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Comparison of methods for urinary albumin determination in patients with type 1 diabetes

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Abstract

We tested the correlation of the albumin-to-creatinine ratio (A/C) in an early-morning urine sample, measured with a commercial kit (DCA 2000®), with the conventional immunoturbidimetric determination in the laboratory and with overnight albumin excretion rate (reference method). Fifty-five type 1 diabetic adolescents had their first-morning urine collected on the 1st and 8th day of the period. Urinary albumin and creatinine were determined immediately using the DCA 2000® kit. Samples were also stored for laboratory analysis. To evaluate the correlation between early-morning urinary A/C ratio and overnight albumin excretion rate, 16 subjects had a timed overnight urine collection. A/C ratios determined with the DCA 2000® kit and by the laboratory method were 13.1 ± 20.5 and 20.4 ± 46.3 mg/g, respectively. A/C results by both methods proved to be strongly correlated ($r = 0.98$, $P<0.001$). DCA 2000®-determined A/C showed 50% sensitivity and 100% specificity when compared to the reference method. Spot urinary A/C of the subset of 16 subjects significantly correlated with their overnight albumin excretion rate ($r = 0.98$, $P<0.001$). Intraindividual variation ranged from 17 to 32% and from 9 to 63% for A/C and overnight albumin excretion rate, respectively. In conclusion, an early-morning specimen should be used instead of timed overnight urine and the A/C ratio is an accurate, reliable and easily determined parameter for the screening of diabetic nephropathy. Immediate measurement of the A/C ratio is feasible using the DCA 2000® kit. Intraindividual variability indicates the need for repeated determinations to confirm microalbuminuria and the diagnosis of incipient diabetic nephropathy.

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• Screening

Introduction

Diabetic nephropathy (DN) is the most frequent single cause of end-stage renal disease in many countries (1). In the São Paulo metropolitan area, DN is the third known cause of admission to end-stage renal disease programs (2). A proportion of genetically predisposed diabetic subjects will develop renal complications, especially if metabolic and environmental risk factors are associated (3,4). The cumulative risk of DN in type 1 diabetes is estimated at 30-40% after 15 years (5). The National Kidney Founda-

tion (6) recommends that diabetic subjects >12 years have their urine tested for albumin excretion once a year.

Hyperfiltration, microalbuminuria (incipient DN) and macroproteinuria (overt DN) characterize the clinical stages of DN. Sensitive assays for protein detection in urine such as radioimmunoassays and immunoturbidimetry have facilitated the characterization of microalbuminuria, defined as urinary albumin excretion (UAE) between 20 and 200 µg/min, which is potentially reversible with certain therapeutic measures (7). Microalbuminuric diabetic patients, particularly those with type 1 diabetes, are at high risk for progression to overt DN when compared to those with UAE <20 µg/min (8,9). This cutoff was established on the basis of prospective studies (10,11) in which UAE >20 µg/min was found to be a strong predictor of disease progression. In addition to the association with retinopathy (12,13), microalbuminuria has also been considered to be an independent risk factor for coronary heart disease and cardiovascular mortality (14). The prevalence of persistent microalbuminuria in children and adolescents with type 1 diabetes has been approximately 4-8% in cross-sectional studies (15,16), but substantially higher frequencies have also been reported (17).

The properties of the ideal type of sample to be used for UAE determination are still controversial. The "gold" standard requires timed overnight or 24-h urine collections that are cumbersome and subject to timing and collection inaccuracies. Approximately 30% of the urine collected needs to be recollected due to procedural errors (18). Even in timed collections, high intraindividual variability of UAE up to 50% is found (19). Physical activity, dietary protein content and blood glucose control may account for the variability (20). Confirmation of increased UAE in repeated 24-h or overnight urine samples is recommended to diagnose DN.

A strong correlation between creatinine-adjusted albuminuria in random samples and 24-h albumin excretion rate has been reported (21). An albumin-to-creatinine ratio (A/C) of 30 mg/g creatinine has 100% sensitivity and specificity for microalbuminuria diagnosis. Sterile urine as well as lack of physical activity - that may transiently elevate UAE - are required for an adequate interpretation of this result (22). Creatinine adjustment of the albumin concentration in random urine samples, glycemic stability and resting minimize UAE variability. Therefore, the A/C ratio obtained in early-morning specimens should be an appropriate index for the screening of microalbuminuria due to easy collection, low cost and high sensitivity. Assessment of the A/C ratio has been widely used (21,23), although some investigators disagree regarding the advantages of concomitant determination of urinary creatinine levels (24).

