study from Kosmadakis *et al*, they validated the use of morning and afternoon timed samples as an alternative to 24-hour urine samples (13). In this study, The Figure demonstrates the strong correlation of the measured 24-hour urinary protein excretion and estimated 24-hour urinary excretion in the morning 12-hour timed sample. The strong correlation was observed in both females and males on assessing gender, with the stronger correlation in the females who may have had less variance in creatinine levels due to lower muscle mass compared to males. Urinary creatinine concentrations are proportional to body surface area, hence the males that are known to have greater muscle mass will have greater creatinine excretion (14).

Urinary protein excretion is considered to be a modifiable risk factor in the management of renal disease (15). Therefore, the convenience and reliability of a smaller timed sample would be useful in the monitoring of such a condition. Table 2 outlines that the best correlated predictor of the estimated 24-hour urinary protein excretion was the 12-hour AM timed sample (r = 0.846, $p < 0.01$, r = +0.795, $p < 0.01$ and r = 0.965, $p < 0.01$) for the whole sample, males and females, respectively. Therefore, the use of the smaller timed sample is a very good alternative for measuring proteinuria as opposed to using a 24-hour sample collection. No correlation was found with the morning spot sample which is unlike most observations in other studies (6, 16). This difference may be chiefly due to the fact that previous studies have focused on Caucasian or Asian participants. Therefore, race and ethnicity might play an important role in determining the renal health of patients and might also be a key factor for the accurate assessment of proteinuria (17, 18).

CONCLUSION

The use of the morning spot sample for the estimation of urinary protein, as observed in other studies, was not corroborated by the findings of this study. The use of a 12-hour timed sample, specifically the morning sample, rather than the 24-hour collected sample, may be more appropriate and a convenient way to assess proteinuria in the Afro-Caribbean population. This method may allow for a quicker assessment of proteinuria which not only allows earlier diagnosis of renal disease but may also reduce the clinical cost of management of the disease. A more robust and randomized population sample as well as a method to correct for high creatinine variability is required to further enhance the significance of the obtained results.

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