

The Validity of Random Urine Specimen Albumin Measurement as a Screening Test for Diabetic Nephropathy

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Abstract

To assess the validity of urine albumin concentration (UAC) and the urine albumin:creatinine ratio (UACR) in a random urine specimen (RUS) for screening diabetic nephropathy in Korea, a total of 105 ambulatory diabetes mellitus patients (male:female, 52 : 53), ages 40–75 years (median 59 years) collected 105 RUSs after completing a timed 24 hour urine collection. Albumin was measured by immunonephelometry. According to the timed urinary albumin excretion rate (UAER) measured in the 24 hour collection (criterion standard), samples were classified as normoalbuminuric (UAER < 20 μ g/min; n=50), microalbuminuric (UAER 20–200 μ g/min; n=30), and macroalbuminuric (UAER > 200 μ g/min; n=25). The receiver operating characteristics (ROC) curve of UAC and UACR in a RUS for screening of microalbuminuria (normo- and microalbuminuric samples; n=80) and macroalbuminuria (micro- and macroalbuminuric samples; n=55) were plotted. Pearson's coefficients of correlation of 24 hour UAER vs. UAC and UACR were 0.81 and 0.75, respectively ($P < 0.001$). The point of intersection with a 100%-to-100% diagonal for microalbuminuria were as follows: 31.0 mg/l for UAC and 32.5 mg/g for UACR; for macroalbuminuria 181 mg/l for UAC and 287.3 mg/g for UACR. The sensitivity and specificity of the cut-off points for microalbuminuria were 77% and 82% for UAC and 77% and 92% for UACR. The sensitivity and specificity of the cut-off points for macroalbuminuria were 84% and 90% for UAC and 88% and 90% for UACR. In present study, no difference was observed when comparing the performance of UAC and UACR based on a statistical comparison by McNemar test. The repeated measurements of UAC and UACR in the same individual were statistically similar and were correlated with each other. Based on these results, albumin measurements (UAC and UACR) in a RUS were considered as a valid test for screening diabetic nephropathy.

Key Words: Diabetic nephropathy, microalbuminuria, random urine specimen, Korea

INTRODUCTION

Diabetic nephropathy is the major health problem in patients with diabetes. The natural history of diabetic nephropathy has generally been viewed as a descending path from normoalbuminuria to end-stage renal disease (ESRD) through an intermediate stage marked by microalbuminuria and overt proteinuria.^{1,2} It appears that the development of each stage of diabetic nephropathy is determined by a somewhat different set of risk factors. Whereas the level of glycemic control is most likely the dominant factor in the occurrence of microalbuminuria³, progression through the more advanced stages is determined by such risk factors as hypertension,⁴ hypercholesterolemia, and unidentified genetic factors.

The definition of microalbuminuria is somewhat

different according to various authors: It was defined as urine albumin excretion rate (UAER) of more than 30 μ g/min by Viberti et al,⁵ more than 15 μ g/min by Mogensen and Christinen,⁶ or more than 40 mg/L by Parving et al.⁷ Kaplan defined it as UAER of 30–300 mg/24 hour or 20–200 μ g/min.⁸ In this study, microalbuminuria is defined as UAER of 20–200 μ g/min, and macroalbuminuria as more than 200 μ g/min.

Once in the advanced stage of diabetic nephropathy, patients are at high risk of death due to cardiovascular disease as well as renal failure. Whereas the risks of microalbuminuria and proteinuria seem to be similar in insulin dependent diabetes mellitus (IDDM) and non-insulin dependent diabetes mellitus (NIDDM), the competing risk of cardiovascular death in the latter preempts the development of ESRD.^{5,9} Diabetic nephropathy has two distinct but interconnected stages (incipient diabetic nephropathy-microalbuminuria and overt diabetic nephropathy-macroalbuminuria). In addition, preventive and therapeutic programs have altered the natural course of diabetic nephropathy.

The microalbuminuria phase is characterized by

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potential reversibility if proper therapeutic measures are used. Macroalbuminuria is a more advanced stage with a progressive, virtually inexorable decline in renal function. Therefore, the screening test for identifying the stage is very important for early intervention. The urinary albumin excretion rate (UAER) is the parameter between incipient and overt diabetic nephropathy.

