

Full Length Research Paper

Prediction of microalbuminuria by using spot urine samples and regression analysis to convert spot microalbumin values to 24 hours microalbuminuria

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Early detection of diabetic nephropathy relies on tests for urinary excretion of albumin. The two methods used to determine if a patient is microalbuminuric are the 24-hour albumin excretion rate (AER) and the spot albumin: creatinine ratio (ACR). However, the strength of correlation of ACR in comparison with 24 hr AER needs to be established and hence we intended to study the utility of ACR in estimating microalbuminuria and predicting diabetic nephropathy. A total of 100 subjects with type2 diabetes mellitus (T2DM) and an equal number of non-diabetics in the age range 40-60 years were included in the study. Urinary albumin was estimated in both the 24 hours and spot urine samples by immunoturbidimetric method and urinary creatinine was analyzed by Jaffe's method. Urinary ACR was calculated by dividing the urinary albumin concentration in micrograms by the urinary creatinine in milligrams. The average ACR and AER were 24.4 ± 12.6 $\mu\text{g}/\text{mg}$ of creatinine and 24 ± 12 mg/day respectively in T2DM and 9.6 ± 1.6 $\mu\text{g}/\text{mg}$ of creatinine and 9.4 ± 1.6 mg/day respectively in non-diabetics. The combined regression analysis showed Y (24 hour Micro Alb) = $.363 + .948$ (Spot ACR). The study clearly brought out that the ACR provides an equivalent result compared to 24 hours AER.

Key Words: Diabetes mellitus, Albumin creatinine ratio, Albumin excretion rate, spot urine sample, 24 hours urinary albumin

INTRODUCTION

Diabetic nephropathy is one of the leading causes of end-stage renal disease. Early detection of diabetic nephropathy relies on tests for urinary excretion of albumin. Conventional qualitative tests (chemical strips or dipsticks) for albuminuria do not detect the small increases in urinary albumin excretion seen in early stages of nephropathy. For this purpose, tests for "microalbuminuria" are used. Microalbuminuria is defined as excretion of 30–300 mg of albumin/24 h (or 20–200 $\mu\text{g}/\text{min}$ or 30–300 $\mu\text{g}/\text{mg}$ of creatinine) on two of three urine collections [American Diabetes Association, 1999]. The two main methods used to determine if a patient is

microalbuminuric are the 24-hour albumin excretion rate (AER) and the spot albumin: creatinine ratio (ACR). Because of the inconvenience of 24-hour urine collection, the ACR has been adopted to estimate urine albumin excretion rates. The ACR has been shown to be convenient, and efficient in screening patients for microalbuminuria when compared with 24-hour collections and has been suggested for diabetic and hypertensive patients [Afkhani et. al 2008]. Currently, the AER is used extensively to determine the presence of microalbumin and has even been utilized as a gold standard when ascertaining the efficacy of other diagnostic tests [Lum, 2000; Tabaei et al., 2001]. However, there is a lack of consistent comparisons between the ACR and 24-hour AER. A morning void sample is said to have better prediction of microalbuminuria and also urinary albumin to creatinine

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ratio in a single urine sample has been proposed to provide an estimate of microalbuminuria by adjusting for variability in urine concentrations [Witte, 2009]. But there are certain studies which say that the spot ACR does not consistently identify patients with microalbuminuria and the 24-hour albumin excretion rate should be used instead for this purpose [Johns et al., 2006]. Overnight collection of excreted urinary albumin has been advocated to screen for microalbuminuria and increased urinary AER, because the sampling technique is more convenient than 24-hour collection and reduces the variability of urinary AER caused by posture and physical activity. Compliance with overnight urine collection has been reported to be excellent. First morning voided samples also allow reliable estimates of microalbuminuria [Witte, 2009].

Recently a conference on the clinical use and measurement of urine albumin was organized by the Laboratory Working Group of the National Kidney Disease Education Program and the International Federation of Clinical Chemistry (IFCC) to examine the current practices in measuring urine albumin and using the resulting data in kidney disease management. The conference objectives were to increase understanding of the issues that must be addressed to enable standardization of measurements and clinical practice guidelines based on urine albumin excretion. Key issues were to identify clinical needs for standardization of (a) urine collection methods, (b) urine albumin and creatinine measurements based on a complete reference system, (c) reporting of test results, and (d) reference intervals for the ACR [Miller et al., 2009].