In order to achieve the greatest benefits in slowing the progression of incipient DN, intensive control of blood glucose (25,26) and blood pressure (27) must be instituted early. DN screening procedures should be accurate and simple enough to facilitate their large-scale use. Reagent strips have the advantage of offering immediate, although semiquantitative, results. A new test for immediate and quantitative determination of the A/C ratio, the DCA 2000® system, has shown to have high accuracy for the detection of microalbuminuria (28), and may represent an interesting alternative for DN screening in populations or even in clinical settings.

The objectives of the present study were: a) to evaluate the accuracy of albuminuria (A/C ratio) determined with a commercial kit (DCA 2000® microalbumin/creatinine assay) as compared with the conventional immunoturbidimetric laboratory method (reference method); b) to analyze the concordance of the urinary albumin values obtained under two different conditions of urine

collection (early-morning urinary A/C ratio and overnight albumin excretion rate).

Patients and Methods

The urine samples analyzed in this study were obtained from type 1 diabetic subjects who attended an 8-day educational summer camp organized by the Federal University of São Paulo and Juvenile Diabetes Association. The study was approved by the Institutional Ethics Committee and informed consent was obtained from the parents of the participants. Sixty type 1 diabetic adolescents aged 13-24 years were initially invited to participate in at least one of the study protocols. Inclusion criteria were glycated hemoglobin <9% and fasting capillary glycemia ≤180 mg/dl on the day of urine collections. None of them suffered from any other major disease apart from diabetes and none took any medication other than insulin. All subjects had normal blood pressure and funduscopy and none showed symptoms or signs of diabetic neuropathy. Clinical nephropathy was first excluded by quantitative analysis. During the camp period, urine samples were retested for the presence of protein and cells using a dipstick technique (Multistix®, Bayer Laboratories, Leverkusen, Germany). Blood cells in urine were interpreted as possible urinary tract infection and subjects with this feature were excluded from the protocol. The educational program included an appropriate diet according to American Diabetes Association recommendations, twice daily exercise, capillary glucose monitoring (Advantage® reflectance meter, Roche Laboratories, Indianapolis, IN, USA), at least four times a day and intensified insulin therapy. The mean value of daily blood glucose was used to monitor daily glycemic control.

Of the 60 subjects initially selected for the study, 3 girls were excluded due to menses and 2 subjects due to unstable glycemic control. Fifty-five subjects (31 girls and 24 boys) had their first-morning urine collected on the 1st and 8th day of the period. Urinary albumin and creatinine were determined immediately using the DCA 2000® microalbumin/creatinine assay system (Bayer Diagnostica, Barcelona, Spain). Samples of these urine specimens were also stored at -20ºC up to 8 days until laboratory analysis by an immunoturbidimetric assay.

Results are reported as urinary A/C (mg/g) ratio. In order to evaluate the correlation between the early-morning urinary A/C ratio and the overnight UAE rate (reference method), 16 male subjects (15.6 ± 2.2 years) also had a timed overnight urine collection and samples stored for further determinations. A laboratory immunoturbidimetric assay was used for albumin and creatinine determinations and the results are reported as A/C ratio (mg/g) and albumin excretion rate (µg/min). Intraindividual variation of UAE was assessed in 8 of the 16 subjects, who had overnight (albumin excretion rate) and early-morning urine collections (A/C ratio) for 7 consecutive days.

Urinary albumin determined in the laboratory was measured in duplicate by an immunoturbidimetric assay (UNIMATE 3 ALB®, Roche, São Paulo, SP, Brazil). The intra-assay and interassay coefficients of variation of this method were 3.1 and 5.0%, respectively. Creatinine was determined by a kinetic procedure using Jaffe's alkaline picrate method. The DCA 2000® kit was also used to measure albumin, creatinine and hemoglobin A1c. The microalbumin/creatinine system detects albumin by immunoturbidimetric direct antibody-antigen aggregation and measures creatinine colorimetrically using the Benedict-Behre reaction. Specific hemoglobin A1c was determined by the inhibition of a latex agglutination assay.