It has been suggested that microalbuminuria predicts the progress to ESRD in IDDM or NIDDM and the increased cardiovascular morbidity and mortality in NIDDM.¹⁰

Recently, recommendations for screening and diagnosis of diabetic nephropathy, with special reference to microalbuminuria, have been published.^{11,12} Measurement of urinary albumin concentration (UAC) or urinary albumin:creatinine ratio (UACR) in a random urine specimen (RUS) or first morning urine sample have been recommended.^{13,14}

There are various methods to measure UAER including a RUS, first morning urine, a timed urine collection (24 hour, 8hour, overnight, 3 hour, 4 hour etc). A timed urine collection (24 hour or overnight) is the most sensitive assay for measuring UAER, however, a RUS is more practical and convenient than a timed urine collection.^{10,15,16}

Although there have been various studies of the screening tests for diabetic nephropathy,¹⁷⁻¹⁹ there have been little data regarding the accuracy of the RUS in screening for diabetic nephropathy.²⁰

The aims of this study were to assess the validity of the UAC¹⁶ and the UACR²¹ in a RUS for the screening of diabetic nephropathy and to determine the cutoff points by receiver operating characteristic (ROC) curves²²⁻²⁴ and to evaluate the reproducibility in Koreans.

MATERIALS AND METHODS

Patients

The study was performed at the outpatient diabetes clinic of Severance Hospital (YUMC), between May 1997 and August 1997. Informed consent was obtained from each patient and the protocol was approved by the ethics committee. Every diabetic patient (per World Health Organization criteria, 1980) without evidence of cardiac failure or renal tract disease other than diabetic nephropathy (urinary tract infection, hematuria, abnormal urinary sediment, and/or plasma creatinine increase without proteinuria) was considered for the study.

Methods

The patients were oriented to take timed 24hour urine collection. No specific recommendations were made about fluid intake, physical exercise, or dietary protein intake. Women were not examined during menstruation. A blood sample was taken to measure biochemical parameters and a RUS was taken for UAC and for UACR measurements.

Urinary albumin was measured by immunonephelometry (Behring Nephelemetry analyzer II, Behringwerke AG, Marburg, Germany). HbA_{1c} was analyzed by high performance liquid chromatography (HPLC) (Variant, BioRad, Hercules, CA, USA) and creatinine by Jaffé reaction (Hitachi 747 Automatic analyzer, Hitachi, Nakashi, Japan). The patients collected 105 24 hour UAER's and 105 RUS's. All urine samples were confirmed to be sterile by culture. The 24 hour UAER was considered adequate when creatinine measurements in the same sample were 700–1,500 mg for women and 1,000–1,800 mg for men. Samples were divided into normoalbuminuric (UAER < 20 μ g/min; n=50), microalbuminuric (UAER = 20–200 μ g/min; n=30), and macroalbuminuric (UAER > 200 μ g/min; n=25) groups, according to the criterion standard.

In RUS, urinary albumin was measured by immunonephelometry (Behring Nephelemetry analyzer II, Behringwerke AG, Marburg, Germany), and creatinine was analyzed by Jaffé reaction (CX3, Beckman, Brea, CA, USA).

Statistical analysis

The relationship between UAER vs. UAC and UACR was calculated by Pearson's correlation coefficients. Sensitivities and specificities of RUS measurements (UAC and UACR) as a screening test for microalbuminuria were calculated using normo- and microalbuminuric samples (n=80) and for macroalbuminuria using micro- and macroalbuminuric samples (n=55). The ROC curve method was used to analyze the performance of the screening test. The true-positive rate (sensitivity) versus the false-positive rate (100-specificity) was plotted for each measurement. Sensitive tests are helpful in screening people without complaints, as is the case in the early stages of diabetic nephropathy. Thus, the first point with a sensitivity of 100% was chosen in each curve. A second cutoff point was also determined in each curve by the intersection of the curves with the 100%-to-100% diagonal. The latter point represented the best equilibrium between sensitivity and specificity.

Table 1. Clinical Characteristics of Patients (n=105)

Median age (yrs)	59 (13–85)
Sex (M : F)	52 : 53
Duration of diabetes (yrs)	11.5 ± 7.9
Hypertension history (%)	18.1 (19/105)
Body mass index (kg/m ²)	22.5 ± 2.8
Fasting glucose (mg/dl)	155.5 ± 57.1
HbA1c (%)	10.1 ± 2.2 (%)
According to UAER in 24hour urine	
-normoalbuminuria (<20 µg/min)	50
-microalbuminuria (20–200 µg/min)	30
-macroalbuminuria (>200 µg/min)	25

Values are means ± S.D. UAER, Urine albumin excretion rate.

The statistical analyses of ROC curves were performed, and the differences in results between UAC and UACR were compared by McNemar test. As for the evaluation of reproducibility in a RUS, the relationship between repeated measurements of UAC or UACR in a RUS was calculated by Pearson's correlation coefficients.