Therefore the present study was conducted to

- a. Assess the correlation coefficients of spot ACR values with 24 hrs AER
- b. Validate if spot ACR values can predict microalbuminuria by performing regression analysis with 24 hrs AER

MATERIALS AND METHODS

Design of the study

Diagnostic accuracy

Sample size

A random sample of a 100 subjects in the age range of 40-60 years with type2 diabetes mellitus (DM) (Group 1) on regular follow up at the outpatient department of Medicine and an equal number of age and sex matched non-diabetics (Group 2) were included in the study. The renal status of the subjects with type 2 DM was assessed

by the serum creatinine levels. Non-diabetic status of the subjects was confirmed by their detailed history, fasting blood sugar and HbA1c levels. The study group subjects were requested to provide fasting blood sample, spot urine sample (collected anytime between 8 am – 8 pm) and 24 hours urine sample. This study was approved by institutional ethical committee and a written informed consent was obtained from all subjects.

The urinary albumin was estimated in both the 24 hours and spot urine samples. The two samples were processed immediately and analyzed by two different technicians and the values were unknown to each other. The fasting blood sugar, glycated hemoglobin and serum creatinine were estimated in the fasting blood sample. Urinary albumin was determined by the immunoturbidimetric method [Harmoinen et al., 1985] and urinary creatinine was analyzed by the kinetic Jaffe's method [Rosano et al., 1990] using RANDOX Daytona autoanalyzer. Kits for the estimation of urinary albumin and urinary creatinine was purchased from RANDOX Laboratories Ltd. The assay range for urinary albumin was 4-200 mg/L. Urinary ACR was calculated by dividing the albumin concentration in micrograms by the creatinine expressed in milligrams. Plasma glucose and glycated hemoglobin were analyzed using RANDOX Daytona autoanalyzer [Buckley and Drury 1960; Groche et al., 2003].

Statistical analysis

Arithmetic mean and standard deviation were worked out to assess the level of various parameters in the two groups under study. As the distribution of ACR and AER are known to be skewed, Spearman's rank correlation coefficient and regression analysis were carried out to study the linear relationship between the two. In order to estimate the sensitivity and specificity of ACR compared to AER, the cut-off's used were 30 µg/mg for ACR and 30 mg/day for AER [American Diabetes Association, 2003]. The data entry and all statistical analyses were carried out using Microsoft Excel and Epi info package version 3.5.1.

RESULTS

The group 1 included 100 proven diabetics with fasting glucose levels more than 110 mg/dl and group 2 included 100 non diabetics. The average age of diabetics was 54±8.2 years and non-diabetics were 53±8.4 years. The male and female distribution was 60 and 40 percent. The fasting blood glucose value was 157±58 mg/dL in group 1 and 88±8 mg/dL in group 2. The average spot ACR was 24.4±12.6 µg/mg of creatinine in group 1 and the corresponding 24 hour AER in group 1 was 24±12 mg/day and in group 2 it was 9.6±1.6 µg/mg of creatinine

Table 1. Mean and standard deviation (SD) of parameters included in the study

Parameters	Diabetics	Non-diabetics
	Mean \pm SD	Mean \pm SD
Age (in years)	54 \pm 8.2	53 \pm 8.4
FBS (mg/dl)	157 \pm 58	88 \pm 8.4
HbA _{1c} (%)	6.6 \pm 1.6	4.5 \pm 0.4
24 hour Urine volume (ml/day)	1967 \pm 382	1909 \pm 368
Spot Albumin Creatinine Ratio (μ g/mg of creatinine)	24.4 \pm 12.6	9.6 \pm 1.6
24 hour Albumin Excretion Rate (mg/day)	24.0 \pm 12	9.4 \pm 1.6

Table 2. Spearman's rank correlation coefficient of spot ACR with the other parameters included in the study

Parameter	Diabetics	Non-diabetics
	Correlation coefficient	Correlation coefficient
Age	0.001	-0.385*
Fasting blood sugar	0.109	-0.008
HbA _{1c}	-0.0074	0.112
24 hour Albumin Excretion Rate (mg/day)	0.828#	0.861#

*statistically significant ($p < 0.05$), # statistically significant ($p < 0.001$)

in spot sample and 9.4 \pm 1.6 mg in 24 hour sample (Table 1).