Statistical analysis was performed using the SPSS software package (version 8.0). Correlation between methods of albumin determination (conventional laboratory method and by the DCA 2000® system) and between different urine collection procedures

(overnight and early-morning urine samples) was tested according to the Pearson correlation coefficient (95% confidence intervals) and confirmed by the interclass coefficient. Intraindividual variation of UAE during the 7-day consecutive collections was obtained by the division of standard deviation by the mean value of the A/C ratio. Since no sex-related difference was observed in the correlation analysis, data from both sexes were combined before analysis. The sensitivity and specificity of the A/C ratio in the early-morning urine were estimated using the timed overnight UAE rate as the reference method (1). The cutoff value was 30 mg/g for an abnormal A/C ratio and 20 µg/min for albumin excretion rate (1). The kappa statistic was used to assess the concordance between two methods and two urine collection conditions. Data are reported as means ± SD, with the level of significance set at $P<0.05$.

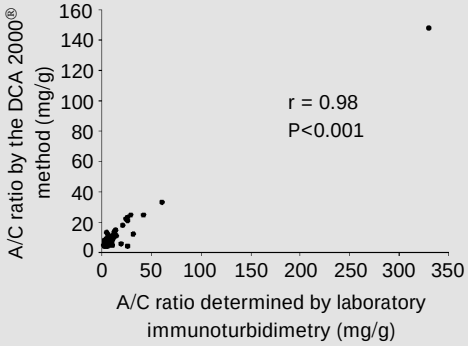

Figure 1. Correlation of the albumin-to-creatinine ratio determined by the DCA 2000® system and by the laboratory method for 55 subjects.

Table 1. Patients classified as normo- (<30 mg/g) or microalbuminuric (30-300 mg/g) on the basis of two different methods of urinary protein determination.

DCA 2000®	Laboratory method		Total
	Microalbuminuria	Normoalbuminuria	
Microalbuminuria	2	0	2
Normoalbuminuria	2	51	53
Total	4	51	55

Results

The 55 subjects submitted to UAE determinations by the DCA 2000® and conventional laboratory method were 16.2 ± 2.3 years old, with a mean diabetes duration of 6.6 ± 4.8 years, BMI of 21.1 ± 3.4 kg/m^2 and systolic/diastolic blood pressure of 107.5/67.3 ± 23.1/9.0 mmHg. Their mean glycated hemoglobin was 8.8 ± 1.9, capillary glucose 117.1 ± 41.0 mg/dl, and the A/C ratio 13.1 ± 20.5 and 20.4 ± 46.3 mg/g, by the DCA 2000® system and by the laboratory method, respectively. Two of 4 microalbuminuric subjects identified by the laboratory method were also microalbuminuric by the DCA 2000® method. Sensitivity and specificity of the DCA 2000® determinations were 50 and 100%, respectively. A/C ratio results by both methods (DCA 2000® and laboratory) were strongly correlated ($r = 0.98$, 95% CI 0.96-0.99, $P<0.001$) (Figure 1) as also shown by the interclass correlation coefficient ($r = 0.98$, 95% CI 0.96-0.99, $P<0.001$). Correlations did not show sex-related differences (data not shown). The DCA-determined A/C ratio showed 50% sensitivity and 100% specificity when compared to the reference method (Table 1). The concordance between different laboratory methods for A/C ratio determination was estimated by kappa statistics ($\kappa = 0.52$, $P<0.05$).

The spot urinary A/C ratio of the subset of 16 subjects (Table 2) correlated significantly with their overnight albumin excretion rate ($r = 0.98$, 95% CI 0.94-0.99, $P<0.001$) (Figure 2). Kappa statistics showed a 0.80 concordance between the two urine collection conditions ($P<0.01$).

Eight of the 16 subjects who had the first specimen collected consecutively for 7 days showed similar initial and final fasting blood glucose (137 ± 45 *vs* 145 ± 38 mg/dl). A lower intraindividual variation of A/C ratio (17 to 32%) was observed when compared to overnight albumin excretion rate (9 to 63%) among such patients.

Discussion

Numerous studies that emphasized the importance of the diagnosis of microalbuminuria not only as a risk marker for DN, but also as a risk factor for macrovascular disease, were based on the detection of UAE in timed overnight or 24-h urine collection (8,14). More recent studies have shown that early-morning urinary A/C ratio, used for screening purposes, is also a predictor of overt DN, being useful to identify patients at high risk (29). Even in type 1 diabetic children and adolescents, a repeated high-normal A/C ratio during the first years of disease has been shown to predict persistent microalbuminuria in prospective studies (30). The present finding of a strong correlation between A/C ratio and the reference method ($r = 0.98$) supports the utility of using a simple procedure to identify high-risk subjects. This result is in agreement with Hutchinson et al. (31) who found a significant correlation ($r = 0.90$) between early-morning urinary albumin concentration and overnight albumin excretion rate. The higher correlation coefficient observed in our study may be due to the fact that albumin values have been adjusted for creatinine concentration. In fact, a further report using receiver operating characteristic curve analysis to determine discriminator values for non-reference methods favored the A/C ratio, since this performed better than albumin concentration in the screening of microalbuminuria (23). Therefore, despite the additional cost of creatinine measurements, the A/C ratio offers several advantages, such as not being influenced by variations in urinary flow rate.

