Screening for Microalbuminuria

A comparison of single sample methods of collection and techniques of albumin analysis

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OBJECTIVE — To evaluate single-sample urine collections to determine their ability to screen patients for the presence of microalbuminuria. Microalbuminuria in patients with type I diabetes predicts the development of diabetic renal disease.

RESEARCH DESIGN AND METHODS — Cross-sectional analysis of single-sample urine collection techniques (first morning void, random upright void) and methods of albumin analysis (RIA, reagent tablet) were compared with conventional 24-h urine collections (RIA). The study included 94 patients (45 males, 49 females; mean serum creatinine 88 μM) with type I diabetes, selected from a screened population of 301 patients from the University Hospital Subspecialty Clinics.

RESULTS — A 24-hour urine collection RIA analysis for albumin revealed 36 normal patients (<30 mg), 27 with microalbuminuria (30–300 mg), and 31 with albuminuria (>300 mg). Random upright urine samples were more sensitive (RIA 89%, tablets 78%) for the detection of microalbuminuria than first morning void specimens (RIA 70%, tablets 60%). Specificity was >80% with both random and first morning voids.

CONCLUSIONS — Screening for microalbuminuria can be performed in the clinic by random upright single-sample urine collections. When reagent tablets are used, these results are available immediately. Patients who screen positive should be confirmed by 24-h or other timed urine collections.

The presence of small quantities of albumin (30–140 or 15–150 μg/min, 30–300 mg/24 h) in the urine of patients with type I diabetes is termed microalbuminuria (1–3). This condition may signal the development of diabetic nephropathy (1–6). It has been suggested that, at this early stage of proteinuria, intervention with rigid glucose control, protein restriction, and angiotensin converting enzyme inhibitors may prevent the progression of diabetic nephropathy (4–14). These interventions may be much less successful if applied later in the course of the disease (4,5). Therefore, accurate and simple methods of screening for microalbuminuria in the diabetic population to identify patients at risk are very important. This study was designed to evaluate and compare single-sample methods for detection of microalbuminuria.

RESEARCH DESIGN AND METHODS

We screened 301 diabetic patients from the Diabetes and Renal clinics of the Duke University Medical Center by review of previous laboratory records and by analysis of urine samples obtained in the clinic. We enrolled 105 patients (35 probable normals, 35 probable microalbuminuria, 35 albuminuria) in the study to determine the best method of screening for microalbuminuria. Each patient provided a 24-h urine sample collected the day before his or her clinic visit. A first morning void spot urine sample was obtained the day of the clinic visit, and a random upright spot urine sample was obtained in the clinic (late morning and afternoon samples). Patients were instructed to avoid strenuous exercise before providing samples. Urine samples were analyzed immediately or were frozen for later analysis.

Of the 105 patients entered in the study, 11 were excluded from the final analysis because of inability to collect an adequate 24-h urine specimen (judged by adequate creatinine in the sample), despite multiple attempts. Characteristics of the 94 patients completing the trial included: a mean age of 44 ± 7 yr.; 25 patients were black, 67 patients were white; 45 patients were male, 49 were female; serum creatinine ranged from

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TYPE I DIABETES, INSULIN-DEPENDENT DIABETES MELLITUS; RIA, RADIOIMMUNOASSAY.

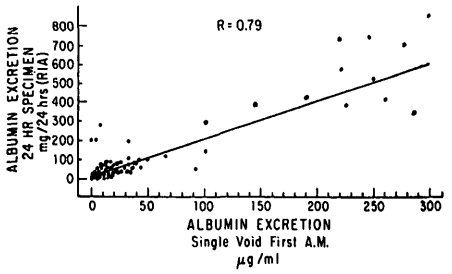

Figure 1—*Comparison of 24-h urine albumin excretions with single-void random upright urine collections. Values >800 mg albumin/24 h not shown.*

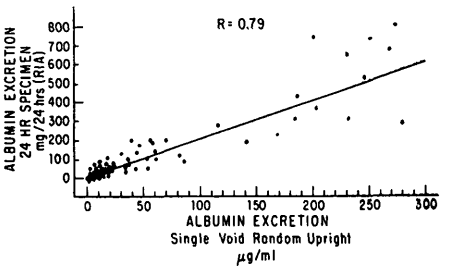

Figure 2—*Comparison of 24-h urine albumin excretions with single-first morning urine collections. Values >800 mg albumin/24 h not shown.*

44–273 μM (0.5–3.1 mg/dl); mean serum creatinine was 88 ± 18 μM (1.0 ± 0.2 mg/dl). The 24-h samples were considered adequate if they provided 10 mg of creatinine/kg ideal body weight for females or 15 mg of creatinine/kg ideal body weight for males.