To study the linear relationship between spot ACR and other variables included in the study the Spearman's rank correlation coefficient was estimated. Age was found to be related to spot ACR in the non-diabetics only. However, 24hr AER was significantly related to the spot ACR irrespective of diabetic status. (Table 2) Subsequently, Linear regression equations were derived for group 1 and group 2 separately as well as combined together for studying the predictive power of spot ACR in predicting microalbuminuria. The percentage variation explained in group 1 was 97.5 and in group 2 it was 89.3. The predicted and observed values were similar in both the groups and therefore the difference between the two i.e. residuals were small and normally distributed. A combined regression equation was also derived for both the groups. The percentage variation explained was as high as 97.5 indicating that spot ACR is almost equivalent to 24 hour AER. The regression coefficients were statistically significant ($p < 0.001$) in all three equations.

Regression Analyses

The Regression Equations of group 1, group 2 and combined for both the groups were as follows

Group 1 (Diabetics)

$$Y (\text{AER}) = .390 + .947 (\text{ACR})$$

Group 2 (Non-diabetics)

$$Y (\text{AER}) = .352 + .947 (\text{ACR})$$

Combined

$$Y (\text{AER}) = .363 + .948 (\text{ACR})$$

The sensitivity and specificity of Spot ACR was evaluated with 24 hours urine sample. The cut off point adopted was < 30 (normal) and > 30 (microalbuminuria). The sensitivity and specificity was observed which was 100 percent indicating complete agreement between the two methods. (Table 3)

DISCUSSION

Microalbuminuria is recommended for studying the progression of type 2 Diabetes mellitus and for early detection of diabetic nephropathy. This test normally requires 24 hour urine sample for estimating the microalbuminuria. Collection of 24hrs urine sample is known to be a cumbersome procedure especially at the community level and outpatient clinical setting. In the eventuality, it becomes necessary to find an alternative method which is easy and acceptable as well as should have the same accuracy. These include spot testing of a morning urine specimen or overnight urine collections for estimation of urine albumin concentration. Since random

Table 3. Sensitivity and Specificity of Spot ACR with 24 Hours AER

		24 hour AER		Total
		≥30µg/mg	<30µg/mg	
Spot ACR	≥30µg/mg	27(100.0)	0	27
	<30µg/mg	0	173(100.0)	173
Total		27(100.0)	173(100.0)	200

urine sample is more convenient to collect, it was proposed to check its efficacy to detect microalbuminuria.

The significance of microalbuminuria is that it is the predictor of clinical proteinuria and chronic renal failure in insulin dependent diabetes mellitus (IDDM), [Krolewski et al., 1995; Mogensen and Christinen, 1984] and the early index of cardiovascular morbidity and mortality as well as diabetic nephropathy in non-insulin dependent diabetes mellitus (NIDDM) [Collins et al., 1989; Lee et al.,1995]. Therefore, it is important to assess the validity of a random urine sample as a screening test of diabetic nephropathy [Gartling et al.,1988; Schmitz and Vaeth, 1988].

In our study, we compared the spot ACR with 24 hours AER in 100 diabetic patients and 100 non-diabetics respectively. The spot ACR showed an excellent performance as a screening test for the diagnosis of microalbuminuria. Our study also highlights the fact that ACR and AER correlates well in both diabetic as well as non-diabetic subjects. The sensitivity and specificity of spot ACR was 100 percent indicating overall concordance. Linear regression equations were derived for studying the predictive power of spot ACR in predicting microalbuminuria which indicates that spot ACR is almost equivalent to 24 hour AER.

Previous studies have also shown that albumin measurements in a random urine sample presented almost perfect accuracy for the screening of micro- and macroalbuminuria in diabetic patients and suggested it as a valid test in screening for diabetic nephropathy [Ahn et al., 1999; Shahbazian and Hosseini-Asl, 2008]. Major Limitation of the study is that fewer cases were having diabetic nephropathy in the study group. This study needs to be repeated with a larger sample size to confirm the strong findings.

CONCLUSION

The study clearly brought out that the spot ACR provides an equivalent result compared to 24 hours AER in both diabetics and non-diabetics which is very highly correlated. The regression function has also shown that the predicted and observed average values were almost similar. Our study concludes that spot urine samples can be effectively used to detect microalbuminuria in patients

of diabetes mellitus especially in the Indian subset of diabetics.

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