Our data also permit the recommendation of early-morning urine collection when choosing spot urine for screening purposes. A random urine sample could be potentially influenced by exercise and diet, leading to false results. In fact, among spot urine collections, the first-urine sample proved to be best correlated with the reference method in studies focusing on this particular aspect of the procedure. Subjects with other factors shown to interfere with the UAE results, such as urinary tract infection, elevated blood pressure, hydration and poor control of blood glucose levels, were excluded from the study, which increased the reliability of our data. The simplicity of an early-morning urine collection should facilitate the screening of diabetic children and adolescents.

Intraindividual variability was shown to be high with both procedures, i.e., early-morning and overnight urine collections. The percentages found in this study were consistent with values reported previously (19). A smaller range of A/C ratio was observed when compared to albumin excretion rate. Harvey et al. (32) also reported significantly lower variability coefficients for the A/C ratio than for albumin excretion rate (27 *vs* 31%, respectively, $P = 0.035$). This was expected since that adjustment for creatinine minimizes the influence of dietary protein

Table 2. Clinical characteristics of 16 subjects submitted to two procedures of urine collection (spot and overnight specimens) for albumin determination.

Clinical characteristics	Mean ± SD
Age (years)	15.6 ± 2.2
Diabetes duration (years)	7.3 ± 5.6
BMI (kg/m^2)	21.9 ± 2.7
Systolic blood pressure (mmHg)	110.5 ± 14.2
Diastolic blood pressure (mmHg)	68.4 ± 11.1
Glycated hemoglobin (%)	6.64 ± 1.04

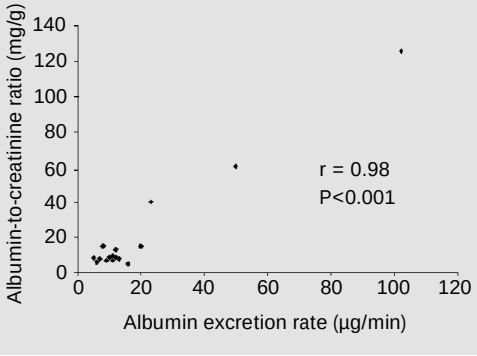

Figure 2. Correlation of spot urinary albumin-to-creatinine ratio and overnight albumin excretion rate determined by the laboratory method of the subset of 16 subjects.

contents and urine dilution. When the albumin concentration is divided by the creatinine levels the number of false-negative subjects tends to decrease (33), a fact that is highly desirable for DN screening. The confirmation of our data regarding smaller intraindividual variation in the A/C ratio, in larger number of subjects could also be helpful when deciding which procedure should be preferred.

Since screening for microalbuminuria is essential to achieve such benefits, it has been recommended as part of the everyday treatment of diabetic patients (34). Therefore, methods providing immediate and reliable results are highly desirable. Based on our findings with the DCA 2000® kit to measure the A/C ratio (reasonable sensitivity and good specificity), we conclude that this may not be an accurate method for the detection of microalbuminuria in field studies or in clinical settings. However, a low sensitivity due to the small size of the sample studied and the low frequency of microalbuminuric subjects cannot be excluded. The strong correlation between the A/C values obtained with the DCA 2000® kit and the laboratory procedure demonstrated in our study is in agreement with other studies (28). The determination performed in fresh urine samples may also have the advantages of lack of storage since it is known that prolonged freezing at -20ºC may decrease albumin immunogenicity and detection in immunoassays (35). However, despite the practical property of the A/C ratio obtained with the DCA 2000® kit, the high cost of this procedure should also be taken into consideration.

We conclude that early-morning specimens should be used instead of timed overnight urine and that the A/C ratio is accurate, reliable and easy for the screening of DN. Immediate measurement of the A/C ratio with the DCA 2000® kit is feasible but may be inappropriate for the diagnosis of the stage of DN. Considerable intraindividual variability indicates the need for repeated determinations to confirm microalbuminuria and the diagnosis of incipient DN.

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