For the purpose of this study, normal subjects were defined as exhibiting <30 mg of albumin in a 24-h urine collection. Microalbuminuria was defined as 30–300 mg of albumin in a 24-h sample, with albuminuria >300 mg in a 24-h urine sample.

All urine samples were analyzed for albumin by RIA (Sigma Diagnostics Assay Kit, St. Louis, MO), reagent tablets (Micro-Bumin Test, Miles, Elkhart, IN), and autoanalyzer for albumin (modified colorometric technique Beckman 700 autoanalyzer, or Cobas Fera autoanalyzer with confirmation by manual sulfasalicylic technique). In addition, urine samples were assayed by means of dipstick reagent (Miles). Microalbuminuria reagent tablets were analyzed as per recommendations from the manufacturer. One drop of urine was placed on each tablet followed by two drops of distilled water. Each preceeding drop should be completely absorbed before the next drop is placed. The test should be read as soon as the final drop is absorbed. Analysis of color reaction using a color chart allowed determination of albumin concentration as negative (no color change), microalbuminuria (light blue-green color), or albuminuria (dark blue-green color). False-positive results can occur in alkaline urine or if the urine contains skin cleansers such as chlorhexidine. All RIAs, urine dipstick analyses, and analyses of microalbuminuria reagent tablets were performed by a single laboratory technician.

RESULTS— Single sample urine determination of albumin by RIA correlated well with 24-h RIA samples (r = 0.79 random upright, r = 0.79 first morning void) (Fig. 1 and 2). Measurement of albuminuria by reagent tablet also correlated well (r = 0.79 random upright, r = 0.74 first morning void) with 24-h urine collections analyzed by RIA. Based on 24-h urine collections and RIA analysis for albumin, 36 patients were considered normal, with <30 mg albumin excreted in 24 h, 27 patients had microalbuminuria (30–300 mg/24 h), and 31 patients had albuminuria (>300 mg/24 h) (Table 1).

When RIA single-sample specimens were used to detect microalbuminuria, values that provided the best correlation with 24-h collections were adopted (20–150 μg/ml single sample = 30–300 mg 24-h specimen). Single-sample urine specimens with concentrations of albumin <20 μg/ml were considered insignificant albuminuria. Quantities of 20–150 μg/ml of albumin reflected probable microalbuminuria; >150 μg/ml reflected albuminuria. Tablet analysis was performed as outlined in METHODS. Using these values, Table 1 shows the distribution of albuminuria in the tested population by method of albumin analysis and sample collection technique. As reported previously, there was a tendency for first morning voids and overnight collections to underestimate albuminuria and for random voids to slightly overestimate albuminuria compared with 24-h urine specimens (15,16). This observation may explain the loss of sensitivity and decrease in correlation of reagent tablets when used on first morning voids. When only patients with microalbuminuria were

Table 1—***Patients determined to have albuminuria by various sampling methods***

	NORMAL	MICROALBUMINURIA	ALBUMINURIA
24-H URINE SAMPLE	(<30 MG/24 H RIA)	(30–300 MG/24 H RIA)	(>300 MG/24 H RIA)
	36	27	31
RANDOM UPRIGHT SINGLE SAMPLE	(NEGATIVE MICROTAB)	(TRACE-+1 MICROTAB)	(+2 MICROTAB)
	34	24	36
	(<20 MG/ML RIA)	(20–150 MG/ML RIA)	(>150 MG/ML RIA)
	32	28	34
FIRST MORNING VOID SINGLE SAMPLE	(NEGATIVE MICROTAB)	(TRACE +1 MICROTAB)	(+2 MICROTAB)
	42	20	32
	(<20 MG/ML RIA)	(20–150 MG/ML RIA)	(>150 MG/ML RIA)
	40	22	32

compared, the correlation coefficient between spot urine and 24-h urine albuminuria decreased (RIA, random upright $R = 0.75$, first morning $R = 0.74$) (reagent tablets, random upright $R = 0.56$, first morning $R = 0.46$). However, the clinical utility is best expressed in the distribution of patients identified correctly (Table 1), especially in the case of the tablets, which are not a continuous variable but are negative, microalbuminuria, or albuminuria.