RESULTS

Clinical characteristics of patients

The study was performed in 105 DM outpatients (male : female, 52 : 53), ages 13–85 years (median 59), with 11.5 ± 7.9 years (mean ± SD) of known diabetes duration, 22.5 ± 2.8 kg/m² of BMI, 155.5 ± 57.1 mg/dl of fasting blood glucose, 10.1 ± 2.2% of HbA_{1c}, 18.1% of the prevalence of hypertension in subjects (19/105). According to the timed UAER measured in the 24hour collection (criterion standard), samples were classified as normoalbuminuric (UAER < 20 µg/min; n=50), microalbuminuric (UAER 20–200 µg/min; n=30), and macroalbuminuric (UAER > 200 µg/min; n=25) (Table 1).

Correlation of 24 h UAER vs. UAC and UACR in RUS

Pearson's coefficient of correlation between 24hour UAER vs. UAC was 0.81 ($p < 0.001$) (Fig. 1), and that between 24 hour UAER vs. UACR was 0.75 ($p < 0.001$) (Fig. 2).

The cutoff points of microalbuminuria and macroalbuminuria in RUS by ROC curves

To determine the cutoff points for screening of

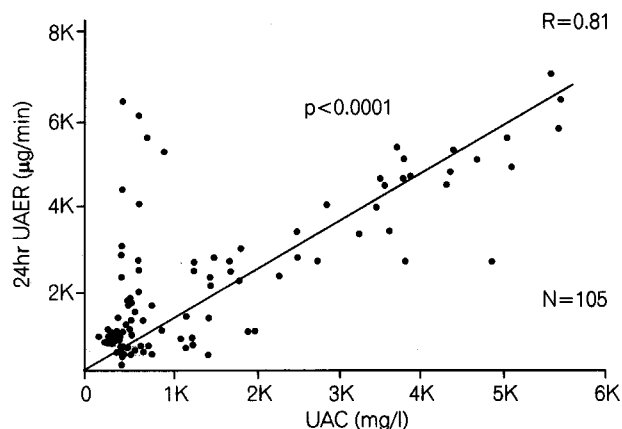


Fig. 1. Pearson's correlation between 24 hour urine albumin excretion rate (24 hr UAER) and random urine albumin concentration (UAC).

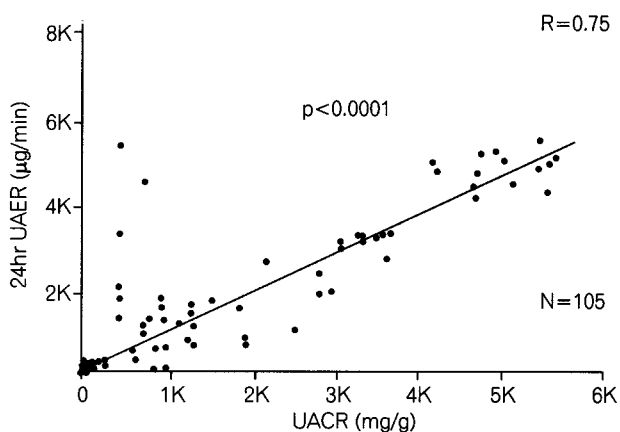


Fig. 2. Pearson's correlation between 24 hour urine albumin excretion rate (24 hr UAER) and random urine albumin:creatinine ratio (UACR).

micro- and macroalbuminuria in a RUS, the ROC curve method was used. The nearest points to the intersection of the curves with the 100%-to-100% diagonal for microalbuminuria were as follows: 31.0 mg/l for UAC and 32.5 mg/g for UACR (Fig. 3), and those for macroalbuminuria were 181 mg/l for UAC and 287.3 mg/g for UACR, respectively (Fig. 4).

The characteristics of cutoff points

Table 4 presents the characteristics of the cutoff points for screening of micro- and macroalbuminuria. The sensitivity and specificity of the cutoff points for microalbuminuria were 77% and 82% for UAC (31.0 mg/l) and 77% and 92% for UACR (32.5 mg/g). Those for macroalbuminuria were 84% and 90% for UAC (181 mg/l) and 88% and 90% for UACR (287.3 mg/g) (Table 2).