Albumin dipstick analysis was used to confirm albuminuria (>300 mg/24 h). When either spot method predicted albuminuria (RIA >150 μg/ml or 2+ reagent tablets) and was confirmed by a dipstick measure of 1+ or greater, overt albuminuria was determined. When spot microalbuminuria methods detected albuminuria and the dipstick was negative, the albumin concentration was <600 mg/24 h, but a timed specimen was required to determine if microalbuminuria was present. There were no instances of a negative microalbuminuria spot sample and a dipstick positive for albuminuria.

Random upright RIA-determined spot specimens were 89% sensitive and 85% specific for microalbuminuria. Random upright specimens analyzed by reagent tablets were 78% sensitive and 89% specific. First morning voids were similarly analyzed (RIA 70% sensitive, 93% specific; reagent tablets 60% sensitive, 81% specific).

CONCLUSIONS — Microalbuminuria is thought to be the first indicator of diabetic renal disease (1–5). It is useful in identifying those patients predisposed to diabetic renal injury. Several studies have suggested that microalbuminuria and perhaps diabetic renal disease in general may be reversible if detected and treated early (4,5). The importance for both prognostic and therapeutic purposes of identifying a reliable method of screening the patients at risk for microalbuminuria is readily apparent. The gold standard for analysis of microalbuminuria continues to be timed urine collections with quantitation of the albumin present. When these are positive on two or more occasions, microalbuminuria probably is present. However, timed specimens, including 24-h urine collections, are notoriously difficult to obtain when performed at home. They frequently are inadequate or overadequate collections, and patients are unwilling to obtain these specimens on a frequent basis. When performed in the office, they are labor and time intensive. For these reasons, it is important to find a mechanism of screening the population at risk to select those patients who need definitive testing.

Several authors have advocated shortened timed clearances to alleviate the problems of 24-h urine collections (4,5,14). Brodows et al. (17) recommended 3-h collections, and the Steno Group used 4-h collections (5). Viberti et al. (1) used overnight collections, and Sochett and Daneman (18) found 1-h timed collections satisfactory. Because of difficulties with timed urine collections, multiple authors have attempted to develop a single sample screening technique. Nathan et al. (19) reported a high correlation with single-void urine samples when albumin-to-creatinine ratios were used. Ellis et al. (20) evaluated multiple techniques and recommended screening by repetitive testing with albumin-to-creatinine ratios in single-void urine samples as an alternative for timed urine collections. Problems with all these collection techniques, given the high volume of patients seen in our outpatient clinics, led us to seek simpler techniques of screening for microalbuminuria.

We previously have found single-sample urine specimens to be quite useful for detection of albuminuria (21). Coonrod et al. (22), Linton and Rowe (23), and Cowell et al. (24) have found spot screening tests to provide adequate specificity and sensitivity for detection of microalbuminuria. In contrast, Kouri et al. (25) found spot specimens to be of limited value.

The goal of a screening test is to be inexpensive, easy to perform, and accurate, while minimizing the number of false-negatives so patients with the condition are not excluded from more intensive testing. We believe this goal is best met in screening for microalbuminuria by using spot specimens, which are easily obtained in the office. Combining albumin and creatinine in the same specimen meets these criteria and probably improves accuracy but requires waiting for the return of both laboratory tests. For these reasons, we feel it is less attractive as a screening test than an instant result test such as a reagent tablet. Selecting a suitable screening test is difficult, and some trade-offs of accuracy for convenience will be made as outlined in the review by Marshall (26). The most accurate tests (timed collections) are the most difficult and time-consuming to perform. Spot or single-sample tests are less accurate, and, to achieve their screening goal, they tend to be weighted toward false-positive errors. The clinician must determine which spot samples are accurate enough to serve as a suitable screen. We believe that spot random upright urine specimens analyzed by reagent tablets and dipstick meet the criteria for a good screening test with the results immediately available.

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