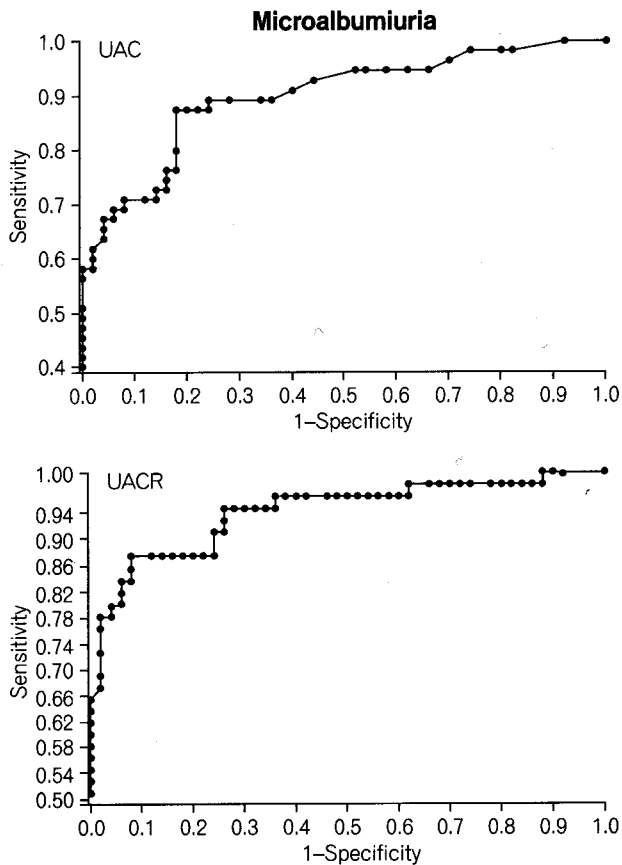


Fig. 3. Cutoff points of urine albumin concentration (UAC) & urine albumin: creatinine ratio (UACR) for microalbuminuria from receiver operating characteristics (ROC) curves.

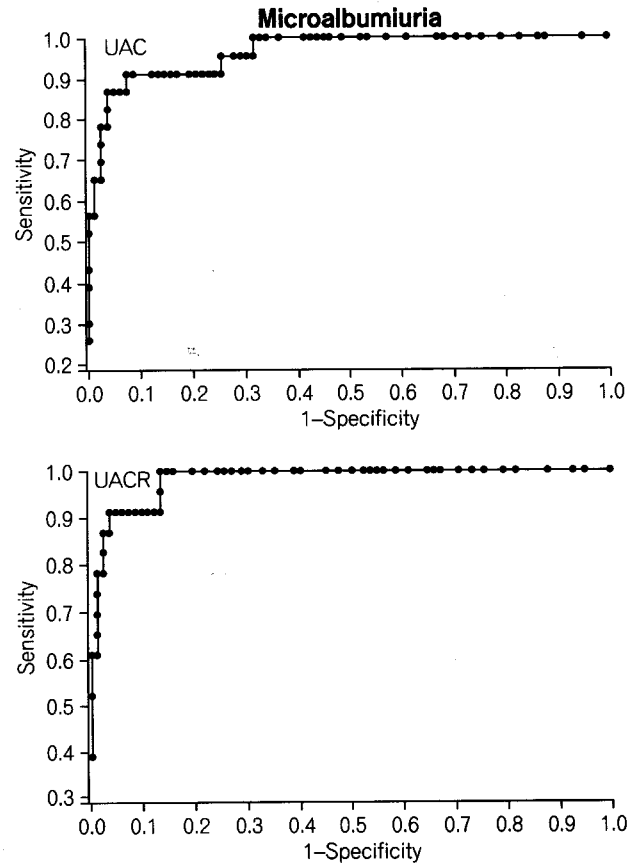


Fig. 4. Cutoff points of urine albumin concentration (UAC) & urine albumin: creatinine ratio (UACR) for macroalbuminuria from receiver operating characteristics (ROC) curves.

Fig. 3 and Fig. 4 depict the ROC curves for UAC and UACR as a screening test for microalbuminuria and macroalbuminuria. The cutoff points for screening of micro- and macroalbuminuria in RUS were determined as follows: 31.0 mg/l of UAC and 32.5 mg/g of UACR for microalbuminuria, and 181 mg/l of UAC and 287.3 mg/g of UACR for macroalbuminuria. The results between UAC and UACR were not different statistically by McNemar test ($p=0.617$, $p=0.157$ for micro- and macroalbuminuria, respectively).

The concordance rates between the classification according to UAER and UAC were 82% in the normoalbuminuria group, 53% in the microalbuminuria group, and 80% in the macroalbuminuria group (Table 3). The concordance rates between the classification according to UAER and UACR were 92% in the normoalbuminuria group, 63% in the microalbuminuria group, and 88% in the macroalbuminuria group (Table 4). The repeated measurements of UAC and UACR in the same individual for the evaluation of the reproducibility were statistically correlated with each other (Pearson's coefficient of

Table 2. Sensitivity and Specificity of UAC and UACR in Microalbuminuria and Macroalbuminuria According to the Cutoff Points Based on ROC Curves

	Microalbuminuria		Macroalbuminuria	
	UAC	UACR	UAC	UACR
Cutoff point	31.0 (mg/l)	32.5 (mg/g)	181 (mg/l)	287.3 (mg/g)
Sensitivity	76.7%	76.7%	84%	88%
Specificity	82%	92%	90%	90%

UAC, Urine albumin concentration in random urine specimen; UACR, Urine albumin : creatinine ratio in random urine specimen.

correlation, 0.653 for UAC and 0.533 for UACR $p < 0.001$).

DISCUSSION

The significance of microalbuminuria is the pre-

Table 3. Concordance Rate between UAC in RUS and UAER in 24 Hour Urine

		By UAC in RUS		
		Normo-albuminuria	Micro-albuminuria	Macro-albuminuria
U	Normoalbuminuria	41	9	0
A	Microalbuminuria	11	16	3
E	Macroalbuminuria	0	5	20
R	Subtotal	52	30	23
				total 105

normoalbuminuria: $41/50=82\%$, microalbuminuria: $16/30=53\%$, macroalbuminuria: $20/25=80\%$.

Table 4. Concordance Rate between UACR in RUS and UAER in 24 Hour Urine

		By UACR in RUS		
		Normo-albuminuria	Micro-albuminuria	Macro-albuminuria
U	Normoalbuminuria	46	4	0
A	Microalbuminuria	8	19	3
E	Macroalbuminuria	0	3	22
R	Subtotal	54	26	25
				total 105

normoalbuminuria: $46/50=92\%$, microalbuminuria: $19/30=63\%$, macroalbuminuria: $22/25=88\%$.

dictor of clinical proteinuria and chronic renal failure in IDDM,^{3,6,25} and the early index of cardiovascular morbidity and mortality as well as diabetic nephropathy in NIDDM.²⁶⁻²⁹ Therefore, it is important to assess the validity of a RUS as a screening test of diabetic nephropathy.^{15,18,30,31}

In this study, UAC and UACR measured in a RUS showed an excellent performance as a screening test for the diagnosis of both micro- and macroalbuminuria. UAC and UACR presented a strong correlation with the 24 hour UAER, confirming data from other authors. In one study where 25 diabetic patients were evaluated, albumin measured in single-void urine samples and expressed as $\mu\text{g}/\text{mg}$ creatinine had an excellent correlation with 24 hour UAER ($r=0.80$).²⁵

In another study, albumin $\mu\text{g}/\text{ml}$ was determined in 94 single-void random upright urine collections from patients with diabetes and correlated well with 24 hour UAER ($r=0.79$).¹⁴

In this study, the accuracy of UAC and UACR analyzed by area under the ROC curves was almost perfect for the screening of micro- and macroalbuminuria because the observed values varied from 0.820 to 0.940.

In another study, the investigators analyzed UAC and UACR in a timed overnight-urine collection and observed that the UACR outperformed UAC in detecting a UAER of 30 $\mu\text{g}/\text{min}$.²¹ Also in this study,

the areas under the curves were not calculated and the comparison between the curves was performed only by visual inspection. In present study, no difference was observed when comparing the performance of UAC and UACR based on a statistical comparison by McNemar test.

The estimated area under the fitted smooth curve ranges from 0.5 (no apparent accuracy) to 1.0 (perfect accuracy) as the ROC curves move toward the left and top boundaries of the ROC graph. The selection of the best diagnostic test is based on the statistical comparison of measurements of the area under the curve.^{22,23} The ROC curve allows for the comparison of the sensitivity and specificity of a test over a wide range of cutoff points and the selection of the best diagnostic criterion for that test. Two criteria were used for the selection of cutoff points to diagnose micro and macroalbuminuria: the first point with 100% sensitivity and the point that represents the best equilibrium between sensitivity and specificity. According to the latter criterion, the observed values of UACR (32.5 mg/g for the diagnosis of microalbuminuria and 287.3 mg/g for the diagnosis of macroalbuminuria) were very similar to the UACR values of 30 and 300 mg/g , respectively, as recommended by the American Diabetes Association in a recent consensus statement.¹⁰ The repeated measurements of UAC and UACR in the same individual for the evaluation of reproducibility were statistically cor-

related with each other (Pearson's correlation analysis, $p < 0.001$).

In conclusion, albumin measurements (UAC and UACR) in a RUS presented almost perfect accuracy for the screening of micro- and macroalbuminuria in diabetic patients, and UAC measured in a RUS was simpler and less expensive than UACR and UAER. It is suggested as a valid test in screening for diabetic nephropathy.

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